Aquaculture 2025

Innovation Through Technology

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WELCOME

Welcome to AQUACULTURE 2025, the Triennial premier international aquaculture conference. On behalf of the World Aquaculture Society, the National Shellfisheries Association, the Fish Culture Section of the American Fisheries Society, and the National Aquaculture Association, it is my pleasure to welcome you to New Orleans – Laissez les bon temp rouler – let the good time roll.

This year's theme is "Innovation Through Technology." This is a recognition of the importance of the research, development, and commercialization continuum in the sustainable growth of aquaculture for global food security. Dr. John Hargreaves, our notable plenary speaker, will be drawing on his 44 years of international experience in research, teaching, training, and development on projects spanning intensive ponds, biofloc systems, partitioned and recirculating ponds, net pens, raceways, recirculating aquaculture systems, and hatchery and nursery systems for his presentation "Do We Need Innovation to Grow Aquaculture?".

This year's Triennial is one of the largest conferences that we have ever organized with over 1,300 abstracts, 110 scientific and technical sessions, and 210 posters. Our extensive tradeshow vendors are enthusiastic to be here to have the opportunity to engage with all delegates again. Make sure that when you stop by their booths to ask them about their state-of-the-art, innovative products and services.

As always, students will have the chance to shine with Student Spotlight awards and presentations, a field trip to the aquarium, the student-mentor breakfast, NSA sales booth, and the always much anticipated student reception. Both the National Aquaculture Association and the National Shellfisheries Association will be hosting their well-attended auctions full of great prizes and fun.

All good things take a lot of time and effort, including support from our Steering Committee (Jay Parsons, Sandy Shumway, Mick Walsh, Paul Zajicek) and Program Committee (Co-Chairs Jay Parsons and Sandy Shumway, and members Steve Allen, Kathleen Hartman and Dan Mosier). As well, the organization of an event this large would not be possible without the tireless work of John and Noah Cooksey and Mario Stael who ensure the meeting and trade show go smoothly. If you see any of these above dedicated folks as you interact with tradeshow exhibitors, network with colleagues, listen in on a presentation, make sure to express your appreciation and thanks for their devotion to this great event.

And thank you for your support. Participate, meet new and old friends and colleagues, and enjoy the events!

Jay Parsons, PhD

Steering Committee Chair
World Aquaculture Society, Past President

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ABSTRACTS

EFFICACY DETERMINATION OF A DISINFECTANT AGAINST CHANNEL CATFISH VIRUS BY IN VITRO AND IN VIVO METHODS

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Channel catfish virus (CCV) poses a significant threat to catfish culture. Lack of effective vaccines and antiviral treatments necessitates effective disinfection strategies to mitigate its spread. In vitro trials indicated the virus to be inactivated at high temperatures, but was infectious at 40ºC. This study evaluated the efficacy of a commercial disinfectant against CCV using both in vitro and in vivo approaches. In vitro experiments assessed the virucidal activity of the disinfectant against CCV in channel catfish ovary (CCO) cells, while in vivo trials evaluated its effectiveness in reducing viral transmission and mortality among channel and hybrid catfish fingerlings. Results indicated that the disinfectant was effective in inactivating the virus at the tested concentrations and improved the survival of fish exposed to the virus. This study provides critical insights into selecting appropriate disinfection protocols to enhance biosecurity in catfish hatchery settings and to mitigate CCV transmission.

ADAPTIVE RESPONSES OF THE MUCOSAL MICROBIOME TO TEMPERATURE VARIATIONS IN REDBAND TROUT (*Oncorhynchus mykiss gairdneri*) POPULATIONS

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Climate change poses a significant survival challenge for cold water fish species such as the redband trout (*Oncorhynchus mykiss gairdneri*), a subspecies of rainbow trout native to freshwater habitats in the Pacific Northwest of North America. While previous studies have focused on the genetic factors influencing their adaptability to warmer temperatures, our research involves understanding host-microbiome interactions as potential mechanisms contributing to phenotypic variation in response to temperature increases.

This study included two seasonal field samplings (summer and winter) and a common garden experiment where trout from different thermal habitats were acclimated to standardized temperature regimes: constant temperatures at 15 °C, 18 °C, and 21 °C; and diel fluctuations of 13 to 17 °C, 16 to 20 °C, and 19 to 23 °C. We collected mucosal samples from the gills, skin, and digestive tracts of trout, along with sediment and water samples from desert and cold montane creeks, and from the common garden system. To investigate bacterial and archaeal community composition—including assessments of alpha and beta diversity and differential abundance—we employed 16S rRNA gene sequencing and analyzed the data using the DADA2, vegan, ANCOM-BC, and phyloseq packages in R. Nanopore sequencing was used to identify microbial functions and determine the relative abundances of bacterial and nonbacterial sequences. Genotyping by sequencing (GTseq) was utilized to genotype single nucleotide polymorphisms (SNPs) in individual fish, enabling analysis of neutral and adaptive genetic markers relative to their habitats.

Results showed statistically significant differences ($p < 0.05$) in both alpha and beta diversity of gill and skin microbiomes among field samples from different populations. In contrast, common garden samples exhibited no significant differences ($p > 0.05$), suggesting that environment (season and temperature) influences mucosal microbiome compositions.

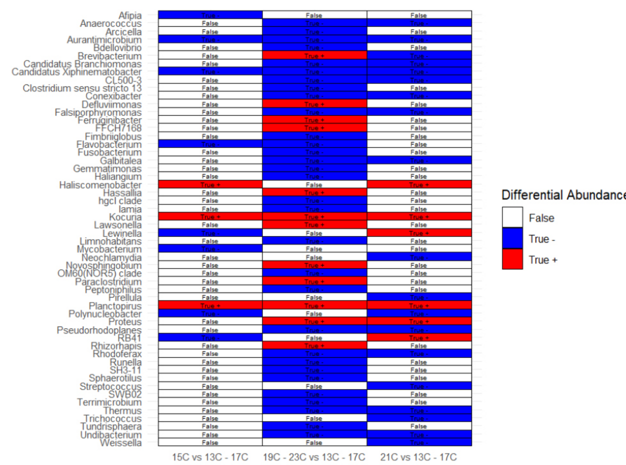

Figure 1: Differential abundance for globally significant (q < 0.05) Genera across acclimation temperature in Common Garden samples

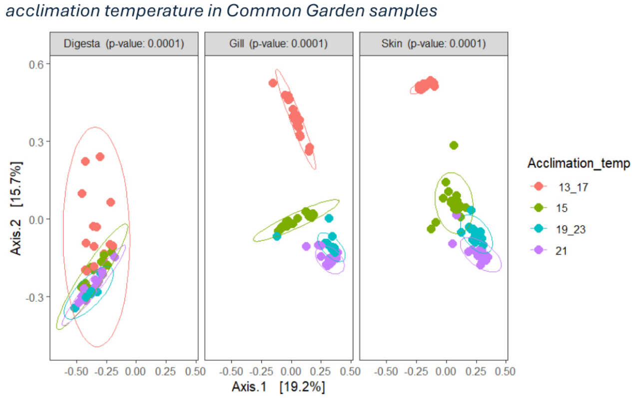

Figure 2: Bray Curtis Beta diversity for Common Garden Microbiome Samples

Future analyses will focus on integrating microbiome and genetic data from both field and common garden samples to elucidate gene-by-environment interactions contributing to the adaptability of redband trout to warmer temperatures.

EVALUATING INCLUSION OF COMMERCIAL PISTACHIO BY-PRODUCT AS A FUNCTIONAL INGREDIENT IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIETS

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The growing demand for sustainable aquaculture requires the exploration of alternative protein sources for fish diets. However, some of these alternatives can have adverse health effects, prompting research into functional feed ingredients to mitigate these issues. This study investigated pistachio shell powder (PSP), a readily available by-product rich in antioxidants, as a potential functional feed ingredient in rainbow trout diets. The effects of PSP inclusion (0%, 0.5%, 1%, and 2%) on the growth performance, intestinal health, and gut microbiota of rainbow trout were evaluated through administration in either a traditional fishmeal (FM) or an ultra-modern plant-meal (PM) based diet.

In a 12-week feeding trial, rainbow trout fingerlings were assigned to experimental diets with varying PSP levels. Growth performance, intestinal morphology, gene expression, total antioxidant capacity (TAC), total phenolic compound (TPC), and gut microbiota composition were assessed.

PSP significantly (P<0.05) improved average weight gain and daily growth index at the 1% inclusion level in FM treatments but did not affect growth in PM treatments. No significant (P>0.05) effects were observed on other growth parameters, intestinal morphology, oxidative stress, or inflammatory gene expression, although a trend toward down-regulation was noted in PM treatments at 2% PSP. Serum antioxidant capacity and phenolic content were unaffected by PSP inclusion, but TPC increased with higher PSP levels in PM treatments. PSP inclusion did not significantly (P>0.05) impact gut microbiota alpha diversity but significantly (P<0.05) altered beta diversity in FM treatments at the 0.5% inclusion level. Although no significant differences were observed in the alpha and beta diversity of gut microbiota of PM treatments, subtle shifts in community composition were observed. Differential abundance analysis revealed taxa-specific responses to PSP, particularly the genus *Candidatus arthromitus* increasing in relative abundance with PSP inclusion in both plant meal and fishmeal-based diets.

Overall, PSP inclusion up to 2% did not negatively impact growth, intestinal health, or antioxidant status. The lack of pronounced effects on gut microbiota and physiological parameters may be attributed to the limited reliance of carnivorous fish on fiber fermentation and complex microbial interactions for energy utilization.

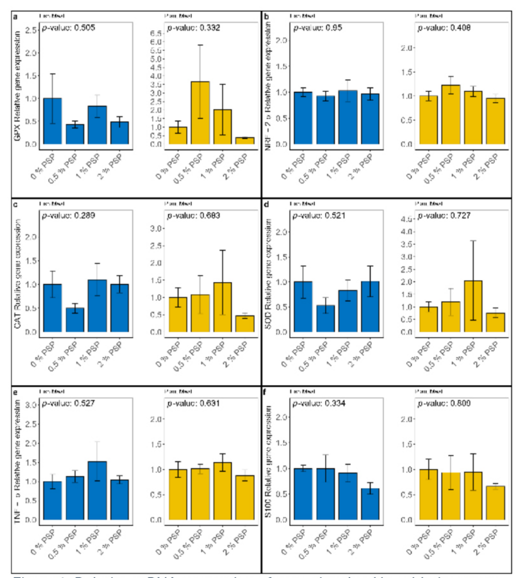

Figure 1: Relative mRNA expression of genes involved in oxidative stress and inflammatory response in the distal intestine of rainbow trout

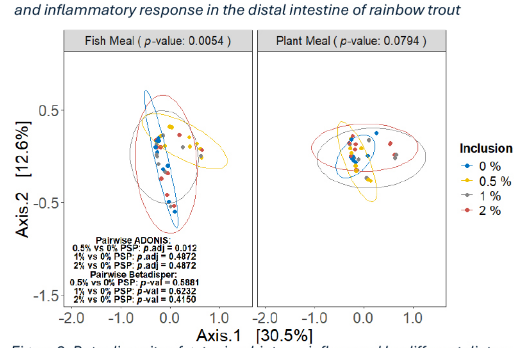

Figure 2: Beta diversity of gut microbiota as influenced by different dietary protein sources and levels of pistachio shell powder (PSP) inclusion

A REVIEW OF BIODIVERSITY AND CHEMICAL CONTAMINANTS IN FISH FROM LAKE NAKIVALE, UGANDA

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Lake Nakivale is a lake in the Western part of Uganda. It is part of the Lake Mburo-Nakivale wetland system. It is located 6km in the western part of Isingiro town. This lake also serves as one of the main water supplies for the district of Isingiro.

Lake Nakivale in Uganda is facing environmental challenges, particularly with chemical contaminants affecting its biodiversity. The lake's water and sediments contain pollutants like heavy metals, pesticides, and microplastics, mainly from industrial activities, agricultural runoff, and improper waste disposal.

Contaminants Found in Lake Nakivale:

Heavy Metals: Lead, cadmium, and mercury have been detected in water and sediments, posing risks to aquatic life and human health

Pesticides: Residues from pesticides like DDT and herbicides have been found in fish and sediments, contributing to ecological disruptions.

Micro plastics: Studies have revealed micro plastics presence in fish and water samples, highlighting concerns about ingestion and food chain contamination.

Impact on Biodiversity: The presence of these contaminants threatens Lake Nakivale's biodiversity, affecting fish populations and other aquatic organisms. The lake's ecosystem is vulnerable to disruptions, and the contaminants' persistence can have long-term consequences.

Conservation Efforts: three key measures to protect the environment: 1) Improved waste management; 2) Sustainable agriculture (ecological and responsible), Use of environmentally friendly cultivation methods, Reduction in the use of chemicals Promotion of biodiversity; 3) Regular monitoring of water and sediment quality. By addressing these concerns, we can work towards preserving Lake Nakivale's biodiversity and ensuring a healthier environment for its ecosystems.

EFFECTS OF COLD HOLDING DURATION AND SUBSEQUENT WARMING REGIME ON THE PHYSIOLOGY OF EASTERN OYSTER SEED

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Shellfish hatcheries are essential in the shellfish farming process, and seed availability can limit the expansion of the shellfish aquaculture industry. Many hatcheries encounter challenges that hinder efforts to maximize seed production. This project represents a collaboration between an academic research team and a commercial shellfish hatchery in Virginia, USA to address an existing bottleneck of additional production of eastern oyster (*Crassostrea virginica*) seed. This hatchery capitalizes on optimal water quality for larval culturing, which is best during the winter and early spring. To take advantage of this high-quality water, the hatchery begins its spawning season in January and has developed a holding system to maintain seed for up to 8 weeks under cold conditions until seed can be moved to outside nursery systems when outside water temperatures exceed 10°C. Prior to moving outside, seed are typically warmed to 27°C. While early season production results in millions of extra seed, the current holding practice can produce losses of up to 50%, manifesting as stunted growth and mortality.

This project addressed whether the length of the cold holding duration (CHD) influences seed physiology and if an acclimation period during the warm-up to 27°C would improve performance. We hypothesized that the existing mortality and growth issues stem from physiological stress during extended CHD and a subsequent abrupt temperature increase. To investigate this, we raised eastern oyster seed at 15°C for three different CHD lengths (8 weeks: CHD8, 6 weeks: CHD6, and 4 weeks: CHD4). Each seed group was then divided and subjected to either a fast warming treatment (reaching 27°C in one day) or a slow warming treatment (gradually reaching 27°C by the end of the warming period) for 8-9 days. We measured oxygen consumption (as a proxy for metabolic rate, MO2), triglyceride (TG) and total protein (TP) reserves, total antioxidant capacity (TAC), and lipid peroxidation (LP) throughout both the CHD and warming periods.

Preliminary results suggest that shorter CHD durations and slow warming may create less stressful conditions for oyster seed. By the end of the CHD, all seed groups exhibited similar MO2. The CHD8 and CHD6 groups experienced a decrease in MO2 towards the end of the CHD, while the CHD4 group maintained consistent MO2 throughout. All groups showed an increase in TG and TP content over time and similar levels, respectively by the end of the CHD. Under fast warming, MO2 in all CHD groups peaked on the first day and then decreased significantly. Under slow warming, the CHD6 group followed a similar pattern to the fast warming group, while CHD4 and CHD8 showed no changes in MO2 over time. There was no significant effect of warming regime on TG and TP levels. However, TG content decreased significantly and TP levels increased significantly by the end of the warming period for most groups. TAC and lipid peroxidation results will be discussed. Overall, these findings highlight the potential for optimizing holding durations and warming protocols to enhance seed health and reduce mortality in oyster hatchery practices.

EVALUATING FRESHWATER SPONGES AS PASSIVE SAMPLERS FOR HEAVY METAL POLLUTION IN LOUISIANA

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Heavy metals, including As, Cd, Cr, Cu, Ni, Pb, Zn, and Al, are persistent pollutants with severe consequences for human health and aquatic ecosystems. These pollutants enter aquatic environments through industrial activities, agriculture, and urban runoff, accumulating in organisms and posing significant risks to the food chain. Monitoring heavy metals (HMs) is challenging due to their bioavailability being influenced by site-specific abiotic factors such as pH, temperature, and organic matter content. In response, the Louisiana Department of Environmental Quality (LDEQ) is working to update aquatic life criteria (ALC) for these contaminants. Identifying natural passive samplers, such as freshwater sponges (FWS), could enhance the efficiency of assessing HMs threats to Louisiana's ecosystems and communities.

FWS, which make up only 3% of global sponge species, are sessile filter feeders with an exceptional capacity to bioaccumulate contaminants, including HMs (Figure1), making them promising passive samplers. While laboratory studies have demonstrated toxicity at low HMs concentrations, sponges in natural conditions accumulate HMs in proportion to environmental pollution levels. In Southern Louisiana, sponges thrive under unique environmental conditions—high temperatures, elevated dissolved organic matter, and salinity—that differ from temperate regions previously studied. Given their year-round presence in Louisiana's FW systems, sponges show significant potential for monitoring HMs pollution.

This study evaluates the feasibility of using freshwater sponges (FWS) as passive samplers by comparing heavy metal (HM) bioaccumulation in FWS tissues with concentrations in water and sediment samples. Samples were collected from Bayou St. John (BSJ) and Daniel Branch (DB) in southeastern Louisiana. HMs were analyzed using inductively coupled plasma mass spectrometry (ICP-MS), and the species of the collected FWS was confirmed via optical microscopy.

Heavy metal concentrations in water were significantly lower than those in both sediment and FWS samples. Water samples were within the safe limits defined by the World Health Organization (WHO) and U.S. Environmental Protection Agency (EPA) guidelines. However, sediment from BSJ showed elevated levels of As (1774.97 mg/kg), while Cr concentrations were notably higher in DB (27.82 mg/kg). Concentration of Al was (1772.28 mg/kg) in BSJ sediment and (19860.93 mg/kg) in DB sediment, and there is no EPA guidelines exist for Al in sediment concentrations.

Heavy metal bioaccumulation in FWS affects their spicule structure. Malformed spicules indicate higher metal adsorption, serving as a visible sign of water quality and pollution. This makes FWS a useful tool for monitoring aquatic environments.

Figure 1: Freshwater Sponges collected from Bayou St. John.

COST-EFFECTIVE CARP FEED FORMULATION USING LINEAR PROGRAMMING

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The aquaculture sector is very competitive around the world. Fish farmers usually get the same price for the same species and of similar size. So, profitability in fish farming depends to a large extent on the cost of production. In commercial fish farming, feed is the major cost item, which accounts for two-thirds to three-fourths of the production cost. Therefore, the industry often seeks to reduce operational costs and improve outputs by optimizing feed inputs. It is a challenging task. Fish feed manufacturers must use an input mix that ensures all nutritional requirements for optimum fish growth, and at the same time, the feed cost is minimal.

Considering the role of feed in the aquaculture industry, this study utilizes a linear programming (LP) technique to identify the optimum combination of different feed ingredients that minimize the feed costs while meeting nutritional requirements for carp fish, namely, Katla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*). We have developed a linear programming model based on the market data collected from Bangladesh and the nutritional requirements noted for carp fish. Carps have a nutritional need for protein (32%) and fat (9%) content while following the local industry standard of moisture levels of 10%. The market data was collected from one of the largest feed manufacturing firms in Bangladesh. Although the data is based on the local market conditions in Bangladesh, the findings have applications beyond Bangladesh as many of the inputs (such as soymeal, corn, mustard oil cake, nutrients, etc.) used for the feed formulation are common for carp production in other countries.

The objective function of our LP model was to minimize the cost of feed ingredients while meeting the nutritional needs of adult carp. The optimization identified that a feed mix containing poultry meal (14.10 units), mustard oil cake (MOC, 58.85 units), and salt (9.39 units) provided the most cost-effective solution, with a total production cost of BDT 4,319 (USD 37.56) per 100 kg of feed. The company that provided us with market data informed us that they spent around BDT 5,000 (USD 43.48) for different feed ingredients to process 100 kg of feed. In other words, the optimum feed mix identified through the LP model has the potential to save feed input costs by 13.6 percent. Bangladesh has a fish feed demand for carp production of about 200,000 metric tons in a year. Thus, with large-scale adoption of the optimum feed mix generated through the LP model, Bangladesh's feed manufacturing industry can save about BDT 1,362 million (USD 11.84 million) in a year to produce feed for carp without compromising feed quality and growth in fish production. We also conducted sensitivity analysis and determined the shadow prices for different feed ingredients.

FROM HATCHERY TO FARM: ADVANCING SHELLFISH AQUACULTURE RESEARCH, EDUCATION, AND COLLABORATION AT ROGER WILLIAMS UNIVERSITY, RHODE ISLAND

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University-affiliated shellfish aquaculture research farms are essential for advancing sustainable aquaculture practices, fostering education, and enabling collaborative innovation. The Shellfish Program at the Center for Economic and Environmental Development (CEED) at Roger Williams University (RWU) offers an integrated system encompassing all three phases of shellfish aquaculture: hatchery, nursery, and grow-out. These facilities form a cornerstone for academic research, industry engagement, and public outreach, with a focus on bivalve species native to Rhode Island waters.

The Luther H. Blount Shellfish Hatchery serves as the program's foundation, producing eastern oyster (*Crassostrea virginica*), quahog (*Mercenaria mercenaria*), and bay scallop (*Argopecten irradians*) seed for restoration programs such as the Town of Warren Oyster Enhancement Program and the Rhode Island Shellfisherman's Association (RISA) Upweller. The hatchery is also an educational resource, offering the Shellfish Hatchery Practicum, a hands-on training opportunity providing RWU students with 100 hours of practical experience in hatchery operations.

The shellfish nursery, featuring an advanced outdoor upweller system, supports research initiatives and offers resources for local oyster farmers within the same biosecurity zone to grow seed to planting size. This system facilitates collaboration between academia and industry while advancing aquaculture sustainability goals.

The FerryCliffe Shellfish Farm, a recently expanded 1.7-acre research and educational site in Mount Hope Bay, accommodates a variety of species, including oysters, clams, scallops, and kelp. This farm employs diverse grow-out gear and enables students to gain practical experience while conducting research and collaborating with innovative companies to test new products and technologies in real-world conditions. Shellfish produced at the farm are donated to university and community events, emphasizing the program's commitment to outreach and service.

The Shellfish Program is further complemented by the Applied Shellfish Farming Course, a 12-week comprehensive training program taught by RWU faculty and expert guest speakers. This course equips participants with the technical knowledge required to manage or establish a shellfish farming enterprise, covering topics from broodstock to market. Together, these facilities and programs not only enhance RWU's capacity for education and research but also position CEED as a critical extension resource for the aquaculture industry. RWU welcomes collaborations with universities, stakeholders, and government agencies to advance shellfish aquaculture research, education, and outreach in the region.

A COMPARATIVE STUDY OF STOCK STATUS AND SUSTAINABLE FISHERIES MANAGEMENT OF *Pomadasys olivaceus* ACROSS PAKISTAN'S COASTLINES

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Fish stock assessment is essential for the sustainable exploitation of fish populations. The stock status of Olive grunt (*Pomadasys olivaceus*) in both coastlines of Pakistan (Balochistan and Sindh) was studied using CMSY, BSM and ASPIC models based on the catch and effort data (2000 to 2022). The findings of B/B_{MSY} in all models of Olive grunt were <1.0 pinpointing the stock is grossly overfished on both coasts. The estimated values of MSY using CMSY and BMS methodologies were 2440mt, 2670mt and 2430mt, 2650mt of Balochistan and Sindh respectively. Also, ASPIC models (Fox and Logistic) showed that the Olive grunt was abundantly over-fished. The Fox and Logistic model estimated the MSY values 1585mt and 1379mt for Olive grunt showing overfished from Balochistan, while 3260mt and 3024mt indicate that the stock is not overfished for Sindh. This study may provide scientific background to the government of Pakistan for establishing management and conservation policies for olive grunt fishery on both coasts of Pakistan, especially focusing on the Balochistan Coast.

WHOLE AND SINGLE CELL TRANSCRIPTOME ANALYSES OF THE HEPATOPANCREAS FROM PACIFIC WHITE SHRIMP *Litopenaeus vannamei* POST EXPOSURE TO *Vibrio* TOXINS

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Vibrio parahaemolyticus is a Gram-negative bacterium commonly found in marine and estuarine environments and is endemic among the global shrimp aquaculture industry. *V. parahaemolyticus* proteins PirA and PirB have been determined to be major virulence factors that contribute significantly to the development of acute hepatopancreatic necrosis disease (AHPND). Our previous work demonstrated the lethality of recombinant PirA and PirB proteins to Pacific white shrimp (*Penaeus vannamei*). To understand the host response to these proteins, recombinant PirA and PirB proteins were administered using a reverse gavage method and individual shrimp were sampled over time. Shrimp hepatopancreas mRNA libraries were generated and bulk RNA sequencing was performed on control and recombinant PirA/B-treated samples.

Differentially expressed genes (DEGs) were identified among the assayed time points (1h up to 6h post challenge), and ranged from approximate 100 at the earliest time points to over 1,000 at later times.

DEGs that were co-expressed at the later time points (2h, 4h, 6h) were also identified and gene associations were established to predict functional physiological networks. Among these were several cell signaling and innate immune function processes (Table 1). From these functional analyses, a candidate gene list was created and included several caspases, integrins, Ras-related proteins and scavenger receptors as potential targets for future examination.

Based on these data, single-cell RNA sequencing of *V. parahaemolyticus* infected shrimp hepatopancreas over time-course of infection studies are underway. Progress on single-cell studies will also be presented.

KEGG Orthology	Number of unique gene transcripts			Description
	2 h	4 h	6 h	
ko04214	7	12	14	Apoptosis, or programmed cell death
ko05100	8	15	12	Bacterial invasion of epithelial cells
ko04625	1	10	18	C-type lectin receptor signaling
ko04013	12	19	12	Mitogen-activated protein kinase (MAPK)
ko04621	8	17	24	NOD-like receptor signaling
ko04145	3	8	16	Phagosome
ko04151	7	29	32	Phosphatidylinositol 3' -kinase (PI3K)-Akt signaling

Table 1. Significant cellular pathways identified among the different time intervals after exposure to rPirA/B.

TESTING THE ABILITY OF ATLANTIC SURFCLAM SUBSPECIES (*Spisula solidissima solidissima* AND *S. s. similis*) TO HYBRIDIZE

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There are two Atlantic surfclam subspecies. The northern subspecies (*Spisula solidissima solidissima*) supports the federal fishery and is abundant on the continental shelf north of Cape Hatteras. The southern subspecies (*S. s. similis*) is predominantly found south of Cape Hatteras, but is also found in shallow, patchy, northern areas, such as coastal Virginia, Long Island Sound, and southern Massachusetts. However, the taxonomic rankings of these clams remain controversial. Recent genetic evidence suggests they could be distinct, but closely related species. Given the surfclam's vulnerability to climate change and its economic importance to commercial fishing and aquaculture industries, the uncertainty around its taxonomic classification must be resolved. The goal of this project was to determine whether the surfclam subspecies have the ability to hybridize. Surfclams were collected from commercial fishing grounds off New Jersey (*S. s. solidissima*) and from a known *S. s. similis* bed in Massachusetts. Breeding occurred in October 2023 and May/June 2024 and resulted in several cohorts of purebred and hybrid progeny. Genetic samples were collected from each of the parental and offspring groups to confirm genetic lineage. Fertilization rate across groups ranged from 95-100%. Larvae were reared in 60 L static cultures maintained at 17-22˚C and a salinity of 29-32. Shell length, shell height, and survival were assessed two to three times weekly during water changes. Growth of the hybrid larvae appeared most similar to the purebred larvae with which they shared maternal parentage. Larvae from all four groups metamorphosed, with most reaching competency at approximately 21±5 days post fertilization. A controlled study of juvenile growth in a flow-through seawater system (18-24˚C, salinity 27-31) showed that hybrid surfclams (*S. s. similis* female x. *S. s. solidissima* male) exhibited significantly greater growth rates than either purebred group. This work demonstrates that *S. s. solidissima* and *S. s. similis* are indeed capable of hybridizing. Future work will include evaluating whether any physiological and thermal tolerance differences exist among the groups and determining whether hybrid clams can generate gametes, backcross with purebreds, and produce F2 hybrids.

ASAP: THE NEW JERSEY APPRENTICESHIP IN SHELLFISH AQUACULTURE PROGRAM

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In New Jersey, shellfish aquaculture is a growing sector of the state's coastal economy. As the industry expands, the demand for a skilled workforce increases in kind. To meet this need, the New Jersey Sea Grant Consortium and the Rutgers University Haskin Shellfish Research Laboratory have developed the Apprenticeship in Shellfish Aquaculture Program (ASAP), a new training program aimed at building a robust pipeline for the New Jersey aquaculture workforce. ASAP is a summer-long program geared towards New Jersey high school students aged 16 and older. Through a combination of in-person instruction, hands-on training, and paid on-farm work experience, ASAP participants learn the inner workings of the shellfish aquaculture industry, gain entry level work skills, and discover what it takes to get food from farm to table. The goal of ASAP is to promote interest in aquaculture career paths in young adults and to build a competent, dynamic workforce. ASAP has four components: 1. School engagement and recruitment; 2. A week-long skill-building "boot camp"; 3. A paid 150-hour apprenticeship on commercial shellfish farms or other aquaculture facilities; and 4. Bi-weekly virtual cohort meetings to connect apprentices with professional development resources.

After a 2022 pilot program, ASAP was fully rolled out in 2024. To recruit students, project coordinators visited 11 public high schools across five counties and engaged with approximately 780 students. During these visits, an aquaculture literacy lesson was presented and specific information about ASAP's summer work program was shared. More than twice as many applicants applied to the program as could be enrolled, and thirteen students were ultimately selected. During "boot camp," the apprentices learned basic shellfish husbandry skills and general principles of permitting, regulation, food safety, marketing, and business planning. After, the apprentices began their 8-week on-farm work experience, working side-by-side with shellfish grower mentors. Growers included five oyster farmers, two clam farmers, and one researcher.

All thirteen apprentices completed the program, received a $3600 stipend, and earned a "Shellfish Farming Practice Certificate." Responses from apprentices and growers was overwhelmingly positive, although participant feedback and lessons learned will be used to improve ASAP in 2025 and the years beyond.

THE EFFECTS OF LOW OXYGEN LEVELS (CHRONIC HYPOXIA) ON THE GROWTH, STRESS RESPONSE AND METABOLISM OF PERFORMANCE-SELECTED DISEASE RESISTANT RAINBOW TROUT (*Oncorhynchus mykiss*)

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In animal agriculture, seafood consumption in the US and globally is on the rise. Aquaculture, an attempt to support the demand for seafood consumption, maximizes the growth of commercially relevant species and decreases the cost to feed through fish husbandry practices that manipulate factors like nutrition, oxygen levels, and temperature. One problem that affects most aquaculture systems is low dissolved oxygen levels. Low oxygen levels are a problem because low oxygen environments can slow the growth of organisms like commercially relevant aquaculture species and lead to the death of fish (affecting or increasing cost and causing loss to fish farmers). Additionally, a previous study conducted using disease-resistant rainbow trout fed high choline diets showed no increase in growth performance but altered expression patterns associated with hypoxia-related genes. Hinting at performance trait selection potentially having implications on other traits such as response to low oxygen environments.

This study will utilize the performance-selected fishes bred for disease resistance (a feature relevant to aquaculture) from the USDA National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, WV. This study will investigate the effects of low oxygen levels (chronic hypoxia) on their growth (i.e., for market relevance: disease resistance), stress response, and metabolism while unraveling if the performance trait of disease resistance they are selected for, affects their response to hypoxia environments as well. Data will shed light on the potential implication of conditioning and generating fishes with these performance traits that can tolerate low oxygen environments with little to no drawbacks on their growth for the aquaculture industry.

UTILIZATION OF WOLFFIA MEAL IN THE DIETS OF GENETICALLY IMPROVED FARMED TILAPIA (GIFT) JUVENILES

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A feeding trial was conducted to investigate the effect of replacing Soybean meal (45% Crude Protein) with Wolffia meal (45% Crude Protein) at 0%, 25%, 50%, 75% and 100% inclusion levels in the practical diets of Genetically Improved Farmed Tilapia (GIFT) juveniles. One hundred and fifty (150) juveniles of GIFT with initial mean weight of 6.86 ± 0.20 g were stocked randomly into fifteen (15) glass aquaria tanks at 10 fish/tank in five treatments with three replicates each. The experimental diets were iso-nitrogenous (40% Crude Protein) and iso-caloric (11.1kcal/kg). Using a complete randomized design, each diet was fed to the test fish for 70days. The highest weight gain was recorded in the fish fed D_5 (25.74±0.30)g containing 100% inclusion level of Wolffia meal and the least weight gain was in the fish fed D_1(13. 50±0.20) with 100% inclusion level of Soybean meal. Specific growth rate (SGR) ranged from 0.67 ± 0.01 (D_1) to 0.97 ± 0.07 (D_5). Feed conversion ratio (FCR) was significantly (P> 0.05) different among the treatments (D_1 - D_5) with the least value in D_5 (1.94± 0.02); protein efficiency ratio (PER) and net protein utilization (NPU) values were significantly (P> 0.05) highest (6.44 ±0.08 and 1.50±0.06) % respectively in fish fed D_5. The overall results indicated that 100% replacement of Soybean meal with Wolffia Meal in the diets of Genetically Improved Farmed Tilapia juveniles gives optimal feed utilization without compromising fish growth.

Growth Performance of Fish Fed Experimental Diets

Parameters	D_1 (0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
Initial Mean Weight (g)	6.86 ± 0.20	6.98 ± 0.08	6.84 ± 0.06	6.75 ± 0.08	6.80 ± 0.06
Final Mean Weight (g)	20.36 ± 0.10	22.2 ± 0.23	23.98 ± 0.50	28.15 ± 0.41	32.54 ± 0.41
Mean Weight Gain (g)	13. 50±0.20	15.22 ± 0.15	17.14 ± 2.44	21.40 ± 1.33	25.74±0.30
Specific Growth Rate (%)	0.67 ± 0.01^{ac}	0.72 ± 0.03^{ac}	0.78 ± 0.04^{ac}	0.89 ± 0.07^{ab}	0.97 ± 0.07^{ab}
Feed Conversion Ratio	3.70 ± 0.18^{ab}	3.29 ± 0.012^{ac}	2.92 ± 0.02^{ac}	2.34 ± 0.04^{aa}	1.94 ± 0.02^{aa}
Protein Efficiency Ratio	3.38 ± 0.02^{ab}	3.81 ± 0.04^{ac}	4.29 ± 0.05^{ab}	5.35 ± 0.08^{ac}	6.44 ± 0.08^{ac}
Net Protein Utilization (%)	1.26 ± 0.02^{ab}	1.27 ± 0.01^{ab}	1.27 ± 0.02^{ab}	1.27 ± 0.03^{ab}	1.5 ± 0.06^{ac}

EVALUATION OF STOMACH CONTENT AND FEEDING HABITS OF *Tilapia zillii* IN LOWER OGUN RIVER, AKOMOJE WATER RESERVOIR, NIGERIA

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Introduction

Fish is a high quality food, apart from its protein contents; it is also rich in vitamins and contains variable quantities of fat and minerals for human health (Bard *et al.,* 1976). Fish is often recommended for cardio-vascular disease patients because of its unique fat, which is composed mainly of Omega- 3 polyunsaturated fatty acid.

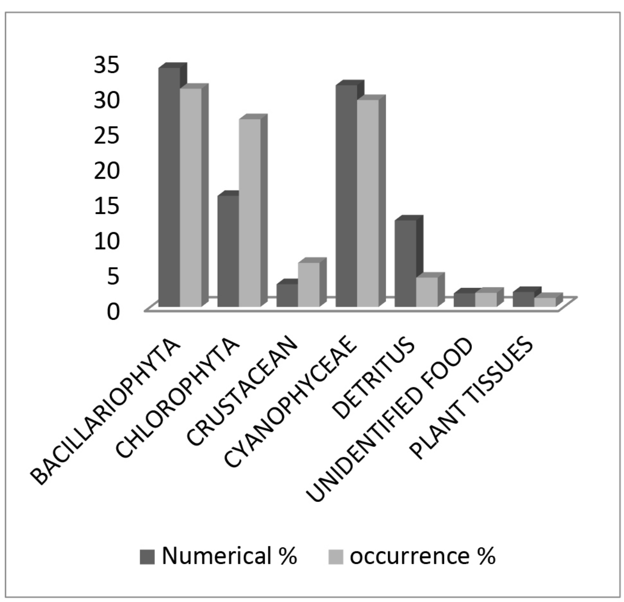
Materials and Methods

The food and feeding habits of *Tilapia zillii* in Akomoje River reservoir, Abeokuta, Ogun State, Nigeria, were examined between the months of August and December 2019. A total number of 125 fish specimens were collected on monthly basis from the commercial landings of fishermen around the water body.

Results

The results of monthly variation in food items show that Bacillariophyta, Chlorophyta, Cyanophyceae, crustacean, detritus, plant tissues, and unidentified food all occurred in varying quantities from August to December 2019. Bacillariophyta (diatoms) was the most important food item in the stomach of *Tilapia zillii* accounting for 14.72% and 78.10% by numerical and frequency of occurrence methods, respectively. Cyanophyceae constituted 11.43% in number and 59.63% in occurrence as the next food item in order of importance. Crustaceans occurred least in order of importance with 2.34% in numbers and 27.12% in frequency of occurrence.

Figure 2: Distribution of food items in the stomach of *Tilapia zillii* from Akomoje water Reservior.



EXPLORING 'STRESS MEMORY' IN FISH FARMING: A NEW APPROACH TO ENHANCE CATFISH RESISTANCE AGAINST ELEVATED AMMONIA

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The economy of commercial-based fish farming is predominantly based on stocking the fish at a higher density and rearing them with a high-protein diet. This scenario, along with limited renewal of clean water (e.g., in recirculatory and pond-farming), eventually results in a build-up of toxic ammonia in the water, which can reduce growth, increase vulnerability to diseases, and even cause mortality. Within the aquaculture system, there is no fast remedy to alleviate high ammonia-induced toxicity, so prevention rather than correction should be the focus.

Therefore, we tested the hypothesis of whether pre-acclimation to a low concentration of ammonia could enable the fish to develop an 'ammonia stress-avoidance' memory, enhancing their tolerance to subsequent lethal and sub-lethal ammonia threats. To test this, we selected channel catfish (*Ictalurus punctatus*), a key species in the U.S. aquaculture industry, as the model organism. Catfish were pre-exposed to 2.17 mg/L (total) ammonia (10% of determined 10-day LC_{50}' value, 21.7 mg/L) for 21 days. Thereafter, each of these pre-exposed and parallel control (without pre-exposure 'naïve') groups were recovered for 7 days in clean water. Following this recovery phase, each group was subsequently exposed to a lethal (100% 10-day LC_{50}) and sub-lethal (25% 10-day LC_{50} for 21 days) ammonia concentration. Results show that during the lethal ammonia challenge, the pre-exposed group had a significantly longer survival time than the naïve group (Fig. 1). This indicates that catfish can develop an 'ammonia stress-avoidance memory' that enables the fish to resist a subsequent ammonia threat. In addition, following 21 days of sub-lethal ammonia exposure, the ammonia excretion from fish was significantly inhibited in the naïve group, which resulted in a significant accumulation of toxic ammonia in the plasma. Interestingly, pre-exposed fish were able to excrete ammonia efficiently and retained ammonia load in the plasma within the basal level. Overall, these data suggest that 'ammonia stress-memory' was evident for both lethal and sub-lethal endpoints. Conclusively, based on our findings, we can state that stocking and rearing the ammonia pre-exposed catfish in aquaculture facilities can be used as an imperative strategy to enhance the fish's ability to alleviate the toxicity induced by the water-borne ammonia.

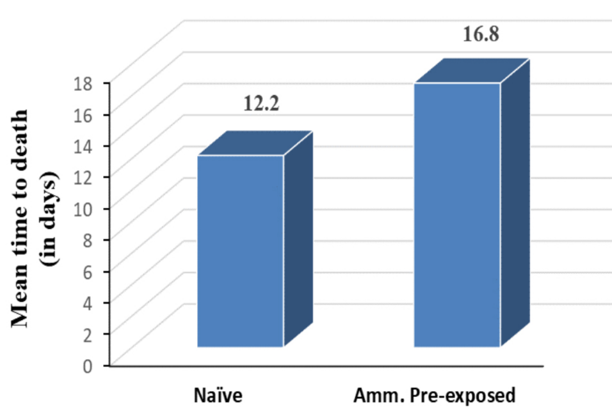

Figure 1. Survival (time-to-death) of naïve and ammonia pre-exposed catfish during lethal dose of ammonia challenge

CHARACTERIZATION OF BACTERIOPHAGE-RESISTANT STRAINS FROM BACTERIOPHAGES TARGETING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)-CAUSING *Vibrio* sp.

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Acute Hepatopancreatic Necrosis Disease (AHPND is a devasting shrimp disease recognized by the World Organisation for Animal Health (WOAH). Strains of the *Vibrio* genus were identified as the causative agent of this disease, particularly *Vibrio parahaemolyticus* (Vp), which produces a binary toxin called PirAB, expressed by the *pirA* and *pirB* genes located on a conjugative virulent plasmid (pVA1).

This disease, first reported in China in 2009 and rapidly spreading around the world, was reported in Mexico for the first time in 2013, causing severe economic losses in the shrimp culture.

Overuse of antibiotics has led to antibiotic-resistant bacteria and weakened immune responses, reducing the effectiveness of treatment. In response, phage therapy has emerged as a promising, environmentally friendly, species-specific, and less expensive alternative to treat bacteria due to its lytic capacity to regulate bacterial populations. However, it has been shown that bacteria can develop resistance through various mechanisms, allowing the proliferation of strains with different genotypes, phenotypes, and levels of virulence, favoring species diversification.

Fig. 1. Normal appearance of *Penaeus vannamei* juveniles (a, b) and shrimp affected by AHPND (c)

In this study, two lytic bacteriophages, which are viruses that infect and kill bacteria, were isolated and purified from shrimp pond samples taken from different locations south of Mazatlán, Sinaloa, with lytic activity against AHPND-causing *Vibrio parahaemolyticus* (M0904), one of the most virulent strains.

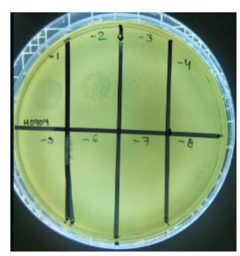

Fig 2. Morphology of plaques formed on a double-layered agar plate by phage against *Vibrio parahaemolyticus* M0904.

Subsequently, the Vp M0904 strain will be exposed to the bacteriophages, and those with phage resistance will be isolated. The complete genome of the phage-resistant strains will be obtained using whole genome sequencing to identify genotypic changes. Simultaneously, they will be biologically characterized to evaluate the phenotypic changes such as motility, biofilm production, and antibiotic resistance. A comparison of the degrees of virulence between the phage-resistant strains and the wild-type strain will be made by *in vivo* experiments with *P. vannamei* juveniles.

CHARACTERIZATION OF BACTERIOPHAGE-RESISTANT STRAINS FROM BACTERIOPHAGES TARGETING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)-CAUSING *Vibrio* sp.

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In this study, two lytic bacteriophages, which are viruses that infect and kill bacteria, were isolated and purified from shrimp pond samples taken from different locations south of Mazatlán, Sinaloa, with lytic activity against AHPND-causing *V. parahaemolyticus* (M0904), one of the most virulent strains. Subsequently, the Vp M0904 strain will be exposed to the bacteriophages, and those with phage resistance will be isolated. The complete genome of the phage-resistant strains will be obtained using whole genome sequencing to identify genotypic changes. Simultaneously, they will be biologically characterized to evaluate the phenotypic changes such as motility, biofilm production, and antibiotic resistance. A comparison of the degrees of virulence between the phage-resistant strains and the wild-type strain will be made by *in vivo* experiments with *P. vannamei* juveniles.

Fig. 1. Normal appearance of *P. vannamei* juveniles (a, b) and shrimp affected by AHPND (c).

MULTI-OMICS ANALYSIS IDENTIFIES CANDIDATE GENETIC AND MICROBIAL MARKERS AND THEIR POTENTIAL INTERACTION TO INFLUENCE FILLET YIELD AND QUALITY TRAITS IN RAINBOW TROUT

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Fillet yield and quality traits are some of the most important traits for genetic improvement in rainbow trout breeding programs. The host genetics and gut microbiome are known contributors to the regulation of these traits. While most studies investigate them separately, a simultaneous holistic look into these two components may provide insights into the interplay between hosts' genetics and the microbiomes and how they influence these traits. We analyzed the host transcriptome, host genome, and gut microbiome of rainbow trout fish families that are divergent for these traits. The transcriptome analysis revealed gene expression patterns and identified several differentially expressed genes (DEGs). To prioritize candidate genes, we performed eQTL mapping, identifying DEGs whose expression patterns are regulated by genetic variants. Further, we performed an association analysis between gene expression and phenotypic traits, and only those DEGs whose expressions are correlated with the phenotypic traits and are regulated by cis-eQTL variants are prioritized as candidate genes. Potential candidate genes for growth traits were identified for body weight (*ubiquitin-protein ligase E3A (UBE3A), collagen alpha-1 (XXV1) chain (COL26A1), protein phosphatase inhibitor 2 (PPI-2)*); muscle yield (*PDZ domain-containing protein 11 (PDZK11)*, *pex5-related protein-like (PEX5-L)*, *Synaptic vesicle glycoprotein 2Ca (SVC2C)*) and condition factor (*Synaptic vesicle glycoprotein 2Ca (SVC2C), Sodium-dependent neutral amino acid transporter SLC6A17, and Meiosis-specific coiled-coil domain-containing protein (MEIOC)*). Our microbiome analysis revealed microbiome biomarkers with the potential to improve body weight, muscle yield, and body condition factor. Integrated gene-microbiome analysis identifies ***Clostridium species (C. beijerinckii, C. intestinale, C.isatidis, C. saccharobutylicum*, *C. taeniosporum*, *C. gasigenes*)** enriched in high muscle yield rainbow trout family to be positively correlated with *PEX5-L*, and *PDZK* genes identified as muscle yield candidate genes. Separately, we performed genome-wide association analysis that revealed candidate genes for fatty acid profiles, muscle fiber size, fiber density, and fillet color. These results provide new insights into the relationship and interaction between host genetics and the microbiome and how these biomarkers could be targeted for selective modification to improve fillet yield and quality traits in rainbow trout.

SHOTGUN METAGENOMICS REVEALS MICROBIAL TAXA LINKED TO FILLET YIELD AND QUALITY TRAITS IN RAINBOW TROUT

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Recent studies highlight the significant role of the gut microbiome in modulating fish growth and nutrient metabolism. Herein, we hypothesize that genetic selection influences gut microbial composition. To explore this, fish were obtained from a breeding program for rainbow trout at the National Center for Cool and Cold-Water Aquaculture (NCCCWA), developed in 2002, that initially selected for growth before shifting to muscle yield. Our study investigates gut microbiome dynamics across fish families selected for fillet yield, using samples from the 2020-year class (3rd-generation families from high (ARS-FY-H) or low (ARS-FY-L) fillet yield genetic lines).

Fecal samples from 60 high-yield (ARS-FY-H) and 58 low-yield (ARS-FY-L) fish were analyzed through shotgun metagenomic sequencing to identify microbial taxa linked to fillet yield, body weight, condition factor, omega-3, fillet color, muscle fiber size, and density.

Alpha diversity Shannon index revealed that ARS-FY-L fish had greater microbial diversity ($P<0.05$), while beta diversity (nMDS) showed minimal clustering for muscle yield genetic lines. However, muscle fiber size comparisons found significant differences in alpha diversity (Shannon index p-value = 0.00314) and moderately distinct beta diversity (bray-curtis, $P = 0.028$, $R^2 = 17\%$). We identified bacterial taxa showing differential abundance between fish belonging to the divergent phenotypes. For example, bacterial taxa such as *Corynebacterium variabile* and *Jeotgalicoccus halotolerans* were differentially abundant between red and white fillet color fish groups, possibly contributing to desired reddish fillet pigmentation through probiotic functions and carotenoid production.

In conclusion, shotgun metagenomics identified microbial taxa associated with different phenotypes, which may serve as biomarkers for fillet yield and quality traits in rainbow trout.

RELATIVE SENSITIVITY OF CYANOBACTERIA TO COPPER

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Copper (Cu) is commonly used in aquaculture ponds to control cyanobacterial blooms. In Alabama, copper is often used when blooms become evident and there is not a strong understanding of how to use dosing to mitigate bloom development. To improve management plans to address harmful algal blooms, I carried out a number of studies characterizing the effects of copper on cyanobacterial species that are commonly found in Alabama catfish ponds. Three cyanobacteria: *Microcystis*, *Anabaena*, and *Oscillatoria* were isolated from a local catfish pond and culturing methods were developed. All three species were exposed to copper using a dose-response format ranging from 0-200 µg/L to identify decreases in cell density, impacts on growth rates, chlorophyll concentrations, and the phycocyanin: chlorophyll ratio. As these algal strains are grown in aquaculture ponds which have consistent copper exposure, *Microcystis* were compared to a lab strain (UTEX 3037). The results demonstrated that Cu affected biological performance of the algal species. Using chlorophyll-a concentrations to determine Cu toxicity, the 96-h EC50 values were 15.7, 19.8, 21.4, and 12.0 µg/L Cu for UTEX 3037, *Microcystis*, *Anabaena*, and *Oscillatoria*, respectively. Based on the results of cell density measurements, the 96-h EC50 values for UTEX 3037 and *Microcystis* were 15.6 and 19.9 µg/L Cu, respectively. These EC50 values indicate that both measurement endpoints gave consistent results. Among the algal species, *Oscillatoria* appeared to be the most sensitive to Cu. These results highlight the need for species-specific knowledge of how different species react to Cu pollution in order to effectively manage aquatic environments. The development of focused mitigation methods will be greatly impacted by the resolution of dose-response connections between Cu exposure and cyanobacterial populations as well as the validation of chlorophyll concentrations as a proxy for algal density. To formulate effective management methods that mitigate detrimental impacts of Cu contamination on cyanobacterial communities, more studies are necessary to fully understand the underlying processes regulating the observed responses.

DEVELOPMENTAL TOXICITY ASSAY OF *Alstonia scholaris (L.) R. BR.* ETHANOLIC EXTRACT IN ZEBRAFISH (*Danio rario*) EMBRYO

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Alstonia scholaris (L.) R. Br. of *Apocynaceae* family, commonly known as "Devil's Tree", has ethnomedicinal importance in the treatment of various diseases like asthma, cancer, jaundice, leprosy, and malaria. *A. scholaris* showed potentiality as an antibacterial, antiviral, antioxidant, anti-fungal agent in various studies. In the present study, the antimicrobial activities of *A. scholaris* ethanolic extract were determined using the broth microdilution method. The developmental and teratogenic toxicity effect of ethanolic extract (EE) of *A. scholaris* was also determined in zebrafish embryos at 500, 250, 125, 62.5, 31.25, and 0 µg/ml concentrations. The toxicity study was done following the OECD guideline 236 for zebrafish acute toxicity testing. Zebrafish embryos were treated with *A. scholaris* EE at different concentrations at 2 hours post fertilization (hpf). During 5-day exposure (till 96 hpf) with EE, developmental and teratogenic features like egg coagulation, mortality, somatic development, eye formation, hatching, yolk sac and pericardial edema, pigment formation, movement, and heartbeats were recorded every 24 hrs. The results demonstrated that ethanolic extract (EE) of *A. scholaris* showed antimicrobial activity against the fish pathogen *Pseudomonas fluorescens* with a half-minimum inhibition concentration (MIC_{50}) of 46 µg/ml and showed broad-spectrum activities against *Streptococcus iniae* (MIC_{50}: 67 µg/ml) and *Flavobacterium columnare* (MIC_{50}:12 µg/ml). During EE exposure for toxicity test with zebrafish embryos, at 24, 72, and 96 hours post-treatment (hpt), mortality was observed 10%, 20%, and 40%, respectively at 500µg/ml concentration. At 96 hpt 15% mortality was observed in 250 µg/ml concentration; no mortality was observed in other concentrations. At 96 hpt, hatching efficiency was reduced by 25% in 500µg/ml concentration. Malformation of varying levels was visible at higher concentrations (125-500 µg/ml). In 500 µg/ml, 100% of embryos were found malformed with various features such as yolk sac edema, pericardial edema, body bending, tail bending, and short body length. No observed-adverse-effect (NOAEL) was observed at 62.5 µg/ml. LC_{50} of *A. scholaris* EE found 254 µg/ml and EC_{50} 276 µg/ml. The teratogenic index (TI) value, the ratio of LC_{50} and EC_{50}, was found less than 1 indicating *A. scholaris* EE is not teratogenic for zebrafish embryos and can be used for further aquaculture welfare and drug development studies.

COMPARATIVE HISTOMORPHOLOGICAL ANALYSIS OF SKIN ADAPTATIONS IN *Erpetoichthys calabaricus* AND *Clarias gariepinus*

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The study investigated the skin anatomy of snakefish (*Erpetoichthys calabaricus*) and African catfish (*Clarias gariepinus*) to elucidate their ecological adaptability to extreme fluctuations in the aquatic environment. *E. calabaricus*, known for its ability to thrive in both aquatic and terrestrial environments. *C. gariepinus*, significant in aquaculture and a model for studying environmental toxicity. Though genotypically unrelated, they are potential models for study of adaptation and resilience in water bodies.

Histological techniques, including staining with H&E, PAS, MT, and Alcian blue, and morphometric analysis were employed to analyze six samples of adult *E. calabaricus* and twenty samples of adult *Clarias gariepinus*.

The histological features of the skin of *E calabaricus* comprise a vascularized hypodermis, a mucous-rich epidermis, and a collagen-rich dermis. *C. gariepinus* shows distinct regional skin differences. The dorsal skin is thicker, containing keratinocytes, melanocytes, and chromatophores, offering mechanical protection, dark pigmentation, and predator resistance. The ventral skin is thinner, lighter, and rich in mucous cells, aiding in defense against pathogens, gas exchange, and osmoregulation. Additionally, scale pockets observed in the ventral region are thought to assist environmental adaptation.

In summary *E calabaricus* demonstrates higher ecological resilience with specialized structures for terrestrial and aquatic survival. Layers of epidermal mucous and sensory cells further enhance adaptability by mitigating environmental challenges. Conversely, *C. gariepinus* exhibits regionalized skin adaptations for turbid aquatic conditions.

The study emphasizes the importance of specifying skin sampling sites to avoid inconsistencies in future research. Both fish have evolved unique structural and functional skin adaptations to their ecological and physiological needs, providing insights into their survival mechanisms across diverse environments.

STERILITY EFFECT OF *Petiveria alliacea* (GUINEA HEN WEED) ROOT BARK MEAL ON THE JUVENILE OF *Oreochromis niloticus* (NILE TILAPIA)

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The sterility potentials of *Petiveria alliacea* root bark meal on the gonads of *Oreochromis niloticus* and its effect on the histology of testis ovary and liver was assessed. Using 150 juveniles of *Oreochromis niloticus*, the sterility effect of *Petiveria alliacea* root bark meal (PARBM) was determined in a 56-day feeding experiments at the inclusion levels of 0g, 15g, 20g, 25g and 30g / kg diet. Each of the treatments were in three replicates and the fish were fed at 4% body weight twice daily. Behavioral responses of fish and physiochemical parameters of water were monitored during the experiment. The results showed that the testis weight was not significantly different. Milt counts and milt volume reduced with increase in the concentration of *Petiveria alliacea* root bark meal. Motility duration also decreased with increase in the inclusion levels. Also, the fish exhibited significant differences (P<0.05) in the final weight of 0g and 20g / kg PARBM level. Ovary weight reduced with increase in the concentration of PARBM. The histology of the testes of fish fed 0g of PARBM/kg diet revealed that primary and secondary spermatocytes were present in seminiferous tubule, while in treatment 15g, 20g, 25g, and 30g of PARBM/kg diet, secondary spermatogonia was seen in the lumen of the seminiferous tubule. Section of testis exposed to 20g showed increase in interstitial cell and necrosis, 25g showed gonadal degeneration and necrosis while section exposed to 30g showed spermatids disintegration and necrosis. Developing oocytes surrounded by a thin wall were observed in the histological examination of the ovary of *O. niloticus* fed 0g of PARBM/kg diet; 15g showed section of ovary filled with developing oocytes, 20g showed developing oocytes and some necrotic oocytes, 25g showed deteriorating oocyte and interstitial cell alteration in ovary development and 30g showed no oocytes in the lumen and alteration in ovary development. Histological observation and gonadal analysis revealed that *Petiveria alliacea* root bark meal can be used as sterility agent in *Oreochromis niloticus* under culture conditions.

Table 1: Reproductive performance *Oreochromis niloticus* fed *Petiveria alliacea* root bark meal.

Parameters	T_1 (0g)	T_2 (15g)	T_3 (20g)	T_4 (25g)	T_5 (30g)
Initial weight	$12.71{\pm}0.75^a$	$10.20{\pm}0.26^a$	$11.70{\pm}0.13^a$	$12.46{\pm}0.61^a$	$10.58{\pm}0.14^a$
Final weight	$20.48{\pm}0.79^a$	$18.81{\pm}0.19^a$	$19.43{\pm}0.16^a$	$20.62{\pm}0.85^a$	$19.61{\pm}0.18^a$
FCR	$2.01{\pm}0.06^a$	$1.45{\pm}0.15^a$	$2.18{\pm}0.86^a$	$1.93{\pm}0.13^a$	$1.82{\pm}0.22^a$
Ovary weight	$1.67{\pm}0.17^a$	$1.35{\pm}0.03^b$	$1.22{\pm}0.02^b$	$1.74{\pm}0.02^a$	$0.81{\pm}0.16^c$
Testis weight	$0.53{\pm}0.002^a$	$0.53{\pm}0.02^a$	$0.53{\pm}0.01^a$	$0.54{\pm}0.01^a$	$0.53{\pm}0.02^a$
Fecundity	$152.33{\pm}0.25^a$	$98.00{\pm}0.10^b$	$84.00{\pm}0.20^c$	$77.33{\pm}0.21^d$	$72.00{\pm}0.20^e$
Egg size	$0.97{\pm}0.02^a$	$0.94{\pm}0.02^a$	$0.87{\pm}0.02^b$	$0.83{\pm}0.03^b$	$0.84{\pm}0.03^b$
Milt count	$1.62{\pm}0.03^a$	$1.48{\pm}0.01^b$	$1.27{\pm}0.03^c$	$0.99{\pm}0.02^d$	$0.90{\pm}0.06^e$
Milt volume	$0.62{\pm}0.03^a$	$0.64{\pm}0.03^a$	$0.66{\pm}0.05^a$	$0.52{\pm}0.02^b$	$0.52{\pm}0.02^b$
Motility	$4.00{\pm}0.00^a$	$6.00{\pm}0.00^a$	$7.00{\pm}0.00^a$	$7.00{\pm}0.00^a$	$8.00{\pm}0.14^a$

Mean with the same subscript are not significantly different (P<0.05) using Duncan multiple range test

MORPHOMETRIC, MERISTICS AND BACTERIOLOGY IN SHRIMP (*Caridean* shrimps) BOUGHT FROM OLOMORE FISH MARKET, ABEOKUTA, OGUN STATE

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Bacterial have been one the major diseases that affect shrimp and give a setback for shrimp farming in aquaculture industry and various fish market. These bacterial infection were been transferred through various medium such as sea water, management practices and handling. A total number of twelve (12) specimen which include carapace, guts and exoskeleton were sampled from Olomore market, Abeokuta axis, Ogun state to investigate the morphometric, meristic and bacteriology in *Caridean* shrimp. This study revealed that there were no significant difference ($P \geq 0.05$) in the morphometric, meristic and total bacterial count of the shrimp stomach, carapace and body. However, the cephalothorax length (CL) showed a significant between the male (0.1-0.5) and female (0.1-1.5). This indicated that the female CL is thrice the male. In the meristic, rostral spine was slightly varied between the male (4-7) and female but had the same value with the total (4-8). All the bacterial identified are resistance to Augmentin, Zinnacef, Erythomyin, Gentanycin and Pefloxacin except for *Bacillus spp* which are sensitive to Gentanycin and Pefloxacin. Therefore, this present study revealed that the female and female Caridean shrimp have similar morphological and meristic features and the identified bacterial can be treated with any of the antibiotics except Augmentin, Zinnacef, Erythomyin, Gentanycin and Pefloxacin.

INVESTIGATING THE POTENTIAL OF MINERALIZED AQUACULTURE EFFLUENT AS A NUTRIENT ADDITION IN AQUAPONIC SYSTEMS

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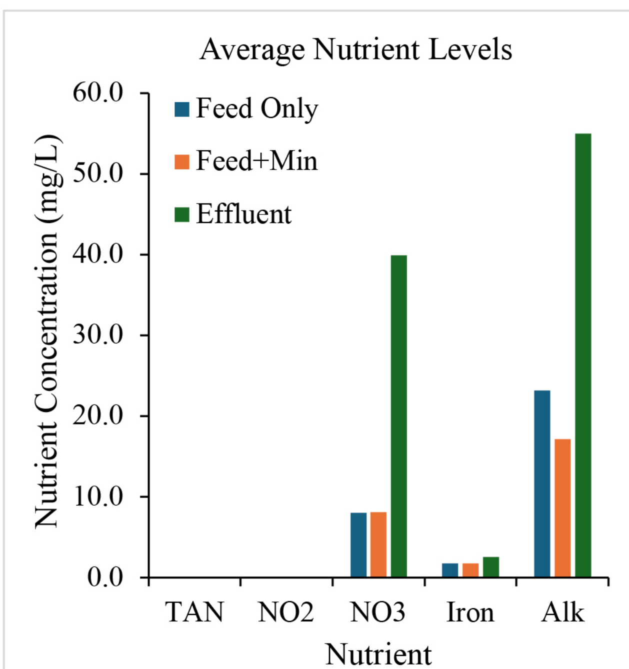
Small-scale aquaponics producers are often challenged by high fish feed costs. To offset this cost, it has been suggested that farmers reduce feed rates at the expense of plant production. However, a significant portion of the nutrients from feed are excreted by the fish and bound in solid waste. Mineralization is a process where aqueous microbes break down organic waste into dissolved nutrients that may be accessible to plants. Using mineralized waste in aquaponics may offset reductions in feed rates and allow farmers to maintain plant production while reducing costs.

This study was designed to explore the effects of added mineralized tilapia effluent on Bibb lettuce (*Lactuca sativa var. capitate)* growth at low feed rates in aquaponic systems. To test the effectiveness of mineralized aquaculture effluent on plant production, a 21-day research trial was conducted. Six 1500L aquaponic systems were stocked with 10 tilapia each (235g avg.) and fed at a rate of 15g of feed per square meter of growing area (2.7m^2) per day. Three tanks were supplied with mineralized aquaculture effluent collected from the solid filters along with fish feed while the other 3 tanks were only provided with feed.

There were no significant differences found in water quality metrics or plant production. Nitrogenous compounds decreased over the study, indicating that feed rates of the fish were inadequate to keep up with the needs of the plants. While the mineralization process increased available nutrients in the effluent, the amount of mineralized effluent added back into the systems was not enough to increase average nutrient levels or plant production relative to the control systems. The low feed rate used in this study likely resulted in a low amount of solid waste generation, which even when mineralized did not generate a meaningful amount of nutrients in the short time frame of this study. Future research will examine the use of mineralization at higher feed rates and over long term.



CONTROL MEASURES FOR BIOSECURITY COMPLIANCE IN FISH FARMS ACROSS SELECTED LOCAL GOVERNMENT AREAS OF LAGOS STATE

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Aquaculture disease outbreaks continue to pose significant threats to the sustainability and profitability of fish farming worldwide. Consequently, biosecurity programs that mitigate the risks of aquatic animal pathogens and diseases have become critical to the aquaculture industry. Biosecurity in aquaculture encompasses a range of practices, procedures, policies, and regulations designed to prevent the introduction and spread of pathogenic organisms, including bacteria, viruses, fungi, parasites, and invasive aquatic species (Dvorak, 2009; Aladetohun et al., 2024). Implementing a biosecurity program remains one of the most effective and cost-efficient strategies for reducing the spread of pathogens and invasive species within fish farms (FIAC, 2010).

This study assessed biosecurity practices in fish farms across three Local Government Areas (LGAs) in southern Lagos State, Nigeria: Epe, Ibeju Lekki, and Eti Osa. A purposive sampling technique was employed to select these LGAs, while cluster sampling was used to identify 62 fish farmers within the study areas. Data were collected through structured questionnaires and interviews, focusing on socio-economic characteristics, aquaculture practices, and biosecurity compliance levels. Key biosecurity measures examined included the isolation of diseased fish, acclimatization procedures, feed quality assessment, effluent and dead fish disposal, and water quality monitoring. The study employed descriptive statistics and chi-square analysis for data presentation and interpretation.

Findings revealed that 66.7% of fish farmers in the study areas did not implement biosecurity measures, while 74.5% lacked awareness and understanding of biosecurity principles. The majority of fish farmers were between the ages of 21 and 38, a demographic with the potential for rapid adaptation to biosecurity innovations if adequately informed. As shown in Figure 1, the lack of awareness was a key factor influencing non-compliance with biosecurity protocols.

This study provides essential baseline data highlighting the urgent need for increased awareness and adherence to strict biosecurity and biosafety measures in fish farms to mitigate the risk of disease outbreaks and ensure sustainable aquaculture practices.

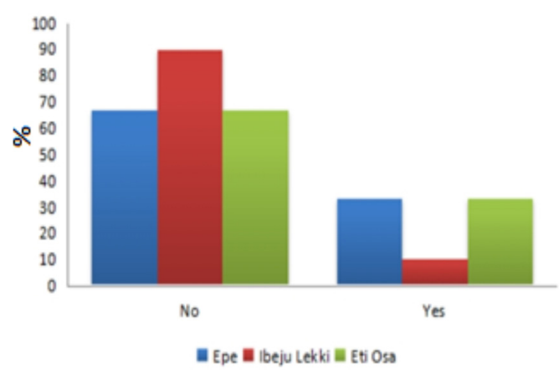

Figure 1: Biosecurity awareness of the respondent

CHANGES IN BAY SCALLOP *Argopecten irradians* IMMUNITY WITH AGE

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The northern bay scallop is vital to the American scallop industry, but since 2019, summer mortality events have caused a decline in their commercial landings in New York. Pathological studies linked these mortality events to an apicomplexan parasite named Bay Scallop Marosporida (BSM), with older scallops being more heavily infected than younger individuals. This raises the question of whether the increased infections are due to a longer exposure time to the parasite or a decline in immunity with age.

To explore this question, scallops from three age groups were collected from an embayment in eastern Long Island, NY, and their immune responses were analyzed in three experiments. In Fall 2023, juveniles (5 months old) and young adults (1 year old) were compared to measure their baseline immune parameters. In Winter 2024, young adults (1 year old) and older adults (2 years old) were exposed to a bacterial cocktail, and their immune responses were tracked over five days. In Summer 2024, young and old adults were assessed for baseline immunity. Immune metrics such as total hemocyte count, percentage of phagocytic cells, percentage of dead cells, and reactive oxygen species production (ROS) were measured in hemolymph samples, while BSM infection levels were assessed in scallop tissues. Some metrics were experiment-specific: percentage of aldehyde dehydrogenase (ALDH)-positive cells (Fall 2023, Winter 2024), bacterial counts (Winter 2024, Summer 2024), and lysosome contents (Summer 2024).

Results showed that young adult scallops had higher immune performances than juveniles and older adults, displayed as higher hemocyte counts, percentages of phagocytic and ALDH-positive cells, and ROS levels, along with lower bacterial counts. The only metric where young adults appear to have underperformed was in having a higher percentage of dead cells compared to older adults. Most results were statistically significant (P < 0.05) and even the non-significant results favored young adults.

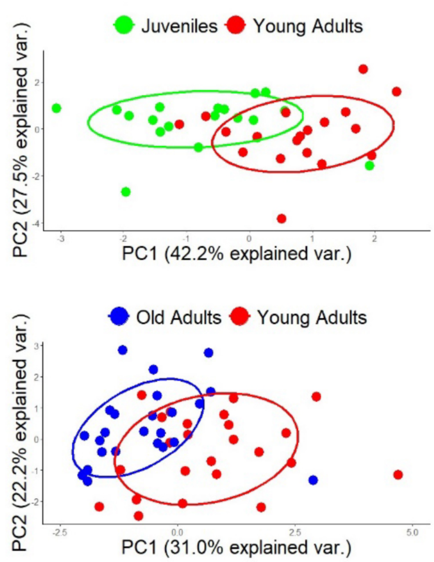

Figure 1: PCA plots showing segregation of scallop age groups based on immune parameters. The top plot (Fall 2023) and bottom plot (Summer 2024) highlight distinct immune profiles in young adults compared to juveniles and older adults.

DEVELOPMENT AND EVALUATION OF PHBV-BIOMASS COMPOSITE PELLETS FOR EFFICIENT DENITRIFICATION IN RECIRCULATING AQUACULTURE SYSTEMS

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Denitrification is a crucial step in maintaining water quality in Recirculating Aquaculture Systems. Conventional liquid carbon sources for denitrification create issues in RAS due to overdose hazard and the requirement of separate loop operation. Our study deals with the development of a unique approach which employs Poly(3-hydroxybutyrate-co-3-valerate) (PHBV)-Biomass composite pellets as a solid carbon source for denitrification in a recirculating aquaculture system. The study investigates the potential of combining PHBV with four biomass materials (saw dust, switch grass, sugarcane bagasse, and rice husk) to make cost-effective and durable pellets. Composite pellets were made with different ratios of PHBV to Biomass (50-90%) by using both a Heated Pellet Pressing Die Set with hydraulic press and a pellet mill. Pellets are submerged for 24 hours for testing physical cohesiveness. The blends pellets passing the physical cohesiveness test are then subjected to comprehensive physical and mechanical evaluation, including wet abrasion resistance in fluidized bed filters.

Based on mechanical properties, basic cohesiveness and wet abrasion tests, a set of finalist pellets is selected. Composite pellets with the best pellet properties and lowest PHBV content are used in denitrification testing experiments. Denitrification experiments were conducted using PVC bioreactor column filled with the selected bio-pellets. The nitrate reduction rate, pellets consumption rate and COD accumulation are quantified. Cost analysis of the selected blends will also be discussed.

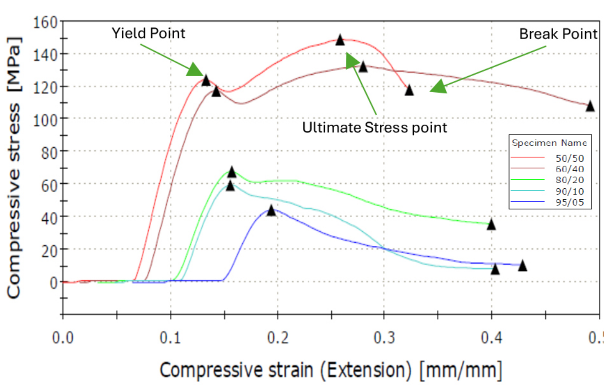

Figure: Stress-Strain curve of Sawdust/PHBV pellets showing the effect of PHBV (wt%).

Table: Summary of Pellets Compression strength of different biomass types and PHBV in different blend ratio.

	Ultimate Yield Strength (Mpa)			
Specimen	Sugarcane Bagasse	Rice Husk	Sawdust	Switch grass
60/40	42.83	38.13	33.81	41.60
70/30	42.36	36.59	25.18	37.10
80/20	17.40	25.86	20.35	33.32
90/10	14.28	18.13	10.74	19.06

PREVALENCE, CHARACTERISATION, AND ANTIBIOTIC RESISTANCE PROFILES OF *Vibrio cholerae* AND *Vibrio parahaemolyticus* IN AFRICAN CATFISH ACROSS SELECTED REGIONS IN IBADAN, NIGERIA

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Antibiotic use in aquaculture contributes to antimicrobial resistance in bacteria from aquaculture sources worldwide. *Vibrio cholerae* and *Vibrio parahaemolyticus ar*e commonly reported zoonotic bacterial pathogens of fish. However, information on Vibrio's prevalence and antibiotic resistance patterns in Nigerian aquaculture is limited. Understanding the prevalence and antibiogram of *Vibrio cholerae* and *Vibrio parahaemolyticus* in cultured fish is essential for assessing human health risks and developing effective mitigating strategies against these risks in aquaculture practices.

A total of eighty-four cultured catfish were collected directly from fish farms from four actively selected regions in Ibadan, Oyo state. Liver, kidney, spleen, and muscle were collected from sampled fish and processed for microbiological analysis at the Food Safety Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan. Sampled organs were enriched in buffered peptone water and incubated at 30□ for 24 hours. The enriched organs were then streaked on Thiosulfate Citrate-Bilesalts Sucrose Agar (Millipore®, Germany), and incubated at 30□ for 24 hours while growth was observed after 24 hours. The isolates were then subjected to biochemical tests and antibiotic sensitivity tests.

The percentage prevalence of *Vibrio cholerae* (45.7%) was higher than that of *Vibrio parahaemolyticus* (23.3%) isolation rate was 68 out of 420; (23.3%). The prevalence of the Vibrio isolates in the regions was: Ido (18%/20%), Lagelu (80%/2.9%), Akinyele (26.7%/5.3%), and Egbeda (30%/32.3%) respectively. Of interest, is the prevalence of both Vibrios in the muscle of the sample fish across the four selected regions. *Vibrio cholerae* and *Vibrio parahaemolyticus* were both resistant to Amoxicillin-Clavulanate (100%), Cefotaxine (100%), and Cefuroxime (100), Imipenem (92%/80%) and Nitrofurantoin (88%/80) respectively. They were both susceptible to Gentamycin (76%; 70%), Ofloxacin (60%; 60%) Ceftriaxone (88%; 50%), and Levofloxacin (76%; 80%) respectively. The multiple antibiotic-resistant index of the isolated *Vibrio cholerae* ranges between 0.33 and 0.78 while that *Vibrio parahaemolyticus* ranges between 0.44 and 0.78.

The presence of multi-drug resistant *Vibrio cholerae* and *Vibrio parahaemolyticus* in table-sized African catfish raised in the active aquaculture regions is an implication of the unwholesomeness of the fish and a concern for fish food safety since catfish is the commonest of the farmed fish in Nigeria and readily available in all fish markets. Therefore, aquaculture biosecurity measures and practising of best management practices should be adopted while regulations on indiscriminate discharge of untreated effluents from industries, hospitals, and households into the waterbodies should implemented.

SALLY LIGHTFOOT CRABS (*Grapsus grapsus)* IN THE EDGE OF PLASTIC POLLUTION

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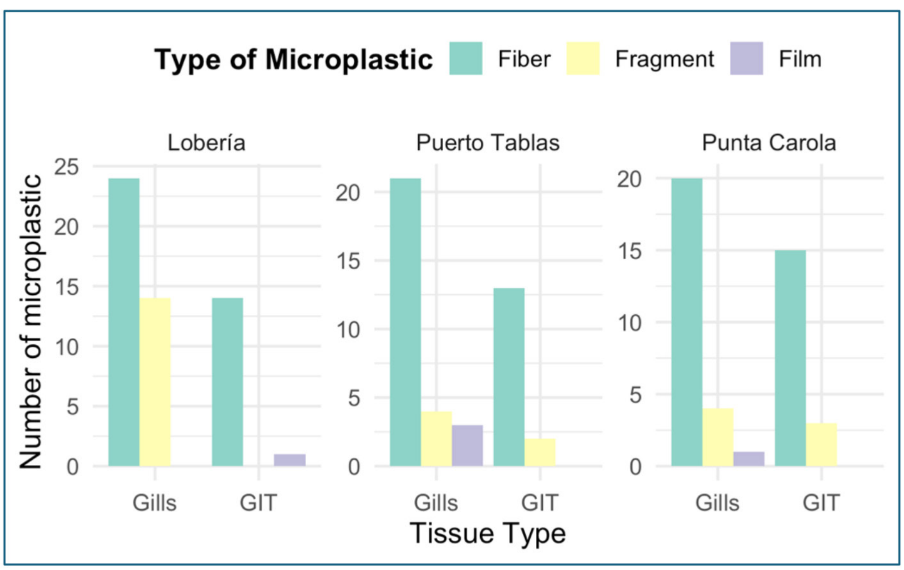
Sally Lightfoot crabs (*Grapsus grapsus*), brightly colored coastal scavengers, are widespread across the Galapagos Islands and along the western coasts of South and Central America. This study evaluated microplastic (MP) pollution in native Sally Lightfoot crabs from three locations on San Cristobal Island (La Loberia, Puerto Tablas, and Punta Carola) in the Galapagos Archipelago. We analyzed the blood chemistry and basic health parameters and presence of MPs in the gills (GILL) and gastrointestinal tract (GIT) of 60 crabs (20 per location), employing Fourier Transform Infrared Spectroscopy (FT-IR) for MP quantification. Fibers constituted the most common polymer type (n = 106), followed by fragments (n = 27) and films (n = 5). A Generalized Linear Model (GLM) analysis assessed the relationships between microplastic presence in the gills and gastrointestinal tract and variables such as sex, weight, length, location, and heart rate. The best-fitting model (AICc = 149.27) included only the intercept, indicating no significant associations between MP presence in gills/GIT and the tested variables. A Chi-Squared test showed no significant differences across locations in terms of MP shape, type, color, length, or polymer composition, suggesting uniform MP properties across sites, likely due to similar pollution sources or transport patterns. However, a Kruskal-Wallis rank sum test revealed a significant difference in MP accumulation between the gastrointestinal tract and gill tissues (chi-squared = 12.534, df = 1, p = 0.0004), indicating differential MP retention in these tissues.



REPETITIVE ELEMENTS FROM THE FIRST SPECIFIC PATHOGEN-FREE (SPF) SHRIMP *Penaeus vannamei* PRODUCED IN THE UNITED STATES: CHARACTERIZATION OF *Outcast-1_Lva* NON-LTR RETROTRANSPOSON SIMILAR TO RETROTRANSPOSON NLRS PUTATIVELY ASSOCIATED WITH ABDOMINAL SEGMENT DEFORMITY DISEASE (ASDD) OF FARMED *P. vannamei* FROM THAILAND

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Limited information is available on the content of repetitive elements [REs] in Penaeid shrimp. A pilot genome sequence (470 Mb) from the first specific pathogen-free (SPF) *Penaeus vannamei* produced by the breeding program of the U. S. Marine Shrimp Farming Program (USMSFP) generated 479 REs representing 269 long terminal repeat (LTR) retrotransposons, 83 non-LTR retrotransposons, and 127 DNA transposons. Sixty elements were also obtained from the genome assembly of *P. vannamei* farmed in China (ASM378908v1: 1.66 Gb). Some REs show similarity to SPF *P. vannamei* microsatellites including the telomeric pentanucleotide $(TAACC/GGTTA)_n$ repeats, the site of insertion of nimavirus *Nimav-1_LVa* (https://pubmed.ncbi.nlm.nih.gov/31947590/). The non-LTR retrotransposon *Outcast-1_LVa* (6,180 bp) is further characterized.

A genomic locus [KC179708, 4101-bp] comprising 4-kb nucleotides derived from the 1,974-6,062 of *Outcast-1_LVa,* shows 96.9% identity to the consensus sequence of *Outcast-1_LVa* stored in Repbase (www.girinst.org). KC179708 locus was putatively associated with Abdominal Segment Deformity Disease (ASDD) of farmed *P. vannamei* from Thailand. Researchers suggested that ASDD may be related to inbreeding and use of eyestalk ablation (EA) in female broodstock. In the ASM378908v1 genome assembly 632 *Outcast-1_LVa* loci are identified in 43 scaffolds and is actively expressed in *P. vannamei* transcriptomes from various developmental stages and adult tissues. Expression changes were observed in ovaries six days after EA.

Considering that the genome size of SPF *P. vannamei* was estimated at 2.89 Gb, a new contiguous whole reference genome for *P. vannamei* is needed to fully characterize its repeatome, study molecular and epigenetics mechanisms involved in growth and disease susceptibility or tolerance/resistance and determine whether expression of *Outcast-1_LVa* is associated with EA and inbreeding.

DNA TRANSPOSONS IN THE GENOME OF THE FIRST SPECIFIC PATHOGEN-FREE (SPF) SHRIMP, *Penaeus vannamei,* PRODUCED IN THE UNITED STATES

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A total of 186 DNA transposons families including one integrated large DNA virus were identified in the genome of Pacific white shrimp *Penaeus vannamei* and are deposited in Repbase (www.girinst.org). Of these, 127 were identified in a small-scale genomic sequence (479 Mb) obtained from the specific pathogen-free (SPF) *P. vannamei* Kona Line produced by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP). These DNA transposons set consist of 42 *DNA*, 7 *DNAV*, 1 *EnSpm*, 11 *Harbinger*, 13 *hAT*, 2 *Kolobok*, 10 *Mariner*, 12 *Merlin*, 1 *MuDR,* 1 *P*, 8 *piggyBac*, 3 *Polinton*, 5 *Sat*, 8 *TE*, 2 *Transib*, and 1 *Zator* families. Additional 59 DNA transposons were also identified from the genome assembly of *P. vannamei* breed Kehai No.1 farmed in China [ASM378908v1) including 4 *DNA*, 1 *Merlin*, 1 *hAT*, 1 *piggyBac*, 33 *Sat*, 2 *TE*, and 17 uncharacterized *REP* families.

Some *Sat* transposons show similarity to microsatellite sequences published from the SPF *P. vannamei* Kona Line, including the telomeric pentanucleotide repeat $(TAACC/GGTTA)_n$. These repeats are also the insertion site of the integrated large DNA virus, a nimavirus (*Nimav-1_LVa,* https://pubmed.ncbi.nlm.nih.gov/31947590/*)* reconstructed to be a consensus sequence totaling ~279 kbp in length, which is designated as *DNAV-1_LVa* and is stored separately in seven segments in Repbase (*DNAV-1a_LVa* to 1g). Homology searches using the five Nimaviridae databases in GenBank showed *DNAV-1_LVa* with 65-75% sequence identity to *Marsupenaeus japonicus* endogenous nimavirus DNA (BFCD01000001, 217,415 bp).

The three whole genome sequence (WGS) databases available for *P. vannamei* (ASM378908v1, 1.7 Gb; CIBNOR_Pvan_1v2, 2.1 Gb; ASM3358929v1, 1.9 Gb) confirm that the telomeric pentanucleotide repeat (TAACC/GGTTA)n of *P. vannamei* is highly abundant and widely distributed in intron and intergenic regions of the genome. *P. vannamei* shares the same telomeric pentanucleotide repeat (TTAGG)n with most insects. The wide interstitial distribution of telomeric repeats is intriguing and may have important implications for the shrimp genome that is also rich in other simple sequence repeats. The evolutionary origin of telomeric repeats is not fully understood, but it has been suggested that the invasion of circular chromosomes by telomeric repeats may have given rise to linear chromosomes of eukaryotes. The wide interstitial distribution of telomeric repeats in the shrimp genome may represent extensive, recent, or active invasion by the pentanucleotide repeat. Considering that the estimated genome size of SPF *P. vannamei* from the United States is 2.89 Gb, a new contiguous, whole reference genome for *P. vannamei* is needed to fully characterize telomeric repeats. Information on the chromosome locations (# of HiC_scaffolds) of the 186 DNA transposons in the ASM378908v1 assembly will be presented.

PUTATIVE ENDOGENOUS VIRAL ELEMENTS (EVE) PRESENT IN FIVE WHOLE GENOME SHOTGUN (WGS) DATABASES AVAILABLE FOR THE PACIFIC WHITELEG SHRIMP *Penaeus vannamei*

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Five whole genome shotgun (WGS) databases are available in GenBank for the Pacific whiteleg shrimp *Penaeus vannamei* (WGS_VDB://DAWKWD01, WGS_VDB://JANIEY01, WGS_VDB://JBFNAF01, WGS_VDB://QCYY01, WGS_VDB://QWLK01). In addition, a pilot sequence (470-Mb) of the first specific pathogen-free (SPF) *P. vannamei* produced by the breeding program of the United States Marine Shrimp Farming Program (USMSFP) generated 441 repetitive elements. Present in these WGS assemblies are DNA transposons [transposable elements and simple sequence repeats (SSRs)] homologous to endogenous viruses like nimavirus *Nimav-1_LVa* (279,905-bp) and white spot syndrome virus (WSSV)-like [*DNAV-1_LVa* (279,384-bp)]. Some SSRs show similarity to *P. vannamei* microsatellites including the telomeric pentanucleotide (TAACC)n microsatellite, the site of insertion of *Nimav-1_LVa.*

Other virus sequences are integrated in *P. vannamei* including portions of infectious hypodermal and hematopoietic necrosis virus (IHHNV; renamed *Decapod penstylhamaparvovirus 1*; AF218266.2, 3,909-bp) and *P. vannamei* solinvivirus (PvSV) (OP265432, 10,447-bp) identified in diseased Brazilian shrimp. BLASTN searches revealed 93% identity of OP265432 to Wenzhou shrimp virus 8 (KX883984, 10,445-bp) and 91% identity to *P. vannamei* picornavirus (OK662577, 10,550-bp). WGS searches identified portions of the 3'-end of OP265432 (92-93% identical) to three sequences [QWLK01003484, QWLK01003486, QWLK01003485] in the contig-level genome assembly ASM373033v1 of *P. vannamei* F1 breed from China (GCA_003730335), but is not present in the large scaffold-based genome assembly of *P. vannamei* breed Kehai No.1 farmed in China (GCA_003789085; 1.7-Gb) or in the recently published assembly ASM3358929v1 (GCA_033589295.1, 1.9-Gb). Similar results were found in the 3'end of Wenzhou shrimp virus 8 strain (KX883984 and OK662577), suggesting putative endogenous viral elements (EVE) of PvSV (PvSV-EVE) present in *P. vannamei* genome. WGS searches of 16 databases for Penaeoidea (taxid:111520) confirmed that these EVEs are specific for *P. vannamei*.

Considering that the estimated genome size for the first SPF *P. vannamei* produced by the USMSFP is 2.83-Gb, a new, contiguous, whole reference genome for *P. vannamei* is needed to confirm presence of these endogenous virus sequences.

PENAEID SHRIMP GENOMICS AND EPIGENOMICS – WHOLE REFERENCE GENOMES NEEDED FOR THE ECONOMICALLY IMPORTANT *Penaeus* SPECIES

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Shrimp is the most important internationally traded fishery commodity in terms of value, with a global market valued at USD 37.6 billion in 2021. Despite its economic importance, research on penaeid shrimp genomics and epigenomics is lacking. So far, only five reference genome assemblies for Penaeid species are available in the Genome Database of NCBI (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=133894): *Penaeus chinensis, P. indicus, P. japonicus, P. monodon* and *P. vannamei*.

The *P. monodon* reference genome sequence is the most complete of all, with an assembly size of ~2.39-Gb, and is the only sequence approximating the expected size of ~2.89-Gb (Jeffery & Gregory, 2019) of the specific pathogen-free (SPF) *P. vannamei* domesticated by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP), the most cultured species worldwide. The assembly size of *P. chinensis* (GCF_019202785.1) is ~1.47-Gb, *P. indicus* (GCA_018983055.1) is ~1.94-Gb, *P. japonicus* (GCF_017312705.1) is ~1.71-Gb, and *P. vannamei* cultured in China (GCF_003789085.1) is ~1.66-Gb.

Research is needed to fill the gaps in the whole reference genomes of Penaeids to address issues like identification of sex-determining genes, susceptibility to diseases caused by viral and bacterial pathogens, growth performance, environmental contamination, interactions of dengue virus with shrimp densoviruses like IHHNV and transposable elements and the impact of climate change, and further for application of molecular genomic assisted breeding techniques in shrimp.

Plans are being discussed for a special issue of the '*Journal of Shellfish Research*' titled 'Penaeid Shrimp Genomics and Epigenomics – Whole Reference Genomes for the Economically Important Penaeus Species' and we welcome reviews or original articles covering genomic, epigenomic, or post-genomic profiling of penaeid shrimp that may provide the clues to solve the mechanisms of pathogenesis, evolution, and resilience to environmental change. For example, whole exomes, genomes and gene–environment susceptible microbiomes, metabolomes, proteomes, transcriptomes, and methylomes can individually and/or collectively inform specific molecular mechanisms, leading to the potential identification of factors or simple sequence repeats, transposable elements, and epigenetic marks to study transgenerational epigenetic inheritance of disease tolerance. This will eventually identify the most effective genomic selection approaches and precise treatments for emerging diseases.

ONE HEALTH EPIGENOMES AND MICROBIOMES SESSION: FROM SOIL TO PEOPLE - RECOGNITION TO STUDENTS AND "OUTSTANDING ONE HEALTH RESEARCHERS IN AQUACULTURE AND FISHERIES" AWARDEES

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At the 'ONE HEALTH Epigenomes and Microbiomes' session we will recognize eleven students, postdocs and researchers who applied for 'Jimmy Alcivar Arteaga Travel Awards' from the FUCOBI Foundation of Ecuador (fucobi.org) to attend the Aquaculture 2025 meeting. Applicants represented two countries: United States (10) and Philippines (1). In addition, four scientists from the United States will be recognized as winners of FUCOBI's 2025 "Outstanding ONE HEALTH Researchers in Aquaculture and Fisheries" awards: Laura Vandenberg, Salvador Rico, Steve Morton, and Sandra Shumway.

The speakers of the ONE HEALTH session will address the following topics: From Bisphenol A (BPA), to phthalates, to per- and polyfluoroalkyl substances (PFAS): How environmental chemicals are harming fish, shellfish and human health and what we can do about it (Vandenberg), Culture-independent meta-pangenomics enabled by long read metagenomics (Minich), Advances in transposable element annotation for genome analysis in aquaculture species (Rodriguez), Community Action for Fresh Water (CAFW): A Rotary International (RI) and United Nations Environment Program (UNEP) initiative to restore, protect, and sustain freshwater ecosystems (Rico), Regenerating coast of Pujada Bay: a community-based mangrove rehabilitation and enhancement in the city of Mati, Davao Oriental, Philippines (Jimenez), Freshwater mussels: global bellwethers and enhancers of aquatic ecosystem health (Kreeger), Characterization of the (green) microbial and physiological diversity on retention ponds in the Lake Ontario watershed (Molee), Participatory science approach to mitigate the effects of harmful algal blooms in Costa Rica (Morton), Reverse transcriptase related proteins are recruited by multiple aquatic organisms to serve in environmental stress response pathways (Yushenova), Enhancing marine ecosystems and promoting sustainable aquaculture (TenBroeck), A review of biodiversity and chemical contaminants in fish from Lake Victoria, Uganda (Roy), The Environmental Sustainability Rotary Action Group (ESRAG): helping to implement sustainable practices on biodiversity, circular economy, food systems, sustainability, climate and pollution (DeWitt) and, How to write effective global grants to apply for funding for your project by Rotary International (DeWitt, Rico).

MANGROVE SHELLFISH SESSION: FROM CARBON STORAGE TO EPIGENETICS - RECOGNITION TO STUDENTS AND "2025 OUTSTANDING ONE HEALTH RESEARCHERS IN AQUACULTURE AND FISHERIES" AWARDEES

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The Mangrove Shellfish session will recognize forty-six students and research associates who applied for 'Jimmy Alcivar Arteaga Student Travel Awards' from the FUCOBI Foundation of Ecuador (fucobi.org) to attend Aquaculture 2025. Applicants represented 16 countries: Ecuador (9), India (7), Mexico (5), USA (5), Kenya (4), Peru (3), China (2), Philippines (2), Uganda (2), Bangladesh (1), Belgium (1), Canada (1), France (1), Nigeria (1), South Africa (1), and Tanzania (1). In addition, four scientists will be recognized as winners of FUCOBI's 2025 "Outstanding ONE HEALTH Researchers in Aquaculture and Fisheries" awards: Daniel Friess, Chistopher Puttock, and Alfredo Quarto from the USA (3) and Jianbo Yuan from China (1).

Speakers will address the following topics: Blue carbon and other ecosystem services in mangrove forests (Friess), The mangrove epigenome (MangroveENCODE) project of the FUCOBI Foundation: A One Health approach to conserving mangroves biodiversity (Rodriguez), What is Rotary and How does Rotary work for shellfish (Puttock), Status of the mangrove forests of Peru (Campos), Heavy metals in mangrove sediment and native species from the genus *Anadara* sp. and *Penaeus* sp. (Echevarria-Flores), Rhizosphere sulfur oxidizing communities dominate plant-microbe interactions in mangrove ecosystems (He), Trace metals in muscle of *Penaeus vannamei* from Ecuador and El Salvador, and *P. monodon* of Philippines (Aveiga), Evaluation of the effect of glyphosate on *P. vannamei:* enzyme activity profiles and oxidative stress as an indicator (Amutha), A One Health analysis of estuarine pollutants affecting mangrove crabs and related consumers in Ecuador (De Cock), What is meant by "mangrove friendly aquaculture?" (Quarto), Some don't like it noisy: shipping noise alters the behaviour and habitat usage of waved whelk *Buccinum undatum* (Gastropoda) (Uboldi), Distribution, population structure and fishery potential of the golden deep-sea crab *Chaceon somaliensis* in the Kenyan coast (Makokha), Trash to treasure: harnessing local resilience to combat ghost nets in Bangladesh (Mamun), A transposable element–epigenetics One Health perspective to understand antimicrobial resistance and contamination by EDCs (glyphosate, metals), microplastics, DEPH, PFAS- and adaptation to climate change (Rodulfo), Integration of chromatin accessibility and gene expression identifies osmoregulation-related transcription factors in penaeid shrimp (Yuan), Shrimp Scampi: A Citizen Science Project - educating about microbes and EDCs using foldscopes (Warren), Taxonomy of shellfish follows the classification by the World Register of Marine Species, only *Penaeus vannamei* Boone, 1931 is accepted - *Litopenaeus vannamei* is not accepted (Alcivar-Warren), Effects of microalgae with normal and high lipids on gonadal development of northern quahogs *Mercenaria mercenaria* (Kallau), Welfare in shrimp *P. vannamei*: eyestalk ablation, sentience and pain perception – a review (Asuncion), Enhancing marine ecosystems and promoting sustainable aquaculture: ESRAG's commitment to a healthier planet (DeWitt) and, Workshop: How to write effective global grants to apply for funding for your project by Rotary International (DeWitt, Rico).

TAXONOMY OF SHELLFISH FOLLOWS THE CLASSIFICATION BY THE WORLD REGISTER OF MARINE SPECIES (WORMS) FOR PENAEUS SPECIES, ONLY *Penaeus vannamei* Boone, 1931 IS ACCEPTED - *Litopenaeus vannamei* IS NOT ACCEPTED

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The taxonomy of *Penaeus* Fabricius, 1798 (Crustacea: Decapoda: Penaeidae) has been reconstructed.[1,2] There has been substantial confusion about their classification over the last few decades[3-4] since the genera scheme proposed by Perez Farfante and Kensley was published.[5] Based on complete mitochondrial genomes and nuclear genes, the phylogenetic relations and genetic differentiation of penaeid shrimps have clearly demonstrated monophyletic status and aptness of single genus nomenclature for shrimp in *Penaeus s,l.*, corroborating findings from phylogenetic analyses in dismissing the six genera nomenclature and favoring reinstating the old genus *Penaeus.*[6-7] The six genera scheme proposed by Perez Farfante and Kensley[5] should be overturned and single genus status for *Penaeus s.l.* be reinstated. Research should continue to confirm taxonomy where both morphological and molecular data are congruent.

In the meantime, let's use the taxonomy classification of shellfish species following the classification by the World Register of Marine Species (WoRMS, 2024).[2] For *Penaeus vannamei* Boone, 1931 the species *Litopenaeus vannamei* (Pérez Farfante & Kensley 1997) is not accepted. The accepted classifications for other Penaeus species are: *Penaeus monodon* Fabricius, 1798; *Penaeus japonicus* Spence Bate, 1888; Penaeus chinensis Osbeck, 1765; Penaeus stylirostris Stimpson, 1871; Penaeus setiferus Linnaeus, 1767; *Penaeus aztecus* Ives, 1891; *Penaeus duorarum* Burkenroad, 1939; Penaeus japonicus Spence Bate, 1888; *Penaeus indicus* H. Milne Edwards, 1837; and *Penaeus merguiensis* de Man, 1888 [in de Man, 1887–1888]. Considering there are more than 20 Penaeus species, taxonomy should be confirmed using both whole genome sequencing and morphological tools. Information on other Penaeid shrimps, and marine molluscan species listed in the MolluscaBase database (www.molluscabase.org/) are also displayed in WoRMS.[2]

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SHRIMP GENOMES AND EPIGENOMES (ShrimpENCODE) SESSION: FROM WHOLE GENOME SEQUENCES OF PENAEID SHRIMP TO EPIGENETICS - RECOGNITION TO STUDENTS AND "OUTSTANDING ONE HEALTH RESEARCHERS IN AQUACULTURE AND FISHERIES" AWARDEES

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The ShrimpENCODE session will recognize ten students and research associates who applied for 'Jimmy Alcivar Arteaga Student Travel Awards' from the FUCOBI Foundation of Ecuador (fucobi.org) to attend Aquaculture 2025. Applicants represented four countries: Mexico (5), United States (3), Brazil (1) and Ecuador (1). Four scientists will be recognized as winners of FUCOBI's 2025 "Outstanding ONE HEALTH Researchers in Aquaculture and Fisheries" awards: Satoshi Kawato from Japan (1), Sonia Soto-Rodriguez and Bruno Gomez-Gil from Mexico (2) and Ximing Guo from the United States (1).

ShrimpENCODE speakers will address the following topics: Endogenous nimaviruses: inhabitants of crustacean repetitive DNA (Kawato), Shrimp shapes a resistance trait against Vibriosis by memorizing the colonization resistance of intestinal microbiota (Yuan), Repetitive elements from the first SPF *Penaeus vannamei* produced in the US: characterization of *Outcast-1_Lva* Non-LTR retrotransposon similar to retrotransposon putatively associated with ASDD of farmed *P. vannamei* (Alcivar-Warren), DNA transposons in the genome of the first SPF *P. vannamei* produced in the US (Alcivar-Warren), Putative endogenous viral elements (EVE) present in five whole genome shotgun databases available for *P. vannamei* (Alcivar-Warren), Development of a disease strategy manual for shrimp hepatopancreatic microsporidiosis (HPM) (Tang), First report of white muscle disease caused by *Photobacterium damselae subsp. damselae* in *P. japonicus* (Langote Alolod), Sex determination and sex differentiation of *P. vannamei* (Guo), Penaeid shrimp genomics and epigenomics: whole reference genomes needed for the economically important Penaeus species (Alcivar-Warren), *Vibrio campbellii* strain M270210 can develop the characteristic clinical signs and mortality of AHPND disease of shrimp (Gonzalez-Benitez), Stepwise involvement of peptidases in the digestive process of *P. vannamei* (Estrella), Exploring the impact of domestication and breeding on the gut microbiota of *P. vannamei* (Cervantes-Echeverria), Application of *Faecalibacterium* genomics for human and shrimp gut health (Sousa), Endogenous virus elements (EVE) of IHHNV (IHHNV-EVE) in *P. vannamei* & *P. monodon* genomes: interactions with a transposable element - comments on issues related to the delisting of IHHN as a notifiable disease by the WOAH (Asuncion), WSSV genomes from Ecuador, Peru, China & Mexico are not integrated in the genome of five penaeid species, but EVE of WSSV (WSSV-EVE) are in the transcriptome of SPF *P. vannamei* domesticated in the US (Galindo), Characterization of bacteriophage-resistant strains from bacteriophages targeting AHPND-causing *Vibrio sp*. (Aguire Juarez), Dengue vectors co-infecting shrimp viruses: epidemiological control challenges in the context of climate change and contamination by insecticides (Rodriguez-Flores), and, Limitations of implementation of RNA interference (RNAi) in shrimp aquaculture (Rodulfo). and, Unlocking the potential of *P. vannamei* breeding through genomic selection (Arteaga-Cedeno).

SINGLE-NUCLEI TRANSCRIPTOME ATLAS OF SPLEEN CELLS FROM CHANNEL CATFISH *Ictalurus punctatus*

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Channel catfish, *Ictalurus punctatus,* are the primary cultured species of the catfish industry. Significant losses in production are attributed to bacterial diseases which are challenging to control and new interventions need to be developed. To gain insight into the *in situ* catfish immune system, we used single-nuclei RNA sequencing.

We produced a single-nuclei atlas from three individual catfish. Libraries were prepared using the 10X Genomics Chromium X system and sequenced on an Illumina sequencer. After aligning to the channel catfish reference genome (Ip Coco_2.0) and filtering for valid nuclei, the cell types were characterized, and trajectory was predicted for B cells.

The spleen atlas included 19,613 cells sequenced to ~34.5 thousand reads/cell, and a median of 665 genes/cell. Both innate and adaptive immune cells were identified including B cells, T/NK cells and myeloid cells (Figure 1). Looking closer at the expressed genes, we characterized immature and mature B cells, plasma cells, γδ T cells, dendritic cells, and macrophages. Several sub-clusters that were not characterized may represent rare cell types.

The splenic cell atlas is a rich resource for examining the immunome of *I. punctatus*. The data will be used to inform future research directions into disease control and interventions.

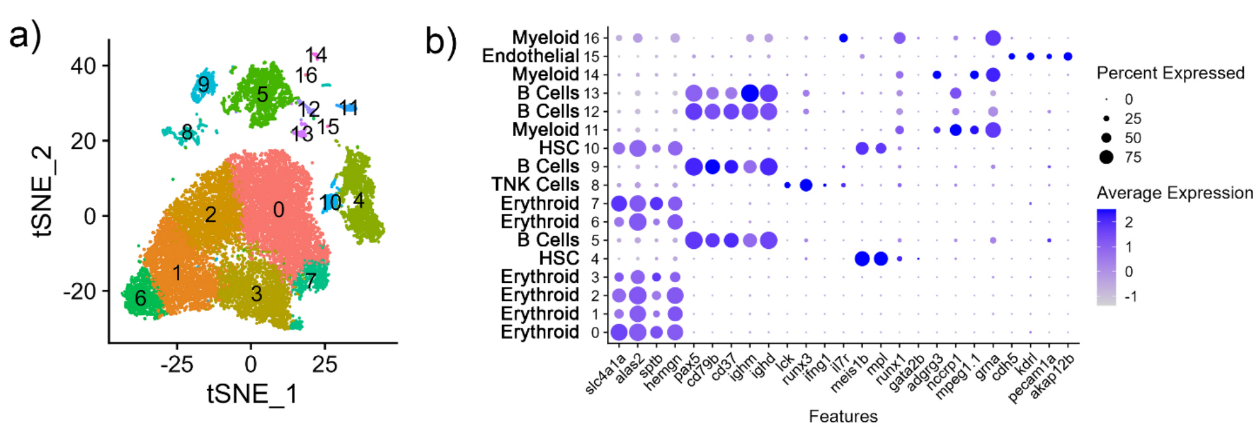

Figure 1: Characterization of spleen cells. a) UMAP plot of cells. b) Dot plot of selected cell type markers. Average expression represented by z-score.

IMPROVEMENT OF EASTERN OYSTER, *Crassostrea virginica*, SPAWNING PERFORMANCE UTILIZING INNOVATIVE METHODS

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The Horn Point Laboratory Oyster Hatchery (HPLOH), based at the University of Maryland Center for Environmental Science (UMCES), has a long history of producing Eastern Oyster (*Crassostrea virginica*) larvae, spat-on-shell, and seed oysters for use in restoration, fishery, research, and educational programs. As all steps in production are continuously evaluated for improvement, one area that HPLOH has been recently focusing on is the spawning performance. So far, the spawning stage at HPLOH has predominantly relied on batch spawns using a traditional mass spawning table for diploid larval production and individual cup racks for triploid spawns. This season (2024), a modified version of our individual cup spawning rack was tested for production of both diploid and triploid larvae, and its performance was compared with the one of the mass spawning table. This customized design promotes the concurrent spawning of up to 100 oysters, allowing temperature, salinity, and ploidy to be controlled, with the ability to produce diploid wild, diploid disease resistant, and triploid strains without sacrificing adult broodstock. This design also involves both flow-through and recirculating water options. The recirculating mode promotes a simultaneous stimulation of all oysters, while the flow-through mode facilitates the control of inflow and outflow water to/from each individual cup, allowing a more efficient egg collection. With a significant increase in the overall egg production, the results of this study highlight the potential for this system to improve both the accuracy of fecundity assessments and the hatchery production of *C. virginica* larvae and spat.

IMMUNE-ANTIOXIDANT TRAIT, *Aeromonas veronii* RESISTANCE, GROWTH, INTESTINAL ARCHITECTURE, AND SPLENIC CYTOKINES EXPRESSION OF *Cyprinus carpio* FED *Prunus armeniaca* KERNEL-ENRICHED DIETS

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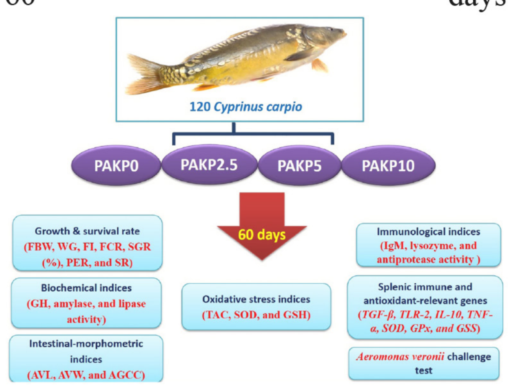
Currently, the intervention of plant by-products in the fish diet has gained tremendous attention owing to the economic and high nutritious value. The current study is a pioneer attempt to incorporate the apricot, *Prunus armeniaca* kernel powder (PAKP) into the Common carp, *Cyprinus carpio* diets, and assess its efficacy on growth, digestion, intestinal morphology, immunity, antioxidant capacity, and splenic cytokines expression, besides the antibacterial role against *Aeromonas veronii* infection.

Apparently healthy fish (N = 120) with an initial body weight of 24.76 ± 0.03g were allotted in 12 glass aquaria (60 L) and randomly distributed into four groups (triplicates, 10 fish/aquarium). The control group (PAKP0) was fed a basal diet without additives. The second, third, and fourth groups were provided PAKP diets with various concentrations (2.5 (PAKP2.5), 5 (PAKP5), and 10 g kg^{-1} (PAKP10)) respectively. After 60 days (feeding trial), sub-samples of the fish (12 fish/group) were intraperitoneally injected with 10^7 CFU mL^{-1} of *A. veronii*.

Results revealed that body weight gain, feed conversion ratio, and specific growth rates were significantly augmented in the PAKP10 group in comparison to the other groups. The dietary inclusion of PAKP at all concentrations boosted the digestive capacity and maintained the intestinal morphology (average villus length, villus width, and goblet cells count) with a marked improvement in PAKP10. Moreover, fish fed on PAKP10 followed by PAKP5 then PAKP2.5 diets had noticeably elevated values of immunological biomarkers (IgM, antiprotease, and lysozyme activity) and antioxidant capabilities (the total antioxidant capacity, superoxide dismutase, and reduced glutathione) as well as significant upregulation of immune and antioxidant-related genes (TGF-β2, TLR-2, TNF-α, IL-10, SOD, GPx, and GSS). Fourteen days post-infection with *A. veronii*, the highest relative percentage survival of fish was observed in PAKP10 (83.33%), followed by PAKP5 (66.67%), and PAKP2.5 (50%).

Our results indicated that a dietary intervention with PAKP could promise growth, digestion, immunity, and protect *C. carpio* against *A. veronii* infection in a dose-dependent manner. This offers a framework for future application of such seeds as a growth promotor, immune-stimulant, and antioxidant, besides an alternative cheap therapeutic antibacterial agent for sustaining the aquaculture industry.

Fig. 1. Schematic flowchart showing the experimental design of the exposure of *Cyprinus carpio* to the various treatments of dietary *Prunus armeniaca* kernel powder (PAKP) for 60 days



EFFECTS OF AQUAPONIC FORMULATED FEED ON NILE TILAPIA (*Oreochromis niloticus*) HEALTH AND ON NUTRIENT DENSITY IN THAI BASIL

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Aquaponics can produce 10x more than agriculture and 5x more than aquaculture, making it the world's solution to food insecurity. With the amount of arable land diminishing, aquaponics has become more popular as we can harvest both fish and plants without the use of fertile soil. In a coupled aquaponics system fish and plants share the same water source, this limits the use of fertilizers and supplemental nutrition for the plants to not affect fish health. There are supplements that can be used but they do come with a heavy associated cost. Optimal Fish Food produces a diet that has been formulated to fortify potassium, phosphorus, and iron that will enter the water column via fish waste. These nutrients are often lacking in aquaponics and will elicit optimal fish and plant growth. To determine if this feed actually works, the overall health of Nile tilapia and nutrient density in Thai basil will be examined. Fish will be fed Optimal Fish Food's Optimal Aquaponic feed and Purina Promax Fingerling Starter 300. Length, weight, blood glucose, packed cell volume, plasma protein, and lysozyme will be measured on day 0 and day 40 for both groups to evaluate fish health. Basil from both groups will be harvested after 48 days of growth. Leaf samples will be sent to A&L Great Lakes Laboratories for nutrient testing and total harvest wet weight and sample dry weight will be used to evaluate plant growth and nutrient richness. Results will be presented at the conference.

EVALUATION OF MUCUS PEPTIDES, BLOOD AND TISSUE PROTEINS, AND CLASSICAL HORMONE MEASURES FOR ASSESSING ENVIRONMENTAL STRESSOR RESPONSES IN TWO CULTURED FISH SPECIES

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Environmental and handling stressors are common to every aquaculture system and affect various cultured fish species differently. Understanding these diverse stress responses is crucial for optimizing aquaculture practices. This study investigates the physiological responses to stressors of two fish species representative of cultured species groups in North America. The species selected, channel catfish (*Ictalurus punctatus*) and alligator gar (*Atractosteus spatula*), were hypothesized to differ greatly in magnitude of responses. We exposed both species to environmental and handling stressors and evaluated their physiological stress responses using a variety of methods, including analyses of blood and tissue metabolites and measurements of the classical stress hormone cortisol. Additionally, we compared the skin mucus peptide profiles of both species following stress exposure. The results will provide a baseline for understanding physiological responses to stress in these species, be useful for comparing a range of stress levels and the techniques used to measure stress, and offer insights that can inform and improve culture practices in aquaculture.

FIRST REPORT OF WHITE MUSCLE DISEASE CAUSED BY *Photobacterium damselae* subsp. *damselae* IN KURUMA SHRIMP *Penaeus japonicus*

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Kuruma shrimp (*Penaeus japonicus*) is a commercially important Penaeid species widely cultured, and highly valued seafood in Japan. However, diseases are limiting factors for *P. japonicus* resulting in massive mortalities and low production output. In August and September 2022, two disease outbreaks were observed in culture farms in Okinawa, Japan. Diseased animals exhibited muscle whitening extending from the abdominal to the distal region. This study reports the first disease occurrence in Kuruma shrimp associated with *Photobacterium damselae* subsp. *damselae* (Pdd) having whitened musculature as a major clinical sign.

After obtaining the RNA and DNA shotgun sequences, we identified nonhost contigs showing significant taxonomic alignment to bacterial species. This led to the investigation of a bacterium as a possible causative agent, which later we characterized its population in the diseased shrimp muscle. The majority of the *16S rRNA* sequence recombinant clones had their highest homology with *Photobacterium* sp. (> 99%) which isolation of any of its species was pursued. The three bacterial isolates (WMD-P1, WMD-P2, and WMD-P3) from the whitened muscle tissue identified as *Photobacterium damselae* subsp. *damselae* (Pdd) were further characterized.

Their genomes consisted of two circular chromosomes with varying numbers of plasmid. Its size ranges from 4.47 Mb to 4.60 Mb with an average GC content of 40.8%, with the predicted number of coding sequences (CDs) ranging from 3816 to 4031. From the genomes, two reported virulence genes encoding for leukocidin pore-forming toxin (*hlyA*) and phospholipase (*pldA*) were identified.

Putative virulence factors are involved in adherence, antiphagocytosis, chemotaxis and motility, iron uptake, quorum sensing, secretion system, and immune evasion. Also, the presence of prophages, genomic islands, and antimicrobial-resistant genes suggests episodes of horizontal gene transfer. Average nucleotide identity (ANI) and pangenome analyses revealed a high genetic relationship of the isolates (>98%) with Pdd from other sources. Moreover, intramuscular injection at 1×10^8 CFU/ml and 1×10^3 CFU/ml produced pathological signs similar to those in naturally infected shrimp after 24 hpi and 10dpi respectively.

Fig. 1. Diseased Kuruma shrimp with apparent whitish discoloration in abdominal to distal muscle portions (a), cloudy meat appearance (b), and whitened muscle cross-section (c).

POTENCY OF CASSAVA LEAF AS PROTEIN SOURCE FOR NILE TILAPIA (*Oreochromis niloticus*)

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Cassava leaves can be used as a source of protein for Nile tilapia. However, as the leaves have high level of anti-nutritional factors that could reduce fish growth. The main aim of this study was to improve its nutrient contents by processing and evaluated its effects on growth performance of Nile tilapia. Fresh cassava leaves were treated with namely: air dried (AD), soaked-boiled sun dried (SBSD), Yeast (YF), Molasses (MF), Rumen-fluid (RF), Yeast-molasses (YMF), Yeast-rumen-fluid (YRF) and Molasses-rumen-fluid (MRF) fermentations.

Then nine iso-nitrogenous and iso-caloric diets were prepared for Nile tilapia. The results of the present study showed that YF resulted in the highest ($p < 0.05$) crude protein (CP) content (CP=30.3%) than others. Furthermore, in most nutrient parameters, rumen fermentation resulted in better nutrient contents than others. Fish fed RF, YMF and MRF based diets showed better apparent digestibility coefficient (ADC) of dry matter and ether extract than others next to the control diet. In addition, fish fed RF based diet showed comparable ADC of CP with the control diet. In most cases, fish fed RF, MRF and YMF cassava leaf-based diets showed better growth performance in terms of FBW, SGR and DWG than others next to control diet. In terms of feed utilization efficiency fish fed RF cassava leaf-based diet showed comparable FCR with fish fed control diet. Similarly fish fed RF and MRF cassava leaf-based diets had comparable PER with control diet. Fish whole body proximate compositions were also affected by the types of experimental diets except for CP content. The cost benefit ratio, gross profit margin, and return on investment were better for fish fed RF based diets than others. In conclusion, rumen-fluid fermentation method is the preferred processing method to improve nutrient value of cassava leaves resulted in better growth performance and profitability Nile tilapia farming.

Table 4 Mean apparent digestibility of nutrient of Nile tilapia fed different processed cassava leaf-based diets (%)

Parameter (% DM)	Control	AD	*SBSD*	YF	MF	RF	YRF	YMF	MRF
Mid Gut									
Dry Matter (DM)	65.1^{c}	51.4^{a}	61.0^{bc}	57.2^{ab}	52.7^{a}	61.8^{bc}	52.8^{a}	62.1^{bc}	$60.7b^{c}$
Crude Protein (CP)	84.2^{e}	70.0^{a}	76.1^{bc}	73.8^{b}	74.3^{b}	80.5^{d}	73.6^{ab}	79.2^{cd}	78.6^{cd}
Ether Extract (EE)	88.5^{e}	75.8^{a}	80.9^{bc}	78.8^{b}	80.0^{b}	85.1^{d}	79.3^{b}	83.8^{d}	83.3^{cd}
Gross Energy (GE)	75.3^{d}	60.9^{a}	68.6^{bc}	64.6^{ab}	62.7^{a}	70.0^{c}	62.4^{a}	70.8^{cd}	69.0^{bc}
Hind gut									
Dry Matter (DM)	73.4^{e}	54.8^{a}	67.3^{cd}	64.7^{bc}	64.9^{c}	73.5^{d}	58.1^{ab}	71.2^{cd}	71.7^{d}
Crude Protein (CP)	89.9^{e}	79.8^{a}	81.1^{ab}	83.7^{bc}	83.6^{bc}	87.2^{de}	80.4^{ab}	85.3^{cd}	86.1^{cd}
Ether Extract (EE)	93.7^{d}	86.2^{ab}	85.7^{a}	88.6^{bc}	88.6^{bc}	91.0^{c}	86.3^{ab}	89.4^{c}	90.0^{c}
Gross Energy (GE)	81.8^{d}	67.6^{a}	75.5^{c}	75.4^{c}	74.8^{bc}	81.6^{d}	69.8^{ab}	79.5^{cd}	79.5^{cd}

[a-e]Mean values within columns with the same superscript are not significantly *($P > 0.05$)* different from each other

Table 5. Growth parameters, feed utilization and whole body proximate composition of Nile tilapia fed different processed cassava leaves

Body parameters	Control	AD	*SBSD*	YF	MF	RF	YRF	YMF	MRF
IBW (g $fish^{-1}$)	11.4^{a}	11.4^{a}	11.6^{a}	11.6^{a}	12.1^{a}	11.7^{a}	11.9^{a}	12.0^{a}	12.2^{a}
FBW(g $fish^{-1}$)	101.4^{e}	73.1^{a}	78.0^{ab}	80.7^{bc}	75.5^{ab}	88.8^{d}	76.1^{ab}	87.1^{cd}	90.1^{d}
BWG (g $fish^{-1}$)	90.0^{d}	61.7^{a}	66.4^{a}	69.1^{a}	63.4^{cd}	77.1^{ab}	64.3^{cd}	75.1^{a}	77.9^{bc}
DWG (g $fish^{-1}$)	0.75^{d}	0.51^{a}	0.55^{a}	0.58^{ab}	0.53^{a}	0.64^{c}	0.54^{a}	0.63^{bc}	0.65^{c}
SGR(%BWdayg^{-1})	1.82^{d}	1.55^{a}	1.59^{ab}	1.62^{bc}	1.53^{a}	1.69^{c}	1.55^{a}	1.65^{bc}	1.67^{bc}
FCR	1.78^{a}	2.31^{cd}	2.33^{cd}	2.23^{bc}	2.35^{cd}	1.98^{ab}	2.55^{d}	2.16^{bc}	2.13^{bc}
PER	2.87^{d}	2.17^{a}	2.21^{a}	2.27^{ab}	2.24^{ab}	2.59^{cd}	2.1^{a}	2.54^{bc}	2.71^{cd}
FCF	1.72^{ab}	1.45^{a}	1.71^{ab}	1.76^{ab}	1.65^{ab}	1.72^{ab}	1.89^{b}	1.70^{ab}	1.68^{ab}

[a-e]Mean values within columns with the same superscript are not significantly *($P > 0.05$)* different from each other

VIDEO DOCUMENTATION OF THE MARINE COMMUNITY USING AN OYSTER AND CLAM FARM AS HABITAT IN BARNEGAT BAY, NJ

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Shellfish growers often observe fish and invertebrates interacting with their aquaculture gear, and there is growing scientific evidence to support these observations. However, gaps remain in our understanding of how farm attributes, such as farm tending activities and biological fouling, contribute to habitat usage. To document and assess these interactions, point-of-view (GoPro) cameras were deployed at an oyster (*Crassostrea virginica*) farm in 2019 and a hard clam (*Mercenaria mercenaria*) farm in 2023 in Barnegat Bay, New Jersey. Underwater video data were collected to evaluate the marine communities associated with these farms and to observe species interactions with aquaculture gear. At the oyster farm, cameras were deployed on 12 days between June and September on oyster cages and floating bags, while at the clam farm, cameras were deployed on 5 days between June and October on clam bed nets. The video data were analyzed using MaxN, a metric that captures the maximum number of individuals of a given species present within each 1-minute segment. Thirty-seven species were identified, with distinct species distributions across the oyster and clam farms. More individuals were observed at both farms compared to adjacent natural habitats, with biological fouling on gear playing a key role in species abundance. Notably, the oyster farm's floating bags, which exhibited high levels of fouling, attracted more individuals than the less-fouled oyster cages, while moderate fouling on the clam farm's predation nets attracted the highest number of individuals. Farm tending activities had a neutral impact on overall abundance, though localized effects were noted. These findings contribute to our understanding of how farm management practices and environmental factors influence habitat provisioning within shellfish aquaculture systems.

TROUT GRANULOMATOUS VIRUS (TGV) INFECTION CAUSES DISRUPTION OF GILL MORPHOLOGY AND IMMUNITY IN RAINBOW TROUT

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Economic losses associated with disease outbreaks threaten the sustainability of the fish farming industry. Recently, we discovered a new nidovirus infecting farmed rainbow trout (*Oncorhynchus mykiss*) in Northern Israel named Trout Granulomatous Virus (TGV). While TGV causes characteristic granuloma lesions in the liver, it can also infect other trout organs such as the gills, spleen and heart. Interestingly, fish infected TGV were also reported to experience anemia. However, the specific impacts of TGV on the gill morphology and immune function are currently unknown.

The aim of the current study was to evaluate and characterize the histopathological damage and immune response in the gills of TGV infected rainbow trout. We sampled eight infected fish from a natural outbreak from a rainbow trout farm in Israel. We used four control uninfected rainbow trout from the Salinas laboratory at the University of New Mexico as baseline controls.

Figure 1. Gill pathology score vs. Ct TGV values detected by qPCR in gills of TGV infected fish from a natural outbreak in a rainbow trout farm from Israel.

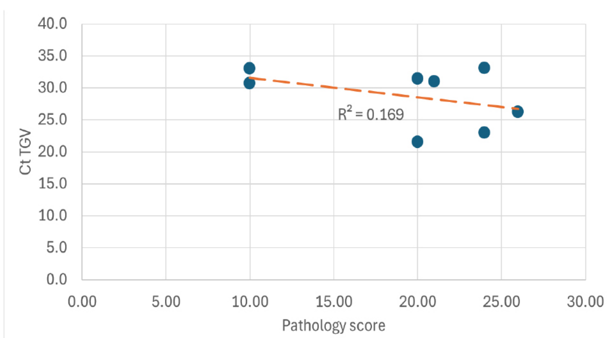

Table 1. Gill Pathological score system used in the present study. Control animals from the laboratory showed no pathology (not included in this table)

Fish	Ct TGV value	Secondary lamella hyperplasia	Edema / Ephitelial lifting	ECG	Cell debris	Goblet cell hyperplasia	Aneurism / hemorrhagy / blood clots	Overall damage
1	31.0	4	3	1	5	3	5	21.00
2	31.4	4	3	5	3	2	3	20.00
3	26.2	5	5	4	4	5	3	26.00
4	30.7	1	3	1	1	2	2	10.00
5	33.1	5	5	4	3	4	3	24.00
6	23.0	5	2	4	4	5	4	24.00
7	21.5	4	5	1	5	3	2	20.00
8	33.0	2	3	0	2	2	1	10.00

(Con tinued on next page)

Histopathological scoring of TGV infected fish revealed a diversity of pathologies including hemorrhagic lesions, edema, lamellar fusion, infiltration of eosinophilic granular cells (EGCs) and goblet cell hyperplasia (Table 1). Interestingly, gill pathology scores were poorly correlated with TGV viral copies in the gills (Figure 1). Immunological staining using anti-trout MHC-II antibodies and anti-trout IgM antibodies revealed different staining patterns in TGV infected compared to control fish. Specifically, three out of the eight infected fish showed high MHC-II expression levels in the endothelial cells at the base of the secondary lamella, a potential indication of vascular dysfunction in the gills due to infection. Other fish showed high MHC-II expression in immune cells, putatively EGCs in the primary lamella. Current efforts are focused on evaluation of IgM, IgT and pIgR expression changes in the gill of TGV infected trout. These results indicate that TGV infection, directly or indirectly, results in severe gill tissue damage and disrupts the gill immune system. Our findings underscore the need for mucosal vaccines against TGV to protect this mucosal barrier in farmed rainbow trout.

THE EFFECT OF DIFFERENT TEMPERATURE MANIPULATIONS ON GROWTH RESPONSES, BODY COMPOSITION, AND SELECTED GENES OF KOI CARP SEED

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The effect of different temperature manipulations on the performance of koi carp seed was studied to ascertain the optimum life stage-specific temperature for its production. Fifteen glass aquarium tanks were used, featuring four different temperature manipulations (26 °C, 28 °C, 30 °C, and a range from 26 °C to 30 °C), along with a control group with no temperature manipulation. Each setup was replicated three (3) times. Larvae obtained through artificially induced breeding were reared at 100 seeds/tank in different manipulated and non-manipulated temperatures for 120 days. Growth data were collected fortnightly while muscle tissue samples were collected for proximate composition, amino acid, fatty acid, and gene expression analysis.

Growth parameters showed that 30 °C and 26-30 °C produced the best performance and significantly differed ($P < 0.05$) from other groups after 120 days post-hatching (dph). The linear regression between temperature and specific growth rate displayed a significantly positive linear relationship at 120 dph. Total amino acid was least concentrated at 26 °C, while control and 26-30 °C had substantial differences ($P < 0.05$) in total essential amino acid. Eicosapentaenoic and docosahexaenoic acids were least concentrated at 26 °C with significant differences ($P < 0.05$). Total polyunsaturated fatty acid in fish at 30 °C showed a remarkable difference while monounsaturated fatty acid had the highest concentration of all fatty acids detected and was highly concentrated at 26 °C. After 60 dph, the HSP70 gene in fish at 28 °C was influenced by temperature while the MYOD gene in the control significantly differed from fish at 28 °C after 120 dph ($P < 0.05$).

This study concludes that an optimum specific temperature of 30 °C and fluctuation not exceeding it could be adopted for koi carp seed production.

Table 1: Effect of different temperature manipulation on growth performance of Koi carp seed at 120-dph

Parameters/Temperature	Control	26 °C	28 °C	30 °C	26-30 °C
Final weight	87.133 ± 13.585^{a}	75.433 ± 11.835^{a}	91.467 ± 12.301^{a}	145.300 ± 4.005^{b}	149.600 ± 3.742^{b}
Weight gain	86.800 ± 13.585^{a}	75.100 ± 11.835^{a}	91.134 ± 12.301^{a}	144.967 ± 4.005^{b}	149.267 ± 3.742^{b}
%Specific growth rate	4.616 ± 0.142^{a}	4.498 ± 0.132^{a}	4.666 ± 0.107^{a}	5.0647 ± 0.023^{b}	5.089 ± 0.021^{b}
% Survival	81.941 ± 5.892^{abc}	71.66 ± 3.499^{a}	76.203 ± 5.852^{ab}	85.185 ± 4.693^{abc}	89.815 ± 0.648^{bc}

Data in the table are mean ± SE, values with different letters across rows in different temperature groups are significantly different at $P < 0.05$

IDENTIFICATION OF MOLECULAR BIOMARKERS AGAINST XENOBIOTICS IN MARINE GREEN MUSSEL *Perna viridis*

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PAHs are one of the major pollutants in aquatic environment. The Coastal marine area having such type of pollutant frequently added by the halting of fishing vessels, oil leakages, drainages from various sources. *Perna viridis* is the sturdy green mussel normally found to live in estuaries and costal marine beds which are the portal entry for land based pollutants. In order to overcome the pollution risk the animals defense come for rescue thus the elevated levels of different types of enzymes as a biomarker whether its elevation rate of hepatic cytochrome P450-associated enzyme activity. The inducibility and activity of phase I reduction nicotinamide adenine dinucleotide phosphate, reduced (NADPH) cytochrome c reductase (CCR), cytochrome c oxidase (COX) and three CYP450 isoforms (benzyloxyresorufin - O-dealkylase [BROD], ethoxyresorufin-O-dealkylase [EROD] and methoxyresorufin-O-dealkylase [MROD]) enzymes were measured in the hepatic S9 fraction prepared from *Perna viridis* collected from three sites: a highly oil-polluted site (Kasimedu fishing harbor, Rayapuram, Chennai [Station1]); a moderately polluted off-shore site, about 3 Km away from this area [Station 2]; and the least oil-polluted site (Vellar estuary, Parangipettai [Station 3], which was a reference site) and also PAHs treated with the same animal at different concentrations in the laboratory. The inducibility and activity of phase I reduction nicotinamide adenine dinucleotide phosphate, reduced (NADPH) cytochrome c reductase (CCR), cytochrome c oxidase (COX) and three CYP450 isoforms (benzyloxyresorufin - O-dealkylase [BROD], ethoxyresorufin-O-dealkylase [EROD] and methoxyresorufin-O-dealkylase [MROD]) enzymes were measured in the hepatic S9 fraction prepared from *Perna viridis* liver , gill and foot tissues were collected from the above sites and also treated with ethyl-naphthalene at different concentrations in the laboratory condition within 24hr duration. The levels of BROD (CYP2B6), MROD (CYP1A2) and EROD (CYP1A1) in the tissues of green mussel were measured. All the MFO enzymes exhibited a hierarchical dose-dependent activity in response to oil pollution in these study areas. Samples from the heavily oil-polluted (Kasimedu Station-1 and Station-2) areas exhibited greater activity of all enzymes than the least oil-polluted (Vellar estuary) reference area. All the sampling sites are located in Bay of Bengal at South east coastal region of India. In the laboratory treated hepatic tissue also elevated the MROD. Among the enzymes analyzed, the MROD activity was best correlated with the level of hydrocarbon contamination. Therefore MROD can be considered as a robust biomarker for petroleum hydrocarbons in *P. viridis*.

EFFECTS OF ZnO NANOPARTICLES ON *Oreochromis mossambicus* USING MOLECULAR ENZYME BIOMARKER

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Certain elements behave differently when their Particle size reduced to nano-level and exerts different impact on the exposed organisms. Such impact could be evaluated through animal models and safe materials can be suggested for various applications including pharmaceuticals. Subsequently create awareness among the public about its effect from the environment. In this study the biochemical assays ie molecular biomarker development (Microsomal /Cytosolic fractions of Muscle ,Gill, Liver , Kidney of fish) were made against ZnO nanoparticles.The specific activity of antioxidant enzymes such as catalase(CAT) ,Superoxide dismutase (SOD) andGlutathione S transferase (GST) and Ethoxyresorufin o dealkylase (EROD) were also measured in response to ZnO nanoparticle on fish showed induction of toxic effects by defense enzyme activity. The level of antioxidant enzymes in liver showed significant decreases with increasing doses. Biochemical and Antioxidant enzymes responses in fish could be used as a biomarker for the early detection of nanoparticles exposure.

UTILIZATION OF MOLECULAR BIOMARKER IN THE DETECTION OF XENOBIOTICS ON WHITELEG SHRIMP *Litopenaeus vannamei* FROM DIFFERENT MANGROVES SITES OF SOUTHERN EAST COST INDIA

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Coastal marine areas are frequently contaminated by pollutants from fishing vessels, oil spills, and other sources. *Litopenaeus vannamei* prawns were collected from estuaries and coastal marine areas near mangrove ecosystems that act as pollutant conduits. To limit pollution, animals use defensive systems with heightened enzyme levels. Enzymes like hepatic cytochrome P450-associated enzymes are used as biomarkers. A study assessed the capacity and function of certain enzymes in the hepatopancreas microsomal fractions of *Litopenaeus vannamei*. The study collected samples from three locations: Kasimedu fishing harbor, Rayapuram, Chennai, a moderately polluted offshore site near mangrove areas, and the least oil-polluted site, Velar estuary, Parangipettai mangrove. The majority of the pollutants is polycyclic aromatic hydrocarbons (PAHs) due to the halting of fishing vessels and other man-made activities. Cytochrome P450 isoforms (CYP450) play the important role of defense mechanism against the PAHs. In this study, we measured EROD (CYP1A1), MROD (CYP1A2), and BROD (CYP2B6) levels from hepatopancreas microsomal fractions of *Litopenaeus vannamei*. The mixed-function oxygenases (MFOs), also known as monooxygenases or hydroxylases, showed a hierarchical pattern of activities in response to oil contamination, with greater activity in highly oil-contaminated sites (Kasimedu Station-1 and Station-2) compared to the low oil-contaminated reference area (Vellar estuary). The hepatic tissues showed enhanced MROD activity, which strongly correlates with hydrocarbon contamination levels, making it a reliable biomarker for detecting petroleum hydrocarbons in *Litopenaeus vannamei*. Antioxidant enzymes like superoxide dismutase, catalase, and glutathione reductase increased in activity, indicating their potential as antioxidants, which neutralize reactive oxygen species (ROS). Among the enzymes analyzed, the MROD activity was best correlated with the level of hydrocarbon contamination. Therefore, MROD can be considered a robust biomarker for petroleum hydrocarbons in *Litopenaeus vannamei*

EXPERIMENTAL EVIDENCE OF GLYPHOSATE HERBICIDE IN *Oreochromis mossambicus* AND ITS BIOREMEDIATION THROUGH MICROBIAL ENZYME-NANOPARTICLE CONJUGATES

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The Glyphosate Glyphosate (N-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide and an organophosphorus compound, it kill the weed that compete with crop. It is affected the non-targeted species that leads to mass mortality of the particular species. The final discharges are in the aquatic system and finally dumped estuary area with mangrove ecosystem . For this reason, the terrible effects lead to the aquatic organisms; finally the entire aquatic biodiversity may be collapses. Bioremediation is very important technique for the current world, because the day by day discharged pollutants toxicated the biosphere and making it unsuitable for life. The chemo- remediations seem to pacify one way but aggravate on the other side. This would indulge the need for bioremediation which is eco-friendly, cost-effective and easily accessible to the environmentalist. Naturally, some micro-organisms are able to play an important role in the degradation of the toxic pollutants. Such microbes can be identified and make use of it to alleviate the emerging pollution problems. Make use of such technology in the abatement of pollution is the need of the hour. The concept of enzyme immobilization and its efficiency of working in the different area is a common phenomenon. The immobilised purified intracellular laccase on amino-functionalized silica nanoparticles have been developed and tested to determine the different environment friendly activity like decolourization of industrial toxic dyes, degradation of Glyphosate. However, the pioneer information and its implements with respect of controlling factors the survival growth and metabolism of micro-organisms in polluted environments. Although these technologies are new and upcoming. It's application and implementation are underway with due refinement. When it is practically efficient and effective then preservation of the pristine global environ become possible. In order to reach the destination, simultaneously need of knowledge about the bioagumentaion process of microbial culture. The cost effective treatment is most important factor for its implementations and prove it be a promising technologies to consider for the remediation of environmental contaminants.

EVALUATION OF THE EFFECT OF GLYPHOSATE ON *Litopenaeus vannamei* : ENZYME ACTIVITY PROFILES AND OXIDATIVE STRESS AS AN INDICATOR

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Glyphosate is a non-selective herbicide that works by preventing the synthesis of proteins in plants. Glyphosate exposure in aquatic organisms can cause oxidative stress, cell damage, developmental impacts, physiological and behavioral changes, plant death, reduced biodiversity, bioaccumulation, and synergetic effects. In this study, we assessed the effects of the relevant concentrations of glyphosate (100-300 ppb) on young *Litopenaeus vannamei* through biochemical endpoints. During 50 days of glyphosate exposure increasing doses of Alkaline Phosphatase Assay (ALP), peaks at 150-200 ppb, and subsequently falls. In contrast, the SOD activity reached a maximum between 250 and 300 ppb. The tissues damaging enzymes GOT and GPT activity was increase rises at concentrations of 250 and 300 ppb. CYP 450 1A1 activities also incresead at 250-300 ppb concentrations. Overall, there was increased levels of tissue damaging enzymes, antioxidant enzymes and defence enzymes . Hepatic tissues of *Litopenaeus vannamei* exposed to commercial herbicide formulations containing glyphosate exhibit altered enzyme activities, suggesting an adaptive and regulated response against herbicide toxicity...

EVALUATION OF THE EFFECT OF GLYPHOSATE ON *Litopenaeus vannamei* : ENZYME ACTIVITY PROFILES AND OXIDATIVE STRESS AS AN INDICATOR

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EVALUATION OF THE EFFECT OF GLYPHOSATE ON *Penaeus vannamei:* ENZYME ACTIVITY PROFILES AND OXIDATIVE STRESS AS AN INDICATOR

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Glyphosate (GLY) is a non-selective herbicide that works by preventing the synthesis of proteins in plants. GLY exposure in aquatic organisms can cause oxidative stress, cell damage, developmental impacts, physiological and behavioural changes, plant death, reduced biodiversity, bioaccumulation, and synergetic effects. In this study, we assessed the effects of the relevant concentrations of GLY (100ppb-300ppb) on young Penaeus vannamei through biochemical endpoints: tissue damaging enzymes (GOT and GPT), antioxidant enzymes (SOD), and defence enzymes were measured.

Glyphosate 41% S.L. (I.P.A salt, 500ml) manufactured by Monsanto India Limited, Mumbai, India was used in this study. GLY was purchased at Theni district, Tamil Nadu. The 41% GLY was used to prepare 100% stock and serially diluted to different concentrations (10ppm, 20ppm, 30ppm, 40ppm, and 50ppm). The effect of GLY exposure was examined to assess the oxidative stress and biotransformation of post larvae PL12 stage P. vannamei collected from a commercial farm near Pattukkottai, Thanjavur District, Tamil Nadu, and acclimatized for one day before the experiment started. After measuring body length (1.760±0.197cm) using Vernier caliper and weight (0.629±0.081g) they were divided into six groups (n=25/tank). Water quality was maintained at pH 7.8, salinity of 28-32ppt and temperature of 27-28ºC throughout the study. Tissue samples were collected every 6hr and the hepatopancreas removed for enzymatic reactions.

After 50 days of GLY exposure, the level of alkaline phosphatase assay (ALP) increased, peaking at 150-200 ppb, they subsequently fell. In contrast, the SOD activity reached a maximum between 250 and 300 ppb. The activity of tissue damaging enzymes GOT and GPT was increased, rising at concentrations of 250 ppb and 300 ppb. The activity of Cytochrome P450 (CYP 450 1A1) also increased at 250-300 ppb concentrations.

Overall, there was increased levels of tissue damaging enzymes, antioxidant enzymes, and defence enzymes. Hepatic tissues of P. vannamei exposed to commercial herbicide formulations containing GLY exhibit altered enzyme activities, suggesting an adaptive and regulated response against herbicide toxicity.

MACHINE LEARNING ANALYSIS OF GENE EXPRESSION PATTERNS IN STRIPED BASS, WHITE BASS, AND THEIR HYBRID FOLLOWING BACTERIAL INFECTIONS

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Striped bass (SB, *Morone saxatilis*) and white bass (WB, *Morone chrysops*) are important aquaculture species and parental contributors to hybrid striped bass (HSB), the fourth largest finfish aquaculture industry in the U.S. Recent studies have examined gene expression changes in the gill and spleen of these fish over time following infection with three pathogenic bacteria that significantly impact cultured and wild populations: *Aeromonas veronii*, *Flavobacterium columnare*, and *Streptococcus iniae*. These studies revealed differences in the expression of pattern recognition receptors (PRRs), cytokines, apoptotic factors, and genes involved in metabolism and bioenergetics, which, when combined with observed survival data, offer insights into the resistance or susceptibility of each group to infection.

In this work, an ensemble machine learning approach was applied to these time series gene expression data to detect key immune response patterns in both timing and magnitude across the causative bacterial agents and SB, WB, and HSB groups. The comparison of SB and WB provides valuable insight into the parental contributions to the hybrid vigor (heterosis) observed in HSB in response to some, but not all, pathogens. Furthermore, incorporating survival data into the machine learning models enables the development of predictive survival models that can be expanded with future data and used for selective breeding and vaccine or treatment development.

INSIGHTS INTO THE FACTORS CONTRIBUTING TO ENVIRONMENTAL, ECONOMIC AND SOCIAL SUSTAINABILITY OF AQUACULTURE

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Globally, aquaculture production has been the food production technology with the highest growth rate in recent decades. While this is a strong indication of economic sustainability, there are significant differences between species and locations. Moreover, the rapid growth has led to the environmental sustainability of the industry being questioned, although the picture is getting nuanced as some sectors have better performance than others. The rapid production growth also leads to societal change, and this is creating a further sustainability challenge for the industry.

A challenge when one is interested in comparing different production systems is that comparable data is generally not available beyond production numbers. In this presentation, we investigate which factors are most likely to contribute to improving aquaculture's environmental, economic and social sustainability. The data were collected using the Aquaculture Performance Indicators (API) for 69 aquaculture production systems that represent over 40% of global aquaculture production quantity and 36% of global production value. The analysis is conducted using regression trees and random forest estimation.

The preliminary results indicate that for all three pillars, general societal factors related to governance and economic conditions are more important than aquaculture specific measures. For both economic and societal sustainability, the ability for collective actions and viable industry organizations are also important.

COMBINED EFFECTS OF TEMPERATURE AND FOOD ON RIBBED MUSSEL *Geukensia demissa* LARVAE

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Ribbed mussels are a key species, increasing salt marsh resilience along the Atlantic coast of North America. Like other marine bivalves, they have planktonic larvae, facilitating broad dispersal. Across their geographic distribution, larvae can experience a wide range of ocean temperatures and food abundance. Climate change is likely to alter local temperature and food availability, which may impact larval survival, growth, and development. Understanding the consequences of climate change driven temperature and food availability will be especially important as they can impact mussel distributions and population dynamics. But, we do not presently understand how these factors impact early life stages. Therefore, we tested the combined effects of temperature and food quantity on survival, growth, and time to metamorphosis in larval ribbed mussels.

We found highest survival at 20°C, but larvae were slow to metamorphose, and lowest survival (no metamorphosis) at 15°C. In general, higher food availability improved survivorship at most temperatures. Larvae developed fastest at 30°C and food availability did not affect development time. Food availability increased larval growth except at the coldest temperature and larvae were larger with increasing temperature. These data suggest tradeoffs between survivorship, growth, and development at different temperatures and will be used to parameterize systems models to understand the impacts of combined factors (including temperature and food availability) during the larval stage on ribbed mussel populations in salt marshes.

TOWARDS COST-EFFECTIVE REDUCTIONS OF ANTIBIOTIC USE IN SALMON AQUACULTURE

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In recent years, many firms in Chile's salmon aquaculture industry have committed to reducing their use of antibiotics. While national data suggests substantial progress, achieving these targets is likely to be both challenging and expensive as there exist few alternatives to antibiotics for treating common infections, such as *Piscirickettsiosis*. In this paper, we explore how the design of antibiotic-use restrictions is likely to impact the productivity of fish farms exposed to a communicable disease. Leveraging insights from both economics and epidemiology, we develop a stylized model of a farm as a forward-looking, profit-maximizing entity where the biomass at harvest is determined by a compartmental model of disease dynamics. Farm managers control disease by applying treatment with temporary effectiveness. Further, we assume that pathogens are transmissible between individuals on the farm and between farms in a region, such that the disease management choices of one farm impact its neighbors. We solve the model as a finite-horizon, deterministic optimal control problem and simulate a selection of restrictions that are parameterized to achieve a targeted reduction level.

Our model suggests that the optimal treatment strategy is a function of both on-farm disease dynamics and ambient disease levels. However, in many scenarios, a reduction in ambient disease pressure does little to reduce optimal antibiotic-use. We also illustrate that, consistent with a theory, a cap on the total quantity of antibiotics used in a production cycle is the most cost-effective policy for achieving an intended target, when compared to more restrictive policies.

CULTURE TANK DESIGN AND KEY FEATURES

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Circular tanks are widely used in aquaculture due to their efficient and flexible hydraulic control, optimal water quality and fish welfare, and adaptability to various additional features. Several key components of the culture tank work together to maximize productivity, reduce labor costs, and ensure fish health. This presentation will cover the general material and geometrical requirements of a properly designed circular fish culture tank and the integration of other key features that are critical to the successful operation of the tank and the system.

Circular tanks naturally facilitate the collection of solids in the center due to their continuous water flow patterns. Proper tank hydraulics are key to ensuring solids are quickly removed from the tank and delivered to the removal treatment process. Mortalities removal is a critical feature of circular tanks, as it helps maintain water quality and prevents disease outbreaks. These systems are often integrated with sensors that detect mortalities, enabling the system to remove them without human intervention.

Efficient and controlled feeding is essential for maximizing growth and reducing waste. Integrated automatic feeders can be programmed to dispense precise amounts of feed at specific intervals. Feed optimization is enhanced with integrated sensors that monitor water quality and feeding activity and adjust feed quantities based on the fish's consumption and metabolic needs.

Tank lighting systems are commonly used in aquaculture tanks to simulate natural daylight cycles, which can promote optimal growth and reduce stress in fish. Lighting can be adjusted in intensity and wavelength to match specific species' requirements or stimulate breeding. Proper lighting can also help guide fish during feeding and prevent stress caused by excessive or insufficient light exposure.

Modern aquaculture systems rely heavily on sensors for real-time monitoring of tank conditions to ensure the health of the fish and the efficiency of operations. These sensors can trigger automated systems, such as Oxygen diffusers or pumps, to adjust water conditions in real-time. Fish behavior sensors use cameras and algorithms to track fish movement, feeding habits, general health and total biomass. This data helps operators make informed decisions regarding feeding, water quality adjustments, or other interventions.

The integration of advanced technologies such as automated feeding, real-time sensors, mort removal, and video monitoring into circular tank designs significantly enhances the efficiency and sustainability of aquaculture operations.

COMMERCIAL WALLEYE (*Sander vitreus*) AQUACULTURE: POTENTIAL AND CHALLENGES

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Commercial Walleye (*Sander vitreus*) aquaculture offers significant economic potential, driven by high market demand and premium pricing in both the recreational fishing and food sectors. However, to realize this potential, the industry must address several biological and economic challenges. Walleye's specific environmental needs and relatively slow growth rates require sophisticated systems, increasing the cost of production. Nevertheless, advances in breeding technology, water management, and feed optimization are beginning to improve efficiency, reducing costs and making Walleye farming more economically viable.

Initial capital expenditures for infrastructure, specialized systems, and labor can be substantial. Yet, as consumer demand grows for sustainably farmed, high-quality fish, there are strong opportunities for profitability. The species' premium market price positions it as a lucrative alternative to more established aquaculture species, offering farmers the potential for high returns on investment. Moreover, the increasing focus on sustainable practices and eco-friendly farming solutions aligns with consumer preferences, potentially enhancing the marketability of farm-raised Walleye.

One of the primary opportunities lies in year-round production. Unlike wild-caught Walleye, which is subject to seasonal availability and regulatory restrictions, aquaculture provides a consistent supply to meet demand throughout the year. This consistency can help stabilize pricing and offer greater market security for producers. Additionally, innovations in recirculating aquaculture systems (RAS) and other efficient water management technologies hold promise for lowering operational costs over time. As the industry matures and economies of scale are realized, Walleye aquaculture could become increasingly cost-effective, making it an attractive investment within the broader seafood market. Overall, the economic outlook for commercial Walleye farming is positive, particularly as technology and sustainable practices continue to improve.

Walleye aquaculture aligns well with the objectives of the National Aquaculture Development Plan (NADP), which seeks to promote sustainable, economically viable, and environmentally responsible aquaculture practices across the United States. As a high-demand species for both recreational fishing and food markets, Walleye represents a valuable candidate for commercial culture, offering opportunities to enhance domestic seafood production while reducing reliance on imports. This fits directly into the NADP's goals of fostering economic growth within the U.S. aquaculture industry and increasing the diversity of farmed species to meet market needs.

POTENTIAL OF *Sargassum fluitans* IN AQUACULTURE FEEDS IN NIGERIA: THE CASE OF *Coptodon zilli* and *Clarias gariepinus*

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One of the major challenges of Aquaculture in Nigeria is high cost of feeds. *Sargassum fluitans* (seaweed) which crept into Badagry coastal waters was assessed for aquaculture potentials for culturing *Coptodon zilli* and *Clarias gariepinus*. *Sargassum fluitans* was analyzed for heavy metals (As, Hg, Cd, Cu, and Pb) and the results showed that *S. fluitans* from Badagry coastal waters is safe for fish and animal consumption. Therefore, *Sargassum*-based feeds were formulated as alternative source of replacement for maize in the diet of *C. zilli* ***and*** *C.gariepinus Sargassum fluitans* were collected by handpicking during low tides from the shores of Suntan Sea Beach, Badagry, Lagos State, Nigeria. The seaweed was washed thoroughly with borehole water, rinsed with distilled water, and sun dried for 1 week. The experimental feeds consists of control (feed ingredients including maize but with no *Sargassum* meal at all), treatment 1(5% of maize meal (MM) was replaced with *Sargassum)*, treatment 2 (10% of MM replaced with *Sargassum*), treatment 3(20% of MM replaced with *Sargassum*), treatment 4(contain only Sargassum as energy source i.e no maize at all).

A total of 180 sub-adult of *C.zilli* and fingerlings of *C.gariepinus* were used for the experiment being evenly distributed in nine (9) tanks (of 1000 litre capacity) and stocking rate of twenty (20) fish per tank. The fish fed with 10% *Sargassum* diet had the least growth performance index (34.60±7.60%), followed by the control (37.15±5.1%) while those fed with *Sargassum* only (100% maize replacement) had highest performance index (40.14±8.57%). However, there were no significant differences in the Specific growth rate and % survival rate across the five feeding trials. The study has indicated that *Sargassum fluitans* is a reliable alternative to maize. *Sargassum* is 100% free and its processing is very cheap. Hence, the *Sargassum*- based feeds formulated and used for this study has been offered to some practicing farmers for further trials on their farm.
Keywords: *Sargassum*- based feed, Safety, growth performance.

DEVELOPMENT STATUS AND TRENDS OF GLOBAL RICE-FISH FARMING SYSTEMS

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Rice fish farming (RF) has been practised for centuries in Asian countries, yet adoption rate remains limited, with many rice producing-nations not integrating aquatic species into their rice fields. This study explores why other countries performs better than others and major limiting factors for implementation and retention of RF. By reviewing RF development status, trends and socio-economic-environment contexts in RF major producing countries including China, Japan, Bangladesh, Thailand, India, Indonesia, Egypt, Nepal, the Philippines, Cambodia, Lao People's Democratic Republic (Lao), Malaysia, Myanmar, Vietnam, and the United States (US). Our study shows that, globally RF production has increased from 88,245.34 tons in 2000 to 423017.58 tons in 2023 accounting for 3.18% of global aquaculture production, occupying 2.24% global paddy field or 0.96% arable land. China is leading in rice-fish production and area followed by Bangladesh and Indonesia. For the last two decades, development trends of RF aquatic food production and area in China, Bangladesh and US has been increasing, however, Thailand, Nepal, Japan, Indonesia, and Egypt have experienced a decrease (Fig.1). Cambodia and Lao primarily practice rice-field fisheries. In Myanmar RF area and production has declined since 2013 due to strict government policy inhibiting transition of the rice-fields to accommodate aquatic species. In contrary, Chinese, Bangladesh, Vietnamese, and Indonesian governments together with non-governmental organizations has been actively promoting RF to enhance ecosystem conservation, income and productivity diversification. Inadequate knowledge and managerial skills, intensification of rice mono-culture, lack of capital and labor, technical and institutional constrains has led to low adoption of RF in various regions globally. Land being a key limiting factor for aquaculture and agricultural expansion, RF therefore is a gateway to specialization, providing nutritious food and economic growth through integration of high-value aquatic species such as red swam craw-fish of China and US, freshwater prawns of Bangladesh, and Tiger shrimp of Vietnam. Integrated RF is therefore an innovative way to sufficiently use resources to increase global agricultural output, alleviate poverty, malnutrition, and hunger at the midst of a changing climate.

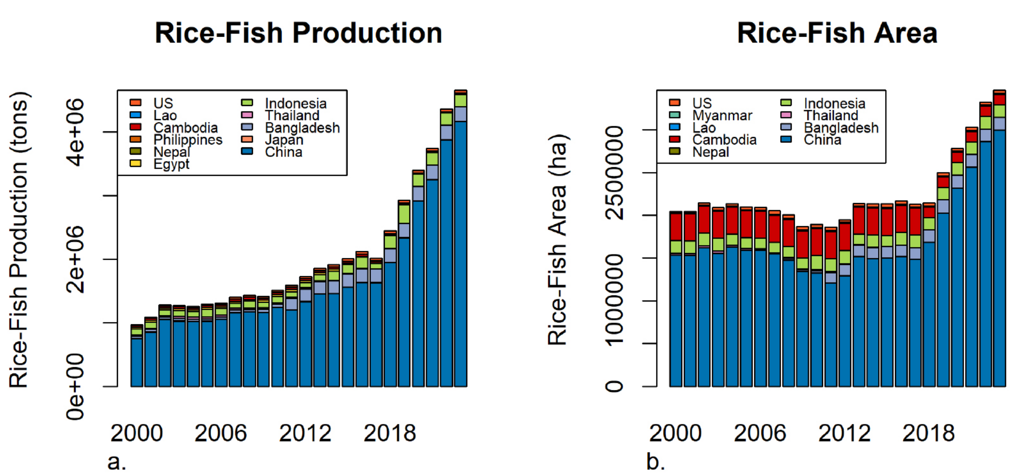

Figure 1: Trends of global rice-fish production (a) and area (b) from 2000 to 2023

EFFECT OF PHYTOGENIC INGREDIENTS ON GROWTH PERFORMANCE AND SUSCEPTABILITY TO BACTERIAL INFECTION IN JUVENILE CHANNEL CATFISH *(Ictalurus punctatus)*

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Phytogenic feed additives, derived from plant-based sources like essential oils and extracts are increasingly utilized in aquaculture. They have the potential to bolster immune responses, enhance disease resistance and improve growth performance in fish. The study investigates the effect of commercial phytogenic feed additives (Actifor Pro, Actifor Power, Fresta Protect, Syrena Boost, and Enviro QS) on disease susceptibility, immunity, and growth performance of juvenile channel catfish (*Ictalurus punctatus*) within an indoor recirculating system. Over six weeks, 750 juvenile channel catfish (14.8 ± 0.4g) were stocked in 30 tanks and fed either a control diet or the phytogenic-supplemented feed. Following the feed trail, fish were immersion-challenged with virulent *Aeromonas hydrophilia* (vAh; ML09-119; 1.86×10^9 CFU mL^{-1}), a pathogen that causes considerable losses in the catfish industry due to motile *Aeromonas* septicemia (MAS).

Growth performance, including weight gain, survival and feed conversion ratio, was similar among treatments, with no statistically significant differences ($P > 0.05$). After exposure to *A. hydrophilia*, for 7 days, cumulative precent mortality (CPM) was higher in the control diet (60%) compared to the phytogenic treated fish. Notably, fish fed with Fresta protect exhibited the lowest CPM (19.6%), while other treatment groups had CPM ranging from 45% to 58%. Survival analysis indicated improved survival probability in the phytogenic-treated groups ($p= 0.009$). Sera lysozyme activity was significant differences across the groups, ($P= 0.009$), moreover, the catfish fed with Fresta protect had higher sera lysozyme activity than the control group.

This study concluded that phytogenic feed additives enhance juvenile channel catfish disease resistance and aspects of innate immunity. Specifically, Fresta Protect showed the most promise toward improving channel catfish survival against vAh. Incorporating phytogenics could be a viable alternative to antibiotic uses in U.S catfish aquaculture.

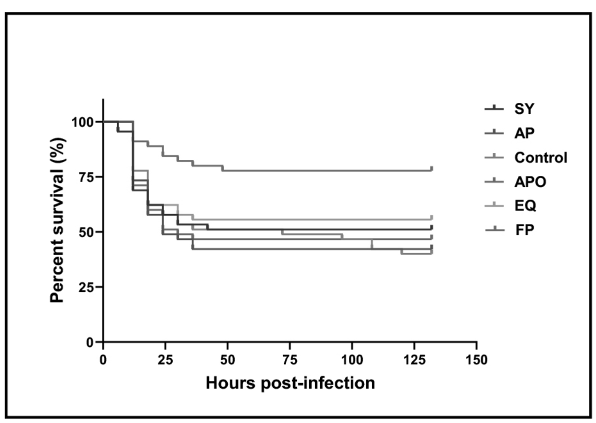

Figure 1: Survival probability (%) of channel catfish (*Ictalurus punctatus)* following immersion in virulent *Aeromonas hydrophila* (vAh; ML09-119) over a 7-day challenge period.

A MULTIREGIONAL EVALUATION OF OFF-FLAVOR DESCRIPTORS AND US CONSUMERS' SENSITIVITY TO GEOSMIN IN WATER

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When working with untrained sensory panelists to study off-flavors, researchers must select the "right" verbal descriptor for the compound of interest. This is an important consideration when investigating recognition thresholds and limits for off-flavor compounds in aquacultural products. Aromatic descriptors attributed to the semi-volatile alcohol geosmin are many, and the compound is often associated with fresh rain, drinking water quality, and off-flavors in aquacultural food products. Though the human olfactory system is known to be quite sensitive to geosmin (reported aroma detection thresholds as low as 5 ng/L in water), the ability to distinguish geosmin from related compounds and an incomplete understanding of sensitivity distributions and acceptable tolerances in food products remain challenges for sensory researchers. This study investigated the most appropriate descriptors for geosmin in water and conducted a qualitative assessment of aroma and flavor sensitivity among 488 untrained assessors. Participants were recruited from university campuses in three different US regions (188 in Louisiana, 114 in New Jersey, and 186 in Ohio). Each assessor evaluated four solutions of (+/–)-geosmin in reverse-osmosis water, at control (no geosmin), low (20 ng/L), medium (200 ng/L), and high (1,000 ng/L) levels, in ascending order. Each panelists selected the best term to describe the aroma [by smelling only] and flavor [by tasting] of solutions, followed by intensity ratings using a 4-point categorical scale (not detected/weak/moderate/strong). Descriptors were presented in a randomly ordered multiple choice list which included *beets*, *dirt*, *earthy*, *moldy*, *muddy*, and *musty*. Concentration of geosmin significantly affected distribution of responses (chi-square test; α=0.05). *Earthy* was the most selected term for aroma and flavor– being selected by 21% and 23% of assessors for high geosmin solutions, with the other five terms selected proportionately across the remaining assessors (chi-square test). Overall, location and native language significantly influenced descriptions of geosmin (multinomial logistic regression). Assessors in New Jersey were more likely to use the terms *beets* and *musty* than their counterparts in Louisiana, while those in Ohio were less likely to select *muddy.* Non-native English speakers were more likely to use the term *moldy* and less likely to use *dirt.* The proportion of assessors reporting no detection of geosmin aroma dropped from 39% (low geosmin) to 12% (medium geosmin) to 9% (high geosmin). Perceived intensities significantly increased upon tasting (Stuart Maxwell test). While *earthy* stood out among other geosmin descriptors, results suggest that sociographic characteristics of potential sensory panelists should be considered when selecting language for metrics. Sensory acuity of subjects and intensity of the stimulus may also affect linguistic expression of perceptions. This understanding is important to assessment of off-flavors such as geosmin, which can impact the acceptance or rejection of foods and beverages.

SMALL, UNENFORCED MARINE PROTECTED AREAS PROMOTE DIVERSITY AND PRESERVE DEMOGRAPHY IN POPULATIONS OF THE SMALL GIANT CLAM *Tridacna maxima* IN FRENCH POLYNESIA

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No-take marine protected areas (MPAs) have been promoted as effective tools for preserving biodiversity and permitting species recovery. However, MPA efficacy can be hindered by small size, enforcement shortfalls, and unfavorable life-history characteristics of target species. In 2004, eight MPAs were established around the island of Moorea, French Polynesia, in response to a decline in fish and invertebrate stocks. One species of particular conservation concern was the ecologically and economically important giant clam, *Tridacna maxima.* Current MPA catch-regulations on Moorea encourage extraction of reproductively mature giant clams, thereby potentially hindering repopulation efforts. In addition, the iridescent coloration of some *T. maxima* morphs confers increased economic value and therefore potentially stronger selective pressures on these genotypes. Whereas several post-establishment surveys have demonstrated positive effects of Moorea's MPAs on fish stocks, little is reported about the recovery status of *T. maxima* giant clams. To address this knowledge gap, I conducted 29 surveys across six of Moorea's MPAs and integrated this data with historical population estimates in order to assess collection-driven demographic alterations and status of recovery in Moorea's *T. maxima* populations.

Although historical surveys revealed similar population growth rates in *T. maxima* between MPAs and controls this likely reflects positive spill-over effects on unprotected sites. Overall, giant clams were 6x more abundant within MPAs and population demography differed greatly between MPA and control sites. Densities of mature and brightly-colored clams were 17x and 6x higher within MPAs, respectively. In addition, brightly-colored adults were only found within MPA sites. Taken together, these results suggest that, despite small size and minimal enforcement, Moorea's MPA network effectively maintains *T. maxima* population diversity and promotes sustainable population demography in this threatened giant clam species.

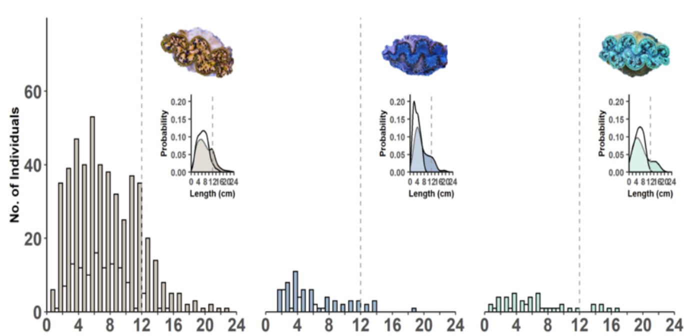

Figure 1. *Population structure differs between MPA and non-protected sites.* Size distributions across all MPA (shaded), and non-MPA (white) sites for *Tridacna maxima* clams with cryptic (brown) and conspicuously-colored (blue and teal) mantles. Size at maturity (i.e., length ≥ 12 cm) is denoted by a dotted line. Insets in each panel show representative morphs as well as respective probabilities of encountering an individual of a given size within (solid fill) or outside (white fill) MPA boundaries. Conspicuously-colored adult clams are present only within MPAs.

UNLOCKING THE POTENTIAL OF *Penaeus vannamei* SHRIMP BREEDING THROUGH GENOMIC SELECTION

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Genomic selection (GS) is considered a powerful tool for genetic improvement of complex traits controlled by many genes, each with minor effects. It offers several advantages such as increasing the rates of genetic gain through increased accuracy of estimated breeding values significantly shorter breeding cycles, and the better utilization of available genetic resources through genome-guided mate selection.

Traditional breeding approach limits the number of variety candidates to be tested, and it is the main cause of the fact that breeding programs are time and cost intensive. Genomic selection offers a more efficient alternative by using molecular markers to predict the genetic value of individuals for specific traits without phenotyping.

In this presentation, we will provide an overview of GS and its implications for shrimp breeding. We will also discuss the current state of the field, the potential for this technology to improve the efficiency and sustainability of the *P. vannamei* shrimp industry despite lacking a chromosome-level whole reference genome sequence.[1-2]

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DESIGNING ECONOMIC RISK ANALYSES FOR VARIOUS SPECIES AND PRODUCTION SYSTEMS IN U.S. AQUACULTURE

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The aquaculture industry is naturally prone to high levels of risk. In the U.S., the diversity of species and production systems adds layers of complexity, exposing aquaculture businesses to various forms of risk. However, existing research that addresses the multiple types of risks encountered by U.S. fish farms across different species and production systems is limited. This study aims to fill that gap by quantifying the economic risks faced by seven distinct species and production systems. Using a comprehensive approach, we will assess economic risk by simultaneously considering production, marketing and financial factors. Farm-level economic data, collected via surveys over recent years, will be used to construct spreadsheet-based risk models. Monte Carlo simulations, performed through Crystal Ball software, help to quantify these risks, with each model undergoing 1,000 iterations to determine variable correlations. The findings of this study will enable aquaculture producers to identify the key variables contributing to economic risk across species and production systems, facilitating the development of more effective risk management strategies.

Table 1. The different species and production systems under evaluation

Species	Production systems					
	Ponds	Raceways	Outdoor tanks and RAS	RAS	Indoor tanks and RAS	Indoor tanks
Catfish	✓					
Tilapia	✓	✓	✓		✓	
Trout		✓				
Redfish	✓			✓		
Hybrid striped bass	✓			✓		
Shrimp	✓					✓
Baitfish/sportfish	✓					

A MASSIVE OPEN ONLINE COURSE (MOOC) ON SUSTAINABLE AQUACULTURE FOR LOW TROPHIC SPECIES

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Climate change and increased pressures on freshwater and land resources have affected our capacity to produce food for the growing population. As a result, food security has become one of the most pressing challenges we are currently facing. In search of a solution, our attention has turned to marine aquaculture. Food coming from marine aquaculture is known to have some of the lowest carbon footprints among animal products. Moreover, producing marine food at a lower trophic level delivers additional environmental benefits through ecosystem services, which are fundamental to creating a sustainable food system.

Together with a number of other academic institutions, UiT has developed an interdisciplinary online course on Sustainable Aquaculture for Low Trophic Species (SALTS) which takes you on a journey of exploring the principles and practices of low trophic aquaculture. The course provides a holistic view of the most recent developments in low trophic aquaculture, focusing on selected species groups, including molluscs, echinoderms, macroalgae and freshwater fish. Throughout the course, we cover various topics, including the biology of the four species groups, the design and operation of the cultivation practices, and their environmental impact. Economic and social aspects of aquaculture and the policies and regulations that govern the industry are also explored, and an interdisciplinary approach is used to deepen the understanding of the subject matter and provide knowledge and skills essential to contribute to a sustainable industry.

So far, SALTS have had more than 600 students, have awarded more than 70 diplomas, and is in the process of being translated into Spanish.

ECOLABELS FOR FARMED SEAFOOD

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Early ecolabeling initiatives for seafood were restricted to wild seafood, and most of the early literature in relation to seafood ecolabels follows this pattern, although with interesting variations such as genetic ecolabels for farmed seafood in anticipation of future market adaptions. The first ecolabels actually used for farmed seafood were organic labeling, which has the potential to fill two information objectives in that they potentially nullifies the advantage of ecolabeled wild seafood and it signals that the product is produced in a sustainable manner, even though the requirements for an organic label generally did not fit aquaculture production practices well.

During the last ten years, a number of studies have appeared investigating the potential premiums associated with the ecolabel of the Aquaculture Stewardship Council (ASC), in addition to studies still focusing on organic, and a few which captures both. The results are similar to the literature for wild fish in that the potential impact at the retail level is highly heterogenous as the price premium vary with country, retail chain and species, and there are several cases where the premium is found to be zero. Moreover, there has been no studies trying to answer the question which markets or market segments have a strong enough preference for ecolabeled fish to actually demand it.

An important discussion in fisheries is whether the ecolabel premium that exists at the retail level moves upstream to the producers and thereby actually creates economic incentives to improve production practices. This is a discussion that has barely started in the case of aquaculture products. However, we will show that to a large extent the producers who are certified with ASC label operates in countries with relatively extensive management systems, and as such that can more easily meet the requirements of the ecolabels.

MARGINS FOR FARMED SALMON

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Most aquaculture producers are exposed to a number of biophysical factors impacting the production cycle. For some species this leads to significant seasonality in the harvesting process, potentially creating market and price cycles. Salmon is a species where there is significant price volatility as well as seasonality in the harvest patterns. For instance, there is a strong seasonal peak in Norwegian salmon production associated with the Christmas season. In this paper we will investigate to what extent the seasonal patterns in harvest in the Norwegian salmon industry are driven by biophysical or market factors. Moreover, we will investigate to what extent regional differences in harvest patterns exacerbate the general patterns or whether they cancel each other out as farmers in different regions use them to their best advantage.

ENDOGENOUS VIRUS ELEMENTS (EVE) OF INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV-EVE) IN THE GENOMES OF *Penaeus vannamei* AND *P. monodon*: INTERACTIONS WITH A TRANSPOSABLE ELEMENT - COMMENTS ON ISSUES RELATED TO DELISTING OF IHHN AS A NOTIFIABLE DISEASE BY THE WORLD ORGANIZATION FOR ANIMAL HEALTH (WHOA)

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Viral diseases cause major economic losses to the shrimp industry, including diseases caused by white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV; renamed *Decapod penstylhamaparvovirus 1*), among others.

Endogenous virus elements (EVE) of IHHNV and WSSV have been reported. The IHHNV genome (AF218266.2; 3,909-bp) is integrated in various chromosomes of *P. monodon* genome from China (PM-nanhai2-001_Lachesis_group23__306, JACBPZ010000025.1) and *P. monodon* from Thailand (JABERT010000007.1) both 95-97% identical. Fragments of AF218266.2 are also present in two genomes of *P. vannamei* farmed in China [(QCYY01000759.1, *Penaeus vannamei* breed Kehai No.1 LVANscaffold_759, nucleotides 87-1851 and 936-2058, 95-96% identity) and *P. vannamei* isolate Guihai-1-2017-001_HiC_scaffold_2, JANIEY010000002.1, nucleotides 1294-2575, 68% identity).

Three IHHNV sequences have been reported from *P. vannamei* of Ecuador [(AY362548.1) 3,775-bp), (OL598344.2) 3,203-bp, and (OM728642.1) 3,902-bp] and portions of these genomes are also integrated in the genomes of *P. monodon* from Thailand (GCA_015228065.1, isolate SGIC_2016 chromosome 7) and *P. vannamei* from China (GCA_003789085.1, breed Kehai No.1 LVANscaffold_759). IHHNV isolates from Peru (like OM728641 4,122-bp) are also integrated in *P. monodon* chromosomes 7 and 35, the number of fragments varying per chromosome, 10 and 51, respectively. Results suggested that the currently farmed *P. vannamei* lines in Ecuador are tolerant to circulating IHHNV genotypes, prompting the industry to request delisting of IHHN disease by the World Organization for Animal Health (WHOA).
Delisting of IHHN disease should be addressed carefully because of the interaction of IHHNV-EVE with a transposable element (*RTE-2_PMon*, (3,656-bp) from *P. monodon* from Thailand that contains fragments of microsatellite markers similar to *RTE-2_PMon*, with potential for re-emerging of IHHNV via horizontal gene transfer. Around 200 members of this family show only 0.5-2% sequence divergency to the *RTE-2_PMon* consensus sequence, indicating its current translocation activity. We should wait until a new genome sequence for *P. vannamei* is available to determine the integration sites and evolution of IHHNV-EVEs, and their association with Simple Sequence Repeats (SSRs) and noncoding RNA potentially associated with slow growth and disease resistance/tolerance.

WELFARE IN SHRIMP *Penaeus vannamei:* EYESTALK ABLATION, SENTIENCE AND PAIN PERCEPTION - A REVIEW

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With the rapid expansion of aquaculture, welfare of fishes and aquatic invertebrates is important. Several jurisdictions have included these taxa under welfare regulation in recent years.[1-3,5] As most of these species are poikilothermic vertebrates and invertebrates, they present unique issues that should be addressed by comercial aquaculture producers and industries providing farmed seafood for the global market. This presentation will review some of these issues, taking into account that regulation of welfare requires use of scientifically validated welfare criteria. Welfare legislation for these taxa should be enacted with strong scientific evidence to avoid generating significant unintended consequences[3], particularly considering the provisions of point 3.1.6.8. of part III to Annex II to Regulation (EU) 2018/848 related to animal welfare in the aquaculture sector. EA and others similar are prohibited in the EU. The relevance of these laws is important for developing countries willing to export animals intended for food to the EU, they must now comply with these regulations. A rigorous science-based approach to the welfare of aquatic organisms should be based on verified, validated, and measurable endpoints that minimize the risk of unintended negative impacts for all stakeholders.[5] Important decisions about their welfare should be based on scientifically robust evidence.

We discuss the ten reasons outlined by Benjamin *et al*.[3] to orient legislators, decision makers and the scientific community. Maintaining high scientific standards is required in order to protect not only aquatic animal welfare but also global food security and the welfare of humans. We will address 'eyestalk ablation' in *Penaeus vannamei,* and review 'sentience and pain perception' in female broodstock submitted to eyestalk ablation, and the challenge associated with the production of *P. vannamei* using this technique from a welfare perspective. We will address two major issues: (1) maturation of female broodstock and the production practice of eyestalk ablation; disease susceptibility and stress-immunity mechanisms, and environmental and biological conditions during grow-out: health-stress-disease axis in ponds, and (2) welfare indicators in decapod crustaceans; the current state on physiological biomarkers; and Operational Welfare Indicators (OWIs): individual and group based, direct and indirect and invasive vs. non-invasive.

The research in shrimp hatcheries from Honduras and Thailand led by Zacarias *et al.*[4] from the University of Stirling will be discussed. Researchers prove that eyestalk ablation is unnecessary because it increases vulnerability to disease. However, we should avoid using feeds prepared with glyphosate-based-herbicides (GBHs)-produced soybeans, these may lead to development of antimicrobial resistance in the offspring and food safety issues.[6] We suggest that retailers should increase shrimp welfare demands from producers, which may require cooperation across the supply chain. The practice of eyestalk ablation should be avoided to meet increasing consumer demands for traceability, sustainability, and animal welfare in the shrimp-farming industry.

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EVALUATION OF SINGLE CELL PROTEIN DIETS FOR THE ZEBRAFISH *Danio rerio*

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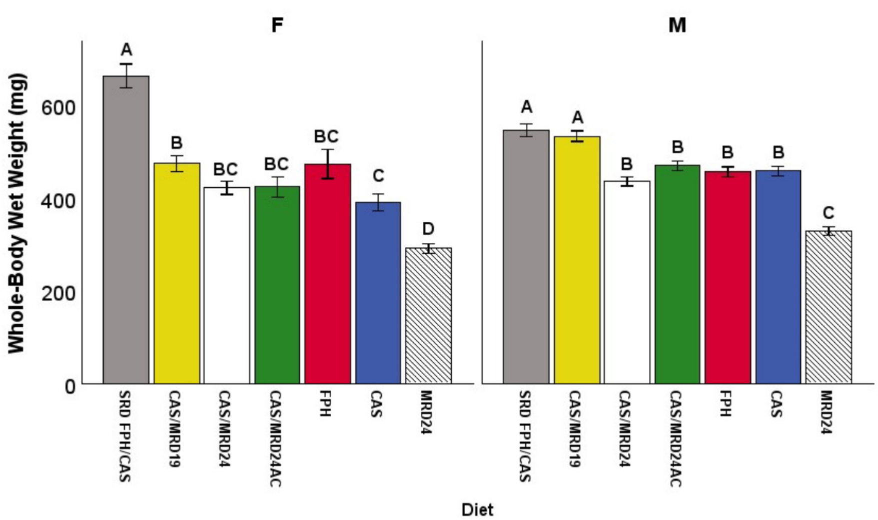
The value of the zebrafish in biomedical research is not questioned. However, knowledge of the specific nutritional requirements of zebrafish is limited. Open formulation diets are being designed to optimize growth and health profiles, including reproductive outcomes. This has resulted in the formulation of a proposed standard reference diet (SRD) designed to support clinically healthy fish. Currently, the protein sources for this diet are casein and fish protein hydrolysate (FPH), a fish meal derivative. Both are high value protein sources; however, FPH is produced from wild-caught fish, so its nutritional composition and level of environmental toxicants may be variable. Other protein sources may improve the nutrient profiles required in an SRD. Recent successes with a bacterial-based source of protein suggest the value of bacteria as a protein source. A reference protein source that is stable in amino acid composition and promotes health will further support the use of zebrafish as a high throughput translational model.

Bacterial protein sources can be produced using agricultural waste products and can be genetically modified to adjust amino acid composition. We evaluated the physiological impact of a commercially-produced, bacterial-based single cell protein (MRD-Pro, Meridian Biotech) when used as a replacement for FPH and CAS. Zebrafish were fed *Brachionus* and *Artemia* nauplii until 35 days post fertilization, then were fed *ad libitum* one of seven experimental diets. The protein content of the diets were as follows: proposed SRD (FPH and CAS as protein), MRD 2019 formulation and CAS, MRD 2024 formulation and CAS, autoclaved MRD 2024 and CAS, MRD 2024 alone, FPH alone, and CAS alone. Zebrafish were housed in recirculating aquatic systems, and the weights and lengths of the zebrafish were tracked every two weeks for 16 weeks. The SRD outperformed all other diets in weight gain, and the diets with only SCP as a protein source underperformed. Diets with SCP included with FPH performed adequately.

Further analysis of body composition, gene expression, plasma lipid circulation, and microbiome are underway. Funded by NIH STTR.



Letters designate significant differences between means (p<0.05). Bars represent mean body weight. Error bars represent one standard error of the mean.

LAND-BASED CULTIVATION OF RED MACROALGAE IN HAWAI'I

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The Hawaiian Islands host over 340 red algal species with new ones being discovered in deep-water expeditions. *Kanaka maoli* (indigenous Hawaiian population) have rich cultural traditions of utilizing macroalgae (*limu*) as food, medicine, and in cultural and religious ceremonies. However, wild stocks are declining due to habitat destruction, urban development and water diversion. In recent years, there have been efforts to expand land-based cultivation of tropical red macroalgae, including for aquaculture feed for urchins and abalone. Current estimates indicate an annual production of about 260 metric tons (MT) of wet weight (WW), predominantly on Hawai'i Island and O'ahu. Key species cultivated include *Gracilaria parvispora*, *Halymenia hawaiiana*, *Agardhiella subulata*, *Devaleraea mollis* (used to be called *Palmaria mollis)*, and *Asparagopsis taxiformis*. Additionally, problematic invasive reds including *Gracilaria salicornia* and *Acanthophora spicifera* are harvested in significant quantities (up to 32 MT annually) from near-shore reefs impacted by eutrophication and repurposed as soil amendment. While most macroalgae are propagated vegetatively, the lack of genetic diversity in these populations increases susceptibility to diseases. Therefore, there is an urgent need to establish a germplasm seed bank for a diversity of economically and ecologically vital cultivars that are also thermally resilient and resistant to diseases and pests. Achieving economically viable production scales is also essential, and this can be supported by new tools and technologies - referred to as precision phyconomy - designed for land-based aquaculture. Finally, as tropical seawater is typically oligotrophic, cultivation of these species in Integrated Multi-Trophic Aquaculture systems can enhance overall productivity and yields.

Figure 1; (Left to Right) *Halymenia hawaiiana* and *Gracilaria parvispora* from tank culture.

TRACE METAL LEVELS IN MUSCLE OF SHRIMP *Penaeus vannamei* FROM ECUADOR AND EL SALVADOR, AND *P. monodon* FROM THE PHILIPPINES

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Natural populations of Pacific whiteleg shrimp *Penaeus vannamei* are threatened by habitat loss and pollutants which may impact genetic diversity and disease resistance characteristics. To protect the habitat of shrimp populations and help develop a sustainable aquaculture industry, baseline information on contaminants in mangroves and shellfish is needed.

We examine the concentrations (µg/g) of 15 metals (Al, Ag, Ba, Cd, Cr, Cu, Fe, Hg, K, Mn, Ni, Pb, S, Se, V) in muscle of *P. vannamei* broodstock from three Ecuadorian provinces (Esmeraldas, Manabi, Guayas) (n=30) and El Salvador (n=8) maintained for breeding purposes under similar environmental conditions in a culture pond in Salinas, Ecuador. Guayas shrimp ranked first in number of metals with highest concentrations followed by El Salvador and Esmeraldas. Manabi shrimp ranked the lowest in metal concentrations. El Salvador shrimp had highest concentrations of four metals (Cd, Hg, Cr, Ni) that could impact reproduction, immune response, and genetic diversity. A literature review of metals contaminating shrimp using the NCBI databases (www.ncbi.nlm.nih.gov/) is also presented. The use of trace metal profiling and multivariate statistics to determine the country of origin of farm-raised penaeid shrimp has been used by the U.S. Customs and Border Protection Laboratory[1]. Metal profiling was also used to determine the potential health risk associated with consumption of shrimp from Ecuador[2].

Consumption of Ecuadorian shrimp does not pose a human health risk. Ramos-Miras *et al.* (2023)[2] studied the presence of nine metals (As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Zn) in shrimps from Ecuador and compared them to such contents noted in other shrimp-production areas in the world to evaluate the possible risks associated with these elements for consumer health, and to relate them to potentially toxic element (PTE) contents in water, sediments and diets, and also to animal biometric parameters. The target hazard quotient (THQ) values for PTEs indicate that the consumption of shrimp muscles from Ecuador does not pose a human health risk because the values of these indices are below 1 in all cases.

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UNLOCKING THE POTENTIAL OF DENITRIFICATION FOR ENHANCED WATER QUALITY IN RAS

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Effective nutrient waste management is a key driver of profitability in recirculating aquaculture systems (RAS), particularly in handling nitrogenous wastes. While nitrifying biofilters are widely established and integrated into RAS, the development of denitrifying biofilters holds promising opportunities for further enhancing system efficiency and sustainability.

Denitrification, carried out by facultative anaerobic microorganisms, converts oxidized nitrogen compounds like nitrite and nitrate into nitrogen gas (N_2). This critical process can be fueled by organic carbon (heterotrophic denitrification) or inorganic sources (autotrophic denitrification), depending on the electron donors available. Heterotrophic denitrification is typically supported by external carbon sources, such as carbohydrates or alcohols, or by endogenous carbon from fish waste.

Beyond its primary function of nitrate removal, denitrification contributes to several vital processes in RAS. It raises alkalinity, thereby compensating for the inorganic carbon lost through nitrification, while also reducing organic carbon discharge. Furthermore, denitrifying organisms play roles in phosphorus and sulfur cycling. Some denitrifiers can accumulate excess orthophosphate, leading to substantial reductions in the ambient phosphorus levels. In marine systems, autotrophic denitrifiers also prevent the accumulation of toxic sulfides by mitigating sulfate reduction.

In today's world of increasingly stringent environmental regulations, denitrification offers a natural, efficient solution to control nitrogen emissions and comply with regulations while minimizing the need for external chemical treatments or costly infrastructure modifications. By leveraging denitrifying biofilters, aquaculture operations can maintain high productivity while meeting environmental standards, reducing their ecological footprint, and enhancing public perception of the industry.

This presentation will delve into the multifaceted benefits of denitrification in RAS, illustrating how the optimization of this process can lead to improved water quality control, resource efficiency, and overall system sustainability.

LESSONS LEARNED AND THE PATH FORWARD FOR AQUACULTURE INTERNSHIPS FOR MASSACHUSETTS

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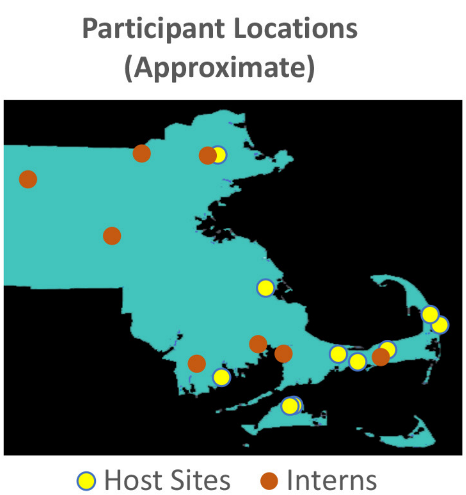
As throughout the US, shellfish aquaculture is a fast-growing sector of Massachusetts' economy, with the farming of oysters presenting the Commonwealth's third most valuable marine product. The vast majority of aquaculture in the state occurs in nearshore waters, nestled in coastal communities with high property values that present multiple barriers to engaging the workforce needed to sustain the sector's growth. In a pilot internship program, the MIT and WHOI Sea Grant programs partnered to seek pathways to overcome some of these barriers, providing the industry with eager and prepared farmhands, and supporting a conduit for often under-engaged communities to explore the professional opportunities that local aquaculture can provide.

The Aquaculture Internships for Massachusetts (AIM) program facilitated a five-week introductory program for eight Trainees recruited from throughout Massachusetts. With wraparound services (housing, transportation, stipends) provided, Trainees received basic training regarding aquaculture, safety training to encourage safe work experiences on farms, and weeks of hands-on exposure to multiple aquaculture-sector operations. From these Trainees, a select few were hired as 7-month interns for aquaculture operations, filling roles as varied as farmhand, municipal biologist, and full partner of a farm.

Many lessons were learned through this program as we aim to increasingly connect aquaculture-sector employers with communities of potential farmhands who seldom have the opportunity to explore aquaculture as a viable path. In this talk, we will outline the program's structure, the successes, and – crucially – the pinch points that must be addressed if aquaculture internships and workforce development efforts are to be successful.



OYSTER-FLIPPING ROBOTS TO EXPANDING WORKFORCE ACCESSIBILITY – AN UPDATE ON THE MIT SEA GRANT AQUACULTURE PROGRAM

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The MIT Sea Grant sustainable aquaculture program has grown in recent years, in part facilitated by the 2021 hire of its first Marine Extension Specialist to be focused on aquaculture. The development of autonomous technology to improve the safety and efficiency of aquaculture operations remains a primary area of ongoing activity. However, we have been broadening our efforts, expanding the aquaculture workforce, supporting farm resilience in the event of oil spills, pursuing better alignment of aquaculture operations around eelgrass with scientifically informed regulations, visualizing the myriads of shellfish jurisdictions in Massachusetts and harvest statuses to be more accessible to all harvesters and constituents, and more. Join us as we lay out our aquaculture portfolio and consider the future directions of this growing program.

THE DEVIL'S IN THE DETAILS: ADDRESSING THE PRACTICAL LIMITATIONS HOLDING BACK THE KELP FARMING INDUSTRY

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As everyone works to transition kelp farming from a nascent to a financially sustainable industry, inefficiencies that are overlooked when operating at a small scale can become major bottlenecks overnight. Drawing from GreenWave's on-the-ground work with a collective of nursery operators, farmers, and processors in Prince William Sound, Kodiak, and Maine, this presentation will focus on the challenges that have surfaced when scaling up production to meet market demand. Unfortunately there is not a one-size-fits-all solution to creating a financially sustainable industry, but rather each region must work to fine tune the details of production and smooth the interfaces between different roles in the value chain to build synergies that contribute to overall success. GreenWave's work thus far has focused on challenges at the three critical interfaces: nursery/farm, farm/processor, and farmer/buyer. We'll highlight how community-driven collaboration can support throughlines of communication up and down the value chain and increase the likelihood of long-term regional success. We'll also share tools and resources Greenwave has developed to address these novel challenges of meeting kelp supply.

Collaborators: Sea Greens, Alaska Ocean Farms, Kodiak Island Sustainable Seaweed, Kelp Island, Noble Oceans, Royal Oceans, Wild Blue Mariculture, Prince William Sound Science Center, Prince William Sound Economic Development District, Maine Family Sea Farms

EGGS, JUVENILES, AND SMOLTS, OH MY!: LONGITUDINAL GROWTH METRICS AT KEY DEVELOPMENTAL STAGES OFFER INSIGHT INTO THE VARIATION OF SELECTIVELY BRED *S. salar* FAMILIES

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The USDA's National Marine Cold Water Aquaculture Center (NMCWAC), located in Franklin, Maine, houses a selective breeding program for *S. salar,* where the goal is to improve N. American lines of Atlantic salmon for commercially important traits, namely, growth, and disease resistance. Selective breeding at the NMCWAC began in 2003, where increases in carcass weight have been successful. While trait improvement is apparent, other performance metrics of survival and growth at earlier stages in production are of great interest, particularly understanding the dynamisms of the two. In 2022 batches of embryos were reared up at the University of Maine, where metrics of egg survival, and egg size were recorded amongst families. 10 families, representing full siblings of the embryonic egg batches have since been followed. Metrics of weight (g), and length (cm) at critical production timepoints were collected; parr (1+ year), and smolt (~1.5 year). Embryonic and larval fish undergo rapid development, where growth rates are at an all-time high. Egg size has been shown to be related to ensuing juvenile growth rates. However, growth dynamics shift, where the once smaller individuals may display compensatory growth rates, that are reflected in growth rates at one year. Combining the suite of size, survival, and growth metrics spanning from early development to later stage fish offers a granular understanding of how growth varies acrost individuals and families within a selective breeding regime. All 10 families and respective individuals will continue to be followed, capturing essential on-grow end points to better inform trends of growth for the NMCWAC breeding program.

A GLIMPSE: WHAT CAN EARLY METABOLISM AMONGST *Salmo salar* FAMILIES TELL US?

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Over the past decade USDA's National Marine Cold Water Aquaculture Center (NMCWAC) has experienced declining egg quality. The NMCWAC located in Franklin, Maine houses an applied selective breeding program, where the primary objective is to improve *S. salar* lines for aquaculture production. Egg quality is crucial as it sets the scene for onsite productivity and provides multiplier eggs. The first-time quality is observed by industry, is termed "eye-up", which coincides with the developmental benchmark where eyes are boldly visible through the chorion. Eye-up marks the first time eggs are handled since fertilization, and survival is determined. To further investigate the intrinsic differences that may influence *S. salar* performance throughout ontogeny we determined routine metabolic rates (RMR) of 16 families. RMR is defined as the energetic cost required to power an individual's baseline living. Metabolism of embryonic and larval fishes was used as a tool to gain insight into the energetics, tolerance, and survival. Utilizing offspring from 2023 broodstock we investigated four developmental timepoints; pre-eye, eye-up, post eye-up, and alevin stages while recording metrics of egg & alevin size (cm^3, weight (g)), mass specific metabolism ($Mg02/g/hr^{-1}$), and survival (% eye up). Trials took place daily for a duration of 3 hours at optimum rearing temperatures (8°C), where oxygen consumption was recorded with oxygen sensors. As a result, small scale variation of metabolism was defined, where differences of rates across families were minute.

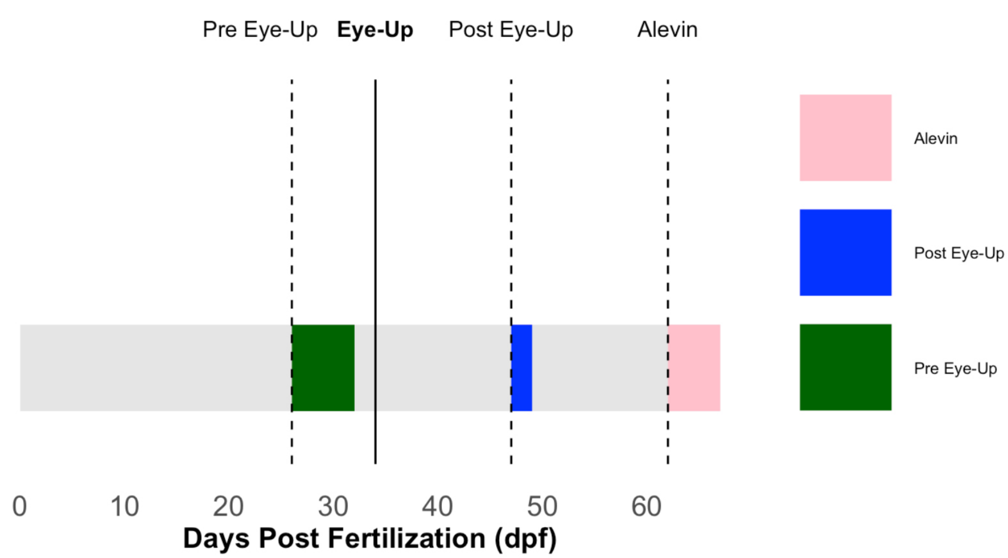

Figure 1. Depicting the 3 distinct early developmental timepoints of *S. salar*, where respiration trials were completed, resulting in mass specific rates ($Mg02/g/hr^{-1}$) of 16 families.

EFFECTS OF PROBIOTIC ADDITION TO CULTURE WATER ON GROWTH, WATER QUALITY, AND DISEASE RESISTANCE AGAINST *Vibrio parahaemolyticus* INFECTION IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei* RAISED IN STATIC BIOFLOC SYSTEMS

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Probiotics are increasingly used in aquaculture to enhance growth, improve water quality, and boost disease resistance in farmed aquaculture species. This study investigated the application of different concentrations of the commercial probiotic added to culture water (via a pelleted product) to evaluate the effects on growth performance, water quality, and resistance to *Vibrio parahaemolyticus* infection. 1 gram of this commercial pelleted probiotic contains >4 billion colony-forming units of naturally occurring bacteria such as *Bacillus subtilis, Bacillus licheniformis*, etc. The pathogen *V. parahaemolyticus* is the causative agent of acute hepatopancreatic necrosis disease (AHPND), which has devastated shrimp farms due to its rapid onset and high mortality rates. Over a nine-week trial, shrimp (1.20 ± 0.01 g; stocked at 160 shrimp/m³) were reared in static biofloc culture systems consisting of 24-156L circular black polyethylene tanks. The shrimp were subjected to various probiotic concentrations weekly (x0, x4, x8, and x16 of the recommended dose, i.e., 500 pellets per 100 kg of feed) as four treatments. Each treatment had six replicate tanks. All shrimp were provided a commercial diet (Zeigler Shrimp Grower SI-35, CP 35%) four times per day via hand feeding.

Following nine weeks of culture, growth metrics (biomass, mean weight, weight gain, and FCR) were not significantly different between treatments. Besides significant dissolved oxygen and temperature variations, no differences were observed in other water quality parameters (ammonia, nitrite, pH, salinity, total solids, and alkalinity). However, during the disease challenge, survival in all probiotic treatments (x4, x8, and x16) was higher (regardless of dose) than that of the control group (x0) ($p<0.05$). This highlights the potential of this commercial probiotic in enhancing shrimp resilience against *Vibrio* spp. infections. These findings suggest that although growth or water quality improvements were not confirmed in this trial, this probiotic appears to positively affect disease resistance in shrimp against *V. parahaemolyticus* infection in biofloc systems.

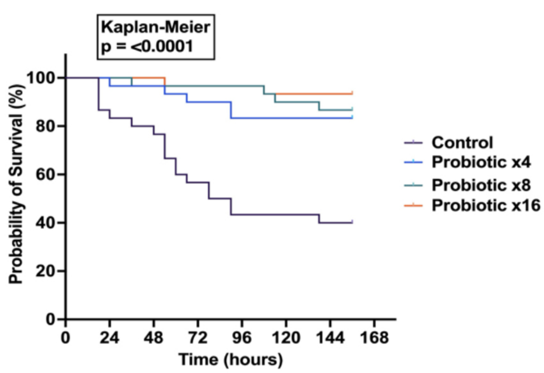

Figure 1: Survival of Pacific white shrimp *Litopenaeus vannamei* in control versus probiotic treatments when challenged with 30 ml of *Vibrio parahaemolyticus* (Vp-A3; 8.6 x 10^8 CFU/ml) via immersion.

AQUACULTURE OUTREACH AT AUBURN UNIVERSITY

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The Auburn University Student Subunit of the United States Aquaculture Society (USAS) promotes aquaculture education, sustainability, and professional networking within the community. Key initiatives include the annual shrimp sale, where fresh, head-on shrimp from the university's Gulf Shores facilities is sold at affordable prices to raise funds and bridge the gap between aquaculture research and the public. The subunit also hosts a student-professional mixer, enabling students to share their research and network with aquaculture experts while gaining insights into state-level aquaculture programs.

Our outreach efforts include family-friendly events like fishing and painting at Ag Round-Up, environmental stewardship through roadside cleanups, and an exciting project with Auburn High School students, teaching them to culture shrimp as part of a USAS grant. By engaging in these activities, the Auburn USAS Student Subunit aims to inspire future aquaculture professionals and strengthen connections within the aquaculture community.

ASSESSMENT OF SOFT-SHELL MUD CRAB (*Scylla olivacea*) FARMING TREND IN THE SOUTHWEST COASTAL REGION OF BANGLADESH

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Soft-shell mud crab (*Scylla olivacea*) farming in the southwest coastal region of Bangladesh has emerged recently as a viable alternative livelihood option for poor and landless coastal families, and vulnerable peoples due to climate change impact instead of live crab and black tiger shrimp farming. Because huge demand from the international market and high returns. This study aims to determine the growth pattern of soft-shell crab farming and future prospects. This assessment includes different farm management practices like crab seed harvest & stocking, feed & labor management, and export earnings. The results divulged that more than 380 farms are currently functioning in the study area, having 278.39 ± 20.90 acres of land accommodation and 5.55 ± 0.42 million seed crabs in demand in each cycle. The active percentage of soft-shell cages decreased from 99.43 ± 2.12% to 67.04 ± 4.75% while the feed demand/cycle increased from 3.00 ± 0.23 mt to 255.84 ± 23.33 mt during the investigated period (Fig. 1). The number of plastic cages increased from 0.05 ± 0.03 million to 5.55 ± 0.42 million whereas the wild harvesting amount decreased from 10.35 ± 2.07 to 4.38 ± 0.83 kg/day/person (Fig. 2) represents an inverse relationship. It was observed that farmers stock wild crablets and catchers reported that the natural stock of crabs is dwindling every year. Crabs fed with crushed Tilapia fish every 3 to 4 days at a ratio of approximately 5–8% of the total biomass of crab. So, crab hatchery development and formulated feed supply are inevitable for the sustainable growth of this sector. The survey shows that approximately 1200 kg of waste is produced annually by a farm with an area of 1 bigha pond. Soft shell farms created significant employment for local women and landless households. In Bangladesh, its export rank is reached above live crab within five years. These is 100% export-oriented product and export to USA, Australia, United Kingdom, China etc. Soft-shell crab farms can develop different types of entrepreneurs like crab hatchery, feed technology, nursery farm, etc. which will create job opportunities for local people.

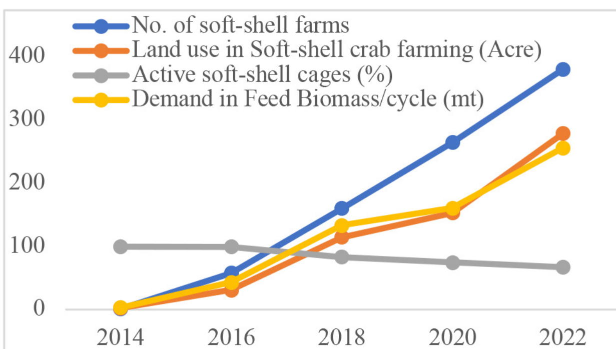

Fig. 1 Current scenario of soft-shell crab farming

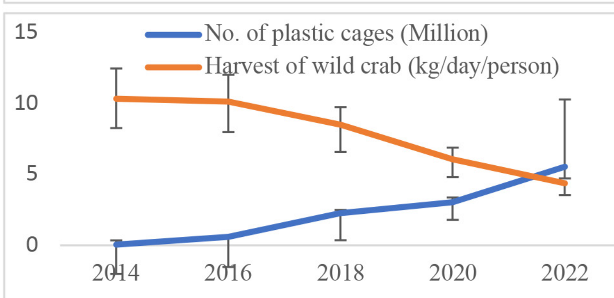

Fig. 2 Relationship of the number of plastic cages and wild harvest amount of crab

DO TEMPORAL PATTERNS IN BIRD DISTRIBUTION AND ABUNDANCE FACILITATE PATHOGEN CONTAMINATION OF SHELLFISH ON FLOATING AQUACULTURE FARMS?

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The spatial distribution and abundance of migratory waterbirds in New England fluctuates seasonally, with peaks observed at floating shellfish aquaculture farms in late summer and early fall when human oyster consumption is typically highest. During this period in 2021, elevated bird numbers at one aquaculture farm in Rhode Island, USA coincided with a Campylobacter outbreak that caused illness in a few people, raising concerns that bird fecal matter may have contributed to the outbreak. In response to this concern, our research aimed to assess the relationship between bird abundance and the presence of fecal coliforms and Campylobacter at floating aquaculture farms in Rhode Island and Massachusetts. We conducted weekly land-based waterbird surveys from July to October each year from 2020 through 2024 to determine the migration phenology for all bird species using floating shellfish gear. In 2023 and 2024, we also collected weekly samples of water and oyster meat from a subset of aquaculture farms where the land-based surveys were conducted concurrently. Water and oyster meat samples were processed to quantify fecal coliform levels and to determine the presence or absence of Campylobacter. In 2023, bird abundance for species that roost on floating shellfish gear (i.e., terns, gulls, and cormorants) was relatively low compared to previous years, limiting our ability to assess the correlation between bird abundance, fecal coliform levels, and the presence of Campylobacter. Fecal coliform levels were below the action level for water samples (geometric mean < 14 MPN/100ml), and Campylobacter was not detected in oyster meats or water. In 2024, bird abundance on floating shellfish gear increased across study sites, providing a promising opportunity to further investigate the relationship between bird abundance and the presence of fecal coliforms and Campylobacter on aquaculture farms. We will present results from the 2024 field season as part of this presentation.

IMPACT OF MICROPLASTICS ON THE LAGOS COAST: SOURCES, PATHWAYS, AND MANAGEMENT STRATEGIES

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Macroplastics (>5 mm) and microplastics (<5 mm) have emerged as pervasive and detrimental pollutants in marine environments, posing significant threats to the blue economy, human health, and marine ecosystems. Macroplastics generally enter the environment through marine litter, inadequate waste management, and discarded fishing gear.

In contrast, microplastics primarily arise from the breakdown of larger plastic items, microbeads, and microfibers shed from textiles. The coastal waters of Lagos, known for their high population density, are severely impacted by pollution and serve as hotspots for plastic waste. However, there is a paucity of data regarding the types, sizes, and quantities of polymers present along the Lagos coast. To address this gap, sediment samples were collected using a Van-veen grab sampler from three locations: Liverpool (LVP), Five Cowrie Creeks (FCC), and Makoko (MKK). Microplastics were isolated from the sediment through wet-sieving and density separation, followed by optical identification using a stereomicroscope.

The distributions of the microplastics were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The findings revealed total microplastic concentrations of up to 37 particles/kg dry weight (dw) at FCC, 65 particles/kg dw at MKK, and 110 particles/kg dw at LVP, with fragmentation of beads identified as the primary source of microplastics at all three locations. The dominant polymer types found in the study area were polyvinyl alcohol and polyamide.

The significant levels of microplastic contamination, particularly at the LVP, raise serious concerns. These pollutants pose risks through seafood consumption, leading to the ingestion of toxins and contaminants in the food chain. Further research is necessary to explore effective methods for these plastics and to develop appropriate remediation strategies for the Lagos coastline.

Table 1: Concentration of microplastic shapes in Lagos Lagoon sediment samples (particles/kg dry weight)

Sample ID	Fiber	Fragment	Pellet	Foam	Bead	Film	Total
Five Cowry (FCC)	12	4	1	3	12	5	37
Makoko (MKK)	29	13	2	15	5	1	65
Liverpool (LVP)	55	19	7	10	17	7	110

GROWING HORSESHOE CRABS FOR ENVIRONMENTAL EDUCATION AND CONSERVATION

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My research updates aquaculture procedures for *Limulus polyphemus* (the Atlantic horseshoe crab) by changing temperature conditions to influence their growth and survivorship. *L. polyphemus* live along the eastern coast of the United States from Maine to the Yucatan peninsula. Horseshoe crabs have blue blood that produces LAL, *Limulus* Amoebocyte Lysate, which is used to detect endotoxins in products like vaccines and other injectables. Furthermore, horseshoe crab eggs provide a vital food source to migratory birds, like the Red Knot, during their stopover locations in the summer. However, the IUCN lists horseshoe crabs as vulnerable due to climate change and being harvested by commercial fisheries and the biomedical industry. In Japan, Taiwan, and China, there are examples of "reintroduction programs" for the other species of horseshoe crab which involve raising horseshoe crabs for a short period of time before releasing them into the wild at a larger size that is more likely to survive. My research aims to create a reintroduction program applicable to the United States. My research project adjusts ideal temperature conditions to optimize horseshoe crab growth over a six-month time period. My experiment raises horseshoe crabs at three different temperature conditions in an aquaculture facility using commonly accessible equipment to assess their growth and mortality over a six-month period. In the first year of the experiment, it was found that higher temperatures result in a faster molting rate but a lower survival rate.

Furthermore, I am partnered with the Maryland Department of Natural Resources for a program called, "Horseshoe Crabs in the Classroom" where we work with elementary, middle, and high school public teachers across the state of Maryland to raise horseshoe crabs in their respective schools. This program creates hands-on scientific opportunities with marine life and introduces students to education surrounding horseshoe crabs and their environmental/economic impacts. This program is restarting after its pause during the pandemic and is being redesigned to increase horseshoe crab survival and including more engagement aspects for the students. We redesigned the horseshoe crab aquaculture setups for school settings and created experiments that can be done at the various educational levels. This program has expanded from five schools in the 2023-2024 school year to ten schools in the 2024-2025 school year. In the coming years, we hope to add more schools from different counties in Maryland to create a more diverse set of schools and students.

Figure 1. Teacher Setup for Horseshoe Crabs in the Classroom.

EVALUATION OF ANTIMICROBIAL PROPERTIES OF AQUEOUS EXTRACTS OF *Senna alata* (L.) Roxb *Mitracarpus scaber* (Zucc) and *Physalis peruviana* (L) ON ISOLATES FROM CATFISH INCUBATOR

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Catfish seed production in the incubator is usually infected by microbes. Public health concern and the resistance of microbes to synthetic antibiotics have occasioned the need to explore alternative natural-based antimicrobial agent for more eco-friendly and responsible aquaculture practice. This study therefore investigated the antimicrobial properties of aqueous extracts from plant samples on isolates from catfish (*Clarias gariepinus*) incubators. Microbes were aseptically isolated from the mid-point of the base of the catfish incubator on the third day of post-spawning operation. Using standard laboratory methods, the microbes were identified and assessed for Zone of Inhibition (ZI), Minimum Microbicidal Concentration (MMC) and Minimum Inhibitory Concentration (MIC) following application of aqueous extracts of *Senna alata, Mitracarpus scaber and physalis peruviana.* Data obtained from the laboratory observations were subjected to analysis of variance, tested at 95% confidence limit using IBM SPSS 22® statistical package.

The results of the analysis (Table 1) showed that the ZI was significantly highest ($p < 0.05$) in *Physalis peruviana* extract and lowest significantly in *Mitracarpus scaber* extract. Exposure concentration of 100 mg/l of the extracts resulted into a significant increase ($p < 0.05$) in the ZI.

Table 1: The Main Effects of Extracts and Exposure Concentrations of Aqueous Extracts on the Zone of Inhibition (mm) of Microbial Isolates from Catfish Incubator.

	Extract				Concentration of Extract (mg/ml)			
Organsims	*P. Peruviana*	*M. scaber*	*S. alata*	Pooled ±SE	100	50	25	Poole d±SE
Staphylococcus spp.	13.67^{a}	11.11^{b}	2.56^{c}	0.25	14.44^{a}	7.56^{b}	5.33^{c}	0.25
Bacillus spp.	8.11^{c}	10.78^{a}	9.22^{b}	0.26	14.89^{a}	10.67^{b}	2.56^{c}	0.26
Klebsiella spp.	0.00^{b}	5.22^{a}	0.00^{b}	0.06	5.22^{a}	0.00^{b}	0.00^{b}	0.06
Escherichia coli	3.22^{c}	5.33^{a}	4.33^{b}	0.20	10.56^{a}	2.33^{b}	0.00^{c}	0.20
Salmonella spp.	25.89^{a}	18.33^{b}	5.00^{c}	0.28	21.67^{a}	14.67^{b}	12.89^{c}	0.28
Pennicillium spp.	12.78^{a}	11.11^{b}	3.22^{c}	0.21	14.00^{a}	7.56^{b}	5.56^{c}	0.21
Yeast	13.11^{a}	12.56^{a}	6.89^{b}	0.26	14.33^{a}	11.33^{b}	6.89^{c}	0.26
Aspergillus niger	13.22^{a}	12.56^{a}	6.22^{b}	0.27	14.22^{a}	10.78^{b}	7.00^{c}	0.27

Mean values in the same row with same superscript are significantly different at $p < 0.05$.

It can be concluded that *Physalis peruviana* showed high potency against microbes isolated from the catfish incubator among the three extracts, at a concentration of 100mg/ml.

U.S. FEDERAL LEGISLATIVE UPDATE ON OFFSHORE AQUACULTURE

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Offshore aquaculture in the U.S. has the unique potential to improve American food security and nutrition, enhance coastal resiliency, create quality jobs, help restore species and habitats, and ensure that seafood (both wild-caught and farmed) continues to be an important part of the global food supply.

Yet, U.S. aquaculture is currently constrained by the absence of an efficient and affordable permitting process, particularly in U.S. federal waters. Stronger America Through Seafood (SATS) has been working to pass bi-partisan legislation to create a predictable and efficient regulatory system to allow the industry to expand to U.S. waters.

Join SATS' Campaign Manager, Drue Banta Winters, for an overview on the federal legislative landscape around offshore aquaculture in the 119th Congress. Learn about SATS efforts to move the bipartisan legislation forward, the shifting dynamics on this issue, and out how you can show your support for US aquaculture to help move legislation forward.

MEASURING ECOLOGICAL CHANGE ON A NEWLY INSTALLED MODERN CLAM GARDEN

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Clam gardens are intertidal features modified by Northwest Coastal Indigenous people to enhance clam habitat for optimal shellfish production. The Swinomish Indian Tribal Community (SITC) recently initiated a clam garden project to address declining clam populations and community concerns regarding climate change and ocean acidification. This effort braids traditional ecological knowledge, contemporary resource management, and climate adaptation strategies to encourage local food security and sovereignty and promote sustainable seafood production.

As the first clam garden built in the U.S. in modern times, SITC's Fisheries Department and Community Environmental Health Program co-designed a long-term monitoring study to examine the ecological changes that occur on the clam garden as it develops over time and socio-cultural changes within the community. For the ecological data, we utilized a "before-after control-restoration" design where (1) the restoration site was the modern clam garden site (Fig. 1) and the control site was a nearby beach that had not been recently modified and (2) before/after was defined by data collected prior to or after we began construction of the rock wall and tending the beach. For each site, we deployed 12 0.25m^2 quadrats along various elevation stratifications and collected data on surficial substrate and benthic species. Next, the top 5cm of substrate was collected in a 4mm sieve and samples were sorted to quantify juvenile clams and other invertebrates. Once subsurface substrate data was collected, we then dug the quadrat to 0.3m depth to sample all adult clams. We also measured elevation change by deploying sediment elevation tables at both sites at 1.5m, 0.6m, and -0.6m tidal elevations relative to mean lower low.

Initial results revealed that the control site likely has higher species abundance and diversity compared to the clam garden, which would be expected because the control site is likely a relic clam garden or other human-modified shoreline. Post-installation, we found high levels of sedimentation at the high-elevation site at the clam garden, indicating that the rock wall had changed some aspects of sediment delivery in a localized area. These results and more will be discussed, with a particular emphasis on monitoring ecological change in a biocultural restoration project.

Fig. 1 Community members building the clam garden wall

ASSESSING CHANGE IN COMMUNITY COMPOSITION WITHIN AND BETWEEN NATIVE OYSTER, *Ostrea lurida*, RESTORATION SITES

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Olympia oysters (*Ostrea lurida*) are the only oyster native to the west coast of North America and are considered functionally extinct in many areas, including Puget Sound. While myriad restoration projects for this species exist throughout the coast, most research associated with these projects is aimed at understanding biological metrics of Olympia oysters such as recruitment, dispersal, density, and survival. Despite the presumed importance of this ecosystem engineer, few studies have investigated biological communities associated with Olympia oyster beds and how those communities change in reestablished sites. In order to better understand changes that occur in community composition, with a particular emphasis on salmonid prey, we asked if the reestablishment of Olympia oysters alters the composition of epibenthic communities through time and between or within embayments. Using an epibenthic suction sampler, we gathered 12 samples on and 12 samples off reestablished oyster beds (n=4) in both March and April (when smolts are using these critical habitats) from 2015-2023 depending on the site. All invertebrates samples were processed through 106 µm sieves, preserved, and later identified to the species level (or higher taxonomic categories if necessary) in a laboratory. PERMANOVA results using a Bray-Curtis dissimilarity measure indicated that the epibenthic community composition was significantly different between locations and across years. Additionally, the interaction between location and substrate type (oyster vs. bare) was significant; however, the independent substrate term did not suggest a difference in epibenthic communities between oyster and bare plots. NMDS plots show a dissimilarity of epibenthic community composition between samples and that samples clustered by location, with some clustering based on substrate type within each embayment (Figure 1). This suggests that the main driver of variation between epibenthic communities is actually the embayment. While there was no general independent effect of substrate across all sites (oyster vs. bare), within each embayment the biological communities did vary between oyster beds and bare substrate. This work demonstrates the importance of site specific variation and establishes that ecosystem services provided by this oyster may vary on a localized scale.

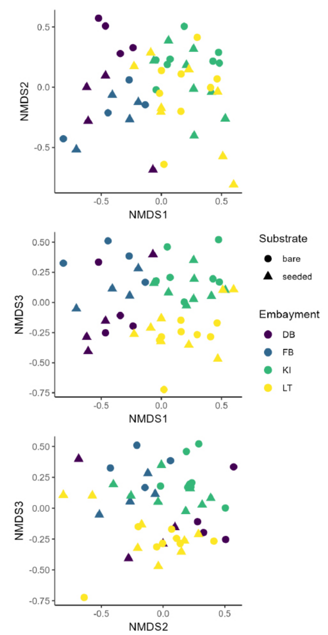

Fig 1: Nonmetric multidimensional (NMDS) scaling plots of epibenthic assemblages based on Bray-Curtis dissimilarity (stress=0.15, k=3). Points represent individual samples, with lagoon location shown in color and substrate

PERFORMANCE EVALUATION OF DIPLOID AND TRIPLOID ARCTIC CHARR

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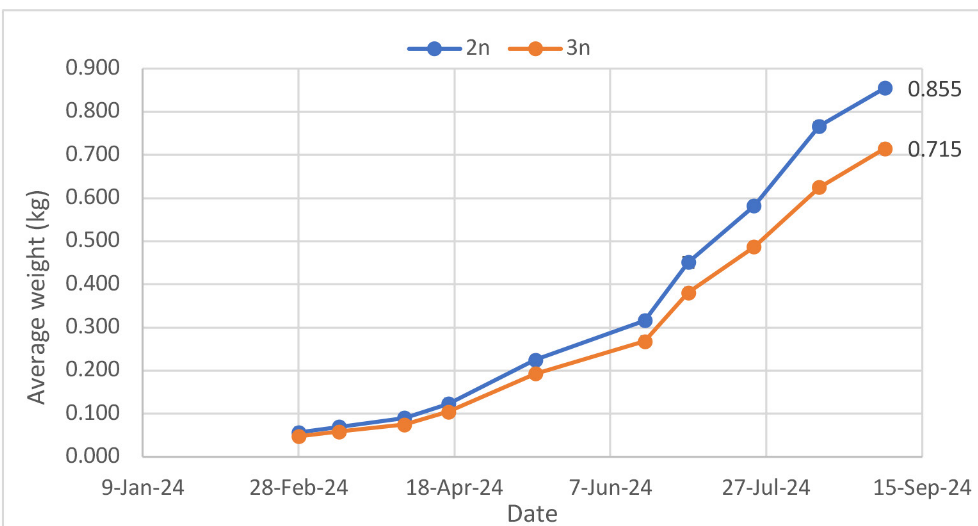
Valorēs implements a breeding program to enhance the performance of Artic charr in commercial aquaculture, now advancing into its 10th generation of selection this fall. Diploid progeny face hurdles in attaining marketable size due to premature sexual maturity, impacting flesh quality and feed conversion. To counter this, Valorēs began producing triploid Artic charr in 2016. Triploids, being sterile, channel energy away from gonad development into growth, offering advantages like reduced escapement risks and safeguarding intellectual property. Despite potential benefits, the financial viability of triploid production remains unexplored. This project is divided in two phases: separate rearing of diploids and triploids until market size, followed by performance assessment during harvest and processing. Metrics include survival rates, growth, feed conversion, maturation rate, and product quality (fillets yields, pigment, lipid and protein contents).

Phase one presented here aims to gather comprehensive data on survival, growth, and feed conversion across different ploidy levels over two years in commercial conditions. This project evaluates triploid fish performance under modern aquaculture conditions. Results will benefit stakeholders invested in Artic charr aquaculture, ensuring the success and viability of Arctic charr breeding program and its industry.



ANALYZING ENVIRONMENTAL IMPACTS ON THE PERFORMANCE OF EASTERN OYSTERS *Crassostrea virginica* IN NORTH CAROLINA OYSTER FARMS

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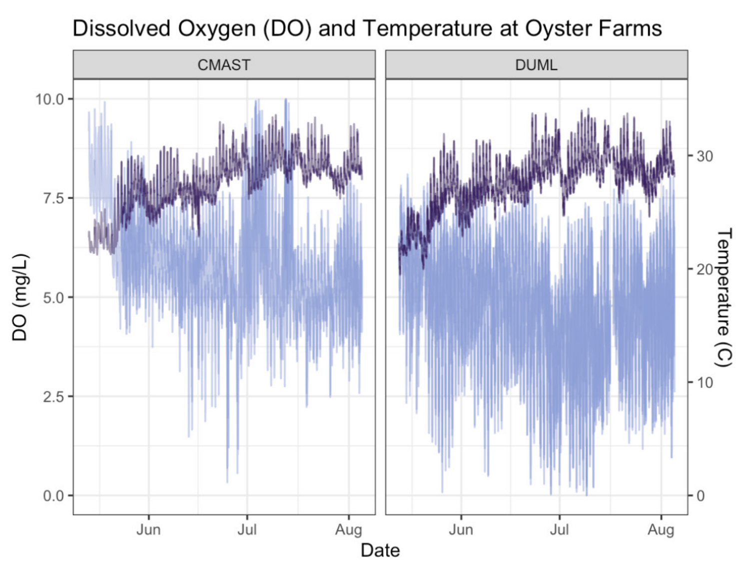
Oysters support commercial and recreational fisheries, and hold considerable economic and cultural value in North Carolina. Environmental stressors associated with climate change can interact synergistically with other stressors (e.g., predation, pathogens) to negatively impact oyster growth and reproduction. This project aims: 1) To monitor and evaluate multiple environmental parameters concurrently with oyster performance at the Duke University Marine Lab (DUML) and NC State's Center for Marine Science and Technology (CMAST) oyster farms, particularly during the summer when environmental stressors may be high; 2) To assess the relationship between changing environmental conditions, genetic lines, and farming practices with oyster performance; and 3) To develop an effective platform to communicate findings with regional oyster farmers and other interested parties in an attempt to further resiliency efforts.

We sought to analyze different characteristics of the two farms that may have impacted oyster performance. To achieve this, we monitored environmental data while assessing impacts on five genetic lines of oysters. Densities of oysters in bags and flipping frequencies were also examined as factors. Data loggers were deployed and maintained to continuously monitor *in situ* environmental conditions within both oyster farms. Temperatures and salinity at both sites were fairly similar, but dissolved oxygen (DO) levels had greater oscillations at the Duke farm, with values more frequently dropping below 1 mg/L (Figure 1). In addition, pH values were also more variable at the Duke farm. We intend to analyze these environmental data points with mortality and growth measurements that were collected at each site over the study period to better understand oyster performance.

Figure 1: DO levels and temperature at the Duke and CMAST oyster farms. DO levels are represented in light purple and temperature is dark purple.



EVALUATION OF A NOVEL WATER TREATMENT TECHNOLOGY FOR BOD, TSS AND PHOSPHOROUS REMOVAL

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Introduction

A novel technology for treating wastewater contaminated with organic matter was developed. The treatment process is mainly based on biological microorganisms distributed in aerobic and anaerobic chambers.

Pilot experimental reactor

A pilot experiment was conducted to evaluate a 5 m^3 plug flow reactor to treat a fraction of the rotofilters backwash. Two flow regimes were evaluated with hydraulic residence times (HRTs) of 24 and 12 hours.

The experimental pilot system PFR had 20 meters length. Water samples were collected at 0 m (affluent), 5 m, 10 m, 15 m, and 20 m (effluent), along the axial water flow direction.

Operation results of the reactor

Regarding to BOD and TSS, around 91% depletion was observed for all treated water samples. The parameters related to phosphorous showed a depletion between 54.0% and 99.8%.

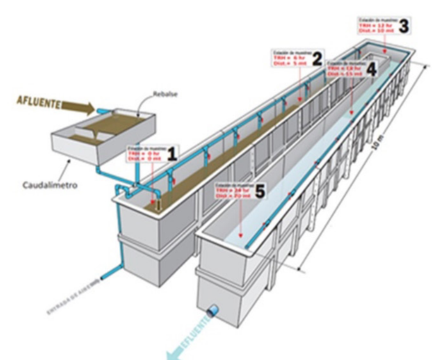
Conclusion

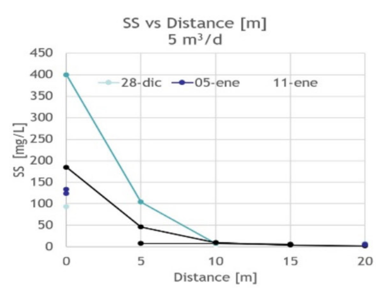
No sludge was generated throughout the entire evaluation. Hence, there was no need to recirculate or to dispose any sludge. Effluent water from the process was recirculated back to the production system.

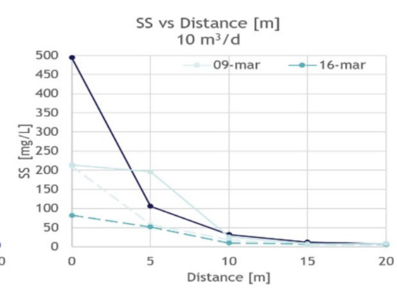
Acknowledgement

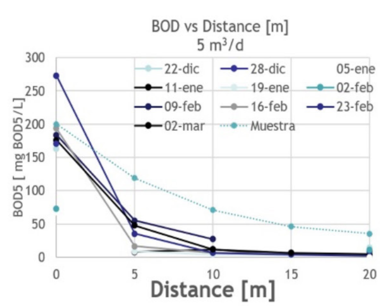
Project grant FONDEF IT20i0095, Project grant SDG ANID GRC22003, and Project grant CYTED P422RT0090

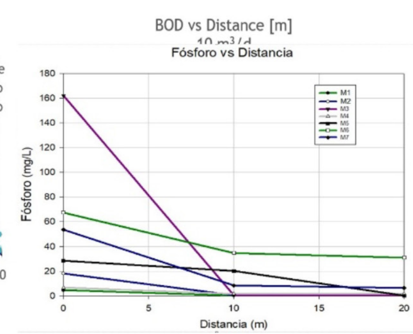
Pilot experimental reactor











CORALS, MARINE SHELLFISH, AND MANGROVES OF TANZANIA

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Mangroves are prime nesting and resting sites for hundreds of shorebirds and migratory bird species, including kingfishers, herons, and egrets. Mangrove roots provide support for filter- feeders like mussels, oysters, and barnacles. The toxic metal levels in four species of fish (*Labeobata, Sillaginopsis panigus, Platycepalus fuscus*) and shrimp (*Penaeus monodon*) from Bangladesh have been reported and the associated health risks to consumers reported (Hossainet al. 2022). The findings revealed that only Pb in finfish and Pb, Cu, and Zn in shrimp samples exceeded the national recommended limits, representing possible risks to consumers. *P. monodon* shrimp had higher levels of metals than fish species. Limited information on heavy metals has been reported for oysters, blue crabs, corals, saltmarsh from the Msimbazi River in Dar es Salaam, Tanzania. A preliminary investigation on the extent of metal pollution (Hg, Cr, Cu, Zn, Fe, Ni, Cd, Mn, Al) was made at Tanzania fisheries Research Institute from samples of sediments and biological indicators. Coral reefs are very important in Tanzania, both ecologically and socio-economically, as major fishing grounds and tourist attractions. These reefs have been partially to severely degraded by human (primarily destructive fishing practices) and natural (particularly coral bleaching) causes. These immediate human causes have been brought about by various socioeconomic root causes, particularly poverty and lack of proper management. The Global Coral Reef Alliance (GCRA) has taken the lead in documenting coral bleaching events worldwide and relating them to climate and weather anomalies as well as locally derived environmental stresses. The goals of this project were to (1) examine metal levels in oysters and saltmarsh, (2) assess the survival of oysters and saltmarsh using the Biorock method mineral accretion and (3) perform an economic analysis of innovative methods to enhance productivity in the cultivation of oysters, seaweed, mussels, and fish. Metals (lead, mercury, arsenic and cadmium) were analyzed in oyster and saltmarsh at the laboratory of Tanzania fisheries Research Institute. Biorock method Accretion Technology increases shellfish settlement while reducing hydroid fouling, keeping oysters and saltmarsh alive and growing under conditions that would otherwise be toxic. This method stimulated a greater settlement of larvae of different marine species. Each hectare of mangrove generates up to US$50,000 a year in mangrove shrimp farming associated with fish and blue crab bringing an estimated US$19 million in income annually to local small holder fish farmers.

SENSITIVITY OF CYANOBACTERIA TO HERBICIDES COMMONLY USED IN AQUACULTURE PONDS

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It is well understood that water quality and eutrophication are major drivers of harmful algal blooms (HABs). Issues arising from nutrients loads, water circulation, and temperature also encourage blooms of harmful algae, such as *Microcystis* and *Anabaena*, in aquaculture ponds. Copper sulfate, an EPA-approved algicide, is often used to treat harmful algal blooms. While effective, repeated annual use of copper can accumulate in aquaculture ponds and potentially make the pond too hazardous for commercial production. Use of alternative herbicides has been proposed however they are less effective than copper. As herbicides have different mechanisms of action, using multiple herbicides simultaneously may be more effective in controlling cyanobacteria blooms. *Microcystis aeruginosa* and *Anabaena* spp. were exposed to herbicides commonly used in aquaculture ponds to establish benchmark doses for each chemical. Results demonstrated that both genus' had comparable dosimetry with some exceptions. Using the effect concentration (EC) where growth was inhibited by 50%, binary chemical mixtures were investigated to determine if herbicide mixtures were more effective than single herbicide use. Herbicide mixtures were designed following the contaminant mixture radial design using EC50 values to represent 1 toxic unit. Five mixtures were prepared based on different ratios of two herbicides (100:0%, 75:25%, 50:50%, 25:75%, and 0:100%) and serial dilutions of each mixture were investigated to determine if herbicides were additive, synergistic, or antagonistic. Results of the study will be used to propose alternative herbicides regimes which may be more effective in controlling harmful cyanobacteria blooms.

Table 1: Effect Concentrations (EC50) of select herbicides towards cyanobacteria.

Effect Concentration (EC50) for Select Herbicides					
	Anabaena		*Microcystis*		
	Chlorophyll (µg/L)	Phycocyanin (µg/L)	Growth Rate	Chlorophyll (µg/L)	Phycocyanin (µg/L)
Bispyribac Sodium	1.55	0.92	>50	>50	>50
Copper	0.016	0.024	0.04	0.025	0.032
Diaquat Bromide	0.67	0.89	0.25	0.14	0.28
Diuron	0.002	0.003	0.028	0.02	0.027
Endothall	>50	>50	60.96	63.32	58.21
Imazapyr	>100	>100	>100	98.56	96.42
Fluridon	>10	>10	>10	>10	>10
Flumioxazin	>0.1	0.02	>0.1	>0.1	>0.1
Penoxsulam	< 0.625	< 0.625	3.18	0.74	0.49
Triclopyr	>10	>10	>10	>10	>10
Topramezone	>50	>50	>50	>50	>50

DRAFT PROGRAMMATIC ENVIRONMENTAL IMPACT STATEMENT FOR THE IDENTIFICATION OF AQUACULTURE OPPORTUNITY AREAS IN U.S. FEDERAL WATERS OFF OF SOUTHERN CALIFORNIA

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Abstract: The National Oceanic and Atmospheric Administration's (NOAA's) National Marine Fisheries Service (NOAA Fisheries) West Coast Region is developing a programmatic environmental impact statement (PEIS), in accordance with the National Environmental Policy Act (NEPA), that analyzes the potential impacts to the human environment that could result from identifying Aquaculture Opportunity Areas (AOAs) in federal waters in the Southern California Bight and evaluates the impacts of siting aquaculture in those locations. The intent of this PEIS is to support long-term planning for offshore aquaculture. This PEIS considers a long-term planning effort that is not a regulatory or permitting action and does not propose to authorize or permit any specific aquaculture-related activities or propose to approve any individual aquaculture projects.

REIMAGINING PLASTIC-BASED AQUACULTURE: AN INVESTIGATION INTO VIABLE MATERIALS

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Aquaculture, specifically shellfish and seaweed aquaculture, is often celebrated as among the most sustainable food production systems in the marine world. Oyster and seaweed aquaculture techniques do tread lightly on the environment, and are beneficial to ocean health in many ways — but the fact remains that a reliance on plastics in every step of cultivation has an enormous negative environmental impact. Aquaculture contributes to more than half of the seafood consumed globally, and is only projected to increase and with it, industries dependency on plastic. In order to have a truly sustainable aquaculture industry, every step of production needs to be examined and improved. Currently, aquaculturists rely on almost 100% plastic materials to cultivate and grow food. Deer Isle Oyster Company strives to change this, through re-thinking the materials we put in the water to grow shellfish and sea vegetables. Over the past four years, we have aimed to identify durable, competitive, viable, and ecologically friendly materials for aquaculture systems, with a focus on oyster surface cultivation. At the most basic level, our goal has been to figure out which materials are the best for building grow-out bags — in performance, supply, scalability—and that meet our ecological goals

SOY AQUACULTURE ALLIANCE (SAA): ADVANCING SUSTAINABLE AQUACULTURE RESEARCH AND DEVELOPMENT GOALS

Amrit Bart*, Easton Kuboushek, Erica Curles

The Soy Aquaculture Alliance (SAA), established in 2011, is dedicated to expanding U.S. aquaculture and promoting the use of U.S. soy in diets for fish, crustaceans, and other farmed aquatic species. Soy-based feeds provide a nutritional, sustainable, efficient, and consistent-quality solution that supports domestic aquaculture growth. SAA collaborates with academic researchers and industry leaders to fund solution-driven research and assist farmers through education and training.

Each year, SAA funds research proposals addressing key priorities, including practical on-farm projects, commercial-scale and proof-of-value studies, reducing soy anti-nutritional factors, environmental sustainability, market analysis, and technological advancements. Research focuses on species such as shrimp, salmon, trout, hybrid striped bass, largemouth bass, and catfish.

In partnership with the United Soybean Board (USB), U.S. Soybean Export Council (USSEC), and the World Initiative for Soy in Human Health (WISHH-ASA), SAA amplifies its impact by addressing research gaps, exploring new markets, conducting on-farm proof-of-value projects, and leveraging resources. Through research, education, and innovation, SAA drives sustainable aquaculture practices, increases soy-based feed adoption, and aligns with global sustainability goals. These efforts enhance aquaculture's efficiency, environmental performance, and resilience, supporting a sustainable food system.

Year	**Topic (s)**	**Soy Products**	**Species**
2023-2024	A nutrigenomic strategy Advanced soy diets for catfish Soy lecithin use for catfish Soy ingredients- largemouth bass Feed efficient rainbow trout families	SBM Soy lecithin Soy protein	RB trout Channel Catfish Channel Catfish LMB RB trout
2022-2023	Aqua Feed Formulation Soybean utilization- insect meal Feed efficient rainbow trout families Soy Based Marine Fish Feeds High Protein Soybean Meals	SBM Soy Protein S-E SBM High protein-SBM	Atlantic salmon Florida pompano California yellowtail
2022-2021	Aquaculture Nutrition and Feed Formulation Soybean utilization using insect meal Select for feed efficient rainbow trout families Soy-Based Marine Feeds for Florida Pompano Selectively Bred CY, High Protein SBMs	SBM Soy Protein S-E SBM High protein-SBM	Atlantic Salmon RB Trout California yellowtail

Research proposals funded through SAA in the last 3 years (2022-2024)

MICROBIAL ASSOCIATIONS IN GREENHOUSE GAS EMISSIONS FROM PRAWN (*Macrobrachium rosenbergii*) FARMS

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Microbial communities are the forefront drivers of Greenhouse gas (GHG) emissions in aquaculture systems, presenting both challenges and opportunities for sustainable management. However, the relationship between microbial assemblages and GHG emissions in prawn farms remains largely unexplored. To demonstrate the linkage between microbial communities and GHG emissions, we measured GHG emissions (CO_2, CH_2, and N_2O) from 35 prawn farms using the floating chamber method, characterized microbial community structures through high-resolution Illumina metabarcoding, and quantified functional genes related to GHG dynamics in aerobic sediment (AeS) and anaerobic sediment (AnS), and water using real-time qPCR technique.

The highest CO_2 emissions were observed in polyculture pattern, extensive system, and low-saline farms, whereas methane emissions peaked in high-saline and semi-intensive farms. Microbial richness correlated positively with CO_2 emissions and negatively with methane emissions. Methanogenic archaea were strongly linked with methane production, but their association with CO_2 consumption was insignificant. Methanotrophic guilds were closely associated with methane breakdown; however, the minimal role of ANME lineage in CO_2 production signifies the ANME-driven methane oxidation as a promising approach to lower the carbon footprint in prawn production. The majority of bacterial and archaeal classes were associated with N_2O consumption, though the N_2O-reducing class Chloroflexi showed a positive correlation with N_2O emissions, contrary to existing literature. The abundance of *mcrA* and *pmoA* genes was influenced by the dissolved oxygen levels. While the lowest abundance of *mcrA* was observed in water, it showed a significant association with methane emissions and CO_2 consumption. This suggests a broader functional capacity of *mcrA* beyond classical limits and warrants further investigation into its aerobic physiology. Methane breakdown was strongly associated with the presence of the pmoA gene in both AeS and water. Interestingly, the amoA gene, when present in AeS and AnS, correlated more strongly with methane consumption, despite its usual association with N_2O emissions, which was observed only in water. The anaerobic nosZ gene contributed substantially to N_2O reduction in prawn farms.

This multidisciplinary study advances our understanding of microbial associations in GHG emissions, offering evidence that simpler on-farm management practices, such as oxygen supplementation, could substantially influence microbial communities and reduce emissions.

AQUSENS: AI-POWERED PLATFORM FOR AUTOMATED IDENTIFICATION OF HARMFUL ALGAL BLOOMS AND OTHER PATHOGENS IN WATER

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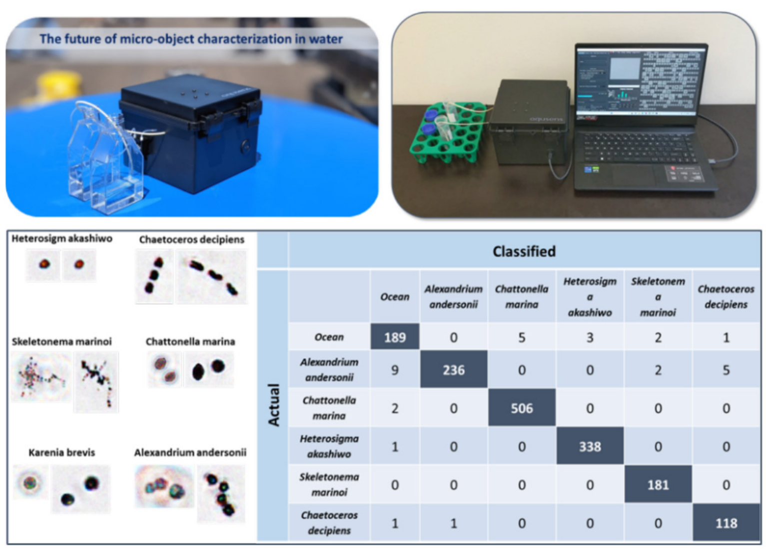
One of the most significant challenges in aquaculture is to mitigate or avoid production losses due to disease agents caused by parasites (such as sea lice), algae, bacteria, viruses, and fungi. This creates a critical need for the early detection of dangerous microorganisms and disease-causing agents in the water. Currently, the samples are collected manually and analyzed by trained technicians in a laboratory, often centralized, using conventional lens-based microscopes. This approach is expensive, laborious, time consuming and limiting scalability of operations. Furthermore, delays with disease agent identification often eliminate many mitigation strategies forcing approaches leading to production losses or pre-mature harvesting.

To address this critical need, Lucendi has developed Aqusens – an AI-based holographic microscopy platform capable of rapid automated monitoring of water samples for the presence and accurate quantification of dangerous organisms, such as harmful algae, sea lice and others. Unlike conventional lens-based microscopy systems, Aqusens relies on capturing interference patterns as objects are passing through a pulsating light field. These patterns are then processed and characterized by deep learning. With this novel approach, Aqusens can identify objects anywhere from 2 micron to 4 mm range and above. The system screens the water with unprecedented 100 mL/hr throughput, which can be increased to liters per hour depending on the application needs. For every object Aqusens generates an intensity and phase images which are then analyzed by custom AI to determine the object type and to compute concentration. Collected data can then be immediately shared with key decision makers to provide advanced warning, optimize operations, and save costs.

Aqusens is currently deployed at several in-field facilities. It was validated in the laboratory studies demonstrating accurate identification of various harmful algae and sea lice. Aqusens can also be customized for specific operational conditions and applications, and is developed to support scalability and safety of the operations.



	Ocean	Alexandrium andersonii	Chattonella marina	Heterosigm a akashiwo	Skeletonem a marinoi	Chaetoceros decipiens
Ocean	189	0	5	3	2	1
Alexandrium andersonii	9	236	0	0	2	5
Chattonella marina	2	0	506	0	0	0
Heterosigma akashiwo	1	0	0	338	0	0
Skeletonema marinoi	0	0	0	0	181	0
Chaetoceros decipiens	1	1	0	0	0	118

Figure 1: Aqusens is an AI-powered cost-effective, high-throughput platform for automated identification and characterization of harmful micro-organisms in water. It can be deployed in-field with minimal maintenance requirements and provide advanced awareness to optimize

AQUSENS: AI-POWERED PLATFORM FOR AUTOMATED QUANTIFICATION OF LIVE FEED

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Many fish hatcheries rely on live feeds (i.e., microorganisms, such as copepods, rotifers, and artemia). One of the critical capabilities missing today is to enable automated live feed counting and characterization. Introducing automation, will result in elimination of manual processes, will improve accuracy, will promote standardization, and will reduce errors and culture crashes.

Existing solutions to live feed quantification and characterization are based predominantly on manual counts performed by trained experts on conventional microscopes. Furthermore, re-purposing existing lens-based imaging flow cytometers for automated live feed characterization is not ideal due to the high cost of the equipment and performance limitations of these solutions, including low-throughput processing, significant maintenance requirements, issues with adapting for large size range of organisms that is the case with live feed species.

Lucendi has developed the Aqusens platform, shown on Figure 1, which is capable of rapid and label free evaluation of the live feed samples with throughputs of anywhere from 100 mL/hr – 5 L/hr, depending on use case needs with sufficient resolution to characterize organisms in a diverse size range from single microns to millimeter scale. Furthermore, since the Aqusens is built primarily from the off the shelf components, the cost of the hardware solution is significantly lower than any of the existing alternatives.

Aqusens AI infrastructure is capable of automated live feed characterization by type (i.e. copepods, rotifers, artemia, etc.), and by phase of development (i.e. egg, nauplii, copepod). Aqusens can also identify cross-contamination to avoid production inefficiencies and losses.

Aqusens has been deployed in-field and was demonstrated to provide continuous live feed sample monitoring and quantification that is at least on par or better and more repeatable than that of the human operator.

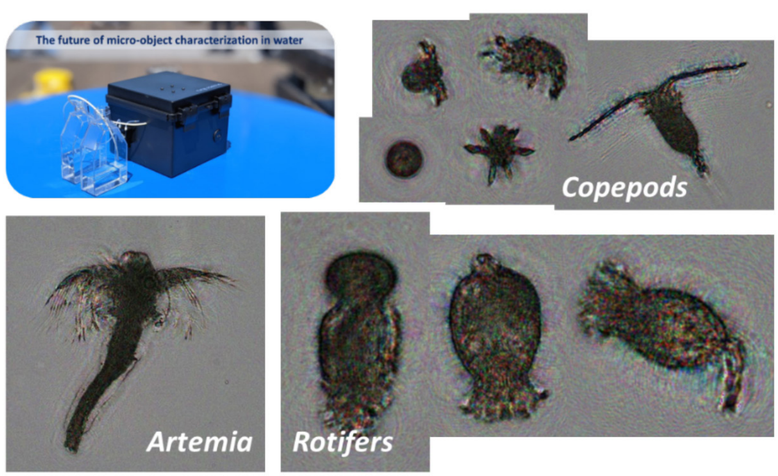

Figure 1: Aqusens platform leverages AI and unique holographic imaging flow cytometry to provide automated high-throughput accurate identification of live feed organisms, including classification of the development phase, to optimize hatchery operations and empower

SALINITY REGIME AND PREDATION RISK DRIVE ALLOCATION TRADE-OFFS IN JUVENILE EASTERN OYSTERS *Crassostrea virginica*

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Eastern oysters (*Crassostrea virginica*) are pivotal members of estuarine ecosystems and support productive aquaculture markets. Extreme precipitation events (drought and flood) driven by climate change are altering estuarine salinity. Increased estuarine salinity reduces oyster feeding rates while increasing energy expenditure, but also increases colonization of oyster predators that are limited by lower salinity. In high predation environments, juvenile oysters induce morphological shell defenses (i.e., thicker, stronger shells) that confer greater predation resistance. However, morphological defenses require increased allocation of energy for shell development, which may reduce growth rates or allocation for the development of somatic tissue. Energy acquisition through filter feeding is reduced at suboptimal salinity, and predator-exposed oysters may feed less than non-exposed oysters (Figure 1). Simultaneous exposure to suboptimal salinity and greater predation risk may drive tradeoffs in energy allocation for shell defenses and somatic tissue. If oysters reduce feeding rates due to salinity stress or predation risk, they may grow slower or produce less body tissue relative to shell tissue (condition index). Alternatively, salinity-induced reductions in feeding may leave predator-exposed oysters unable to devote adequate energy to morphological defenses, which may increase their susceptibility to predation in suboptimal salinity regimes.

Here, I exposed juvenile oysters to three salinity regimes in the presence or absence of predatory cues from the carnivorous gastropod *Melongena corona*. I quantified oyster feeding rates on microalgae (*Tisochrysis lutea*), oyster growth, and survival over eight weeks and measured tissue weight, shell weight, and shell thickness of predator-exposed and non-exposed oysters. I hypothesized that oysters grown in suboptimal salinity regimes will grow slower and have lower condition index (ratio of tissue mass to shell mass) than oysters grown in favorable conditions, and that predation risk will further reduce condition index by signaling predator-exposed oysters to produce heavier, thicker shells than non-exposed oysters. My results will benefit aquaculture by providing an example of how the presence of predators and suboptimal abiotic conditions can interact to alter the allocation strategies and development of a common aquaculture species, which can ultimately influence its growth, survival, and contribution to desired aquaculture outcomes.

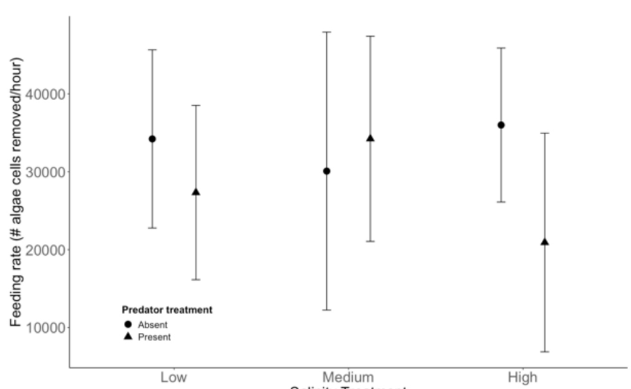

Figure 1: Feeding rates of juvenile oysters (*C. virginica;* circles = non-exposed, triangles = predator-exposed) in low (15-20 ppt), medium (22-27 ppt), and high (39-34 ppt) salinity. Bars represent 95% confidence intervals.

IMPACT OF DHA AND ORGANIC AND INORGANIC MINERALS IN DIETS FOR NILE TILAPIA BROODSTOCK AND THEIR EFFECTS ON LARVAL GROWTH

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The global aquaculture is expanding rapidly, with tilapia being the second most produced fish worldwide. However, the availability of high-quality fry remains a challenge in the production chain. Stress from adverse conditions negatively impacts fish health, leading to losses and increased disease susceptibility. Nutritional management, particularly through additive supplementation, offers a solution to enhance fish performance. Proper nutrition of broodstock affects the quality of eggs, larvae, and fry, benefiting future generations. Supplementing diets with fatty acids like DHA and organic minerals can improve metabolic health and boost resistance in tilapia offspring.

Therefore, 60 male and 180 female Nile tilapia were placed in 20 cages (3×2×1m) placed in earthen ponds. The broodstock were fed four different diets, containing two levels of DHA (0% and 1%) and two sources of mineral premix (100% inorganic Zn, Mn, and Se; and 50% organic Zn, Mn, and Se). The broodstock underwent reproductive management with continuous mating and egg collection every five days. The collected eggs were incubated, and after hatching, 12,000 8-day-old tilapias were placed into aquariums (60L) operating as a recirculating system (28.0 ± 1.0ºC). The offspring obtained from each of the four dietary treatments were fed for 34 days with five different diets, containing two levels of DHA (0% and 1%) and two sources of mineral premix (100% inorganic Zn, Mn, and Se; and 50% organic Zn, Mn, and Se), as well as another diet containing 2% DHA and 100% organic Zn, Mn, and Se. At the end of the rearing period, growth and survival were assessed. Subsequently, 21 fry from each aquarium were subjected to a bacterial challenge by immersion with *Streptococcus agalactiae* (2.26×10^5 CFU/mL) in a recirculating system. After 24 hours of exposure, the fish were counted, and the survival rate was assessed. The results were analyzed using two-way ANOVA and Tukey's test (Statistica 7.1).

Although the effects of DHA are more evident in health parameters, the greater vigor of these larvae may be associated with the greater availability of nutrients during vitellogenesis, consequently constituting a better-quality calf. It is concluded that feeding tilapia broodstock with diets supplemented with 1% DHA and inorganic minerals improved the growth and vigor of the offspring.

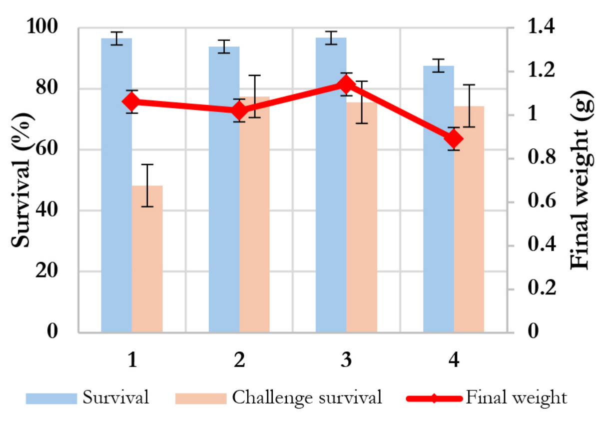

Figure 1. Final weight, survival and challenge survival ($p<0.05$) of Nile tilapia larvae from breeders fed organic and inorganic minerals, with 0% or 1% the addition of DHA. Treatments: 1- 0% DHA and inorganic minerals, 2- 0% DHA and organic minerals, 3- 1% DHA and inorganic minerals, 4- 1% DHA and organic minerals.

IN VITRO INCUBATION OF PATHOGENIC BACTERIA AND BLOOD OF CATFISH FED DIETS SUPPLEMENTED WITH ORGANIC OR INORGANIC IRON

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The channel catfish (*Ictalurus punctatus*) is a freshwater species native to the U.S. and northern Mexico, known for its rapid growth and disease resistance, making it ideal for aquaculture. However, bacterial infections are a challenge for the industry, particularly affecting *I. punctatus* and its hybrids. Iron supplementation is commonly used to prevent deficiencies and maintain health. Organic minerals, being more bioavailable, improve absorption and avoid negative dietary interactions. The dietary iron supplementation became a standard practice by the catfish industry to prevent idiopathic catfish anemia. This study aimed to evaluate the *in vitro* growth of bacteria in the blood and serum of fish fed with organic and inorganic iron diets.

For this study, 120 channel catfish, weighing ~250 g, were allocated to three aquariums of 200 L in a recirculating system with heating and aeration. The animals were fed for six weeks three experimental diets: 200 mg organic iron/kg (ORG) and 1,000 mg inorganic iron/kg (INORG), along with a control treatment containing a basal 70 mg inorganic iron/kg. Weekly, blood from four fish was collected, two fish for whole blood and two for serum. Each weekly collection was considered as a block in a manner that all tanks were exposed to the dietary treatments twice, totaling six blocks. Four bacteria of importance to the catfish industry were selected: *Edwardsiella ictaluri* (Ei), *E. piscicida* (Ep), *Streptococcus dysgalactiae* (Sd), and *Aeromonas hydrophila* (Ah). A standard for each bacterium was generated and diluted to ~1 × 10^4 CFU/mL in sterile phosphate buffer saline (PBS), with an optical density of 0.170 at 600 nm. The total blood and serum were subjected to a bacterial survival test, where 50 µL aliquots of each were pipetted into 96-well plates, incubated in triplicates with 3 µL of each bacterium suspension. The plates were incubated at 28°C with shaking at 150 rpm for 1 h. Afterward, six 10 µL aliquots of blood or serum were plated on TSA (Tryptic Soy Agar), incubated at 30°C. The Ah plates were evaluated after 24 h, and the others were evaluated after 48 h. The evaluation involved counting the number of colonies and comparing them to the negative control, which consisted of PBS, to estimate the bacterial survival percentage in each blood and plasma sample. The results were analyzed using one-way ANOVA and Tukey's test (Statistica 7.1). No significant effects were found for bacterial survival; further studies are needed, considering a longer feeding period.

Table 1. Bacterial survival (%) in total blood and serum of catfish fed diets supplemented with organic and inorganic iron.

	Iron supplementation			
	Control	**ORG**	**INORG**	***p-value***
		Whole Blood		
Ei	73.9±24.3	57.6±20.3	132.3±86.9	0.59
Ep	122.8±28.3	94.1±40.8	127.79±22.52	0.72
Sd	234.1±82.6	231.3±45.1	283.0±102.8	0.87
Ah	399.9±200.9	142.1±63.35	512.0±427.3	0.63
		Serum		
Ei	191.6±122.2	136.8±90.2	228.2±153.1	0.87
Ep	910.3±828.2	94.6±14.5	324.2±220.6	0.87
Sd	952.6±458.7	590.3±344.5	691.7±197.4	0.75
Ah	897.2±494.9	254.6±65.3	822.9±238.7	0.32

THE INFLUENCE OF COASTAL WATER QUALITY ON THE SUCCESS OF MICROALGAE GROWTH AND QUEEN CONCH LARVAL DEVELOPMENT

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Water quality is crucial for conserving and culturing queen conch (*Aliger gigas*), native to Florida and the Caribbean region. Key parameters like temperature, salinity, dissolved oxygen, alkalinity, pH, and nutrients critically impact the development of queen conch veliger larvae and their microalgal food. Suboptimal conditions can hinder the growth and survival to metamorphic competency in aquaculture and affect proliferation of the microalgae cultures.

Consequently, it is crucial to create tools for quickly detecting if water conditions are suitable for queen conch aquaculture, especially during the larval stages. These tools include laboratory analyses for nutrients, heavy metals, and pollutants in water sources. Other methods such as growing microalgae for 4-7 days and growing conch veligers for at least 1 week or for three weeks to metamorphosis in various water sources can help identify potential issues with the water source.

Characterizing water quality is key for designing appropriate treatment systems. For example, Jamaica and Curacao hatcheries needed basic filtration to handle fine suspended particles that affect conch larval movement and normal development as well as algae culture growth. To stabilize production, especially in summer, hatchery locations like Grand Bahama, Great Exuma, and Jamaica use chillers and/or air conditioning to maintain optimal temperatures at 28 °C. More advanced systems, like Naguabo's hatchery in Puerto Rico, employ comprehensive filtration for incoming seawater and stored water, including 100- to 1-micron filters, activated carbon, UV lights, and chillers (Fig. 1).

Understanding and managing these water quality parameters is vital for the success of microalgae culture growth and queen conch larval development, which contributes to the sustainability and efficiency of aquaculture practices used for restoration purposes of this species.

Figure 1. Filtration and chiller system at Puerto Rico, Naguabo Aquaculture Center

EFFECTS OF REGION, TIDAL HEIGHT, SEASON, AND CLAM SIZE ON FECUNDITY OF THE SOFT-SHELL CLAM *Mya arenaria* L. IN MAINE, USA

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Soft-shell clams, *Mya arenaria* L., represent an iconic fishery in Maine, USA that typically ranks 2nd or 3rd annually in commercial value of all marine species harvested. The fishery is in a crisis as dockside landings have plummeted by 55% since 2000-2004, reaching an all-time low (5.2 million pounds) in 2023 with previous all-time lows occurring four times since 2017. Clams are co-managed by Maine's Department of Marine Resources (DMR) working together with local communities. While adjacent communities may have different management practices, all are governed by the 2-inch clam law that regulates the taking of clams to those animals greater than or equal to 2 inches in shell length (SL). We wish to work with clammers and the clam industry to increase production by exploring specific aspects of the soft-shell clam reproductive cycle.

Beginning in 2023, we initiated a comparative field experiment in a community within three geographic regions of the Maine coast (southwest: Brunswick; midcoast: Bremen; downeast: Jonesport). Soft-shell clams ranging in SL from 30-50 mm were dug by us and those ranging from 51-100 mm were purchased directly from clammers or from clam buying stations located near (within 10 km) each study site. In March 2023, we placed 24 clams into 1-ft x 1-ft x 6-inch deep wooden boxes filled with ambient sediments (N = 42 boxes across each of three tidal heights: upper, mid, and lower intertidal) protected with a vinyl-coated wire (10.5mm aperture to exclude large crushing predators, birds, and fish) over a polypropylene mesh (3.2 mm to exclude smaller predators). Beginning the first week of May, clams were collected weekly for 19 weeks from two boxes at each tidal height at each study site, and within 24 hours were induced to spawn using a thermal shock technique. When this technique was successful, the eggs from a female were counted using a FlowCam. For animals not stimulated to release gametes, one group from each tidal height (N=6) was taken to estimate weekly condition and gonadal index, while another group (N=6) was taken every other week for histological analysis of gonadal tissue. In March 2024, we used 18 clams, and began the 19 weekly collections in mid-April.

We discovered region-specific spawning schedules, and determined that reproductive senility does not occur (the relationship between SL and number of eggs released was described by a power exponential function). Some commercial buying stations in Maine do not buy clams exceeding 3-inches (76.2 mm) in SL from harvesters. We sampled the commercial catch over 24 months at one station in the downeast and another in the southwest region. Approximately 5% of clams exceed 3.5 inches, which should be considered a coastwide upper size limit. Within five years of implementing this new size restriction, it would be possible to devise a sampling program to test whether more recruits settle and survive to the commercial fishery. If no improvement is forthcoming during that period, then the upper size limit would be revoked.

THE DEVELOPMENT AND COMMERCIALIZATION OF A BLUE MUSSEL *Mytilus edulis* HATCHERY IN MAINE, USA TO STRENGTHEN DOMESTIC MUSSEL PRODUCTION

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Blue mussel, *Mytilus edulis*, aquaculture in Maine is poised for expansion with many mussel farms increasing production to keep up with consumer demand. Yet, the majority of mussel farms in Maine are currently reliant on wild seed collection to propagate their farms. The spatial and temporal differences in annual wild mussel recruitment poses a risk to Maine mussel farms, and may act as a barrier to expansion. To present options for growers who would otherwise rely on wild seed collection, which was normal husbandry practice for the past 30 years, the Downeast Institute (DEI) has developed a method using hatchery technology to produce seeded mussel ropes positive and eyed larvae for the Maine mussel industry. This development has provided Maine mussel farmers with another, less variable approach to mussel production and has led to significant improvements in mussel genetics and yield. A major component of the hatchery process is the development and maintenance of select broodstock chosen for several attributes such as phenotype, fast growth and high meat yield. DEI focused breeding efforts to create a line of mussel broodstock that produce a unique golden, striped phenotype, which adds value to the final product and offers new approaches to marketing the product. We will discuss how the hatchery process was created and how the relationship between a marine research institution and an industry partner helped spur innovation and the rapid development of commercially viable hatchery-reared mussel seed.

SINGLE-NUCLEI RNASEQ ANALYSIS OF AN OSHV-1 INFECTION IN PACIFIC OYSTERS REVEALS CELL SPECIFIC INFECTION AND RESPONSES

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Ostreid Herpesvirus OsHV-1 remains one of the most significant issues facing Pacific oyster aquaculture, and continues to cause disease in oyster growing regions worldwide. Our understanding of how the virus interacts with oysters has developed, but the exact locations and routes of infection remain cryptic. Here we performed an immersion challenge between Pacific oysters and OsHV-1 µVar and assessed the whole-animal response over a 72-hour infection cycle with a bespoke single nuclei RNA-seq analysis. New methods of nuclei isolation were optimised before cells were processed with Parse Biosciences Evercode and sequenced by Illumina. Across eight samples, we generated 2.2 billion reads in 22,000 nuclei. Results allow us to characterise gene expression in a range of cells from different tissues, including muscle, haemocyte, mantle, hepatopancreas, adductor muscle, digestive epithelia and gill (Figure 1). We were in addition able to characterise viral transcripts and host responses in cells undergoing infection. Results demonstrate that infection at all stages is highly specific to one cell type and that the response previously observed in whole tissue bulk RNA-seq is likely driven by gene expression changes in one cell type. Within this presentation we will describe some of the challenges associated with performing single nuclei RNA-seq in animals for which there is limited genome annotation and some of the methods that can be used to overcome these.

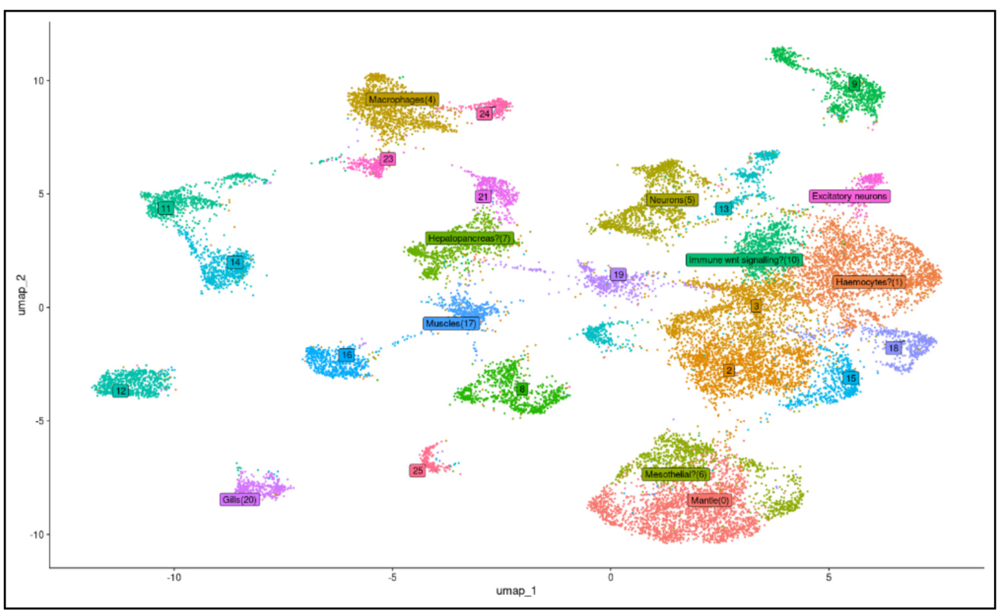

Figure 1. UMAP plot of oyster cells based on differential gene expression within each cluster. Each point represents a single nuclei and clusters are largely representative of cell types.

REVITALIZING LAKE ECOSYSTEMS: SEDIMENT REMEDIATION, NUTRIENT DYNAMICS, AND BENTHIC ACTIVITY IMPROVEMENTS USING TRYMARINE TECHNOLOGY IN BRICKHOUSE LAKE

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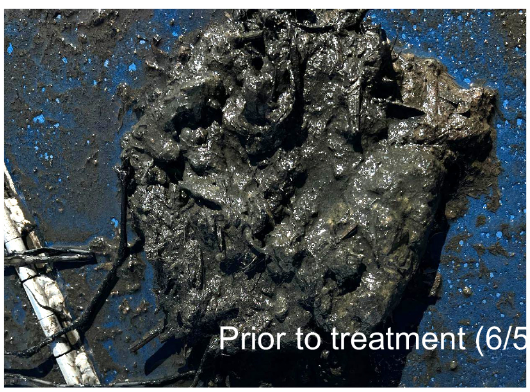
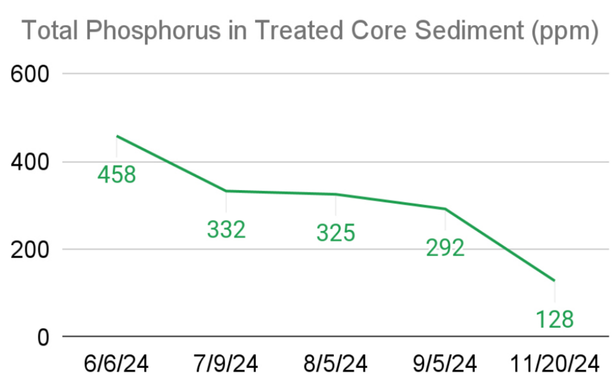
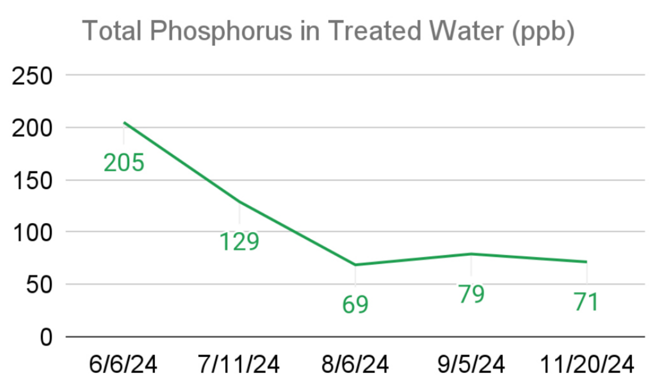
This study examines the specialized photocatalyst-based product, TryMarine, for nutrient remediation and revitalization of aquatic ecosystems, assessing its efficacy in a single aquatic environment – Brickhouse Farm Lake. Monthly measurements of sediment nutrients (TN and TP), texture, aerobic bacterial activity, benthic diversity, and microalgae were conducted across treated (10 acres) and adjacent untreated areas (30 acres). A tenfold increase in aerobic plate counts was observed at the bottom of the treated zone, accompanied by fluffier sediment and increased moisture content in the core sediment sample. Benthic organisms increased thirteenfold, and species diversity rose 160% in the treated area compared to the untreated area. Sediment permeability improved by one foot, allowing deeper water infiltration. Adjacent untreated areas showed gradual improvement, likely due to water turbulence. These findings demonstrate that TryMarine can stimulate healthier sediment and food web reactivation, positioning it as a promising candidate for sustainable aquaculture and ecosystem restoration.







CHALLENGES AND OPPORTUNITIES FOR SUSTAINABLE MARINE AQUACULTURE DEVELOPMENT FOR SRI LANKA

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Globally, wild-catch fisheries are reaching their limits, making aquaculture essential for meeting seafood demand. Aquaculture now supplies over half of the world's seafood, though its growth and impact vary significantly across regions. For example, Sri Lanka, where annual per capita seafood consumption is 30kg, produces only 3% of its aquatic food through aquaculture, significantly lagging behind other South Asian countries. Although Sri Lanka's aquaculture output reached 42,000 metric tons in 2020, the industry's growth has been slower compared to the rest of the region.

Partnered with Sri Lankan government agencies and universities, we undertook an expert consultative process with public and private partners to develop an evidenced-based consensus for country-wide marine aquaculture planning. Further, we undertook essential pilot studies that were concentrated on generating genetic resources as well as biosecurity, and disease management strategies for key aquaculture species, including edible oysters (*Magallana bilineata*), Asian sea bass (*Lates calcarifer*), and seaweed (*Kappaphycus alvarezii*).

Despite large productive areas and promising native species, Sri Lankan aquaculture has been underdeveloped due to a 30-year civil war and lack of knowledge. Key challenges hindering expansion include inconsistent seed supply, regulatory complexity, gaps in value chains, and insufficient infrastructure and technical training. Despite recent advancements in species like Asian sea bass, prawns, and seaweeds, disease outbreaks, habitat degradation, and water quality issues remain. Edible oyster and seaweed farming represent value chains with the potential to empower women in rural communities, offering sustainable livelihoods and promoting economic inclusion. Currently occurring in small, isolated pockets, these practices could be expanded to have a more widespread impact across Sri Lanka. Further, the project generated baseline genetic resources for *M. bilineata* to help re-establish oyster aquaculture. These genetic insights will underpin biosecurity and translocation guidelines, supporting sustainable breeding and aquaculture practices. Additionally, we identified disease threats to developing oyster aquaculture and expanding the existing seaweed aquaculture industry. Seaweed farming was found to be impacted by 'ice-ice' disease and like other industries, low genetic diversity was observed in the cultivars of *Kappaphycus alvarezii* farmed in Sri Lanka. The project emphasizes the need to develop reliable juvenile supplies for smallholder farms, establish biosecurity policies, and address market access challenges, particularly for oyster exports.

REDUCING TRANSBOUNDARY RISK OF INFECTIOUS SPLEEN AND KIDNEY NECROSIS VIRUS *Megalocytivirus pagrus 1* IN AUSTRALIA

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Viruses of the genus *Megalocytivirus* are globally emerging pathogens that are widespread in a diverse range of aquaculture and fisheries industries affecting both food and ornamental fishes. To help reduce taxonomic confusion, a binomial virus name of *Megalocytivirus pagrus 1* was assigned to represent the three recognized genotypes of the former species *Infectious spleen and kidney necrosis virus* (ICTV, 2024). This includes infectious spleen and kidney necrosis virus (ISKNV; Genotype II), red sea bream iridovirus (RSIV; Genotype I) and turbot reddish body iridovirus (TRBIV; Genotype III). RSIV and ISKNV have been listed by the World Organization for Animal Health (WOAH) since their emergence. Recently revision from the WOAH Aquatic Standards Committee has specified the three genotypes of *M. pagrus 1* as being notifiable. Of particular importance to Australia, barramundi (Asian seabass; *Lates calcarifer*) is a natural host for ISKNV (in marine and freshwater environments), RSIV and TRBIV with significant mortality events observed across South Asia. As an iconic Australian species, barramundi is important for aquaculture, recreational and cultural fishing practices. Further, several other endemic Australian freshwater finfish species, including some endangered species are also at risk from ISKNV.

Australia is considered free of megalocytiviruses and has strict import controls and biosecurity measures to support this status. In 2003, there was one mortality event associated with ISKNV at an aquaculture enterprise farming Murray cod (*Maccullochella peelii*) (Lancaster *et al.* 2003). Since, then there have been numerous detections of ISKNV associated with the trade of ornamental fish in apparently healthy and diseased fish (e.g. Rimmer et al., 2015, Becker et al., 2017). Incursions of *M. pagrus 1* have been contained and eradicated through state-managed biosecurity responses and to date, there have been no detections in wild fishes in Australia. ISKNV DNA has been detected in frozen seafood products imported to Australia (Landos et al., 2021), but its viability in these products remains untested.

Despite being studied for nearly three decades, we actually have a poor understanding of the transmission risk posed by fish infected with ISKNV but are not showing any signs of disease (i.e. subclinical infection). The objective of this study was to assess the risk pathways for ISKNV incursion into Australia via live ornamental fish trade and frozen seafood products. By evaluating the minimum infective dose, we aimed to inform risk assessments and to quantify the potential for transmission from ISKNV-infected fish. Further, we evaluated the risks from frozen fish products contaminated with ISKNV. These findings will provide essential data for biosecurity assessments, helping to inform evidence-based policies to mitigate the transboundary spread of ISKNV and protect economically and ecologically important fish populations.

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INDUCED DEFENSES: HOW CHANGES IN OYSTER SHELL MICROSTRUCTURE SHAPE ECOLOGICAL INTERACTIONS AND THE SUCCESS OF REEF RESTORATION EFFORTS

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Induced defenses are well-known to increase prey survivorship in the presence of predators and carry growth costs. However, our understanding of the mechanistic underpinnings of these cost-benefit interactions and how they shift across landscapes is limited. We investigated how raising a model foundation species, oysters *Crassostrea virginica*, under commercial hatchery conditions with cues from a common predator can alter shell microproperties, improve survival, and govern growth patterns spanning 55 sites across the Alabama coastline. Oysters exposed to predator cues developed a 4% harder and 16% thicker foliated layer in their shell which increased the crushing force shells could withstand by 52% (Figure 1). Oysters induced to grow defenses under these methods had 68% greater survivorship over controls after a year when used to build a new ~42 m^2 reef (Figure 2). Developing these defenses had marked costs in shell size and soft tissue mass initially. However, growing caged oysters across the coastline with citizen scientists found that these differences were essentially nonexistent after six months in the field. Instead, oyster growth was highly dependent on local conditions, with average shell size and soft tissue mass varying 120% and 570% respectively within the region. Our findings provide insights on how minute physiological changes can structure ecological interactions and highlight the applications of a new culturing technique.

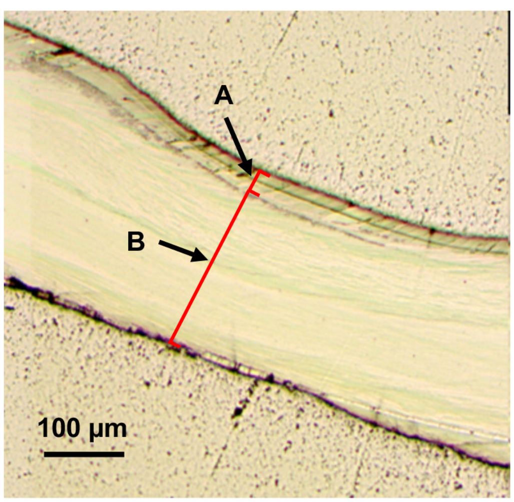

FIGURE 1. Cross section of oyster shell showing the A) prismatic and B) foliated layers

FIGURE 2. Reef built with alternating columns of induced and control oysters.

MARINE BIOFILTER COMBINATIONS IN RECIRCULATING AQUACULTURE SYSTEMS

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Marine-integrated aquaculture recirculating systems hold immense potential to resolve food, energy, and water dilemmas on a global scale. Recirculating aquaculture systems help to conserve water; however, significant energy, cost, and environmental savings may be obtained by trying different biofilters. For instance, periphyton (the slimy coat of algae and bacteria that grows on surfaces) biofilters demonstrate their ability to save energy and recover nutrient resources. This study delves into the testing of different aquaculture biofilter combinations such as periphyton with halophytes (H+P), periphyton with moving bed biofilm reactors (P+M), and periphyton only (P^2). Two pilot-scale RAS (2500L/RAS) were utilized at a red drum stocking density of 14-18 kg/m^3 (Figure 1). The experiment was run in two trials (spring and summer) for four replicates. Fish mortalities were low, with the food conversion ratio between 1.1 and 2.0. All trials maintained NO_2^-, NO_3^-, and CO_2 below toxic limits. The P^2 trials maintained a stable alkalinity and pH balance; however, the M+P and H+P systems required an alkalinity addition of 100-200 $CaCO_3$ mg/L per trial. Periphyton in all trials added DO to the water (at an average of +3.95 ± 6.52 mg/(L*d)), thus adding energy savings. The M+P trials removed NH_3/NH_4^+ at a higher rate; however, they also operated at higher energy and required more DO. Seasonal effects of spring and summer were found to influence several parameters including the periphyton growth rate, pH, ammonia concentration, and DO concentration. All systems recovered nutrients, although the H+P and P^2 had the highest recovery rate. Periphyton was found to include valuable lipid content (4.55 ± 2.24 % of dry weight) with the detection of Ω-3 fatty acids. The H+P system produced periphyton for aquaculture use and halophytes for human food. Sea purslane growth rates (1.0431 ± 0.3361 g/day/plant) were high in all trials. The microbiome revealed denitrifiers, ammonia-oxidizing microbes, nitrite-oxidizing microbes, and valuable algae such as *Chlorella.*

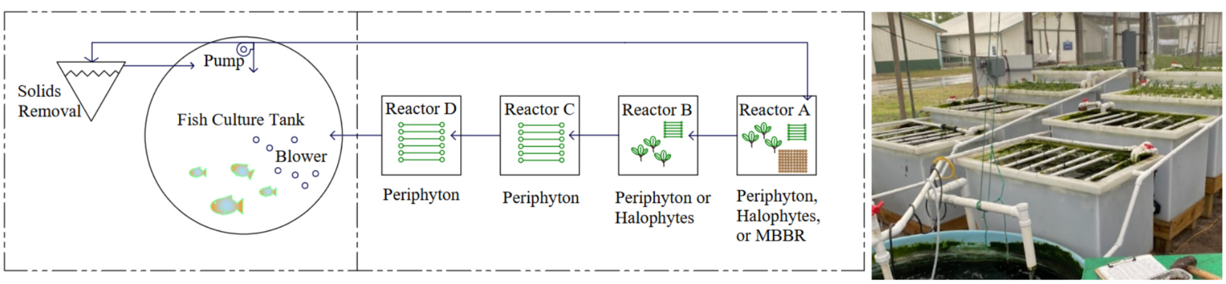

Figure 1. Schematic Aquaculture Biofilter Combinations in RAS. This consisted of a culture tank and solids removal unit on one side and the ABC on the other. In the M+P trials, Reactor A was an MBBR with the remaining reactors as periphyton. In the H+P trials, Reactor A and B were halophyte biofilters, with the remaining reactors as periphyton. In the P^2 trials, all reactors were periphyton. The picture displays the biofilters of two separate RAS.

PATHOGENS RESERVOIRS FOUND IN RECIRCULATING AQUACULTURE SYSTEM MARINE BIOFILTER COMBINATIONS

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Fish pathogens in marine recirculating aquaculture systems (RAS) can be the source of major costs due to loss of fish. Pathogens can reside in the biofilms of RAS, including in moving bed-biofilm reactors (MBBRs). The microbial communities of periphyton may reduce the number of pathogens in the system, preventing their establishment in the RAS biofilms. In contrast, periphyton may serve as an additional reservoir for pathogens. This study delves into the testing of different aquaculture biofilter combinations such as periphyton with halophytes (H+P), periphyton with moving bed biofilm reactors (P+M), and periphyton only (P^2). The microbiome was quantified using 16S rRNA next generation sequencing. The microbiome revealed denitrifiers, ammonia-oxidizing microbes, nitrite-oxidizing microbes, and valuable algae such as *Chlorella.* Classification of pathogens showed that significantly fewer OTUs containing fish pathogens were found in the periphyton biofilters in comparison to the MBBR (Table 1). To gain higher resolution, the *hsp70* gene was used to examine pathogen groups in periphyton biofilters, conventional MBBRs, and in the effluent water from the biofilter.

Table 1. Summer 16S Prokaryote Analysis of Pathogens*

		MBBR		Periphyton	
		average	sd	average	sd
Vibrio	RAS1	1215	292	70	27
	RAS2	123	13	29	17
Pseudomonas	RAS1	314	37	74	21
	RAS2	89	7	21	6
Acinetobacter	RAS1	33	8	49	21
	RAS2	141	24	73	52
Clostridium	RAS1	56	8	63	21
	RAS2	762	64	178	12
Mycobacterium	RAS1	491	68	88	34
	RAS2	313	28	259	224
Total**	RAS1	3107	518	427	149
	RAS2	1434	138	563	316

* Total OTUs per sample were 28298 ± 457 (100±1.6%)

**Total taken is the sum of 10 pathogen clades, the 5 listed in the rows in addition to *Shewanella*, *Pseudoalteromonas*, *Flexibacter*, *Flavobacterium*, and *Sporocytophaga*.

THE ROLE OF WORKFORCE DEVELOPMENT AND TRAINING IN ECONOMIC DEVELOPEMNT – A PRIVATE SECTOR PERSPECTIVE

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The development of an economic sector is impacted by a number of factors. Regulations, access to capital, business climates, market demand, available technologies and technical knowledge all play important roles in the growth and size of a sector. Like any business or economic sector aquaculture is impacted by all these factors. Unlike some other sectors aquaculture typically involves the husbandry of living animals and plants in what may be at times a hostile and dangerous environment. Lack of skills and knowledge can result in farm failures, increased production costs, worker injuries and compromised animal and plant health and welfare. Many U.S. aquaculture producers compete with products produced in other countries that have invested significantly in workforce development and training. The U.S. aquaculture sector has not grown at the same rate as those countries and lack of workforce training and farm management expertise has been one factor.

Training programs that target the specific needs of the farming community and support the professionalization of the aquaculture sector can contribute significantly to the growth of the sector. The necessary skills and knowledge that the workforce and/or business owners need in aquaculture are extensive, multidisciplinary, highly technical and at times unique. Traditional academic programs provide important pieces in the assembly of the necessary knowledge but often lack hands on training and may inhibit cross-disciplinary education. The various forms of non-academic training programs are reviewed and how they link to the sectors economic development are discussed. Training needs for workers vary depending on their level in the business, specific farm responsibilities and where they are in their career development. Although beginning farm owners need many of the same skills as farm workers, owners require additional training in order to become successful. Training programs should be designed to address these varying needs and facilitate the progression of individuals through their career development. Regular surveys of the private sector to identify training and skills needs should be conducted to ensure programs are producing graduates with the appropriate skills and knowledge. Economic development professionals should recognize the importance of training programs and view them as necessary long term investments. Economic development agencies should find ways to fund training programs at sustainable levels and include them in long term development plans. Companies should invest in professional development for their employees and provide clear career pathways for advancement for interested employees.

WORKING TOWARD A SUSTAINABLE FRAMEWORK FOR COOPERATIVE SHELLFISH HATCHERY AUDITING THROUGH THE FY23 AQUACULTURE WORKFORCE TECHNOLOGIES AND EDUCATION TRAVEL GRANT PROGRAM

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Shipping shellfish seed, oftentimes across state or national jurisdictions, is a cornerstone of today's shellfish aquaculture industries. All jurisdictions maintain regulations to eliminate, reduce, or mitigate biosecurity risks associated with moving aquatic animals, however regulatory compliance and enforcement remain major obstacles for industry and resource managers. The Regional Shellfish Seed Biosecurity Program (RSSBP) was created to collaboratively establish biosecurity standards and hatchery best management practices to assist all parties involved with permitting shellfish seed transfers. With support from the NOAA Sea Grant Aquaculture Workforce Technologies and Education Travel Grant Program, the RSSBP is hosting workshops and a focus group with participation from federal agencies (NOAA and USDA), state Sea Grant aquaculture extension specialists, RSSBP hatchery auditors, industry representatives, and members of the RSSBP project team. The goal is to expand the existing hatchery auditing framework to broaden program participation across diverse hatchery models, notably smaller local to regional facilities that at times can represent the bulk of seed availability for many shellfish farms. This presentation will discuss outcomes from this focus group and chart a path forward for broad participation in the RSSBP.

THE NC SEA GRANT SHELLFISH MARICULTURE AND DEMONSTRATION CENTER AT CARTERET COMMUNITY COLLEGE AND NORTH CAROLINA STATE UNIVERSITY

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North Carolina Sea Grant oversees water column and bottom leases at the Shellfish Mariculture and Demonstration Center, located at Carteret Community College and the North Carolina State University Center for Marine Sciences and Technology. The leases provide research and training opportunities for high school, undergraduate, and graduate students, and researchers across various institutions, as well as a platform for extension, engagement, and philanthropy with coastal communities and groups. Research projects have included evaluations of various top and bottom culture shellfish grow-out methods, shellfish crop diversification in North Carolina including bay scallops and sunray venus clams, and investigations into Sudden Unusual Mortality Syndrome in eastern oysters. These research studies often include close collaborations with other universities and groups maintaining shellfish leases of their own, making the regional coordination of university research farms a high priority for impactful scholarship.

LET’S GET READY TO TUMBLE: BAG ROTATION AND *Crassostrea virginica* GROWTH ON INTERTIDAL AND SUBTIDAL FARMS

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Tumbling is important for the shaping of oysters and forming a deeper cup, creating a marketable and meatier oyster. However, this process is labor intensive and often requires expensive, gas-powered, and noisy equipment that can be a barrier for farmers wanting to grow their business. This project aims to use the energy from the fluctuating tides to tumble bags on a suspended longline system - a design that works alongside the environment while reducing farmers’ labor. Previous experiments have explored tidal energy for shaping an oyster during the final four-month “finishing” period; however, this study begins a long-term project that will follow the oyster growth from spat to market size.

There was no difference in shell growth among the three farm systems. However, other research with larger oysters suggests that differences in oyster performance start to appear after three months. Intertidal bags followed a complete 180º motion that followed the tides. The sensors were limited to 180º motion, not allowing us to assess if the bags were achieving a 360º rotation. Shallow subtidal bags achieved a 100º motion. Subtidal bags had greater average acceleration (~10.9 m/s^2) than intertidal bags (~10.3 m/s^2).

No biofouling was observed on the control or intertidal bags. There was small but noticeable biofouling on the subtidal bags one week after deployment. The biofouling on the subtidal bags covered only half of the bag, suggesting that the bag was not completely flipping and was only exposing one side to the sun.

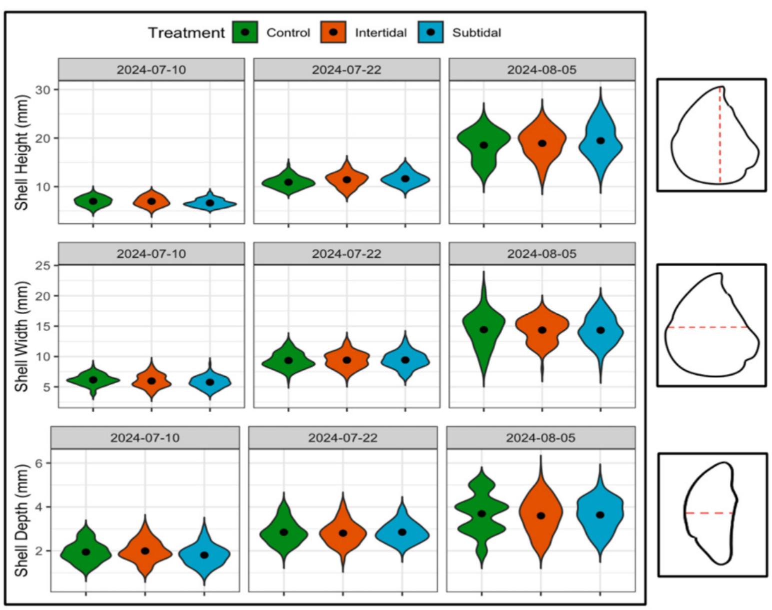

Figure 3: Violin plots represent shell performance for the three treatments (n= 90 per treatment).

PRODUCTION OF WALLEYE *Sander vitreus* FINGERLINGS IN A RECIRCULATING AQUACULTURE SYSTEM

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The Quad Cities Spray Canal Project has operated since 1984 to produce advanced sport fish fingerlings for stocking Pools 13 and 14 of the Mississippi River. Walleye were produced extensively in a 57 acre canal around the Quad Cities Nuclear Generation Station. Target production has been 175,000 walleye fingerlings per year. Over time the canal became less suitable for fish culture due to extensive sedimentation and deterioration of the canal liner. A recirculating aquaculture system (RAS) was installed in 2022 for the production of walleye fingerlings. Starting with the 2023 production season the old canal was abandoned and walleye were raised from fry to fingerling in the RAS system.

The RAS system consisted of three circular self-cleaning larval tanks 96" in (244 cm) diameter, 54" (137 mc) tall, with an operating volume of 5.7 m^3 each. The tanks had black interior walls and light grey flooring. A cleaning arm on the wall and floor would sweep waste to a collection trough where it could easily be siphoned out of the tank. Two spray bars were mounted over the tank to provide surface spray to break up any oils on the surface and allow walleye fry to inflate their swim bladders. The recirculation system consisted of a drum filter with 60 micron screens, a 2,839 L (750 gal) divided filtration sump, a 2 cubic meter moving bed bioreactor (MBBR), a 121" (307 cm) tall by 48" (122 cm) diameter degassing tower, an 18KW inline titanium heater, a 960 watt UV Sterilizer and an Oxygen Cone supplied by a liquid oxygen tank.

Each tank was stocked with 150,000 fry 3 days post hatch, for a rate of about 26 fry/liter. Initial temperature was 15.7 ^{o}C and was gradually increased to about 21 ^{o}C over the culture period. Light was maintained at 50 Lux, 24 hours per day. Concentrated preserved *Nanochloropsis* algae was added to the sump twice per day, 800 ml per addition, to maintain turbidity and reduce cannibalism. Each tank was equipped with two Arvo Tec feeders, and fish were fed every 5 minutes, 24 hours per day. Fry were started on Otohime B2 feed and progressed through Otohime C1, C2, and Bio Vita fry 1.2mm. Mortalities were counted daily and feeding rates were adjusted daily.

A total of 239,968 walleye fingerlings were harvested from the three RAS tanks in 2023. This represents a 53.3% return. A total of 258,669 fingerlings were harvested in 2024 representing a 57.4% return. Production targets were met in both seasons. Potential problems and pitfalls will be discussed.

A HIGH-RESOLUTION LOOK AT MILK CONCH *Macrostrombus costatus* FECUNDITY AND EGG MASS MORPHOMETRICS

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A captive breeding population of 16 milk conch (*Macrostrombus costatus*) was established at Florida Atlantic University in February 2024. Milk conch are similar to the important fishery species, queen conch (*Aliger gigas*). The overarching goal of the project is to develop techniques to reliably produce captive laid egg masses and then transfer this technology to queen conch to assist with restoration efforts. The project has successfully demonstrated that milk conch will lay egg masses in captivity when they are fed primarily from diatom-rich algal turf scrubbers and maintained on a sand substrate with flow through seawater.

The objective of this part of the project was to develop a method to determine the number of eggs in captive laid milk conch egg masses. Counting eggs individually would be highly impractical, as a single egg mass can contain upwards of 250,000 eggs, highlighting the need to efficiently estimate egg quantity. To facilitate this, for one month (June 15 to July 16, 2024), egg masses collected during this period the following values were measured: egg mass displacement, eggs per mm, and egg strand and egg capsule diameters. In addition to these measurements, 10 egg masses were uncoiled to measure total strand length and percent fertilization in the egg mass. Use of a high-resolution microscopy provided accuracy of these measurements along with observations of early embryonic development (Fig. 1), recorded at a level of precision not previously achieved.

From the 37 egg masses that were collected during the one-month observations, the average eggs per mm was 12.8, average egg strand diameter was 801.5 microns, and average egg capsule diameter was 238.2 microns. The percent fertilization for the 10 egg masses that were uncoiled was 98.6%, and the total egg strand length for the 10 masses ranged from 438 – 2005 cm long, which is equivalent to 60,500 – 279,500 eggs, respectively.

Based on this technique it is now possible to calculate the number of eggs per ml from egg mass displacement, which will allow for rapid determination of the number of eggs per egg mass (average of 7,125 eggs per ml of displacement). This will assist with understanding the productivity of a captive breeding population of conch and assist with hatchery rearing. In the future, these methodologies can be transferred to the queen conch to assist with conservation and restoration of the species throughout the Caribbean.

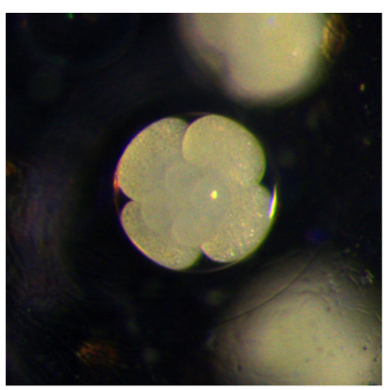

Figure 1. Novel image of 8 cell milk conch *Macrostrombus costatus* embryo displaying asymmetric, spiral cleavage.

PERFORMANCE AND MICROBIAL COMPOSITIONS OF JUVENILE BLUEGILL (*Lepomis macrochirus*) FED TWO DIFFERENT PROTEIN DIETS

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Bluegill is a popular game fish in North America. Despite its popularity among anglers for its tasty and flavored meat and increasing demand for fish, its current food is made for largemouth bass and requires two growing seasons to reach food size. This study investigates growth characteristics, gut microbiota compositions, and gene functions of juvenile bluegill fed a commercial and an alternative diet in a recirculating system. In a completely randomized experimental design, with three tanks per diet, bluegill with an initial average weight of 12.67±0.14 g was fed for six weeks. The fish were fed two times daily to apparent satiation, and growth parameters were monitored. The results revealed the ability of the fish to utilize both diets, but the responses differed between the two diets. Growth, feed conversion ratio, and specific growth rate were significantly affected by dietary treatments ($P<0.05$). The hypolipidemic effect was observed; elongation and desaturation of the C18:2n6 and C18:3n3 pathways were affected by dietary treatment affected by diets ($P<0.05$). The 16S rRNA data revealed Fusobacteria, Firmicutes, Proteobacteria, and Bacteroidota as the major bacterial phyla in the bluegill intestinal samples, and their relative abundance differs in the two diets. More information about the effect of the dietary treatments on gut microbiota and predicted functional gene families using PICRUSt2 will be presented.

DIGITAL CAMPAIGN STRATEGIES TO ADVANCE PUBLIC SUPPORT FOR AQUACULTURE IN MAINE

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Historically, the US aquaculture sector has shied away from proactive communications, focusing instead on responding to the criticisms that have plagued the sector for decades. Even when aquaculture businesses and organizations tackle communications projects, they typically consist of myth-busting fact sheets, FAQ pages, and crisis communications tactics. While these can be useful, relying on reactive PR alone is not enough to turn the tide of public opinion. Tactics like these have put the US aquaculture sector on the defense, perpetuating its “guilty until proven innocent” status. Furthermore, the sector is challenged to break out of its echo chamber and reach new audiences, rather than preaching to the proverbial choir. It is clear that a new approach, coupled with significant investment, is desperately needed to help this sector advance in the 21st century.

Maine has recognized this need and taken action. This lightning talk will showcase how Maine is developing and implementing effective digital campaigns that tell the story of Maine’s aquaculture farmers and reach beyond typical followers to engage new audiences. Topics covered will include broad strategy development, target audiences, key messages, and objectives related to the campaign, as well as specific types of content, tools, metrics, and analytics used to measure success and fine-tune strategy over time.

ELEVATING WATER pH TO MITIGATE IRON TOXICITY: A SUSTAINABLE SOLUTION FOR CATFISH FARMING IN IRON-CONTAMINATED WATER

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The success of fish farming heavily relies on maintaining optimal water quality in production systems. Among various water quality factors that impact fish productivity, waterborne high iron is a significant concern. In many regions of the United States, well water and underground water sources used for fish farming, often have high levels of iron, which can adversely affect fish health and growth. To achieve maximum growth and overall fish fitness, iron-induced toxicity must be alleviated.

Therefore, this research was undertaken to investigate the potential mitigation of iron toxicity by raising the pH of the fish culture water. Channel catfish (*Ictalurus punctatus*) was used as the test species as it is the leading aquaculture industry in the United States. To provide primary information on the sensitivity of catfish to iron toxicity under long-term realistic fish culture operation, a 10 day-LC_{50} test was conducted and calculated as 17.32 mg/L iron. For determining the protective effect of elevated pH levels on iron-induced toxicity, three levels of water pH, viz. 7.8 (control), 8.3, and 8.8 were tested against high iron (Fe, 4.33 mg/L representing 25% of 10-day LC_{50}). Catfish were randomly divided into six groups in triplicate. The groups were (i) pH7.8(Control), (ii) pH8.3, (iii) pH8.8, (iv) pH7.8(Control)+Fe, (v) pH8.3+Fe, and (vi) pH8.8+Fe. Following the two-month trial, weight gain (%) reduced significantly in all Fe-exposed groups (irrespective of pH levels) compared to Fe-unexposed groups. However, relative to pH7.8(Control)+Fe, the Fe-exposed fish reared at a higher pH of 8.3 (pH8.3+Fe) exhibited enhanced ammonia excretion capacity (Fig. 1) and reduced toxic ammonia accumulation in plasma. Hemoglobin content and hematocrit were also highest in pH8.3+Fe compared to all other groups. In addition, exposure to Fe at control pH (pH7.8+Fe) incited hepatic oxidative stress based on an over-accumulation of malondialdehyde (MDA) along with a significant inhibition in superoxide dismutase (SOD) and catalase (CAT) activities; whereas in pH8.3+Fe and pH8.8+Fe, the MDA content restored to basal level accompanied by high CAT activity. Severe histopathological lesions were noted in gills at pH 7.8(Control)+Fe, which was also complemented by an over-accumulation of iron in the gills and plasma. In conclusion, although higher pH levels did not improve growth performance under iron exposure, raising the pH to 8.3 helped fish maintain ammonia homeostasis, blood health, anti-oxidant capacity, iron balance, and gill structure, suggesting a viable approach to managing iron toxicity in aquaculture systems.

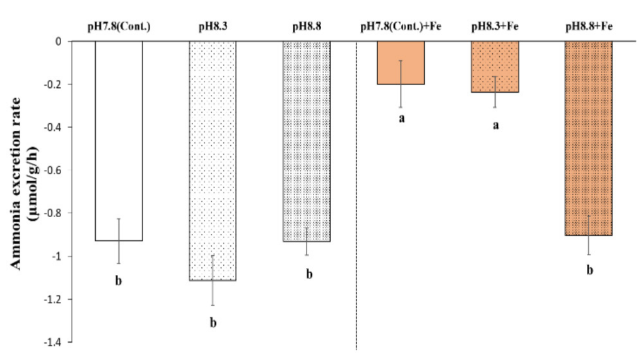

Figure 1. Ammonia excretion rate (µmol/g/h)

GERM CELL TRANSPLANTATION FOR THE CREATION OF XENOGENIC BLACK CRAPPIE *Pomoxis nigromaculatus* USING FRESHLY PREPARED AND CRYOPRESERVED OVARIAN GERMLINE CELLS FROM WHITE CRAPPIE *P. annularis*

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Germ cell transplantation techniques have broad applications, including the production of live fish from cryopreserved germ cells, the conservation of endangered fish species, and the production of hybrid fish. However, no studies have explored interspecific germ cell transplantation between closely related species, such as White Crappie (WC) and Black crappie (BC).

This study investigates the efficiency and viability of ovarian germline cells (OGCs) transplantation from diploid WC into the triploid BC female to advance the production of hybrid crappie using a xenogenic method. The objective of this study was to assess the viability, integration, and proliferation of transplanted freshly prepared and one-month cryopreserved OGCs in the gonad of the triploid BC females. OGCs were isolated from age-1 diploid WC females, tagged with PKH-26 green-fluorescent dye, and transplanted into triploid BC females using a catheterization procedure. First, freshly prepared OGCs were first transplanted into one group of nine triploid BC females. Next, OGCs were cryopreserved for one month in liquid nitrogen using a Recovery™ cell culture freezing medium. The survivability rate of the cryopreserved OGCs was 80.28%. After one-month, cryopreserved OGCs were transplanted into a second group of nine triploid BC females. The presence and viability of the implanted OGCs were checked using a BD Accuri™ C6 Plus Flow Cytometer by randomly sacrificing three fish each at 15, 30, 45-days post-implantation.

Flow cytometry results indicated that both freshly prepared and cryopreserved transplanted OGCs were able to integrate and proliferate in the gonads of the host triploid BC females, creating a xenogenic fish. These findings suggest that OGCs transplantation between diploid WC and triploid BC female can be viable technique for producing xenogenic crappie for the production of hybrid crappie. The results also demonstrated the feasibility of long-term cryopreservation of OGCs for the hybrid crappie production and the conservation of female genetic material.

MATERNAL CHOLINE INTAKE IMPACTS OFFSPRING EPIGENETIC LANDSCAPE IN RAINBOW TROUT *Oncorhynchus mykiss*

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Rainbow trout (*Oncorhynchus mykiss*) are an important commercial fish species in the United States, with a total value of fish sales received by U.S. trout growers in 2023 totaling $27.2 million. Understanding the mechanisms of growth regulation of trout can lead to improved commercial production advances. One proposed strategy is to alter one-carbon metabolism through dietary interventions that alter epigenetic pathways regulating growth performance. We recently demonstrated that maternal dietary choline intake positively affects offspring growth performance. This project focused on evaluating the effects of maternal dietary choline intake on global DNA methylation profiles and related transcriptional changes in rainbow trout offspring. Three experimental diets were formulated to test different levels of choline intake: (a) 2065 ppm choline (Low Choline, 0% supplementation), (b) 5657 ppm choline (Medium Choline, 0.6% supplementation), and (c) 9248 ppm choline (High Choline, 1.2% choline supplementation). Six rainbow trout families were fed experimental diets beginning 18 months post-hatch until spawning; their offspring were fed a commercial diet. Reduced representation bisulfite sequencing (RRBS) was utilized to measure genome-wide methylation in offspring immediately after hatching. When comparing to the Medium Choline offspring, differential DNA methylation occurred more in the Low Choline offspring than High Choline, especially in genic features like promoters. The differentially methylated CpGs ($q \leq 0.01$) were identified evenly between CpG islands and shores in the genome, mostly found in the introns of genes. Genes such as *fabp2* and *leap2B* associated with protein binding, fatty acid binding, DNA binding, and response to bacteria were differentially methylated and detected as differentially regulated genes by previous RNA-seq analysis. Although these findings indicate that levels of dietary choline available in broodstock diets alter offspring DNA methylation, most differentially expressed genes were not associated with differential DNA methylation, suggesting additional mechanisms playing a role in regulating gene expression in response to maternal choline intake.

CLOSING THE LIFE CYCLE OF THE NATIVE CALIFORNIA CLAM *Tivela stultorum* FOR COMMERCIAL AQUACULTURE PRODUCTION

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The Pismo clam (*Tivela stultorum*) is a large bivalve with a native range from central California through Baja, Mexico. At its peak in the early 20th century, annual commercial landings for the Pismo clam were recorded near 700,000 lbs. Following closure of the commercial fishery in 1947, a large recreational fishery persisted until many populations drastically declined in the early 1980s. Recent studies show that populations remain at historically low densities in much of southern California. Clams, in general, represent one of the highest in demand bivalves in terms of market value and consumer preference ($122 million/year). Pismo clams represent a potential native California aquaculture species that can help meet some of the growing demand for seafood and boost the California aquaculture industry. In 2022, the presenting team successfully spawned and reared the Pismo clam through juvenile stages (Figure 1) at Holdfast Aquaculture, a crucial step towards developing a commercial aquaculture framework for this species.

Here the authors will report on Pismo clam pilot study datasets and new preliminary results from a Sea Grant-funded project that has four major goals: 1) determine ideal micro-algal feed concentrations and compositions for larvae; 2) demonstrate hatchery production on a commercial-scale; 3) generate protocols for *ex situ* broodstock conditioning; 4) develop an out-planting strategic plan with stakeholders. The project aims to produce a hatchery manual for commercial-scale production of the Pismo clam. Additional extension of the project will engage regulatory agencies and industry constituents regarding the appropriate path for the eventual introduction of this California native species into commercial production and food systems, while also exploring synergies with conservation efforts.

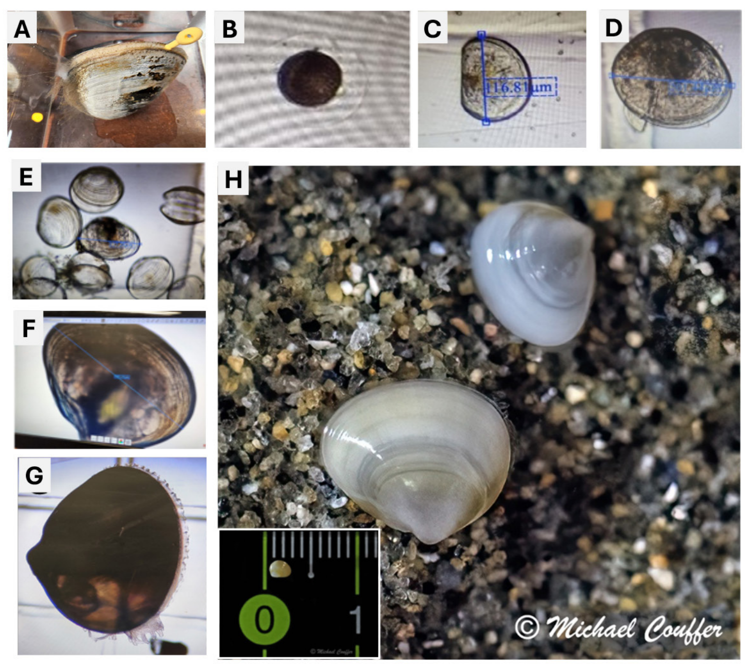

Figure 1. Results from pilot-scale spawning of Pismo clams at Holdfast Aquaculture. (A) Male pismo clam (15.9 cm) spawning. (B) Fertilization (C) D-hinge veliger larva, 3 days post-fertilization (dpf). (D) Pediveliger with foot, 14 dpf. (E) Post metamorphosis, 22 dpf. (F) 56 dpf. (G) 70 dpf. (H) 2-3 mm juvenile clams 86 dpf.

ESTIMATING GROWTH RATES OF PISMO CLAMS *Tivela stultorum* ON PISMO BEACH, CALIFORNIA

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Pismo clams (*Tivela stultorum*) supported an iconic recreational fishery in California until populations declined in the 1980s. Legal sized clams were absent from Pismo Beach, the historic epicenter of the fishery, from the early 1990s until the last few years. The clam population on Pismo Beach appears to be recovering after recruitment events in 2015 and 2021 and legal sized (114 mm) clams are becoming more abundant. As the population recovers, current estimates of key fishery metrics, such as growth rates, are essential to determining if existing fishery regulations can adequately support this recovery. The original estimates of growth rates for Pismo clams were conducted in the 1940s and found that they reached legal size in 6-7 years. A more recent aging study (2018-2019) on Pismo Beach using shell aging found that the time to legal size was 14-15 years. To understand this discrepancy in growth rates between the historic estimates and current estimate, we conducted four growth rate experiments to gain a better understanding of the current growth rates of Pismo clams on Pismo Beach. We used our long term monitoring data to conduct a cohort analysis, ran tank and caging experiments, and conducted a mark and recapture study. Comparing the estimated growth rates from these four approaches allows us to develop robust estimates of growth rate for the current population, compare this to historic estimates, and contribute to the management of this recovering recreational fishery by helping set expectations for population recovery and growth to harvestable sizes.

INTEGRATED MODELING APPROACHES FOR ANALYZING NET PEN AQUACULTURE ENVIRONMENTAL STRESSORS

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There are a number of environmental stressors associated with the operation of offshore net pen aquaculture facilities that pose impact analysis challenges for related consent processes. A selection of example approaches are presented where integrated modeling was used to determine the spatial and temporal magnitude of the impact or risks associated with:

- releases of fecal matter and residual feed,
- dispersal of pharmaceutical / therapeutics dosing, and
- the spread of disease.

DHI have carried out numerous studies of the deposition of fecal matter and residual feed impacts by applying calibrated and validated 3D deposition modeling techniques. This typically entails integration of a MIKE 3 FM Hydrodynamic, MIKE PT, and MIKE ECO Lab - open equation solver - water quality models. The methodology includes 3D hydrodynamic (HD), particle tracking, and biogeochemical modeling of the deposition, transport and mineralization of organic matter covering several sequential production cycles (see figure 1).

The dispersal of pharmaceutical/therapeutic dosing for open cage aquaculture can be approached with different levels of modeling complexity, integrating the capabilities of a MIKE 3 FM hydrodynamic and MIKE ECO Lab to model the dispersion of pharmaceuticals, therapeutics, and antifouling agents. The approach can rely on more robust advection dispersion and substance decomposition modeling techniques or be adapted to consider additional environmental complexities.

The dispersal of copepodite larvae from salmon lice net pen aquaculture is a representative example of integrated disease transmission impact and management related modeling. Carried out initially at a project level, with follow-up development of a regional web-accessible operational system, the modeling allows for an analysis of both dispersal and infection (see figure 2). The web system is based on extensive data collection combined with HD and agent-based modeling (MIKE ABM Lab).

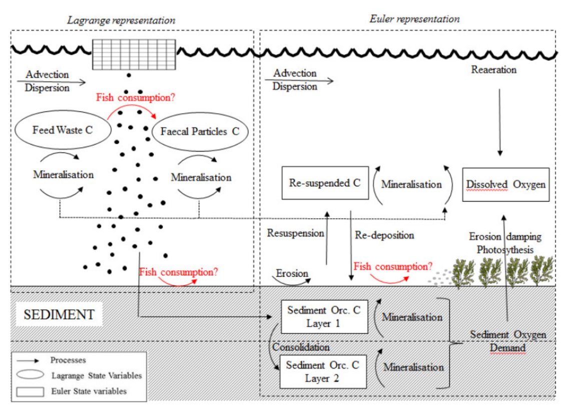

Figure 1 Example of key variables included fecal matter and residual feed modeling

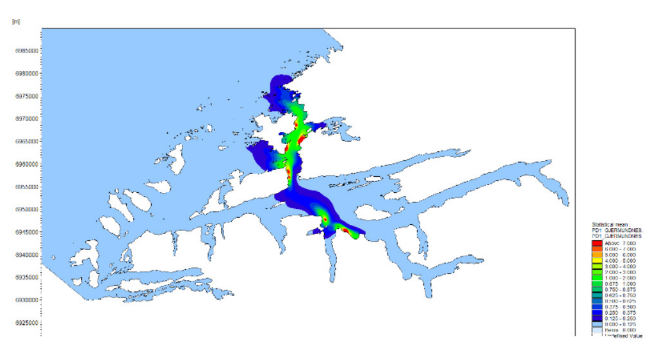

Figure 2 Example of Salmon lice copepodite larvae modeling results

ALASKA AOA NOTICE OF AVAILABILITY

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US Federal Sessions - Aquaculture Opportunity Areas
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In early 2025, NOAA Fisheries (NMFS) announced a 30-day public comment period on the preliminary results of the marine spatial planning study including the identification of Draft Aquaculture Opportunity Area (AOA) Options in Alaska state waters through a Notice of Availability (NOA). The Alaska Draft AOA Options occur within the 10 AOA study areas.

NOAA's National Centers for Coastal Ocean Science (NCCOS) collaborated with NMFS to initiate a marine spatial planning study to identify Draft AOA Options within state waters of the Southeast, Southcentral, and Southwest Alaska as part of the AOA identification process. The preliminary result of the marine spatial planning study used a scoring and ranking process to narrow the suitability analysis results to Draft AOA Options that have high relative suitability scores. In Alaska, the Draft AOA Options include areas:

- up to 2,000 acres per study area for subtidal shellfish aquaculture
- up to 2,000 acres per study area for seaweed aquaculture
- up to a 100 acres per study area for intertidal/shallow subtidal shellfish aquaculture

All Draft AOA Options occur within Alaska state waters (<3nm from shore) up to 61 m (200ft) depth; and meet identified species environmental tolerances and gear thresholds.

The NOA requests comments regarding the features, activities, mitigations or concerns within or around the Alaska Draft AOA Options.

This presentation highlights the process of identifying AOAs in Alaska state waters, and the new step on gathering public feedback on the preliminary results of the spatial analysis and Draft AOA Options. This feedback will help inform NOAA as it works with Federal, State, and Local agencies, appropriate Regional Fishery Management Councils, and in coordination with appropriate Tribal governments to identify AOAs.

EMPOWERING AQUACULTURE'S NEXT GENERATIONS: EDUCATIONAL PATHWAYS TO EXPANSION WITHIN WORKFORCE DEVELOPMENT

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Minorities In Aquaculture (MIA) is at the forefront of driving diversity and inclusion in aquaculture, setting a precedent for how demographic data can be leveraged to transform an industry. In this presentation, we will explore how MIA is leading efforts to empower underrepresented individuals to become the next generation of aquaculture professionals. By providing access to essential resources, education, and networking opportunities, MIA is closing the gap for minority groups, ensuring that the future of aquaculture is inclusive and sustainable. Delving into the importance of these initiatives for the growth of a U.S. sourced aquaculture industry and the broader seafood supply chain. MIA's data-driven approach, community-building programs, and partnerships contribute to building a workforce that reflects the diversity of the communities it serves. Through this, MIA not only fosters equitable growth but also ensures that the U.S. aquaculture sector can thrive in the global marketplace with a rich talent pool, by focusing on the critical support systems and resources required to advance diversity in the sector and understand why Minorities In Aquaculture is essential in shaping the future of this vital global industry.

THE FUTURE OF SEAFOOD: HOW AQUACULTURE CONNECTS LOCAL AND GLOBAL COMMUNITIES

Imani Black

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Aquaculture has become interconnected into our global seafood resource. This industry has helped many coastal and inland communities supply food resources, and while the United States imports a large proportion of its seafood resources, it's important that we effectively communicate the importance of aquaculture. Aquaculture has the power to help the world in its goal to sustainability, and in order for this to happen, we must allow people to understand, support and advocate for sustainable seafood in their own communities.

GENETIC DIVERSITY OF U. S. SELECTED WHITELEG SHRIMP (*L. vannamei*) POPULATIONS

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U. S. shrimp breeding companies play a major role in driving genetic progress in global shrimp genetics and productivity as industry based genetic diversity (GD) underpins genetic progress. To date an assessment within and among companies has not been performed. Quantifying GD aids in future selection options and guides gene banks in germplasm collection strategies.

For this study, we obtained 878 animal samples from three corporations, denoted as C1 (n = 278), C2 (n = 300), C3 (n = 300). DNA extraction and genotyping were performed using 192 SNP evenly spaced across the shrimp genome. Quality control of a call rate threshold of 80% (SNP and animals) and a minor allele frequency of < 0.05, left 171 SNP and 878 animals for analysis. Data analyses included: principal component analysis, STRUCTURE, effective population and Fst.

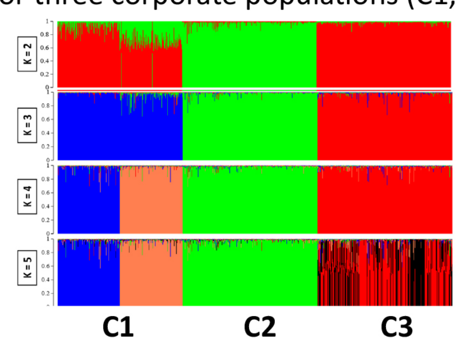
First and second principal components (PC), accounting for 19.5% of the variation, and suggested corporate populations were genetically distinct. This finding was supported by estimates of Fst (Table 1) and STRUCTURE results (Figure 1). STRUCTURE analysis suggested within company substructure for C2 and C3. Low Ne for C1 and C2 (Table 1) suggest future management of GD is needed to preserve selection options.

Our findings suggest corporate lines are genetically diverse from each other. Within corporation GD based upon Ne estimates suggest vigilance should be employed to minimize inbreeding and genetic relationship.

Table 1. Fst among and within corporate populations and Ne size in parentheses.

Corporation	C1	C2	C3
C1	0.11 (76)		
C2	0.20	0.03 (28)	
C3	0.17	0.22	0.06 (10)

Figure 2. STRUCTURE results for three corporate populations (C1, C2, C3) and K = 2 to 5.



UNLOCKING THE POTENTIAL OF SEAWEED: NOAA'S CONTRIBUTIONS TO A SUSTAINABLE U.S. AQUACULTURE INDUSTRY

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Ensuring access to healthy food while protecting our global ecosystems has never been more important. As we confront the challenges of climate change and the lessons learned from the COVID-19 pandemic about the fragility of our food supply chains, we must plan for a future built on resilience. To keep our marine ecosystems resilient while protecting the socioeconomic interest of coastal communities and guaranteeing seafood access for all, we must invest in and enable a robust domestic aquaculture industry. Aquaculture is a key component of our seafood systems and is one of the most climate-friendly and resource-efficient ways to achieve food security. With limited arable land and freshwater to expand terrestrial farming, human populations will increasingly turn to coastal and marine resources, and to technologies that more efficiently use space, water, and nutrients to source food and reduce climate impacts.

Seaweed farming is the fastest-growing aquaculture sector in the United States, but several challenges must be addressed to achieve the goals of increasing seafood production and reducing reliance on imports. This keynote presentation will provide an overview of current barriers facing the domestic seaweed aquaculture industry and highlight the strategic efforts NOAA Fisheries is making to overcome them. By expanding partnerships and collaborations, prioritizing needs-based research, conducting extension and outreach to various groups, and consulting on regulatory processes, NOAA Fisheries plays a vital role in supporting the growth and success of U.S. aquaculture.

ATLANTIC SEA SCALLOP IMMUNE ONTOGENY AND ITS RELEVANCE TO HATCHERY SURVIVAL RATES

Nichole Blackmer*, Jennifer Perry, Sue Ishaq, Kyle Brennan, Brian Beal , Brea Salter, Tessa Houston, Sarah Zuidema, Kyle Pepperman, Anne Langston Noll, Christopher Davis, Sydney Avena, Meredith White, Cody Jourdet, Damian Brady, Erin Grey, Mark Dixon, Gary Wikfors, and Timothy Bowden

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In recent years, Maine bivalve hatcheries have attempted to produce a consistent supply of Atlantic sea scallop (*Placopecten magellanicus*) juveniles, but they continue to experience sudden mass mortalities of larval scallops with unknown causes. The immune system plays a critical role in protection against foreign substances, such as pathogens. There is currently a knowledge gap about the sea scallop immune system and its development during vulnerable larval stages. Further research on sea scallop immune ontogeny and immunocompetence can provide insight into underlying reasons behind the mass mortalities and what types of immune functions are available for larval protection.

This project seeks to understand the immune ontogeny and immunocompetence of hatchery-raised sea scallop larvae through gene expression analysis of immune markers and its relevance to larval survival and hatchery related events. Several genes related to immune function have been identified, such as ferritin, and amplified through RT-qPCR using sea-scallop specific primers. Expression patterns of immune-related genes were measured at various stages (early D-stage veliger through early pediveligers) in larval samples collected from a Maine hatchery. This could reveal insight into how larvae are interacting with the hatchery environment, when coupled with hatchery data. In addition, transcriptomic analysis of bacterially challenged adult sea scallops is underway and should provide further information about how sea scallops respond to a pathogenic *Vibrio* species. *Vibrio* are common hatchery pathogens. Knowledge about the immune system development and events during larval development in the hatchery environment can be used to improve management processes, which could then improve larval survival.

COMPARING THE EFFECTS OF DIFFERENT LIGHTING TYPES ON GROWTH AND PHYTOCANNIBINOID PRODUCTION OF HEMP IN AQUAPONIC SYSTEMS

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Hemp, a U.S. legal classification for cannabis cultivars that produce a low quantity of THC, is gaining popularity in the United States as a source of CBD, a non-psychoactive compound purported to have a range of beneficial effective for its consumers. Due to the current regulatory environment in the United States, cannabis is generally produced in close proximity to markets (owing to a prohibition on interstate transport) and cannabis grow operations are required to maintain a heavy security infrastructure around the zone of production. Aquaponics, the combined culture of fish and plants in a recirculating aquaculture system, is a technology that may be particularly suited to culturing cannabis in these circumstances. This is of particular relevance to producers with indoor, urban operations. Indoor operations rely on artificial lighting for plant production, and the rapid growth of the industry has flooded the market with different styles of lighting at a wide range of costs.

To this end, an experiment was conducted at the Kentucky State Aquaculture Center's replicated aquaponic research systems to test a variety of light sources, differentiated by their purchasing and operating costs. A hemp cultivar of cannabis sativa was cultured in the aquaponic systems using a blend of common commercial production techniques. These included planting in an expanded clay media, continuous drip irrigation, and partial submersion of the planted container in the nutrient-carrying recirculating water. A variety of LED (SpiderFarmer, MarsHydro, NeoSol) and High-Pressure Sodium (Efinity) lights were employed in each growing system, and plants were analyzed afterward for THC/CBD content, terpene content, flowering mass, and overall biomass. Power consumption metrics and plant production:electrical use ratios were also recorded.

Results showed that the light source employed had significant effects on several plant production and electric use ratios, including kWh per gram of flower bud and electrical cost per gram. Differences in the majority of THC/CBD content appeared to be negligible, except for % CBD content. Given the amount of focus high-end lighting systems receive as determinants of quality for cannabis, our findings suggest that more affordable lights may be used by aquaponic cannabis producers without sacrificing production.

Light	Height (cm)	Plant Weight (g)	Flower Weight (g)	kWh per g of flower	Electric cost ($) per gram
MH	41.0 ± 6.7	159.9^{a} ± 82.8	57.7^{a} ± 34.1	42.7	1.7
NS	41.3 ± 10.5	118.1 ± 64.3	41.6 ± 27.5	62.7	2.5
SF	36.7 ± 11.6	103.4 ± 79.0	33.9 ± 32.0	81.1	3.2
HPS	35.1 ± 13.0	77.5^{b} ± 66.9	25.7^{b} ± 26.4	181.1	7.2

Table 1, figure 1. *Lights listed in order from top to bottom: Mars Hydro LED: MH, NeoSol LED: NS, Spider Farmer LED: SF, Efinity High Pressure Sodium: HPS. Electrical cost per gram of flower calculated using national average of pricing per kWh.*

MICROBIAL DYNAMICS AND FUNCTIONAL POTENTIAL OF BIOFILTER COMMUNITIES FOLLOWING CHEMICAL DISINFECTION IN ATLANTIC SALMON RECIRCULATING AQUACULTURE SYSTEMS

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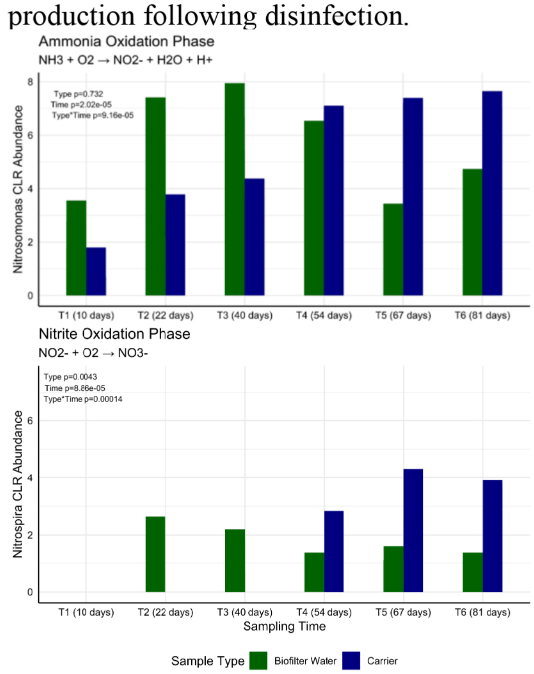
Maintaining water quality in recirculating aquaculture systems (RAS) is critical to fish health and production efficiency. Biofilters play a central role in removing nitrogenous waste via microbial nitrification and denitrification but can also act as reservoirs for pathogens, raising concerns about pathogen carryover between production cycles. Chemical disinfectants, such as sodium hydroxide (NaOH) and peracetic acid (CH_3CO_3H), are used to mitigate these risks, though they also eliminate beneficial nitrifying bacteria. This study evaluated microbial diversity, functional capacity, and succession of biofilter communities following disinfection in a large-scale Atlantic salmon *Salmo salar* RAS facility.

16S rRNA gene sequencing identified taxonomic shifts during biofilter re-maturation and revealed delayed recovery of key nitrifiers, including *Nitrosomonas* and *Nitrospira*. Pathogen-associated genera, such as *Flavobacterium* and *Aeromonas*, were detected throughout the sampling period, highlighting concerns about persistence or re-introduction.

Long-read metagenomics provided a transkingdom perspective, identifying microbial taxa beyond bacterial profiles, including archaea, fungi, and other eukaryotes. Functional metabolic analyses and metagenome assembly to strengthen aquaculture-specific microbial databases. From two pooled metagenomic samples, 35 high-quality metagenome-assembled genomes (MAGs) were recovered. Shared nitrogen cycling genes, such as *amoB*, *hao*, *nirK*, *nirS*, and *nosZ*, were detected across MAGs and biofilter zones, though zonal differentiations in composition and function were detected as well.

This study demonstrates biofilters support diverse and resilient microbial communities but emphasizes vulnerabilities, including delayed recovery of nitrifiers and persistence of potential pathogens post-disinfection. Refining disinfection protocols and improving biofilter management strategies is essential to enhance microbial recovery and reduce pathogen risks within RAS production.

FIGURE 1 Centered log ratio abundance of *Nitrosomonas* and *Nitrospira* in biofilter water and media samples over 81 d of production following disinfection.



CAUCASUS AGRICULTURAL DEVELOPMENT INITIATIVE (CADI): BOLSTERING TROUT CULTURE EDUCATION, EXTENSION, AND OUTREACH IN THE REPUBLIC OF GEORGIA

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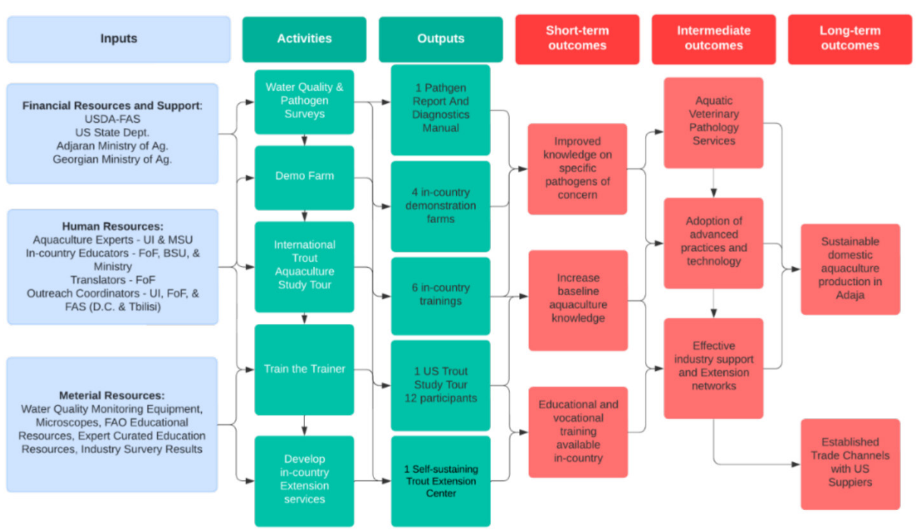
The Republic of Georgia faces rural socioeconomic challenges due to low agricultural productivity and profitability, despite employing 40% of the workforce. The Adjara region, with significant potential for inland trout farming, is constrained by high investment risks, disease prevalence, and a lack of technical expertise. The CADI program was established to address these issues through training, demonstration farms, and novel diagnostic techniques.

Program activities included field demonstrations, molecular pathogen surveys, and a U.S. trout aquaculture study tour for 12 Georgian stakeholders. A 2018 pre-program survey identified critical needs, including technical expertise, training programs, and disease diagnostic services. Post-program evaluations (2026) will assess the program's impact on farmer knowledge, disease management, and industry growth.

Outcomes include improved diagnostic capacity, adoption of advanced management practices, and the establishment of a self-sustaining aquaculture extension center. These efforts aim to sustain industry growth in Adjara and foster trade opportunities with U.S. suppliers. Feedback from stakeholders, including the USDA-FAS and the Georgian Ministry of Agriculture, ensures alignment with local needs. Early evaluations demonstrate progress toward empowering Georgian trout farmers through education and innovation.

FIGURE 1. Extension logic model overview of CADI trout project in the Republic of Georgia.



EXPANSION OF THE PIER TO PEER PROGRAM FOR OYSTER (CRASSOSTREA VIRGINICA) AQUACULTURE FARMERS IN THE SOUTHEASTERN U.S.

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Oyster aquaculture is a growing industry in the United States and particularly in the southeast, from North Carolina to Texas, which have seen tremendous growth in the past decade – which led to the formation of the 501c3 non-profit organization, Oyster South, dedicated to promoting and advancing oyster aquaculture throughout this region. Eastern oyster (Crassostrea virginica) harvest was estimated at 78 million pounds and in 2018, sales in the U.S. were valued at 134 million dollars (USDA 2019). To help facilitate industry growth within this broad region, states have adopted regulations to allow leases to use of gear from on bottom, within the water column and at the surface. North Carolina was among the first dating back to 1989 and Georgia and Texas were the last two states to adjust regulations in 2019 and 2020, respectively (Table 1) (Revell and Hill 2021). These changes in rules have allowed oyster farms to become established throughout the Oyster South region along with the development of aquaculture training programs. While many states now offer aquaculture training programs (i.e. Alabama, Florida, Georgia, South Carolina, North Carolina) in the Gulf of Mexico and southeast Atlantic, there is no substitute for new farmers to meet and interact with established shellfish farms to gain experience to help them become more successful. We will provide an update trips funded and share results from farmers on what information was gained by meeting with peers in the industry.

THE SHELLFISH RESEARCH LABORATORY SHELLFISH TRAINING AREA AT THE UNIVERSITY OF GEORGIA MARINE EXTENSION SERVICE AND GEORGIA SEA GRANT

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The shellfish research laboratory (SRL) at the University of Georgia Marine Extension and Georgia Sea Grant was established in 1985 with the mission to develop clam aquaculture in coastal Geogia. To complement the creation of a shellfish hatchery at SRL, that was established in 2015 to support development of commercial oyster aquaculture, a two-acre shellfish training and research site in the Skidaway River was proposed in 2019. Characteristics of the proposed site include semi-diurnal tides (~3m), a site footprint spanning intertidal and sub-tidal zones at a depth range of 0.0 – 1.0m at MLLW, firm sandy-soft mud bottom, water temperature range of 10-34°C, and salinity range of 14-24 PSU. Site gear capacity includes space for 50 floating cages, 100 FlipFarm baskets, 25 bottom cages, and 100 soft 1.2m x 1.2m clam bags.

Though the proposed site was permitted for temporary use to provide hands-on experience using conventional oyster and clam farming gear to participants in an eight-week aquaculture course during 2023, it has not attained its full potential due to delays in permit review for permanent use. State permits approving use of the site have been issued to the SRL by the Georgia Department of Natural Resources. However, due to the proximity of the training site to the intracoastal waterway (ICW) federal permits are still in review with the Army Corps of Engineers (USACE) with an anticipated issuance date of 2025. The training site is ideally located for educational purposes since it is highly visible from land and sea via a fixed dock on the Jay Wolf Nature Trail located on the University of Georgia Skidway Island Campus that overlooks the site.

INTERTIDAL GROW-OUT TECHNIQUE, NOT EELGRASS *Zostera marina*, INFLUENCES PERFORMANCE OF PACIFIC OYSTERS

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Colocation of farmed shellfish in seagrass, although not permitted in some regions of the USA, has been proposed as a means to reach co-benefits of habitat and food production. Seagrass could benefit bivalves through protection from predation or abiotic stressors but conversely can reduce water flow and food delivery. At 10 farm sites in Washington state (USA), we tested the performance of Pacific oysters (*Crassostrea gigas*) by outplanting them in ground culture or off-bottom culture across a range of seagrass densities. Within each farm site, ground and off-bottom beds were selected with each of three categories of seagrass (none, sparse, dense). For outplanted oysters over 3–9 months, many aspects of oyster performance improved when oysters were elevated above the sediment. Relative to ground culture, off-bottom oysters had better survival (85% vs. 69%), 7% larger size, and 48% higher condition after 3 months in summer, and the survival advantages persisted over winter. Oyster survival on-bottom was especially impaired in finer sediment. No oyster performance differences were associated with seagrass, except for 9-month results, available for five of 10 farms. After 9 months at these five farms, oyster survival showed a small negative effect of dense seagrass, and shell size showed a small positive effect of sparse seagrass. Consequently, seagrass may not provide a boost to colocated intertidal shellfish, but we found little evidence of trade-offs in which maintaining seagrass would reduce yield of farmed oysters. Moving oysters out of the boundary layer and away from soft sediment improves both survival and tissue growth aspects of yield.

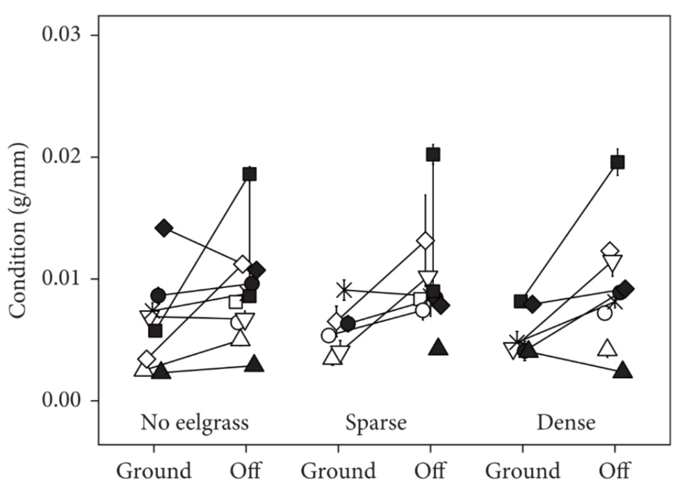

Figure 1. Oyster condition as dry flesh mass per shell height after 3 months (September). Error bars show SE of multiple samples (cages, bags, or glued cultch) within shellfish aquaculture beds across three levels of eelgrass (no, sparse, dense), with lines connecting on and off-bottom culture deployment in each site.

DEVELOPING A QUALITY MANAGEMENT PACKAGE FOR RECORDKEEPING IN AN OYSTER NURSERY

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Oyster aquaculture plays a vital role in the Gulf of Mexico's economy and culture. However, the region's shortage of oyster hatcheries and nurseries poses a risk to the industry's sustainability, as farmers may face unreliable access to oyster seed (juvenile oysters). A potential solution is for farmers to establish their own nurseries and raise seed. This project aimed to facilitate farmer-run nurseries by advancing quality management techniques. Accurate recordkeeping is essential for effective quality management in oyster nurseries. In an oyster nursery, records of water quality, the number of seed, and seed growth and survival rates must be kept. In upweller systems, where seed grow quickly, overcrowding is a concern, so careful density measurements are crucial. Farmers estimate seed densities and counts by weighing a subsample and calculating the total seed count and density based on the sample's weight. Counting seed daily is often too time-consuming, and results can vary by operator. To address this, a cost-effective quality management package consisting of software and hardware components was developed.

The software component consists of image analysis software that automatically counts the number of seed in an image (Figure 1). This provides an alternative counting method that does not require a microscope and allows seed counts to be more time-efficient. The software was coded in Python (v3.9.13, Python Software Foundation, 2024). When using the software, the time required for the seed-counting step was 65% faster than manually counting. Furthermore, the total time to perform all seed counting steps decreased by 10% when using the software compared to manual counting. The hardware component is in development and will include a series of scoops of different volumes to collect seed in different size classes. These collection tools allow farmers to quickly transfer a known seed volume into the second device type, a counting chamber. The counting chamber will be made of material that allows for high-contrast images of the seed, to facilitate better software results. In addition, the chamber will have an imprinted grid to provide a reference for manual seed-size estimations and will be able to accommodate a range of size classes.

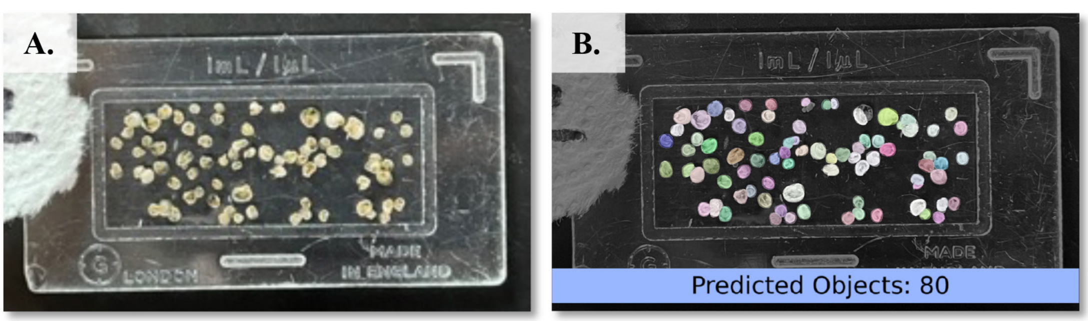

Figure 1. The original image taken during the seed-counting process (A) and the same image annotated by the software with the predicted seed count displayed (B).

ADVANCING QUALITY MANAGEMENT AND SEED CONDITIONING PRACTICES IN AN OYSTER NURSERY

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Oyster aquaculture is an integral part of the economic and cultural landscape in the Gulf of Mexico. However, the region's lack of oyster hatcheries and nurseries could threaten the industry's sustainability because farmers may not have reliable access to oyster "seed" (a juvenile life stage). One solution is for farmers to build their own nurseries and raise seed. This project aimed to facilitate successful farmer-run nurseries by advancing quality management techniques in oyster nurseries. Farmers need to raise high-quality seed to plant on their farms in a time and resource-efficient manner. Farmers cannot control all aspects of seed quality. However, farmers may be able to influence seed quality and prevent mortalities on the farm during the nursery grow-out phase with seed conditioning practices. While not common in North American oyster farming, farmers have practiced seed conditioning or "hardening" for decades in Japan and Korea. Seed hardening entails raising nursery-aged oysters in stressful environmental conditions to increase survival rates during the farm grow-out stage. This study will examine if incorporating desiccation practices into routine nursery operations can decrease farm mortality without significantly increasing the nursery labor required.

This ongoing study began in July of 2024 at the Louisiana Sea Grant Hatchery in Grand Isle, LA. Two conditioning treatments were tested to raise diploid oysters: a 16-hour, overnight desiccation treatment (the stress treatment) and a control treatment. The trial began after oyster seed reached a size of 2 – 4 mm (R2 size class) and were transferred into silo upwellers. From 7/11 to 8/06, the total number of seed in each size class (R2, R4, and R6) for each treatment was counted weekly (Figure 1). By the second week of the experiment, 7/23, over 30% of seed in the control treatment had grown into the R4 and R6 size classes. Conversely, an average of 90% of seed in the stress treatment remained in the smallest size class, R2, for the duration of the nursery experiment. After four weeks in the nursery, a portion of seed from both treatments was transferred into floating cages to track growth and mortality on the farm. Seed will be measured and counted monthly for one year to track growth and mortality.

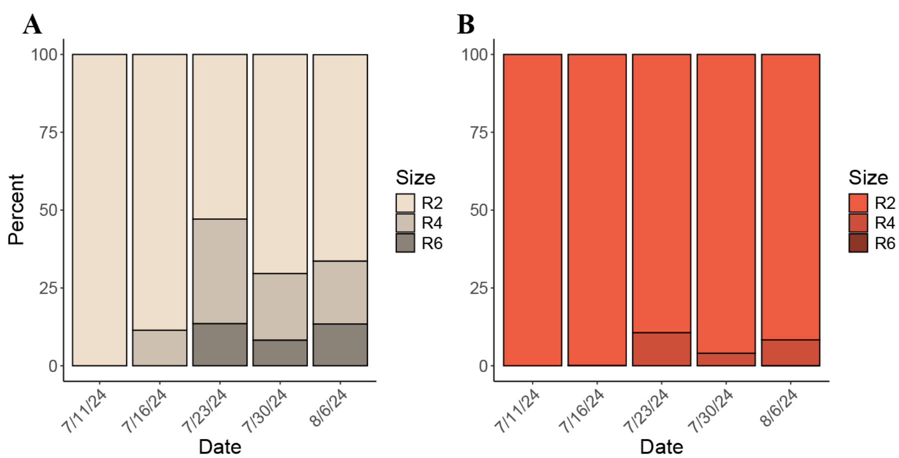

Figure 1. The percent of seed in each size class (R2, R4, or R6) in the control (A) and stress (B) treatments during the 4-week nursery experiment. Seed counts for each size class were evaluated with subsampling, therefore, percentages may fluctuate from week to week.

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN FINFISH AQUACULTURE FEEDS AND ACCUMULATION BY RED DRUM *Sciaenops ocellatus*

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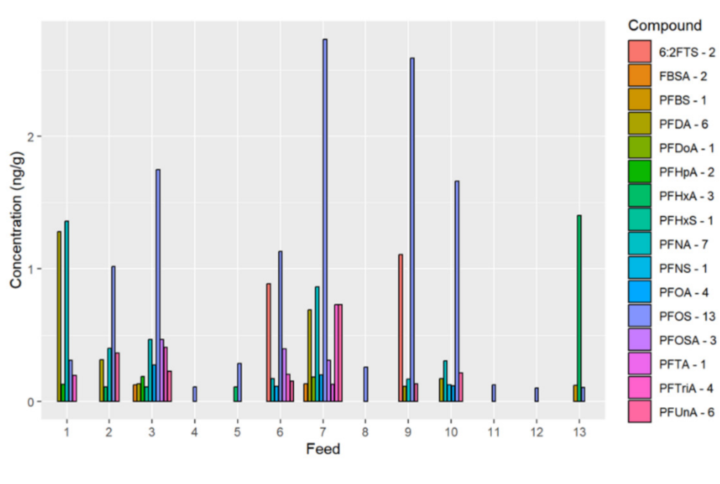
Per- and polyfluoroalkyl substances (PFAS) are a group of ubiquitous, highly stable chemicals used in many consumer and industrial products that can have negative health effects in humans including cancer and liver disease. According to the European Food Safety Authority (EFSA), an estimated 86% of dietary PFAS exposure to European adults comes from consumption of contaminated seafood. Fish bioaccumulate PFAS at higher concentrations than the environment with diet suspected to be a contributor of PFAS bioaccumulation. However, to our knowledge there are no studies examining aquaculture feeds as a potential source of PFAS exposure for finfish raised for consumption. This study: (1) developed a liquid chromatography tandem mass spectrometry method for the measurement of 30 PFAS in commercial aquaculture feeds (2) conducted a feeding trial using low and high PFAS feeds at different ration levels to assess accumulation of 30 PFAS in the filets and livers of juvenile red drum (*Sciaenops ocellatus*), and (3) estimated human PFAS dietary exposure from aquaculture fish filets.

Sixteen PFAS were detected across the 13 feeds, with perfluorooctanesulfonate (PFOS) being the highest concentration detected. In both the filets and the livers, only PFOS was detected above the reporting limits with a maximum of respectively 0.164 ng/g and 0.904 ng/g. Fish given the higher PFOS diet exhibited a higher percentage of detection in their fillets compared to fish given the lower PFOS diet. However, there was a significant difference in size between the feed groups, suggesting a possible growth dilution effect. PFOS did not differ among rations. Using the filet concentrations, estimates of dietary exposure to PFAS did not exceed the tolerable weekly intake limit set by the EFSA.

This study provides an assessment of commercially available feeds for PFAS and the accumulation of these contaminants by fish raised in via aquaculture. While fish in this study were not raised to market size and later developmental changes to metabolism could alter PFAS accumulation, the filets of juvenile red drum in this study did not bioaccumulate PFAS in amounts that would cause concern for human consumption.



PLANNING FOR SUCCESS: INTERACTIVE FINFISH AND OYSTER BUSINESS MODEL TOOLS TO ENHANCE FARMER'S ECONOMIC AND FINANCIAL DECISION MAKING

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While more than half of the seafood in the world is sourced from aquaculture, the U.S. is experiencing slowing or declining growth in its aquaculture sector in recent years. Contributing to this decline are economic and market conditions such as competition from cheaper imports, rising input costs, a complex regulatory environment, and a lack of information on investment, market risk, and profitability.

To help tackle some of these challenges, we developed three customizable, interactive business plan models for pond, raceway, and oyster production to allow producers to understand their current or future financial health, profitability, risk, and costs on their farms. These models ask producers to enter their farm information, production characteristics, gross receipts, input costs, and investments for capital goods and equipment allowing for each model to consider the unique characteristics of an individual farm. Then, the model walks the user through each output which includes an enterprise budget, sensitivity analysis, income statement, balance sheet, financial indicators, loan repayment schedules, and amortization schedules. Farmers can then save and print their outputs to have a record of the information that they entered.

The links to these models will be hosted online to allow them to be accessed by a broad audience. Additionally, these models will be featured at in-person training workshops held around the country as a part of an effort to increase financial literacy and enhance economic decision-making for U.S. aquaculture farmers.

THE USE OF NON-INVASIVE ULTRASOUND TECHNOLOGY TO MONITOR CARDIAC ACTIVITY IN RED ABALONE *Haliotis rufescens*

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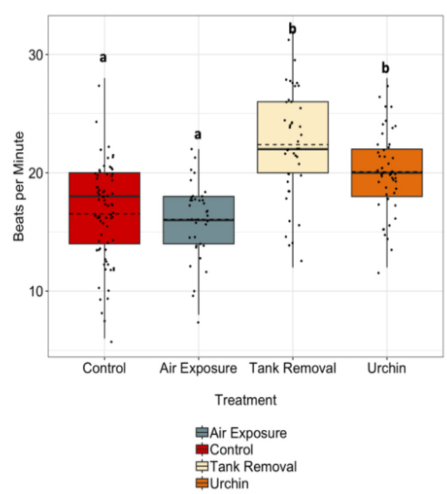
Abalone (*Haliotis* spp.) are an edible, herbivorous group of marine snails with over 100 species found worldwide, seven species of which are located along the rocky coast of California, United States. All California species of abalone are listed as endangered or critically endangered species by the International Union for Conservation of Nature (IUCN). Abalone conservation and restoration efforts for these abalone species have been implemented, including captive breeding and out planting programs. Traditional abalone stress assessments, such as cardiac exams, involve drilling holes into the shell inorder to insert electrodes above the heart or require the attachment of sensors to the shell causing excessive handling stress to the animal that can gonad maturation state and can possibly lead to mortality.

The objective of this study was to investigate the application of non-invasive ultrasound technology for cardiac assessments under control conditions, during routine animal care, and other various potential stressors experienced in a conservation and production aquaculture facility.

In this study, cultured red abalone (*H. rufescens*) were used as a proxy for the endangered black abalone (*H. cracherodii*) to assess cardiac activity. Monitoring cardiac activity using non-invasive ultrasound imaging technology can be a useful tool to inform abalone captive breeding and restoration programs to enhance animal welfare for these endangered abalone species.



ENDOPROTEASE HATCHING ENZYMES IN PEA CRABS *Pinnotheres pisum*

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Brachyuran crabs extrude fertilized eggs covered with glue and attach them to feathery pleopods under their tails. Eggs are brooded during embryo development and are physically and enzymatically cleaned until egg hatching. Peptide chemical signals coordinate egg hatching and larval release which is synchronized between the larvae and the female. In many crabs, release occurs in synchronous bursts that last about a minute and involves enzymes that cleave glues while eggs burst due to biological, osmotic, and physical pressure. Ovigerous pea crabs, which are oyster parasites, were removed from oysters into 0.2 micron gravity filtered aged sea water. Next, we incubated individual crabs in a sea water mixture of 3 pure proteins at 0.5 to 1 um for 20 minutes. The addition of proteins to the females with eggs about to hatch stimulated hatching. Proteomics identified peptides from pure proteins generated by maintenance proteases and hatching proteases. Based on carboxyl terminal amino acids of peptides, there is an ensemble of at least 5 endoproteases. Because we cleaved pure proteins, we know the absolute identity of the sequences around the cleavages, which means we can define enzyme specificity.

Preliminary analysis suggests the presence of serine proteases in addition to many unidentified proteases. The identification of the non-serine proteases is actively being investigated.

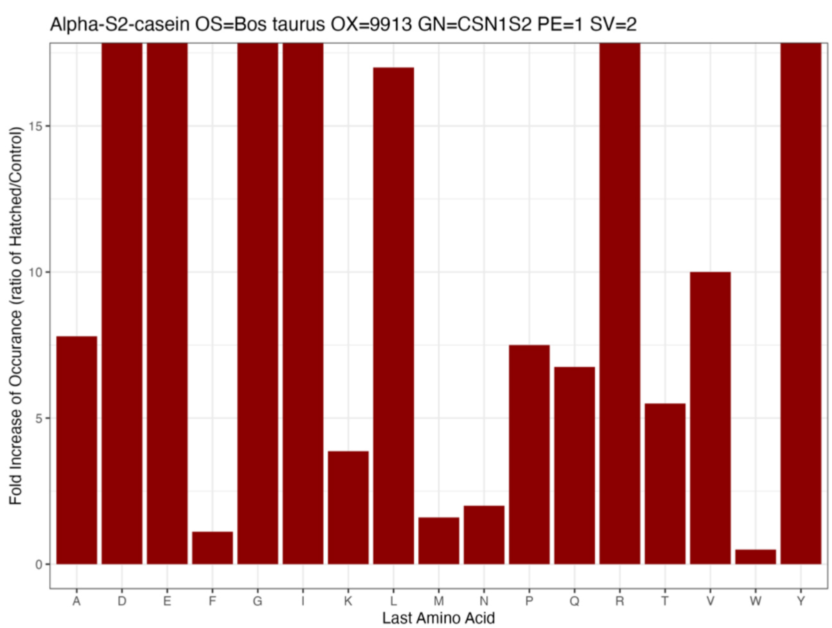

Figure 1: The number of fold increase in the frequency of the last amino acid between the hatching pea crab treatment to the control.

eDNA METABARCODING TO SURVEY GULF OF MAINE FOR SHELLFISH

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Environmental DNA (eDNA), which is DNA left in the environment through the defecation or shedding of organic material (fur, skin, feces) from organisms, has proven to be an efficient and cost-effective tool in biomonitoring and biodiversity assessment in several ecosystems. Here used eDNA metabarcoding to survey wild populations of fish and shellfish such (e.g., longfin squid *Doryteuthis pealeii*, shortfin squid *Illex illecebrosus*, scallops, and sea urchins), in the Gulf of Maine (GOM) during the Maine's Department of Marine Resources (DMR) inshore trawl surveys in spring and fall 2023. Specifically, we compared traditional trawl survey results to those from two eDNA collection methods: passive metaprobes attached to the codend of the trawl or by filtering slush water from the trawl. From eDNA samples we amplified fish, invertebrate, and cephalopod eDNA with general primers and sequenced the amplicons to infer species distribution. The goal of this study was to compare traditional trawl and eDNA surveys in terms of species composition and abundance of key shellfish species and determine whether and how eDNA surveys can be used to survey wild shellfish populations in the GOM. Results of this comparative study, as well as applications to aquaculture of cephalopods, bivalves, and urchins, will be discussed.

LEPTIN REGULATION OF METABOLISM AND ITS IMPLICATIONS IN THE ADAPTIVE STRESS RESPONSE IN TILAPIA *Oreochromis mossambicus*

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Leptin is a pleiotropic hormone known to influence numerous physiological processes including appetite, energy expenditure, and reproduction in vertebrates. In mammals, leptin is produced by adipose tissue works primarily as an adipostat. It circulates in proportion to fat deposition and inhibits appetite while stimulating lipolysis and fatty acid oxidation to prevent excessive lipid accumulation. Its function on energy homeostasis in fish remains poorly understood despite leptin's well-conserved anorexigenic actions. Here we discuss the putative function of leptin on metabolism and its implications in the stress response in teleosts fishes through studies in the euryhaline tilapia, *Oreochromis mossambicus*. The liver is typically the predominate site of production in fishes and data suggests that leptin may act to regulate carbohydrate catabolism in these and other ectothermic vertebrates. Our work in the tilapia shows that recombinant tilapia leptin A (rtLepA), the predominant paralog in fishes, and its mRNA levels in the liver acutely rise with systemic glucose during seawater challenge and increase with fasting as well as under hypoxic conditions. The hormone increases plasma glucose and decreases liver glycogen *in vivo* in tilapia, suggesting it promotes glycogenolysis. Results suggest that LepA may be involved in the adaptive stress response by mobilizing energy reserves, namely carbohydrates.

Both insulin and the classical stress hormones, epinephrine and cortisol, play roles in regulating glucose availability and interact with leptin in tilapia to maintain glucose homeostasis under normal anabolic states as well as during stress-associated catabolic states. LepA synthesis and secretion from hepatocytes declines as ambient glucose levels increase, suggesting a negative feedback inhibition whereby leptin stimulates glucose release (glycogenolysis) during the initial stress response and glucose subsequently acts to directly inhibit *in vitro* leptin synthesis and secretion. Cortisol stimulated hepatic LepA secretion and suppressed *lepa* mRNA *in vitro*, while epinephrine, a major adrenergic stress hormone, stimulates LepA secretion. The response was accompanied by increases in glucose release likely indicating a classical glycogenolytic effect of the adrenergic hormone. These data suggest hepatic LepA is sensitive to ambient glucose and is stimulated by both catecholamines and glucocorticoids.

Additional studies utilizing the tilapia pituitary transcriptome identified numerous metabolic pathways regulated by leptin. Orthogonal testing showed the hormone induces glycolysis by increasing the activity of key glycolytic enzymes and their transcript levels. Liver *lepa* abundance increases with hypoxia in the tilapia and leptin affected hypoxic responsive pathways within the transcriptome likely associated with enhanced anaerobic glycolysis. Collectively, research suggests leptin plays an integral role in conjunction with the classical stress hormones to promote carbohydrate catabolic processes critical to the adaptive stress response in fishes.

ALTERNATE DAY FEEDING ON GROWOUT OF DOMESTIC STRIPED BASS (*Morone saxatilis*) IN RECIRCULATING AQUACULTURE

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Striped bass (*Morone saxatilis*) is an emerging new cultivar in the U.S. due to its ability to grow rapidly up to 1.3 - 2.3 kg (3 - 5 pounds) within a 24-month production cycle. The feasibility of cultivating this species has increased with successful domestication and many years of selective breeding for improved growth characteristics. Relatively little is known on the growout of this species in Recirculating Aquaculture Systems (RAS), including feeding strategies that might reduce feed and labor costs. Our research suggests that feeding striped bass every other day (3x/week) instead of daily (5x/week) to satiation may improve feed efficiency while having little impact on growth performance, particularly at larger body sizes. Fish grown from 475 to 800 g on alternate day feeding had a similar and at body weights > 850 g an improved FCR relative to fish fed daily. We further tested if feeding fish on alternate day at a reduced feeding rate could improve feed conversion efficiency and the amount of feed required to grow fish to market size relative to daily fed fish. We investigated the effects of feeding daily (7x/week) vs. alternate day (3-4x/week) on striped bass growout. Fish (500 g) were fed daily througout a 253 day period. Fish on the alternate day feeding regime were fed at the following rates relative to fish fed daily to satiation: 50% for 0-89 days and 75% for 90-253 days. Alternate day fish were subsequently shifted to daily feeding at 100% rate to that of the daily fed group until reaching 2 kg body size. The results show that 0.5 kg striped bass fed daily grew very rapidly to 2 kg (6 g/day) over only 253 days. By day 89 fish fed daily had significantly higher weights and lengths then alternate day fed fish that persisted throughout the course of the study. However, fish fed at 75% rate showed a similar specific growth rate and 17% improved FCR during the 90-253 day growth interval relative to daily fed fish suggesting that feeding at this rate on alternate days may improve production efficiency of striped bass in RAS.

To this end, we have undertaken an additional on-going growth trial of domesticated striped bass that will be grown to a final semicommercial density of 0.7 kg/L (0.6 lbs/gallon) at a 2 kg harvest size. Striped bass were raised in RAS from 4.5 g to 890 g on daily satiation feeding in freshwater supplemented with NaCl (3-5 ppt, 21-24C). Fish were then fed on alternate days (3 times/week, MWF) at 78% of the rate of daily fed fish (6 times/week, MTWTFS) for 3 months (N = 4 tanks/group, 84 fish/1900-L tank). Weight of fish fed on alternate day was 6.9% lower (1.352 ± 0.003 kg) than daily fed fish (1.454 ± 0.004 kg, Mean ± SEM), while FCR was similar among groups (1.73 alt day vs 1.72 daily). Mortalities were 1.78% and 0.60% for the alternate and daily fed groups, respectively. Total feed consumption was 22% less for the alternate day group. These results suggest that a significant savings in labor costs is possible with implementation of an alternate day feeding strategy in larger striped bass (> 800 g) grown in RAS with relatively little impact on production. The feed management protocol will be carried out until fish reach 2 kg to further ascertain if production efficiency can be improved. This work was supported by NOAA-National Sea Grant (StriperHub) R/22-AQUA-06.

EFFECTS OF SEX, FAMILY, AND SIZE GRADING ON GROWTH, FEED CONVERSION, VISCERAL FAT, AND WEIGHT LOSS DURING FEED DEPRIVATION IN DELTA SELECT STRAIN CHANNEL CATFISH

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Feed conversion efficiency is an important determinant of profitability in catfish production. Understanding factors affecting feed conversion efficiency (FCE) at a physiological level could lead to improvement of FCE at production scale. Effects of sex, family and size grading within full-sib families on juvenile channel catfish (*Ictalurus punctatus*) growth, feed consumption, feed conversion, visceral fat accumulation, and weight loss during feed deprivation were determined. Correlations between traits were determined for each treatment within a trial.

In Trial 1, fingerlings from 5 channel catfish families were graded by weight into small, large and random mixed-size groups, fed for 8 weeks, subjected to 2 weeks of feed deprivation, and then euthanized to determine sex. In Trial 2, all-male, all-female and mixed-sex groups of channel catfish fingerlings were subjected to the same feeding regime and then euthanized to confirm sex and weigh visceral fat. Effect of fish sex, family, and size group within family were determined for mean increase in weigh, percent wait gain, residual feed intake, feed conversion ratio, weight-loss during feed deprivation, survival; and correlations among measured traits.

Males had a higher percent weight gain, better feed conversion, and less percent visceral fat than females; mixed-sex fish were generally intermediate between all-male and all-female groups. Feed conversion was positively correlated with visceral fat percentage in the all-female treatment (i.e. fatter fish had poor feed conversion), but feed conversion and visceral fat were not correlated in the mixed-sex or all-male treatments. Family had a significant effect on weight increase, percent weight gain, feed intake, feed conversion efficiency, and percent weight loss during feed deprivation. Within families, large fish had better growth, feed conversion efficiency, and lower percent weight gain than small fish. Mixed-sized fish were generally intermediate to small and large fish treatments for all measured traits. The correlation among weight loss during feed deprivation and feed conversion ratio for treatment effects was only marginally significant or not significant. Survival was near 100% in all treatments, and treatment had no effect on survival.

Family, fish size within family, and sex all affect feed conversion in juvenile channel catfish. The basis for the effect of fish sex (males superior to females for percent increase in weight, feed conversion, and less fat) should be determined in future research projects.

ADVANCING U.S. OFFSHORE AQUACULTURE ENGINEERING

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As demand for seafood production in the United States increases, offshore aquaculture will become more widely adopted. Due to the extreme weather events experienced in offshore conditions, offshore aquaculture will require rigorous engineering efforts to enable safe, long lasting aquaculture production systems. This presentation will provide a high-level overview of a typical ocean engineering design process that can be used to support the development of sustainable offshore aquaculture systems. This presentation will also identify the gaps in literature around this topic and present a plan to work towards robust offshore aquaculture engineering guidance for the U.S.

DETERMINING SITE SUITABILITY AND PROCESSING POTENTIAL AT ALASKA'S SALMON HATCHERY RELEASE SITES FOR THE INTEGRATION OF MARICULTURE SPECIES

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Each year nearly 1.7 billion salmon are released into the ocean from 29 different hatcheries around the state of Alaska. Most of these fish are reared in ocean net pens for 3 or 4 months and are fed intensively, creating increased nitrogen and phosphorus outputs as well as adding to the nutrient load in the benthic environment near the rearing fish. These excess nutrients can be a detriment to the environment and to the Aquaculture organizations if they become out of compliance with their Alaska Pollutant Discharge Elimination System (APDES) permit. This detriment can alternatively be considered an opportunity. Currently in the State of Alaska there is substantial money and effort being put into the development of the mariculture industry and these hatchery release sites have the space and nutrients to help move the needle on mariculture production while benefiting their organizations, the environment and potentially the rearing fish. In this project we supplied 6 Private nonprofit hatchery organizations with the equipment necessary to help determine site suitability for mariculture integration at 18 different salmon release sites all around Alaska. Hatchery personnel installed loggers and launched Acoustic Doppler Current Profilers (ADCPs) to measure current (speed and direction), oxygen levels, salinity, and temperature. In addition, they went out bi-weekly to collect water samples to measure nutrient levels and light attenuation. Most of these sampled sites show promising site suitability with the possible exceptions of two sites that showed very low salinities at 3 meters in late spring. This information can be used to inform Aquaculture organizations and interested farmers on placement of future farms and provide insight into how to best mitigate excess nutrients in the environment.

ELECTRO EUTHANASIA AS A HUMANE ALTERNATIVE TO TRADITIONAL METHODS FOR EUTHANASIA OF ZEBRAFISH *Danio rerio*

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Zebrafish are the most utilized fish in research, and it's estimated that five million fish are used for research annually. Every year, vast quantities of Zebrafish must be euthanized, usually achieved via immersion in chemical solutions or rapid chilling. Aversive behavior is often seen, and extended exposure periods are needed for these methods to be effective. AVMA humane slaughter guidelines state that electrocution is also an acceptable euthanization method, provided that the electric current is sufficient to immediately induce insensibility before or simultaneously with killing the fish. Electro-euthanasia was shown to be effective on zebrafish at 12 hours and 5 days after fertilization with no aversive behavior. This research aims to validate the manufacturer's recommended settings and develop standard operating procedures for electro-euthanasia for zebrafish across various developmental stages.

Wild-type zebrafish and two electrical systems, WASP3 and EFS-WASP1 (Electro Fishing Services, Ltd. Ireland) were utilized. All developmental stages were exposed to a continuous and pulsed direct current with a rectangular DC waveform at a frequency of 50 pulses per second with a 50% duty cycle. All tests included handling and non-handling controls. All trials included five replicates (n=5 replicates per treatment). The voltage gradient was reduced every five successful trials until 100% mortality was not achieved. If 100% mortality was not achieved, trials were discontinued.

Pre- and post-hatch embryos (<5 days post fertilization [dpf]) were transferred to a 33mm^3 electrode chamber. Pilot testing began with manufacturer-recommended settings (Voltage gradient of 30V/cm and a 60 sec exposure period). Trials (n=5 replicates per treatment, 10 fish per replicate) began with a voltage gradient of 30V/cm and a 120 sec exposure. Trials were assessed immediately after for signs of survival and at 5 min, 30 min, and 12 hours post-exposure. 100% mortality was achieved for embryos 4 dpf following a 90 sec exposure to 30 V/cm. Embryos 5 dpf required a 120 sec exposure period to a voltage gradient of 25V/cm. Trials for adults (n=5 replicates, 3-5 fish per replicate) began with the manufacturer's recommended voltage gradient of 2.8V/cm. 100% mortality was observed in Adult zebrafish with lengths 21-25mm and 26-35mm, following a 150 sec exposure to 2.0V/cm. Adults 36-40mm required only a 90 sec exposure to 2.0V/cm to achieve 100% mortality. Results of these experiments show that electricity is a viable alternative to currently utilized methods of euthanasia.

FINANCING SMALL SCALE AQUACULTURE FARMS: A MODEL FROM MAINE

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Coastal Enterprises, Inc. (CEI) is a nonprofit 501(c)(3) community development financial institution (CDFI) founded in 1977. Our mission is to build a just, vibrant and climate-resilient future for people and coastal communities in Maine and rural regions. We do this by integrating finance, business expertise and policy solutions in ways that make the economy work more equitably.

Through a dedicated Fisheries & Aquaculture Program, CEI staff has been able to create a workable model in providing not just lower-interest financing to Maine's marine aquaculturists, but to combine expert training for entry-level fishermen considering a career in aquaculture. Supported by multiple assets both internally, privately, statewide, and federally, CEI has been able to not only to raise capital to deploy directly into industry, but to also provide the resources necessary for aquaculturists to become "bank-ready" both on and off the water.

Presenter will share unique loan products developed at CEI that is assisting farmers to grow and expand their operations in the Gulf of Maine. Our "Sea Farm Loan" provides financing to individuals looking to expand an aquaculture venture, but who have not been able to access conventional sources of capital. Term loans are made to eligible growers for purchasing boats, gear and equipment, renovating or building infrastructure, acquiring land, supplying operational capital and restructuring debt. Our "Marine Green Loan" was created to support Maine fishermen and aquaculturists looking to transition vessels to hybrid retrofitting, and power to more renewable and climate friendly sources - electric engines, batteries, solar arrays for charging, upwellers, and shoreside charging and electrical connections.

During this presentation, CEI will share how a combination of financial funding sources and custom loan products – in concert with expert technical consultant and business development resources – has created a truly impactful accelerator for Maine's aquaculture sector, and how this model could be duplicated in other coastal states and communities seeking to provide better financial resources to their aquaculturists nationally.

CLOUD-BASED DATA APPLICATIONS FOR STREAMLINING NATURAL RESOURCE MANAGEMENT

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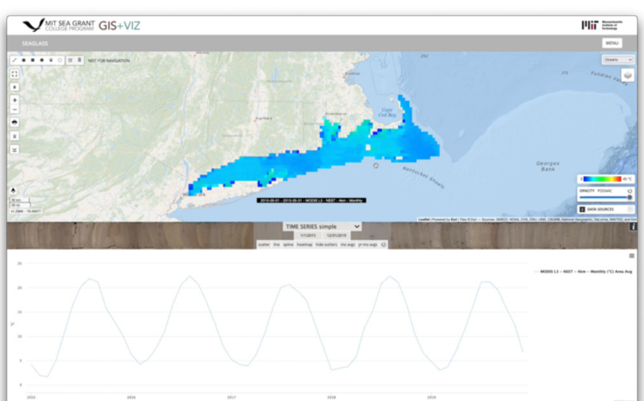
MIT Sea Grant (MITSG) is currently utilizing the *Seaglass* framework with Massachusetts towns for a cloud-based system that streamlines operations in shellfishing management, facilitates sharing of shellfish data, and increases transparency and accessibility to permit holders. This system will help users understand what embayments can be fished, the rules behind a closure decision, and the data supporting rules. MITSG developed the *Seaglass* geospatial data analysis and visualization system initially for the Nantucket Natural Resources Department (NNRD), for analysis and visualization of its entire 40-sensor dataset, and to streamline its analysis workflow. NNRD uses *Seaglass* for data queries, auto-calculation of annual health indices, analysis, visualization and download, which has resulted in reduced employee hours and reduced error in data reporting. Workflow time was reduced from 240 hours to 30 hours, for a time savings of 88%. *Seaglass* provides for immediate dissemination of engaging and mobile-friendly data visualizations to the public as well, addressing the need for data diversification and outreach effort by research communities. MITSG has also worked with the National Marine Fisheries Service to develop new features that will improve workflows in habitat assessment, and with scientists to develop modeling and simulation workflows using satellite and in-situ data via application programming interfaces (APIs).

Seaglass utilizes data services and APIs for direct access to a wide variety of data, including satellite data hosted via DAACs such as PO.DAAC and OB.DAAC, as well as point-based services like Ambient Weather and NCEI. Users can also upload their own data into the system database via a simple, adaptive procedure, and can utilize and download data from this database or any of the internally-linked data services. All data uploaded into the system database can be made available via the system's own API, for use by external analysis and visualization systems. The emphasis on data services and APIs allows for more efficient queries of remote databases and more timely sharing of data collected, reducing wasteful duplication of data and overhead of data transfer. This approach allows data generators can focus on storage, and make their data available via a robust and secure data service for web developers and UX designers to use in building interfaces.



ASSESSING A DECADE OF GROWTH: ECONOMIC IMPACTS OF AQUACULTURE IN MAINE

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While some may look to Maine as leaders in U.S. aquaculture, its economic growth has not been assessed in nearly a decade. This study uses data from a sector-driven survey to assess the economic impacts of Maine's aquaculture sector in 2023. The findings paint a picture of the various types of aquaculture occurring in Maine, their impacts, and the related businesses and services that have grown to accompany the sector. This allows for comparisons to be drawn from the previous, 2014-based study, and highlights successes and shortfalls in the Maine aquaculture sector's growth. Preliminary findings show notable increases in the farm gate value and total economic impacts of the shellfish sector, led by oysters. The survey also reveals projections for Maine's aquaculture growth over the next ten years, along with the identification of barriers to growth.

This presentation will cover the methodology of the impact assessment, and lessons learned from it. It will also use Maine as a case study to show where other efforts in Maine have led to notable economic impacts, along with other areas that are lacking.

WHO WANTS TO EAT SEAWEED - HOW AND WHY? A NATIONWIDE STUDY ON U.S. CONSUMER PREFERENCES FOR SEAWEED

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Some work, such as that led by the Maine Aquaculture Innovation Center, has responded to the question as to who is eating seaweed in the U.S. This study builds on top of such work to assess what types of seaweed products people prefer, and why. A nationally representative panel was contracted through Centiment, a marketing research firm, to target consumers from across the country (n=2,035). Brayden worked with an industry advisory team, consisting of 6 seaweed-related businesses, to draft a survey that answered their questions about seaweed consumers.

The survey's focus lies around 10 individual products that include seaweed. The products were selected by the industry advisory board. They were then rated by survey respondents as to their likelihood to purchase them. Following each product, respondents were also asked both why they would purchase the product, and why not. This reveals not only which types of seaweed products were preferred – but why there were preferred as well. Preliminary findings show that respondents preferred products that were convenient, ready-to-eat, and had a base product that they already liked (e.g. seaweed teriyaki sauce - meaning they already liked teriyaki sauce, and thus liked the combined product). Additional questions explored respondents' seaweed consumption patterns, diet, sociodemographics, and more.

EXPANDING MAINE'S BLUE ECONOMY – A FINAL REPORT ON MAINE'S AQUACULTURE MARKETING NEEDS, SUPPLY CHAIN LOGISTICS, AND CONSUMER PREFERENCES (NOAA-FUNDED)

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This provides a final update to the NOAA-funded work entitled Expanding Maine's Blue Economy. The work explored how Maine's aquaculture marketing needs, supply chain logistics, and consumer preferences could be better understood to increase both in-state seafood production and sales across the U.S. It combines efforts and data across multiple institutions (Maine Aquaculture Association, Gulf of Maine Research Institute, University of Maine School of Economics, and Maine Sea Grant) and using multiple modes of research – surveys, interviews, distribution mapping, and more.

A preliminary update was given at the 2024 Aquaculture America meeting. This presentation provides a final report, including completed supply chain interviews and analysis along with a completed consumer survey analysis, added to the already existing work. It will also detail the science communication and outreach methods employed to share the work widely and effectively within Maine's seafood community. It will also include lessons learned from the project, to be shared for others interested in similar assessments for their own state.

THE USE OF PROBIOTICS TO MITIGATE ATLANTIC SEA SCALLOP (PLACOPECTEN MAGELLANICUS) MORTALITY FOLLOWING CHALLENGE WITH PATHOGENIC VIBRIO SPECIES

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The Atlantic sea scallop (*Placopecten magellanicus*) supports one of the most economically important fisheries in the northeastern United States. As the demand for sea scallop meat increases, aquaculture productions must supplement wild harvest efforts to reduce our dependence on importation. Scallop farmers rely on the acquisition of juvenile sea scallops, known as spat, to continue production, but wild spat collection is inconsistent and limited to a short season. Hatcheries in the state of Maine could reliably produce spat year-round, but struggle with mortality events, presumably induced by pathogenic Vibrio species. Probiotics have decreased mortality amongst infected bivalve larvae in other industries and could be implemented to decrease larval sea scallop mortality.

Seven probiotic bacteria were identified from literature in similar industries and cultured for use in sea scallop hatcheries. These bacteria were screened *in vitro* for potential probiotic effects on sea scallop larvae. Bacterial competition assays were used to examine the inhibition of a model pathogen, *Vibrio pectenicida*, by probiotic candidates. Forty-eight-hour challenge trials involving larval sea scallops and *V. pectenicida* (10^5 CFU/mL) were also conducted to test the effectiveness of applied probiotics on challenged and non-challenged larvae. Two probiotic candidates displayed beneficial impacts to Atlantic sea scallop larvae. *Alteromonas macleodii* (10^5 CFU/mL) increased survival amongst challenged larvae (RPS 46% ± 11) and *Pseudoalteromonas espejiana* (10^4 CFU/mL) amongst non-challenged larvae (RPS 46%).

The effect of both promising probiotic treatments on larval sea scallops were tested at a hatchery scale with *in vivo* challenge trials. *A. macleodii* had a negative impact on larvae growth and survival. *P. espejiana* improved the rate of larval sea scallop growth and development during the late straight hinge to early pediveliger stage, where larvae mortality typically occurs. This study can improve hatchery protocols through the implementation of the probiotic *P. espejiana*. Further optimization of hatchery protocols in tandem with probiotic inoculation may prove critical to the success of Atlantic sea scallop hatcheries in Maine.

OVERWINTERING SURVIVAL IN JUVENILE OYSTERS: UNDERSTANDING THE ROLE OF ENERGETIC PHYSIOLOGY FOR *Crassostrea virginica* SEED

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Crassostrea virginica is an economically important oyster species that extends from the Gulf of Mexico to the Gulf of St. Lawrence where it experiences negative temperatures for 3-4 months in winter. In response to these low temperatures, adult oysters decrease the unsaturation index of their polar lipids during the fall to adjust the fluidity of their cell membranes and progressively reduce their metabolism to a near standstill in winter. During this dormant phase, oysters stop feeding and mobilizes their energy reserves to maintain their vital physiological functions. However, the metabolic strategies of oyster spats are still little studied, although they represent a crucial stage determining the production of adults and the economic performance of hatcheries. To better understand the energetic physiology of oyster seeds, we conducted two experiments from September 2021 to May 2022 and from September 2022 to May 2023 to follow the seasonal lipid dynamics and energy reserves of Crassostrea virginica seeds from fall acclimation to the end of winter dormancy. During this period, we analyzed the energy reserves and fatty acid composition of polar lipids of three size classes of juveniles from hatchery and wild origins. The results revealed homeostatic adaptation mechanisms in autumn, characterized by a gradual increase in polyunsaturated fatty acids within cell membranes in response to declining environmental temperatures (Figure 1). However, this lipid remodeling is reversed below a threshold of 2°C, marked by a sharp reduction in polyunsaturated fatty acid levels, potentially indicating the onset of spat dormancy (Figure 1). This dormancy, implying a halt in acclimatization to colder winter conditions, may partly explain the high mortality rates observed among juvenile oysters along the St. Lawrence coast.

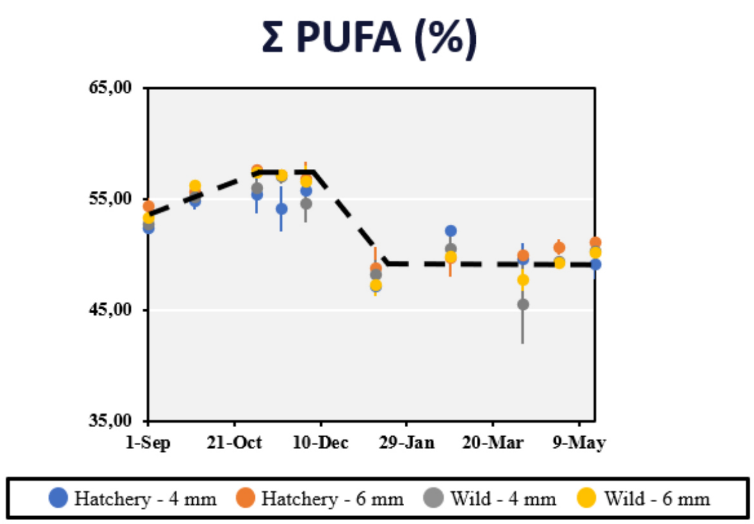

Figure 1: Relative contribution of the sum of polyunsaturated fatty acids in the polar lipids of 4 and 6-mm oyster seeds of hatchery- and wild-origin.

WHAT WILL WE GROW HERE? AN ANALYSIS OF CANDIDATE SPECIES FOR OPEN OCEAN AQUACULTURE IN THE UNITED STATES

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As a nascent industry in the United States, open ocean (also referred to as "offshore") aquaculture has the potential to positively contribute to domestic seafood supplies. However, it also has the potential to cause environmental damage if the risks and impacts of the farms are not well understood. Due to a lack of domestic operations, examples of commercial open ocean aquaculture farms in other nations can provide insights into which species might be most suitable for open ocean cultivation, and the specifics of cultivating them offshore. Broad studies of the environmental benefits, risks, and innovations for open ocean aquaculture have been conducted, but lack the granularity of how the environmental and fiscal sustainability of an open ocean farm, which will likely vary depending on species cultivated.

The purpose of this report is to highlight what species appear to be viable candidates for open ocean aquaculture cultivation, with a focus on those that may be optimal for farming in the U.S. This report identifies three categories for viable species: finfish, shellfish, and seaweed and describes each species' specific needs, ecological risks and benefits, and current commercial potential. The species analyzed in each category were selected for at least one of three reasons: 1) Whether they have actually been farmed in U.S. waters or abroad, 2) repeated mention in literature and by industry advocates as an ideal open ocean species, and 3) physical compatibility with the open ocean and exposed conditions. The finfish species that seem most likely to be farmed in U.S. open waters in the near term are: **cobia** (*Rachycentron canadum*), **kanpachi** (*Seriola dumerili* and *Seriola rivoliana*), **Atlantic salmon** (*Salmo salar*), and **steelhead trout** (*Oncorhynchus mykiss*). The species that appear to have the most potential to be farmed in the future, but haven't yet been grown at commercial or semi-commercial scales, are: **nenue** (*Kyphosus vaigiensis*), **California yellowtail** (*Seriola dorsalis*), **mutton snapper** (*Lutjanus analis*), **Florida pompano** (*Trachinotus carolinus*), **Atlantic cod** (*Gadus morhua*), *red drum* (*Sciaenops ocellatus*), **bluefin tuna** (*Thunnus thynnus* & *Thunnus orientalis*), **red snapper** (*Lutjanus campechanus*), **sea bream** (*Pagrus pagrus*), and **mahi-mahi** (*Coryphaena hippurus*). **Blue mussels** are the highlighted shellfish, and **giant kelp**, **bull kelp**, and other Australian native algae are the featured macroalgal species.

Full report can be found here.

EVALUATION OF HORMONE EFFICACY FOR INDUCED SPAWNING OF SOUTHERN FLOUNDER *Paralichthys lethostigma*

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Southern Flounder (*Paralichthys lethostigma*) are distributed throughout inshore waters along the Southeastern Atlantic and Gulf of Mexico coastline. They have been heavily targeted by fisherman in recent years which has led to severe population declines. This has prompted more stringent management regulations and stock enhancement programs in multiple states. The South Carolina Department of Natural Resources initiated a stock enhancement program for the species in 2021 and has been heavily focused on applied research to improve production. One such area the Department has focused on is spawning through evaluation of potential hormones for spawning induction.

From January through March of 2024, staff at the Waddell Mariculture Center and Marine Resources Research Institute evaluated the efficacy of multiple spawning hormones including Ovaprim®, Ovaplant-Liquid, and a type II gonadotropin releasing hormone analogue (GnRH IIa). All three hormones were administered to female Southern Flounder at a standard dose of 10 µg/kg. An individual female (n = 12) of each treatment was paired with a single male and observed for spawning up to 120 hours post administration. Females were also assessed at 48 and 72 hours post administration to determine if eggs could be stripped from females. Standard artificial fertilization protocols were applied for all strip spawns. Spawning frequency, egg output, and egg and larval viability parameters were recorded for every spawn.

All hormones were effective at inducing females to spawn volitionally as well as be stripped of eggs, with no significant differences detected in spawning frequency. However, floating egg output was significantly higher for stripped females administered Ovaplant-Liquid (62.51 ± 8.25 eggs/g) in comparison to females administered GnRH IIa (31.34 ± 6.56 eggs/g), but similar to Ovaprim® (49.88 ± 6.95 eggs/g). In addition, hatching success of floating eggs stripped from females was significantly higher for females treated with Ovaplant-Liquid or Ovaprim® (51.54 ± 1.47% and 50.37 ± 1.47%, respectively) compared to those treated with GnRH IIa (39.46 ± 1.41%). Overall, more fertile eggs were produced from Ovaplant-Liquid or Ovaprim® treated females compared to GnRH IIa by the conclusion of the experiment. These results will ultimately lead to better production protocols for spawning Southern Flounder in captivity.

COORDIANTING SHRIMP DISEASE SURVEILLANCE TESTING FOR STATE AND FEDERAL PROGRAMS TO REDUCE REDUNDENT TESTING EXPENSES FOR HAWAII'S SPF BROODSTOCK PRODUCERS

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The Hawai'i Shrimp Health Certification Program (SHCP), is administered by the Hawai'i Department of Agriculture. The disease surveillance and biosecurity practices upon which the program is based were initiated in the early 1980's when intensive shrimp culture began to be explored in earnest in Hawai'i. The SHCP's disease surveillance guidelines are based on the World Organization for Animal Health's (OIE) aquatic animal health code; as is also the case for the USDA administered program for shrimp. The state and federal programs are both voluntary, and periodically require updates or changes. Despite significant programmatic overlap and both programs being based on the same WOAH framework, there are some differences between the programs that can lead to confusion about regulatory compliance requirements (such as specific disease surveillance testing needs, document submission, etc.). By working closely with colleagues at the federal level and with producers, we effectively reduced redundant testing by coordinating the timing of disease surveillance testing for the state program with what the producers needed to maintain compliance under the federal program.

INSIGHTS ON TRANSFORMATIVE STRATEGIES TO GROW AQUACULTURE IN AN URBAN AREA

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With millions of people in close proximity, a number of cities in the United States are ripe for the development of high value local aquaculture industries. This on both the commercial and artisanal levels. However, how does one grow aquaculture in urban areas?

By definition, transformative planning creates marked changes in institutions and people including performance, direction or impact. So, perhaps the problem needs to be redefined, not only to support the local food system. But also perhaps, to examine how a new industry could address the wide variety of urban issues it may impact?

Over the past 10 years our work in Phoenix Arizona has provided a number of valuable insights on strategies necessary for successful industry development. These include the recapturing the value in brown fields, community and family economic development, family nutrition and health, STEM education, new opportunities for diverse populations (DEI), mitigating climate change including heat islands, reducing food deserts and food swamps, skills training and job creation, assisting the unhoused and mitigating poverty, new financing (access to capital), new business assistance institutions, and much more.

INSIGHTS ON ADAPTING AQUAPONIC FOOD PRODUCTION TO INCREASING LOCAL TEMPERATURES (AQUAPONICS IN THE HEAT)

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Backyard and open air (non-CEA) aquaponics remain a popular application of the technology. However, many parts of the world are experiencing the impacts of climate change, in this case elevated temperatures. For example, locations including Phoenix, Las Vegas, Philadelphia, Boston, Washington DC and many others have recently suffered record temperatures and record number of high heat days. Successful outdoor agriculture is adapted to the environments that are best suitable for the plant and animals that are desired to be produced. Aquaponics is no different. However, there is a dearth of literature available on aquaponic food production strategies in increasingly high heat.

Applying aquaponics to the at times 118^{o} F (47.8^{O} C) + environment of Phoenix Arizona over the past 10 years has provided a number of valuable insights on strategies necessary for successful food production. This includes new construction materials, designs, crops, growing seasons, IPM, production economics and business models.

COMPARISON OF THE 1993 AND 2021 REPRODUCTIVE CYCLE OF *Holothuria cinerascens* (BRANDT, 1835) FROM PARK RYNIE, KWAZULU-NATAL, SOUTH AFRICA

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The sea cucumber *Holothuria cinerascens* (Brandt, 1835) is a species with potential pharmaceutical value that is not currently farmed or cultured in South Africa. To determine its reproductive cycle and make it suitable for farming, this study aimed to monitor the gonad-somatic index (GSI) of *H. cinerascens* from Park Rynie, KwaZulu-Natal, between September 2019 and October 2021 using histology. The GSI was correlated with total monthly rainfall (mm) and mean monthly air temperature (°C) to determine possible cues for gametogenesis and spawning.

The study found that *H. cinerascens* reproduced through sexual reproduction in the summer months (November to February), while asexual reproduction occurred during winter (May to July). The GSI significantly changed over the sampled periods, peaking in January 1992 and January 1993 for the old study, and December 2019 and November 2020 to February 2021 for the new study. Both studies showed weak positive correlations between mean monthly air temperature (°C) and GSI, with a stronger correlation observed in the new study. The total monthly rainfall was also correlated with GSI in the new study but not in the old one.

The gonads of *H. cinerascens* developed and matured during summer months, followed by regression to winter months when they completely disappeared. Gonads showed clear sexual dimorphisms, with male gonads having white hues and female gonads peach hues when mature. Female gonads developed oocytes along the internal walls of tubules in September, which had regressed by December, and were full of eggs by March. Mature eggs ranged between 100 and 120µm in diameter.

Out of the sampled *H. cinerascens*, 39 out of 133 were found to be fissiparous, with 20 out of these lacking gonads. Fissiparous individuals tended to be longer and heavier compared to non-fissiparous counterparts, with those exceeding a length of 17.6 cm being more likely to be fissiparous. The plane of fission was consistently located at 8.35 cm (SD±2.43 cm) from the mouth, accounting for approximately 44.04% (SD±12.88%) of their total body length.

Overall, this study provides valuable insights into the reproductive cycle and characteristics of *H. cinerascens* in South Africa, which can inform future farming practices and conservation efforts.

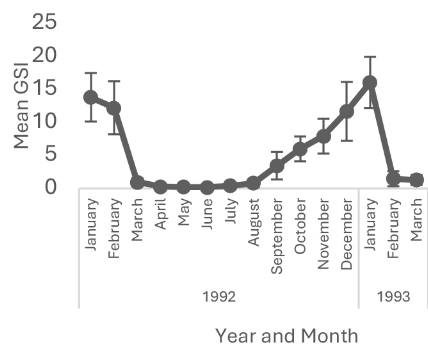

Figure 1: Mean (±SD) monthly GSI for H. cinerascens (Brandt, 1835) for the indicated months (Old study)

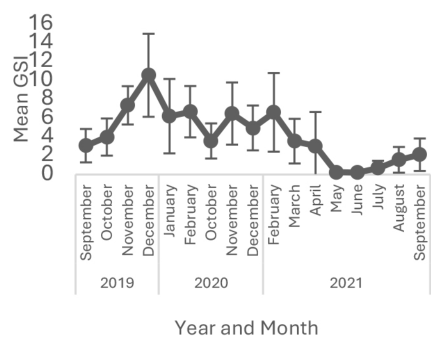

Figure 2: Mean (±SD) monthly GSI for H. cinerascens (Brandt, 1835) for the indicated months (New study)

CAN MANGANESE NEUROTOXICITY TO THE DOPAMINERGIC SYSTEM OF THE BIVALVE MOLLUSC *Crassostrea virginica* BE REVERSED BY WASHING

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Gill lateral cell (GLC) cilia of Crassostrea virginica are innervated by a serotonin-dopamine innervation from their ganglia. Serotonin is an excitatory neurotransmitter causing GLC cilio-excitation, while dopamine (DA) causes cilio-inhibition. Manganism is a human neuropathology caused by chronic exposure to elevated environmental levels of manganese (Mn). Symptoms of Manganism are similar to Parkinson's disease, but unlike Parkinson's patients, those with Manganism are unresponsive to L-DOPA. The neurotoxic mechanism of Mn toxicity is unclear and successful clinical treatments for Manganism remain lacking. Recently, p-aminosalicylic acid (PAS) was reported a potentially effective treatment. While PAS had been used as an anti-inflammatory drug for tuberculosis, it also has strong chelating properties. Our lab previously showed placing C. virginica in artificial sea water (ASW) containing Mn impaired their DA system and prevented the cilio-inhibitory effect of DA on GLC. We also found this Mn neurotoxicity could be prevented and reversed by chelation, using various agents including taurine, carnosine, EDTA and PAS. Since chelation therapy targets metal removal from tissue, we were interested to determine effects of washing Mn exposed oyster tissue in reversing Mn neurotoxicity on the animal's DA system. We hypothesize washing Mn from animals, by changing their water, would reduce tissue Mn levels and reverse the neurotoxic effects of Mn on the DA system. To test this, oysters were placed in containers of ASW containing 0.5mM of Mn for 3 days. Some Mn treated animals were then "washed" by placement in containers of ASW without Mn for 3 more days. Control animals were similarly treated, over the six-day period, in ASW without Mn. After the experimental period, controls, Mn treated, and Mn treated/washed animals had their gills excised and GLC cilia beating rates, in response to DA (10-5 - 10-3M) determined by stroboscopic microscopy. In control animals, applying DA to isolated gill decreased GLC cilia beating rates over 90%, from 16 to 1 beats/sec. In Mn treated animals, the DA response was severely impaired with beating rates decreasing only 20%, from 19 to 15 beats/sec. In Mn treated/washed animals the DA response was impaired to a lesser extent, with beating rates decreasing by 43%, from 15 to 8.5 beats/sec. The study demonstrates washing alone was slightly effective in reversing the neurotoxic effects of Mn on the oysters' DA system. It is possible a longer or more intensive (changing the water several times a day) wash period may be more effective. We plan to continue the study by increasing the washing protocol. These findings are helpful in furthering the understanding of the mechanism of Mn neurotoxicity and demonstrates bivalve gill is a useful model to study regulatory mechanisms of cilia activity as well as the pharmacology of drugs affecting biogenic amines in nervous systems.

This work was supported in part by grants 2R25GM06003 of the Bridge Program of NIGMS, 0537231071 of the CSTEP Program of NYSED, P120A210054 of the MSEIP Program of the DoEd, and NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University.

SEA VEGETABLES ALLIANCE: SUGAR KELP (*Saccharina latissima*) FERTILIZER FOR *Salicornia* PRODUCTION

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Halophytes' cultivation is in its infancy, and there are many baby steps until it can be quantified as another category of sea vegetables together with seaweed. Seaweed Liquid Fertilizer (SLF) is an eco-friendly option for nutritional supplementation of vascular plants. During sugar kelp (*Saccharina latissima*) harvest, the lower parts (*i.e.* stipes and holdfasts) are left behind for a possible regrowth, but it is not known whether this biomass could be an alternative fertilizer or whether only the upper parts (*i.e.* blades) are beneficial for plant growth. *Salicornia* is the most promising halophytic genus for Marine Aquaculture, and *Salicornia depressa* is a spread species with no cultivation restrictions in New England. Our aim was to identify if sugar kelp fertilizer from distinct parts and concentrations might improve the agronomical features of *S. depressa* for the grow-out in hydroponics systems. In June, sugar kelp was harvested from the Integrated Multi-Trophic Aquaculture (IMTA) site of University of New Hampshire (UNH) and processed by filtration to produce a SLF (plus a preservative). *Salicornia depressa* was collected in a salt marsh (Research license RES 7403; Department of Natural and Cultural Resources Division of Forests and Lands), cultivated in covered Erlenmeyers with full Hoagland solution plus sugar kelp liquid fertilizer and placed in two Percival growth chambers (rotation every 3 days; Figure 1). Our experimental design was bi-factorial: seaweed parts (upper vs. lower) and seaweed concentrations (0, 0.5, 1 and 2%), five replications per treatment. Parameters were analyzed by Analysis of Variance (ANOVA). From all the agronomical features measured for *S. depressa*, root length was significantly affected by sugar kelp fertilizer. This shows that all the parts of sugar kelp for liquid fertilizer can reduce the root length of *S. depressa* in contrast with the control (Hoagland solution; 0% seaweed fertilizer). Our findings evidenced that sugar kelp liquid fertilizer did not enhance the growth, biomass or minimized the reproduction (*i.e.* flowering) of *Salicornia*. However, any sugar kelp part as liquid fertilizer might be used for *Salicornia* cultivation in hydroponics units to avoid root entanglement, for example in Nutrient Film Technique (NFT) system, without affecting commercial traits.

FIGURE 1. *Salicornia depressa* cultivated in controlled condition

A SHELL SHOCKING STORY: SOFT SHELL CLAMS *Mya arenaria* IN THE CHESAPEAKE BAY

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Soft shell clams (*Mya arenaria*) have historically played key ecological roles in the Chesapeake Bay, notably as biofilters, sediment burrowers, and significant prey items. Unfortunately, their natural populations have been severely depleted, which could be due, in part, to diseases caused by parasites and cancers. In response to this depletion, strategic efforts to culture *Mya arenaria* have emerged, like at the Patuxent Environmental and Aquatic Research Laboratory at Morgan State University. Introducing the culturing of *M. arenaria* in Maryland holds the potential for significant expansion of the shellfish industry. The inclusion of *M. arenaria* as a cultured shellfish species could contribute to the economic value of the region, but the current disease threats are not well understood.

This research focuses on examining the parasites, pathogens, and transmissible cancers affecting 1-year-old hatchery-grown ("young") and wild 2-year-old ("adult") *M. arenaria*. Clams were assessed for the prevalence and intensity of *Perkinsus* spp., *Vibrio parahaemolyticus,* and Disseminated Neoplasia (DN).

The study involved a 9-week outplant experiment, initiated in April 2024, across three distinct rivers within the Chesapeake Bay, MD. This approach tested how environmental conditions (water temperature, salinity, and dissolved oxygen), along with different life stages, affect infection levels in *M. arenaria*. Preliminary findings indicate differences in mortality and infection levels across the study sites and life stages. Mortality was higher in the Chester River, which had elevated water temperatures and lower salinity (Figure 1). There were great differences in disease impacts between young and adult *M. arenaria*, based on infection prevalence and intensity of *Perkinsus* spp. and *Vibrio parahaemolyticus*. Disease patterns also varied across sample sites. Further analysis of these results is ongoing, including testing site and age patterns for DN, characterizing *Vibrio* toxicity, and comparing overall disease impacts across sites.

This research provides a comprehensive, up-to-date analysis of diseases affecting *M. arenaria*, with the goal of supporting the potential introduction into the aquaculture industry.

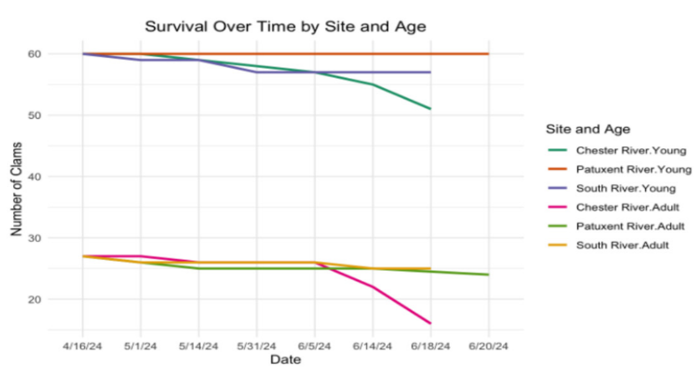

Figure 1: Survival of *M. arenaria* over time, comparing young and adult clams across three study sites. The warm colors represent the young clams, with each site starting with 60 individuals. The cooler colors represent the adult clams, with each site starting with 27 individuals.

EFFECT OF STRAIN VARIATION ON GROWTH PERFORMANCE AND BODY PROXIMATE COMPOSITION OF THREE STRAINS OF *Oreochromis niloticus* L., (1758) UNDER GREENHOUSE CONDITION

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Growth performance and proximate body composition of *Oreochromis niloticus* strains from different Lakes of Ethiopia were evaluated to determine the effect of strain variation on growth and body composition and further select the best performing strain for aquaculture development. Fingerlings of average weight 4.4 g were stocked at a stocking density of 20 fish/m^3 in tanks (1.2 m^3 each) in three treatments with four replicates each. Treatment groups were fed formulated feed with 30% crude protein at a rate of 5% body weight per day. Length and weight measurements were taken on monthly basis. Nutrient content was chemically determined following the procedure of Association of Official Analytical Chemists at the end of the experiment. Growth performance parameters, feed conversion ratio, body proximate composition (except carbohydrate) and survival rate were not significantly different ($p>0.05$) among the strains tested (Table 1 and 2). Generally, based on the current finding, strains of *O. niloticus* either from Lake Chamo, Babogaya or Hashengie can be used for stoking activities and further experiment, with further researches recommended to be conducted in this regard with more strains included.

Table 1: Growth parameters, feed conversion ratio, Fulton's condition factor and survival rate of O. niloticus strains cultured in tanks under greenhouse condition (mean ± standard error)

	Strains		
Parameters	Chamo	Babogaya	Hashengie
Initial weight (g)	4.4±0.2^a	4.4±0.2^a	4.4±0.1^a
Final weight (g)	37.2±2.6^a	37.6±2.1^a	33.3±2.8^a
Initial length (cm)	5.6±0.07^a	5.9±0.06^a	5.9±0.04^a
Final length (cm)	12.6±0.3^a	12.6±0.2^a	11.2±0.3^a
Weight gain (g)	32.8±2.5^a	33.2±2^a	28.9±2.8^a
Daily weight gain (g/fish/day)	0.18±0.01^a	0.18±0.01^a	0.16±0.02^a
Specific growth rate (%per day)	1.1± 0.03^a	1.1±0.02^a	1.0±0.05^a
Feed conversion ratio	4.7±0.14^a	4.6±0.32^a	4.7±0.24^a
Fulton condition factor	1.8±0.04^a	1.9±0.02^a	2.2±0.05^b
Survival rate (%)	100±0.0	100±0.0	84±3.25

Mean in the same raw with similar superscript are not significantly different (p>0.05)

Table 2: Percentage proximate body composition and gross energy content in kcal /100 g of Nile tilapia fillet in wet basis

	Parameters					
Strain	Moisture	Crude protein	Crude Fat	Ash	Carbohydrate	GE (Kcal/100g)
Chamo	78.0±0.06^a	18.2±0.18^a	1.53±0.0^a	1.27±0.1^a	1.0±0.1^a	90.6±0.8^a
Babogaya	78.1±0.06^a	18.6±0.08^a	1.26±0.1^a	1.20±0.1^a	0.84±0.1^a	89.1±0.9^a
Hashengie	77.8±0.23^a	17.6±0.25^a	1.20±0.1^a	1.33±0.1^a	2.07±0.1^b	89.5±0.2^a

Mean in the same column with similar superscript are not significantly different (p>0.05)

UNLOCKING COMMERCIAL SCALE GENOME EDITING

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Genome editing technologies hold transformative potential for aquaculture by enabling precise and efficient modifications to key traits, thereby driving advancements in commercial-scale production. These technologies offer unique opportunities to enhance economically valuable characteristics, including sterility, growth rate, and thermal tolerance, which are crucial for addressing challenges in sustainable aquaculture. However, achieving large-scale adoption requires overcoming significant technical, regulatory, and societal barriers. Here, we present results demonstrating high-throughput microinjection delivered genome editing in tilapia, achieving over 95% editing efficiency in somatic cells across thousands of embryos per day. Additionally, we showcase methods to utilize genome editing for the mass production of sterile fish, directly addressing environmental, regulatory, and societal concerns about genetically engineered organisms. This work highlights recent progress, innovative strategies for scaling genome editing, and the critical role of interdisciplinary collaboration in unlocking its full potential, ultimately fostering a more sustainable and resilient aquaculture industry.

IMPACT OF DIETARY MICROPLASTIC CONTAMINATION ON SURVIVAL, GROWTH PERFORMANCE, AND HEAT SHOCK TOLERANCE IN JUVENILE YELLOW PERCH (*Perca flavescens*)

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Microplastics (MP), defined as plastic particles < 5 mm in one dimension, are emerging contaminants that threaten aquatic organisms. The impacts of MP on fish are shown to vary across studies, influenced by factors such as fish species, type and size of microplastics, and exposure levels and routes. The objective of this study was to investigate the effects of high-density polyethylene microplastics (HDPE-MP) at sizes of 45 µm and 125 µm on juvenile yellow perch, an ecologically important species in the Laurentian Great Lakes and favored for human consumption. A five-week feeding trial was conducted to test four diets containing either 1% or 2% of each size of HDPE-MP, alongside a control diet without added HDPE-MP. Each treatment contains three replicates with 75 fish (30-day post hatch, 49 mg/fish) per replicate raised in an indoor flow-through water system. Water temperatures was maintained between 20-22°C. Results showed that fish survival was unaffected by the MP contaminated diets under the current conditions. However, weight gain decreased with increasing levels of HDPE-MP in the test diets, and fish fed 45 µm HDPE-MP showed lower growth compared to those fed 125 µm at the same concentration. Fasting blood glucose levels were higher in fish consuming the 125 µm HDPE-MP compared to those on the control diet. Prior to heat shock, blood glucose levels were similar across all treatments. Heat shock significantly elevated blood glucose levels in fish across all diets. This preliminary study suggests that smaller MP negatively impact fish growth more significantly when compared to larger MP, while larger MP may disrupt blood glucose homeostasis and heat shock responses. The underlying mechanisms warrant further investigation.

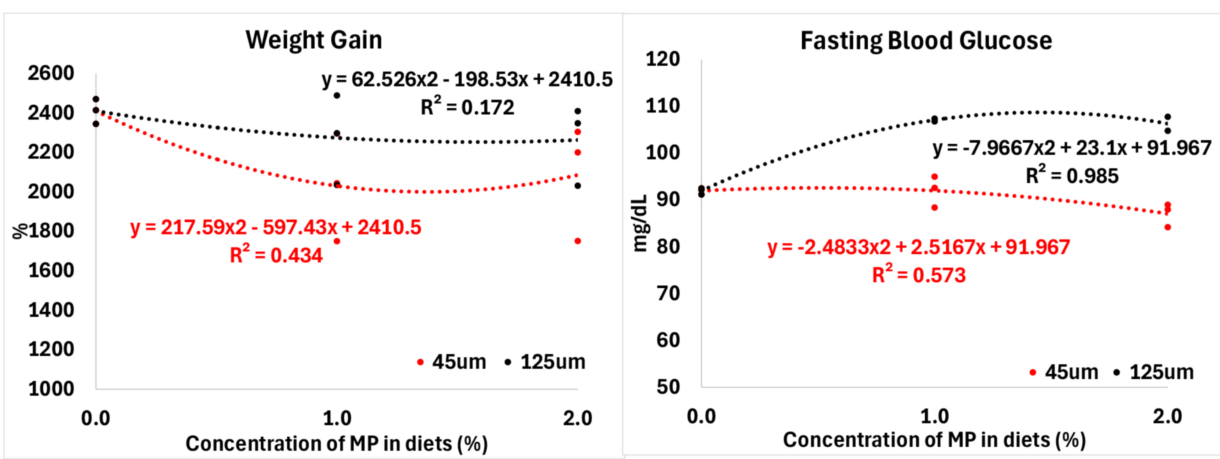

Figure 1. Growth and fasting blood glucose in yellow perch after feeding diets contaminated with different sizes and concentrations of HDPE-MP for 5 weeks.

ISOLATION AND CHARACTERIZATION OF *Vibrio parahaemolyticus* BACTERIOPHAGES FROM OYSTERS

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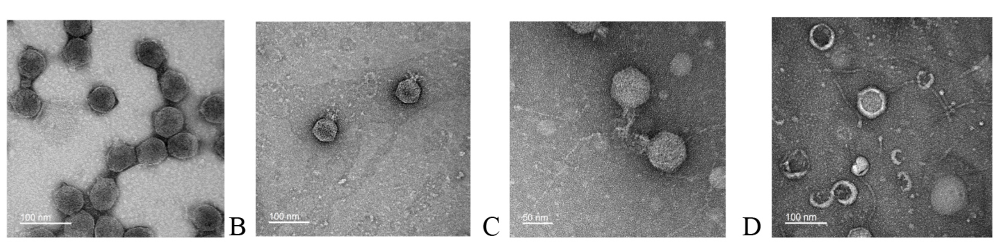
Vibrio parahaemolyticus (VP) is a bacterial pathogen found in brackish and marine water that infects many marine organisms, like oysters and shrimp. Consumption of raw or undercooked seafood contaminated with VP is a primary cause of seafood-borne gastroenteritis in humans. Due to increasing ocean temperatures, VP contamination of oyster beds in the US has spread from around the Gulf of Mexico up the east and west coasts to the northern-most states. Promising new research is exploring the isolation of bacteriophages against VP with a long-term goal to possibly decontaminate oyster beds in order to expand the harvest season and allow for safer consumption of seafood.

Store-bought oysters harvested from the Chesapeake Bay in VA, were used to isolate four bacteriophages. A standard double agar overlay plaque assay was used to identify phage activity. After phage isolation, the genomes were sequenced, and transmission electron microscopy was completed (Figure 1). The genomes and TEM images show four distinct phages. Two of the phages have podovirus-like morphology with shorter tails and approximately 60 predicted proteins, one phage has siphovirus-like morphology with about 45 predicted proteins, and the 4th phage is a mid-sized tailed phage with 100 predicted proteins.

Plaque assays were carried out with the 4 bacteriophages and up to 10 different VP strains. (Table 1). CREW showed the widest host range and was capable of lysing 3 different VP strains. Future work will be necessary to determine the viability of using the bacteriophages for elimination of VP in harvested oysters and/or the environment.

Table 1: *Vibrio* Bacteriophage Host range trials with various *Vibrio parahaemolyticus* strains

	Bacteriophage			
VP Strains	**Smiley**	**Crew**	**OYD**	**REJA**
G12408	Plaques	Plaques	Plaques	Plaques
V1B373	No Plaques	No Plaques	No Plaques	No Plaques
MAVP-26	No Plaques	No Plaques	No Plaques	No Plaques
V1B389	No Plaques	No Plaques	No Plaques	No Plaques
RIMD		Plaques		No Plaques
CT4291	No Plaques	Plaques	No Plaques	Plaques
MAVP-K	No Plaques		No Plaques	No Plaques
G13119	No Plaques		No Plaques	No Plaques
LM				No Plaques
V1B374	No Plaques		No Plaques	No Plaques



A B C D

Figure 1: Transmission Electron Microscopy of *Vibrio parahaemolyticus* bacteriophages. (A) CREW; (B) OYD; (C) SMILEY; (D) REJA.

EXPANSION OF THE REGIONAL SHELLFISH SEED BIOSECURITY PROGRAM (RSSBP) UNDER NOAA SEA GRANT HUBS

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Shellfish seed transfers are a major concern for shellfish farmers, fishers, and resource managers worldwide because of the pathogens they may carry. Developing, understanding, following, and enforcing regulations can be a big impediment for all interested parties. The RSSBP was created to assist all parties involved with permitting transfers of bivalve shellfish seed for commerce, restoration and enhancement. The goal is to incentivize the movement of the most biosecure bivalve shellfish, namely larvae and young seed before they have had much opportunity to contract parasites and pathogens. The Core Elements of the RSSBP provide a science-based pathway to improved biosecurity with respect to pathogens that afflict shellfish.

With support from the NOAA Sea Grant Aquaculture HUBS program, the RSSBP has expanded to the Gulf of Mexico. ZOOM® meetings with representatives from each state agency were key to providing proper extension and outreach to the regulatory community while the Oyster South Symposium has provided a broader outreach effort to all key stakeholders as shellfish aquaculture develops along the northern Gulf of Mexico. Louisiana has integrated the RSSBP into their seed importation/transfer request policies as Mississippi and Alabama contemplate similar language. Texas and Florida have regulations that strictly regulate genetics which limits movements to stocks originating from within their respective state yet consider the program as a positive move towards biosecurity of disease during shellfish transfers.

More information is available at http://rssbp.org as the program continues to grow and mature with support from NOAA Aquaculture and USDA APHIS.

PROJECT AQUAGRID: DEMONTRATING OCEAN ENERGY'S CONTRIBUTION IN ACEELERATING THE DECARBONISATION OF AQUACULTURE'S ENERGY REQUIREMENTS

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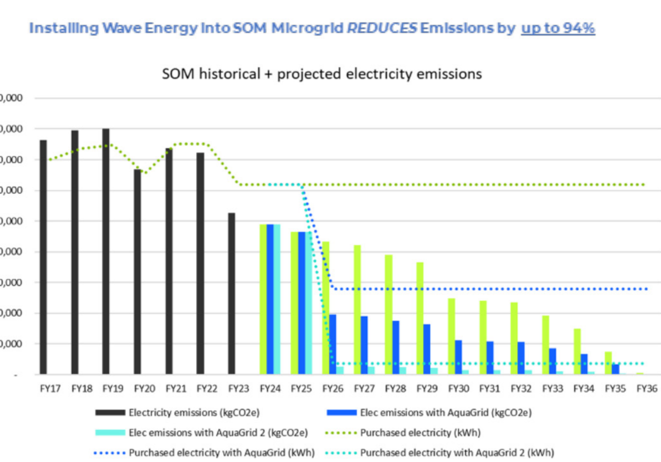
The Aquaculture sector is in a period of disruption, diversification and growth. Marine based aquaculture operators are largely reliant on diesel power generation while shore-based facilities like hatcheries, recirculating, and processing facilities use increasingly volatile and expensive grid supplied electricity, often with diesel powered backup.

Producers are facing increasing regulatory, stakeholder, and market driven pressures to respond to sustainability issues such as reducing emissions and lowering carbon footprints. Securing sources of reliable, affordable, and low risk clean energy is paramount for the future prosperity of the sector in the face of these escalating pressures, identifying pathways to decarbonise operations and supply chains, can facilitate market access (i.e. EU), provide carbon credits and receive price premiums for qualified sustainable products.

Project AquaGrid - Australia's Fisheries Research and Development Corporation (FRDC) requested the development of a scalable alternative energy solution to strengthen resilience in a changing climate; and identify novel decarbonisation pathways. Project AquaGrid was selected as an early mover project within Seafood Industry Australia's (SIA's) overarching 3-year aquaculture decarbonisation program.

The project analysed, modelled and documented the energy requirements of Southern Ocean Mariculture, an abalone producer in Victoria, Australia. Their aim - facilitate a resilient, reliable, cost effective and secure off grid (or with grid redundancy) energy system, to meet their emission reduction (94% reduction achieved), and business growth targets. Modelling successfully looked beyond their existing onsite solar (PV) array, to design and model a microgrid system that met these aims resulting in project implementation.

1 Azura Ocean Technology, Hamilton VIC, Australia
2 Australian Ocean Energy Group, Sydney NSW, Australia
3 Deloitte Australia, Melbourne VIC, Australia
4 Syncline Energy, Melbourne VIC, Australia
5 Climate Kic Australia, Albany WA, Australia
6 Southern Ocean Mariculture, Port Fairy, Victoria, Australia



EFFECTS OF MULTIPLE COASTAL STRESSORS ON EARLY LIFE-STAGE EASTERN OYSTERS FROM NARRAGANSETT BAY

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Coastal ecosystems worldwide are facing an increasing number of anthropogenic stressors, and yet concurrent and cumulative impacts of multiple stressors remain relatively unknown, especially across critical early life stages. Two prevalent and often coupled stressors in shallow coastal waters are hypoxia, or low dissolved oxygen (LO), and coastal acidification (CA), which are driven by diel cycles. Pulses of freshwater are also observed, causing short-term low salinity (LS) conditions and altering carbonate chemistry. Global climate change is expected to alter salinity regimes in estuaries and intensify the magnitude and duration of both LO and CA events.

Here we investigated the responses of larval and juvenile Eastern oysters *Crassostrea virginica* exposed to four different treatment levels: control, combined LO and CA (1 mgO_2/L and 7.0 pH units), LS (10 ppt), and interaction LO/CA+LS. First, oyster larvae, obtained from wild populations in Narragansett Bay, have experienced early (pediveliger stage) and late (eyed-pediveliger stage) larval exposure to the four experimental treatments for 24 and 36h, respectively. Second, the newly settled juveniles were exposed to either ambient conditions or diurnal LO/CA cycling for 11 weeks. This long-term juvenile exposure was associated with regular 48h exposures to the previous four treatments, representing constant, short-term and intense LO/CA and LS events. Both larval and juvenile oysters were sampled for growth, survival and genomic analysis. Preliminary results of the experiment will be presented at the meeting. Subsequently, this study will help determine how short- and long-term exposures to multiple stressors during early life-history stages affect the distribution of genomic diversity across Eastern oyster populations.

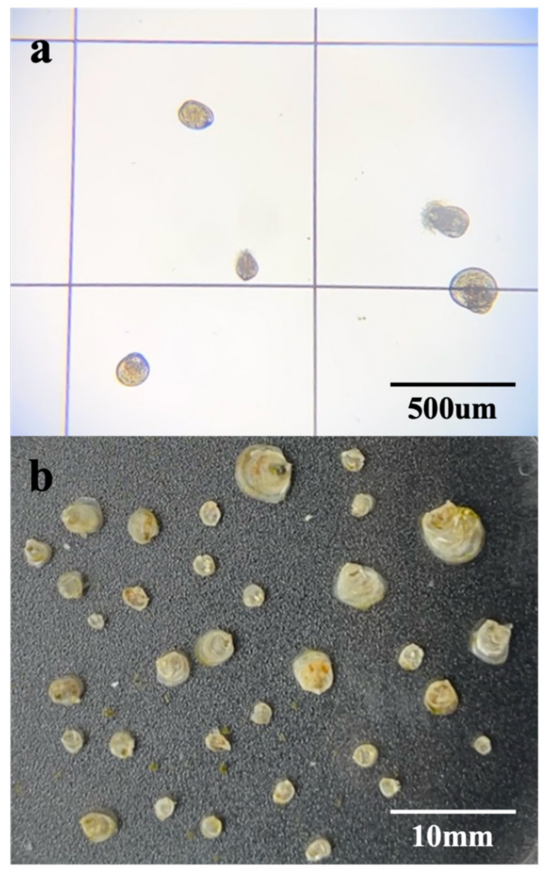

Figure 1. Eastern oysters aged 15 (a) and 114 (b) days post-fertilization under ambient conditions.

SCIENCE-BASED SOLUTIONS TO OPTIMIZE SABLEFISH (BLACK COD) AQUACULTURE TECHNOLOGY

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Sablefish, *Anoplopoma fimbria*, are a high-value species that is farmed to a limited extent in the US and Canada. NOAA's Northwest Fisheries Science Center has been working to optimize culture methods and develop tools and strategies to minimize disease-related impacts. Early work with sablefish addressed all life stages and methods for spawning, egg incubation, larval weaning, and grow-out to market size. Current efforts are focused on optimizing such methods and refining technologies to enhance growth, survival, and reproductive efficiency of broodstock. Specific research efforts linked to improved growth and enhanced survival will be highlighted in this talk. Since sablefish exhibit sexually dimorphic growth, the production of monosex all-female stocks to capitalize on their faster growth has been a high priority. Several studies found that sex differentiation of XX-genotype fish could be redirected towards testicular development instead of ovarian development using dietary 17-alpha methyltestosterone (MT) treatment. This was utilized to produce neomale (i.e., XX-genotype male) F1 broodstock that could be bred with normal female broodstock. The offspring from neomale x female crosses are therefore 100% female. This method is now used routinely, and semi-commercial scale trials have demonstrated the importance of all-female monosex breeding technology to the economics of sablefish aquaculture.

Another bottleneck for this species involves the disease furunculosis, which is caused by an atypical strain of the bacterium *Aeromonas salmonicida*. Although antibiotic treatments can be administered following an outbreak, their effectiveness is limited, and disease prevention through vaccination is desirable. This has been identified as a high-priority need for sablefish aquaculture and the NWFSC has initiated several vaccination projects to explore new vaccine development (e.g. attenuated bacterins), practical delivery strategies (i.e. oral and immersion administration), and assess adjuvant effects when using more traditional vaccine formulations. Progress on the selection of attenuated vaccine candidates will be discussed along with results from the adjuvant study, which showed that protection could be achieved by combining immersion vaccination with an injection booster. This resulted in high antibody titers in the adjuvant groups and strong long-term protection in all vaccinated groups. Production level vaccination using the same approach appears successful as no furunculosis-related mortality has occurred in fish reared to market size in both net-pen and land-based tank systems. Although efforts to eliminate the need for injection delivery are continuing, the above protocol currently represents the most practical disease prevention strategy for furunculosis in sablefish. These advances in culture technologies emphasize the potential for continued expansion of commercial sablefish aquaculture.

EFFECTS OF DIFFERENT pH LEVELS DURING AEROBIC MINERALIZATION OF FISH EFFLUENT ON NUTRIENT RECOVERY

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In aquaponic systems, fish are used to produce nutrients utilized by plants. While the majority of these nutrients are dissolved in the water, a significant portion of fish waste is solid and the nutrients within are unable to be utilized by plants. Mineralization is a process where microbes break down solid waste, releasing nutrients in a recoverable form for plants. This approach not only supports sustainable aquaponic practices but could also reduce costs on external nutrient inputs. While research on nutrient recovery using mineralization has been conducted, there is little information on low cost, low input mineralization methods. This study aimed to compare the effects of pH levels 5, 7, and 9 on the mineralization process in aerobic conditions using simple aeration and fish effluent from Nile tilapia (*Oreochromis niloticus*).

This study utilized 12 replicated aerobic systems, with four systems allocated to each of the three pH treatments (5, 7, and 9). The experiment was conducted over a 21-day period, during which the systems remained closed except for daily water parameter checks, pH adjustments, and comprehensive water quality tests. The parameters of interest were TAN, NO□, NO□, alkalinity, iron, potassium, magnesium, and phosphorus.

There were few differences in recovery between treatments that were not due to amendments to control pH. The large differences in K and Alkalinity between treatments were due to the addition of potassium hydroxide to maintain pH levels. When these additions were controlled for, it was calculated that there was K loss at pH 9. Phosphate loss was greatest at pH 9, possibly binding with calcium and potassium and falling out of suspension. Nitrate, Fe, and Mg recovery occurred at all pH levels, and no significant difference was detected between treatments.

While there were no significant differences found, mineralizing at a pH around 7 offers several advantages. First, constant additions were required to keep pH at 5 or 9. Second, many nutrients precipitate and fall out of suspension at pH 9, leading them to be unavailable for plants. Further research will examine mineralization at different oxygen levels and over longer periods of time.

pH	TAN	NO_2	NO_3	ALK	Fe	K	Mg	P
Five	0.01	0.01	0.2	-60.3^{b}	0.62	5.6^{a}	17.2	-6.9^{a}
Seven	0.01	0.00	4.3	-100.8^{b}	0.14	14.1^{a}	14.6	-7.0^{a}
Nine	0.01	0.01	2.5	16.0^{a}	0.38	-84.5^{b}	13.8	-11.9^{b}

Table 1: Total nutrient recovery or loss during mineralization at pH 5,7 and 9. All units in mg/L.

EVALUATION OF GENETIC DIVERSITY IN PACIFIC OYSTERS FROM THE US WEST COAST USING WHOLE-GENOME RE-SEQUENCING

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The genetic diversity of a population defines its ability to adapt to episodic and fluctuating environmental changes. For species of agricultural value, available genetic diversity also determines their breeding potential. Comprehensive knowledge of the extant gene pool in Pacific oysters (*Magallana gigas*, previously *Crassostrea gigas*) remains fundamental to the development of practices that maintain health and productivity in this species. In this study we use whole genome resequencing to evaluate the genetic diversity within and between naturalized and captively reared Pacific oyster populations from in the US Pacific coast. The analyses included individuals from preserved samples dating to 1998 (Dabob Bay; a.k.a. MBP6) and 2004 (Midori). Two contemporary naturalized populations (Willapa Bay and San Diego Bay), and one domesticated population (MBP30) were also included. We show that despite high overall heterozygosity, there was extremely low genetic divergence between populations. The MBP30 population which was reared in captivity for over 25 years was the most genetically distinct population and exhibited reduced nucleotide diversity, attributable to inbreeding. Fifty-nine significant F_{ST} outlier sites were identified, the majority of which were individually present in high proportions of the MBP30 samples, and which are possibly associated with domestication. Our results show that Pacific oysters in the US Pacific coast may hold enough genetic diversity to sustain health and efficient commercial productivity, but captive populations need to be actively managed to prevent inbreeding depression.

META-ANALYSES REVEAL MICROBIAL BIOMARKERS TO MONITOR THE HEALTH OF PENAEIDAE LARVAE IN HATCHERIES IN NEW-CALEDONIA

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In New-Caledonia, *Penaeus stylirostris* is farmed in a semi-intensive mode and is one of the main economic inputs for the territory. However, for the last decades, this sector has been facing huge larval mortalities occurring at all larval stages in hatcheries, for which no causes have yet been found. Microbial dysbiosis of the rearing water and/or of the larvae are suggested as factors leading to larval death. Indeed, aquacultured animals are reared in water hosting various microorganisms with which they are constantly in close relationships. Microbial exchanges between the animal and the water can occur but little is known about the interactions between the shrimp larvae and their environment, especially in shrimp hatcheries Thus, detecting stage specific biomarkers of healthy and unhealthy larvae for later developing monitoring tools of the rearing might be useful to help the farmers.

To highlight specific microbial lineages and larvae-associated biomarkers we have studied the daily microbial compositions of larvae reared in different conditions along with the storage waters. Illumina sequencing of the V4 region of the 16S rRNA gene coupled to zootechnical parameters and statistical analyses allowed us to link microbial lineages and biomarkers to a given larval stage and mortality rate.

Our results underline that the active microbiota associated with the larvae was highly dynamic through the rearing. Deeper analysis exhibited that various active lineages were specifically associated with a given larval stage and survival rate, while several of them were shared between various conditions (Figure 1). When these taxa were compared to the storage waters microbiota, many of them were previously detected in the natural seawater, emphasizing the great role of the natural seawater on the larval microbiota. The biomarker exploration allowed to identify microbial genera that were specific of healthy or unhealthy larvae; biomarkers that might be used as monitoring tool to survey the larval health and to predict the fate of future rearing.

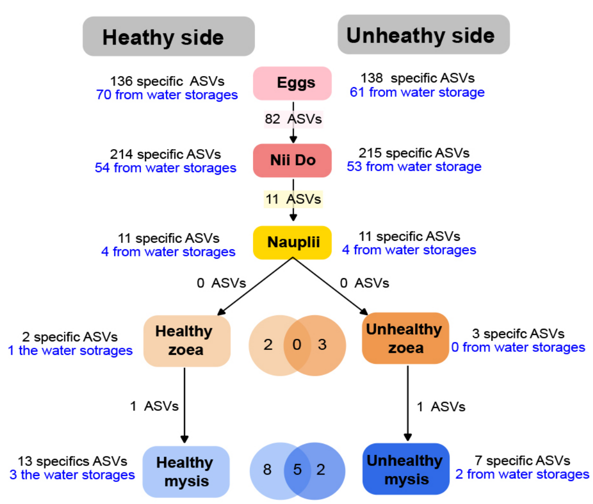

Figure 1 : Shared and specific bacterial genera from the larvae according to their stage and survival. Healthy stands for good survival rate and unhealthy for high mortality rate.

AQUACULTURE TECHNIQUES AND EARLY LIFE HISTORY OF THE HAWAIIAN ENDEMIC POTTER'S ANGELFISH (*Centropyge potteri*)

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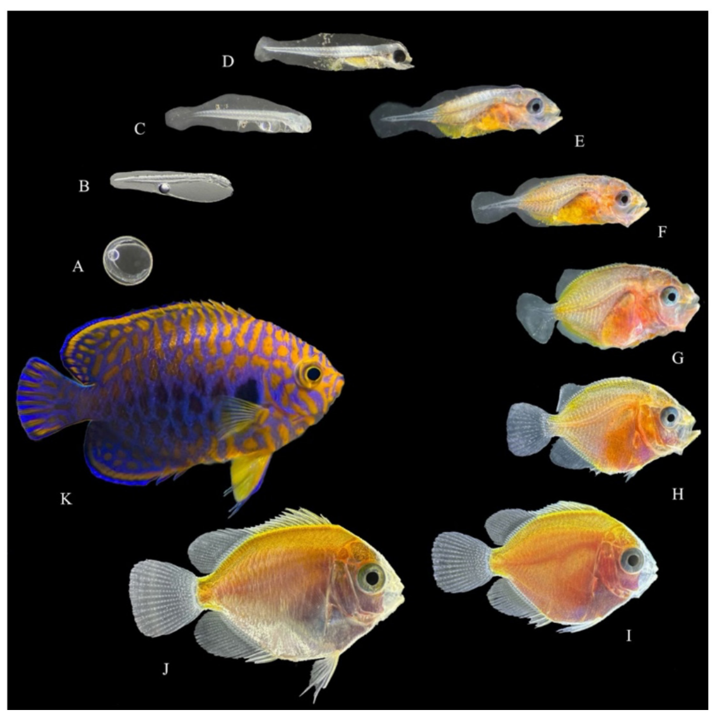
The Potter's angelfish (*Centropyge potteri*) is a striking reef fish endemic to the Hawaiian archipelago, highly sought after by aquarists for its vibrant colors and unique characteristics. This research documents the first commercial aquaculture success and larval growth of Potter's angelfish, providing critical data for future larviculture research of this species and other reef fishes. By iteratively optimizing larval feeding regimes, the project enhanced the survival rates of Potter's angelfish larvae, monitoring morphometric data and survival rates. Larval development closely followed other *Centropyge* species, with flexion beginning between 14-17 days post hatch (dph) and completed by 21 dph followed by settlement behaviors observed at ~60 dph. Survival rates to settlement, varying between 0% and 0.6%, were critically influenced by strategic adjustments in feeding schedules to mitigate acute mortality points.

Spawning was observed in captivity following lunar cycles, with broodstock producing viable eggs nightly from December 2022 to May 2024. Larval rearing trials examined six feeding protocols across various tank volumes, stocking densities, and diet compositions.

Early developmental stages were characterized, revealing critical points for feed transitions and vulnerabilities. Flexion began at 14–17 dph, with post-flexion larvae exhibiting increased body depth and pigmentation. The study observed consistent mortality events during feed transitions, particularly with newly hatched Artemia and dry feeds. These events were mitigated by delayed introductions of Artemia, reducing mortality rates linked to digestive challenges in pre-flexion larvae.

Adult copepods and continuous live algae proved essential for supporting larval development. Larger tanks (1000 L) improved larval survival and reduced stress events compared to smaller volumes (200 L), likely due to stable environmental conditions. Protocol adjustments informed by these findings underscore the importance of diet composition and environmental stability in improving larval survival rates. Future research should explore pathogen control and further optimize diet transitions to advance the commercial viability of *C. potteri* aquaculture. This work has broader implications for sustainable aquarium trade practices, contributing to reef conservation efforts by reducing wild capture pressures on wild populations.



AN UPDATE ON DELAWARE'S PILOT SHELLFISH HATCHERY

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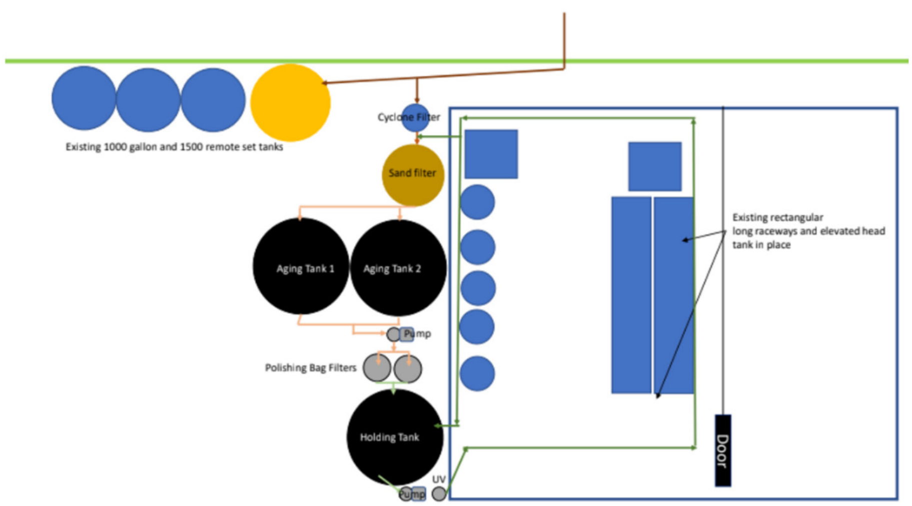
Oyster farming is a new industry in Delaware, with the legislation to permit shellfish aquaculture passing in 2012, the first leases distributed in 2017, and the first cultured oyster sent to market in 2018. Despite initial interest, industry growth has been slow due to factors such as the COVID-19 pandemic, financial and regulatory barriers, and a lack of seed supply. In 2022, the University of Delaware in collaboration with Delaware State University and Delaware Sea Grant established the state's first industry-serving oyster hatchery to provide larvae, seed, and remotely set spat on shell to Delaware growers and restoration projects. Our goal was to address the seed supply barrier. Our hatchery utilizes an existing 800 sq ft building on the University of Delaware, Hugh R. Sharp campus in Lewes, DE at the mouth of Delaware Bay. This facility, equipped with seawater intake pumps, a multistage seawater filtration system, algae grow-out, broodstock conditioning system, larval tanks, and outdoor nursery has the production capacity of up to 50 million eyed larvae per year once optimized. Our small-scale facility is proof of concept toward a larger, industrial-scale facility that can better serve Delaware growers and beyond. The pilot hatchery also serves as a research and development space and a demonstration space for extension purposes. In the 2023 inaugural season, we successfully supplied 105,000 remotely set spat-on shells to a commercial grower in Delaware Bay while providing educational tours to approximately 50 individuals. In 2024, we successfully supplied 650,000 seed oysters to eight Rehoboth Bay growers and 460,000 remotely set spat-on shell to four Delaware Bay growers along with touring approximately 100 individuals. In the pilot phase, the hatchery aims to transfer 1 million seed oysters to Delaware growers annually. Though eastern oysters are the main focus, the hatchery aims to also produce hard clams and scallops as the operation develops.



EVALUATION OF BLACK SOLDIER FLY LARVAE *Hermetia illucens* AS FEED ADDITIVE THROUGH SALMONID SHK-1 CELL LINES: POLYPHENOL ACCUMULATION, ANTIOXIDANT RESPONSE, AND CELLULAR PROLIFERATION

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This study evaluated the accumulation of polyphenols of black soldier fly larvae (BSFL) fed different organic wastes commercially produced and evaluated those larvae as feed additive through cellular antioxidant response and proliferation of Chinook salmon cell lines (SHK-1). The highest polyphenol concentration was achieved by larvae fed plant-based feeds, while the lowest concentration by those fed animal-based feeds. Cell lines analysis similarly revealed that larvae fed plant-based feeds significantly enhanced antioxidant response and cellular proliferation, with comparable results to the protective effects of Vitamin C. These results highlight that BSFL can be fed specific waste streams to produce a functional feed additive with bioactive properties that may enhance animal health through cellular antioxidation and proliferation.

Currently, insect meal is not able to partially or completely replace fish or soybean meal due to its market price, nutritional profile, and volume of production. This study fed BSFL five different waste streams locally and commercially produced: Kitchen Waste, Agricultural Waste, Aquaculture Sludge, Aquaculture Offal, and Mix. Larval rearing parameters such as biomass gain, survival, feed efficiency ratio, bioconversion, and nutritional profile were collected and analyzed. To further investigate the produced raw materials, total polyphenol concentration (Folin-Ciocalteu method) was evaluated, as well as using immortalized salmonid cell lines (SHK-1) to assess antioxidant response and cellular proliferation. Between larval rearing, polyphenols, antioxidant response, and cellular proliferation, clear significant differences were observed between plant and animal-based feeds, with the former showing better results. This novel and sustainable raw material could fall into the additive niche by providing antioxidation and proliferation effects.

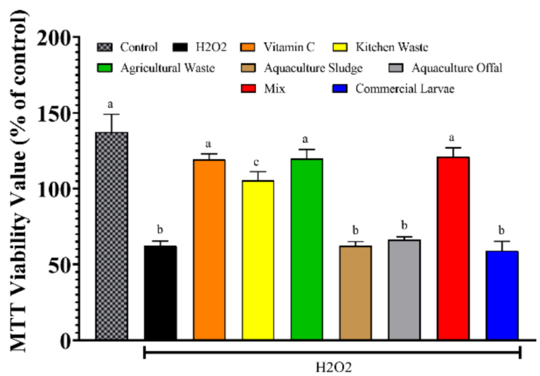

Figure 1. Antioxidant response of black soldier larvae reared on selected feed treatments in presence of H_2O_2. Data is presented as mean ± SEM, n=4. Same superscript indicates no significant difference ($p > 0.05$).

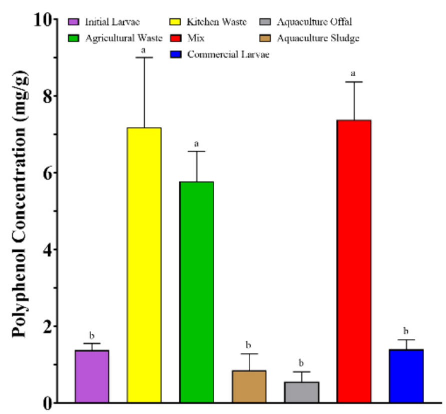

Figure 2. Polyphenol concentration of black soldier fly larvae reared on the selected feed treatments. Data is expressed as mean ± SEM, n=4. Same superscript indicates no significant difference ($p > 0.05$).

STATUS OF THE MANGROVE FORESTS OF TUMBES, PERU IN 2024

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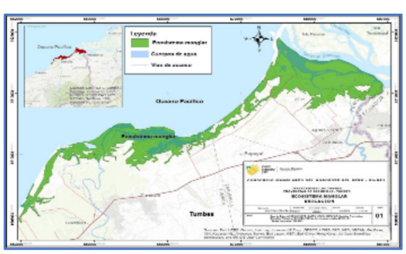
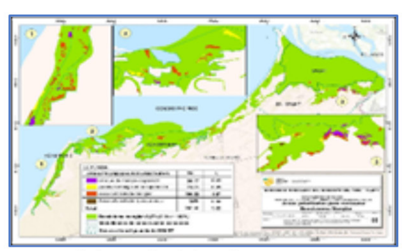
The mangrove ecosystem of Peru is located in the coastal marine zone of the department of Tumbes, on the coast, which ranges from 3°23´22.94» South Latitude on the border with Ecuador (International Canal - Punta de Capones) to 3°34´53.71» South Latitude (Playa Hermosa sector) and between 80°13´15.33" (Border with Ecuador) to 80°32´02.69" (Playa Hermosa sector) West Longitude. It is constituted as the southern limit of the distribution on the Pacific Coast of South America.

The mangrove ecosystem of Tumbes has an area of 8,274.21 hectares, which includes the total area of the Santuario Nacional Los Manglares de Tumbes (Los Manglares de Tumbes National Sanctuary; 2,972 ha), the total of the Áreas de Conservación Ambiental (ACA) (Environmental Conservation Areas (ACA); Manglares del Estero La Chepa-Corrales, 313.54 ha (Mangroves of the La Chepa-Corrales Estuary, 313.54 ha), Manglares Delta del Río Tumbes-Bahía Puerto Pizarro, 1927.84 ha), (the Mangroves of the Tumbes River-Delta-Puerto Pizarro Bay) and other wild areas without conservation status (3,060.83 ha). Part of this area without conservation status (1416.71 ha) is located in the Zona de Amortiguamiento del Santuario Nacional Los Manglares de Tumbes; SNLMT) (Buffer Zone of the National Sanctuary Los Manglares de Tumbes).

The mangrove ecosystem represents 1.78% of the total surface of the department of Tumbes, which has an area of 4,646.67 km2. The main problem of the mangrove forests of Tumbes was the change of use for aquaculture and agricultural activities, which have had a negative impact on the loss of forested areas and the loss of biological diversity. In the upper basin of the Tumbes River, gold mining activity is carried out and in the lower basin agriculture. Lead (Pb) and arsenic (As) heavy metals are detected in the river water that exceed the limits of national legislation. As and Pb are detected in molluscs at levels that do not yet exceed the limits considered to be at risk. Pesticides have not been not analyzed in mollusks and crustaceans because they are expensive. We suggest that (a) the mining activity of the basin in Ecuador make use of clean technologies, (b) that the agricultural activity of Tumbes carry out biological control in replacement of agrochemicals, (c) the shrimp farming activity uses probiotics replacing the antibiotics oxytetracycline, florfenicol, ciprofloxacin, and (d) continue with rehabilitation activities in degraded areas.



Map of the Mangrove Ecosistem in Tumbes, Perú.



Map of the prioritized areas to restore.

STATUS OF THE MANGROVE FORESTS OF TUMBES, PERU IN 2024

Fiorela Campos*, Jorge Echevarria-Velásquez, Jorge Echevarria-Flores, and Acacia Alcivar-Warren

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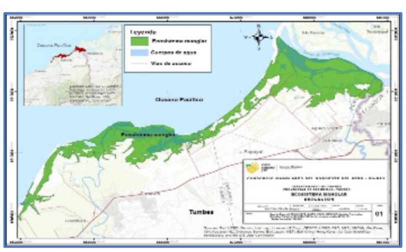
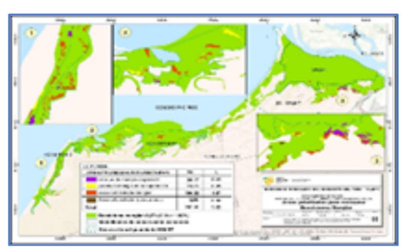


Map of the Mangrove Ecosistem in Tumbes, Perú.



Map of the prioritized areas to restore.

PROPAGATION OF FRESHWATER DRUM *Aplodinotus grunniens* FOR MUSSEL PROPAGATION AND CONSERVATION

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Interest in cultivating freshwater drum (Fig. 1) is growing because they serve as host species for the propagation of various freshwater mussels, including the endangered scaleshell (*Leptodea leptodon*), Texas heelsplitter (*Potamilus amphichaenus*), and Salina mucket (*Potamilus metnecktayi*). While collecting wild-caught fish is an alternative for propagation, it may harm freshwater drum populations and raise disease concerns at propagation facilities. Additionally, wild-caught fish often do not adapt well to laboratory environments, resulting in low survival rates. Despite extensive information on the natural spawning behaviors and life cycle of freshwater drum, there is limited knowledge regarding their propagation in a hatchery setting.

We carried out a range of experiments from 2020 to 2024 using wild-caught fish to propagate drum in both indoor laboratory and earthen pond settings. Research focused on culture methods, feed preferences, gamete assessment, spawning techniques, egg incubation, and larval grow-out trials.

The long-term survival of adult wild-caught fish was suboptimal, with high handling mortality and susceptibility to diseases, particularly *Ichthyophthirius multifiliis*. Furthermore, these fish typically rejected commercial diets and often refused natural foods for long durations. Juvenile wild-caught fish also faced disease susceptibility and had difficulty accepting various food types. However, once acclimated and trained to consume commercial diets, juvenile fish became domesticated and were more tolerant to handling. During the spring, we collected gametes approximately every two weeks to evaluate and stage the eggs for optimal hormone induction timing. Spawning protocols were established using human chorionic gonadotropin (HCG), with dosages and latency periods identified. Eggs were successfully incubated in glass aquariums with added aeration. Hatching began two days post-fertilization at 22°C. Feeding trials tested rotifers, live artemia, and a krill-based commercial diet; however, hatchling survival was 0% across all treatments after two weeks, despite a few fish consuming small amounts of artemia.

In summary, significant progress has been made in developing freshwater drum propagation methods; however, further research is required for consistent propagation in controlled environments to ensure a reliable supply of healthy host fish for successful mussel propagation. Future experiments could integrate spawning in ponds following hormone induction, along with further investigation of effective larval feeding protocols.

Fig. 1. Adult freshwater drum

MASS MORTALITY EVENTS SEVERELY IMPACTING DUTCH MUSSEL PRODUCTION - OVERVIEW AND INVESTIGATION OF POTENTIAL CAUSES

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European mussel production has significantly declined over the last few decades. In the meantime, world aquaculture production is steadily increasing, and low-trophic aquaculture is in high demand as one of the most sustainable protein sources. The decline in European mussel production has several causes; dramatic factors are a decrease in recruitment and the occurrence of high mussel mortalities. Such events have increased in the last decade, particularly in Italy, Spain, France, and the Netherlands. We will present our observations on a series of mass mortality events involving mussels in the Netherlands, similar to those that have been occurring in France since 2014.

Three waves of mass mortality events (MMEs) of blue mussels have occurred in the Netherlands since 2016, all in Oosterschelde Bay, one of the main producing areas for blue mussels. Blue mussels (*Mytilus edulis*) are the country's primary aquaculture product by volume and turnover, making the industry regionally significant. The first MME in 2015/2016 was unprecedented in scale and duration. This was not an isolated incident; a second MME occurred in 2019, followed by a third in 2023/2024. In 2016 and 2019, about 40-60% of mussels died, while in 2024, the estimated mortality reached around 85%.

No single putative cause has been found, but the mussels seem to be in a weakened state and more vulnerable to a variety of stressors. Histological analysis shows a high prevalence of granulomas and granuloma-like structures since 2016 (FIGURE 1). *Francisella halioticida*, which has been identified as a causative agent of francisellosis in mussels, was detected in most mussel stocks, with a higher prevalence in mussels that experienced mortality; however, the role of this bacterium remains unclear. Concurring MMEs of other species in the region, like the European lobster (*Homarus gammarus*) also suggest a wider problem with ecosystem health.

We will conclude with an overview of the research we currently have underway to investigate possible causes and to identify stressors, and with an outline of areas where there may be opportunities for collaboration.

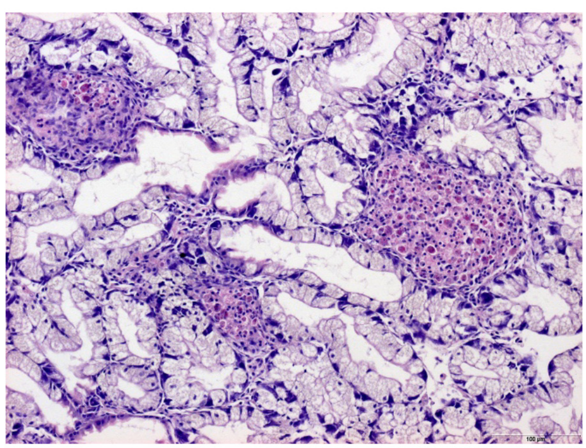

FIGURE 1 Histopathology of granuloma-like lesions in the digestive diverticulum of a mussel.

EFFECT OF THE USE OF NATURA PRO IN DIFFERENT FISH SPECIES: MOVING FROM MEDITERRANEAN AQUACULTURE TO THE REST OF THE WORLD

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Introduction
In 2022, INVE Aquaculture revolutionized the industry with the introduction of Natura pRo and Natura ExL, premium feed lines designed to reduce reliance on rotifers. This innovation results from decades of dedicated research and collaboration with the scientific community to understand marine larvae's physiology and nutritional needs.

By reducing dependency on live prey, we improved the efficiency of larval rearing and create more sustainable practices. This innovation helps hatcheries maintain high standards of fish health and growth while ensuring consistent feed quality.

The development of the Natura feed line involved extensive research and testing. The feeds are designed to be attractive to larvae, with the proper particle size and behaviour in water to encourage capture and ingestion. Be easily digestible and assimilable by the larvae's undeveloped digestive system. To be efficiently convert into energy to support proper growth and survival.

Trials conducted at INVE's research center demonstrated that these feeds could significantly reduce the need for rotifers resulting in a reduction by up to 80% for sea bream and completely for sea bass when using the "Green Water Technique; without compromising the growth or survival rates of the larvae.

Materials and methods
Following the success in European hatcheries (Sea Bass and Sea Bream), the Natura feed strategy was tested on tropical species such as Barramundi, Yellowtail Amberjack, Snapper, and Totoaba. Over the past two years, INVE's Technical Support Team has conducted commercial validation trials, tailoring feeding protocols to specific hatchery needs. This approach has confirmed the versatility and effectiveness of the Natura feed line across different species and hatchery conditions.

In all the experiments the treatments were Control (following the hatchery protocol) vs Natura protocol (developed by INVE TS to reduce Rotifer consumption using Natura).

Data collection and observations were related to Rotifers consumption, survival at first grading, length until first grading, deformity levels and Natura acceptance from first feeding.

Being the tests done in different geographical areas, the parameters of the larval rearing like stocking densities, thermo-photoperiod and grading were moving depending on the area and the fish species.

Results
The results were impressive, showing not only high levels of feed attractivity but also good fry quality, growth and survival rates, reduced cannibalism and better stress resistance compared to traditional feeding protocols.

In Natura protocol Rotifer consumption was reduced by 50 to 74% depending on the fish species, growth and survival were comparable with Control. Fish fed with Natura had similar or even better quality compared with Control.

(Continued on next page)

Chart 1: Rotifer Consumption and Reduction Levels

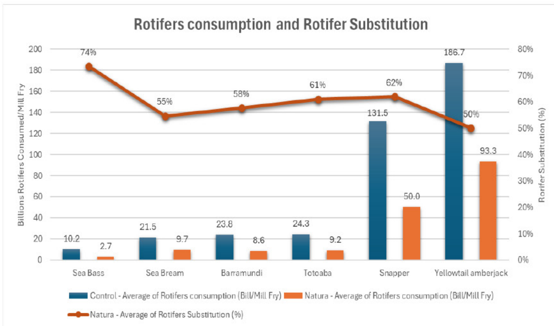


Chart 2: Survival at First Grading

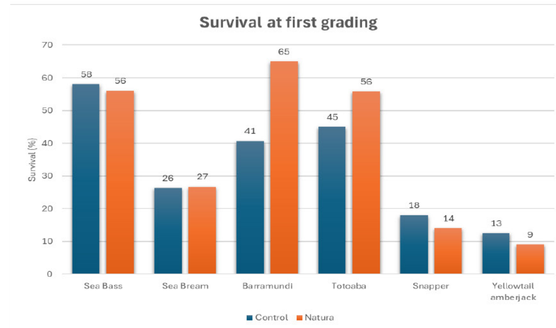


Chart 3: Length at First Grading

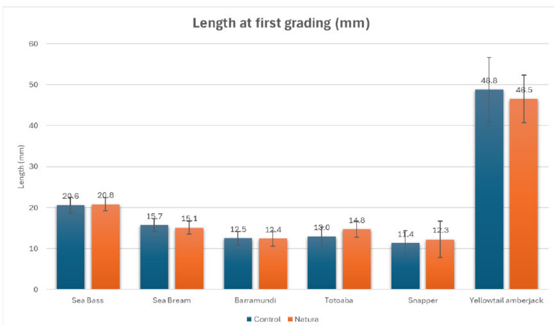
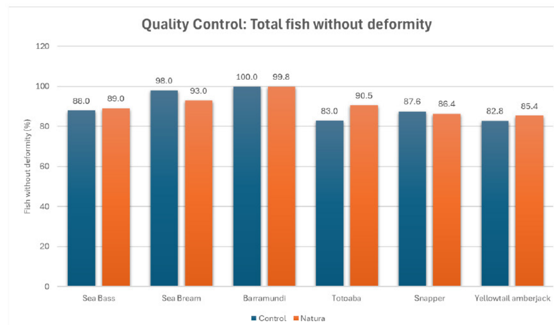


Chart 4: Quality Control: Total fish without deformity



Conclusions

Using Natura feeds in hatcheries worldwide has shown remarkable results. We have seen improved survival rates and growth in various species, and we've also reduced the environmental impact of live prey cultivation and helped the farmer in case of issue with the rotifer production. This progress is crucial for securing sustainable food sources for our growing global population and ensuring the long-term success of aquaculture operations.

Ongoing refinement of dietary formulations will improve digestibility, and understanding metabolic and biological processes will be crucial. This incremental approach aims to fully substitute live food, emphasizing the need for sustained research and innovative thinking to meet the demands of improved aquaculture practices.

EFFECTS OF ORGANIC AND INORGANIC MINERAL PREMIX AND MENHADEN OIL ON NUTRITION AND HEALTH OF CHANNEL CATFISH *Ictalurus punctatus* FINGERLINGS

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Catfish play an important role in the American aquaculture, as it is the most cultured fish in the US. To farm animals with greater vigor and resistance, the feed can potentially be improved by using organic mineral premixes.

The dietary treatments consisted of the inclusion of 50% organic and inorganic mineral premix, 100% organic mineral premix, and 50% organic mineral premix + menhaden oil. The control group received the basal diet with the inclusion of 100% inorganic mineral premix. Four hundred and eighty channel catfish juveniles (*Ictalurus punctatus*) were equally distributed in 16 aquaria (30 fish/tank), operating as a recirculating aquaculture system and treatments were distributed in a completely randomized design. Fish were fed to apparent satiation twice a day and at the end of the feeding trial, fish were group weighed to calculate the production performance. Three fish per aquarium were anesthetized (~100 mg MS-222/L) for blood collection and hematological analyses. The remaining fish from each dietary treatment were transferred to a flow through system and acclimated for 7 days in fiberglass tanks (22 L). Fish were challenged with an LD50 of *Edwardsiella ictaluri*, where 70 mL of the direct BHI broth was introduced into each tank to generate a dose of 6.2×10^{6} CFU/mL. The data were subjected to analysis of variance in one-way ANOVA, post-hoc testing was performed using the Tukey test. There was a significantly higher feed intake (56.2 g/fish) and hepatosomatic index (1.25%) for fish fed control diet (100% inorganic mineral premix). Fish fed different types of premixes did not influence resistance to *E. ictaluri* infection (Figure 1; P=0.10). Although nutrition plays an important role in fish health a better production performance was observed for the control group, which was formulated with 100% inorganic minerals.

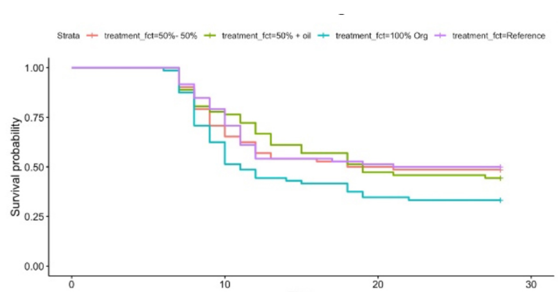

Figure 1. Survival rate of catfish challenged with *E. ictaluri* after supplementation with organic and inorganic mineral premixes and menhaden oil.

EVALUATING THE USE OF AGRICULTURAL BY-PRODUCTS AS CARBON SOURCES FOR DENITRIFICATION IN RECIRCULATING AQUACULTURE SYSTEMS

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Nitrate buildup in recirculating aquaculture systems (RAS) poses risks to fish health and water quality, threatening environmental sustainability. This study explores agricultural by-products as cost-effective carbon sources for heterotrophic denitrification, offering a sustainable alternative to traditional nitrate management methods. By evaluating carbon release dynamics and material composition, this research provides insights for optimizing nitrate removal processes in RAS, promoting more effective and sustainable aquaculture practices. Four by-products—water weed, moringa husks, almond hulls, and wine corks—were processed and tested for carbon release over 120 hours in distilled water. Chemical oxygen demand (COD) was measured, and compositional analyses of cellulose, hemicellulose, and lignin were conducted. Carbon release kinetics were modeled using second-order and Ritger-Peppas equations. Almond hulls exhibited the highest carbon release, followed by water weed, with moringa husks and wine corks showing significantly lower release rates. The Ritger-Peppas model indicated diffusion as the primary release mechanism, contributing to steady carbon availability essential for stable denitrification. The kinetic models provide insights, helping to optimize carbon dosing in denitrification applications.

Table 1 - Cellulose, Hemicellulose, and Lignin Content of materials analyzed.

Carbon Source	Cellulose %	Hemicellulose %	Lignin %
Moringa husks	35.7	14.8	34.0
Water weed	27.1	17.2	11.2
Almond hulls	29.3	25.7	17.1
Wine cork	4.8	9.4	83.0

Table 2 – Second-order model fitting parameters.

Carbon Source	Equation	R^2	c_m	K	$t_{1/2}$
Moringa Husks	$1/c = 0.0035/t + 0.0196$	0.768	51.020	285.714	0.1179
Water Weed	$1/c = 0.0049/t + 0.0147$	0.988	68.027	204.082	0.332
Almond Hulls	$1/c = 0.0024/t + 0.0054$	0.812	185.185	416.667	0.444
Wine Corks	$1/c = 0.0784/t + 0.0574$	0.990	17.422	12.755	1.366

Table 3 – Ritger-Peppas model fitting parameters.

Carbon Source	*Equation*	R^2	n
Moringa Husks	$M_t/M_\infty = 0.843\ t^{0.0713}$	0.894	0.071
Water Weed	$M_t/M_\infty = 0.418\ t^{0.110}$	0.971	0.110
Almond Hulls	$M_t/M_\infty = 0.678\ t^{0.1288}$	0.903	0.128
Wine Corks	$M_t/M_\infty = 0.427\ t^{0.2681}$	0.957	0.268

GROWTH OF *Mercenaria campechiensis* FROM OFFSHORE VIRGINIA

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The genus *Mercenaria* is restricted to the Gulf of Mexico and the Atlantic coast of the United States and is considered to be represented by two species: *Mercenaria mercenaria* and *Mercenaria campechiensis*. The former occupies a more northerly range from New England to the Carolinas. The latter is described as ranging from Cape Hatteras into the Gulf of Mexico, although there are limited records for collections as far north as offshore New Jersey. The two species are generally differentiated by external ribs and a thickened shell in *M. campechiensis* versus a smoother shell in *M. mercenaria,* although this feature is not definitive.

Historical records describe a fishery for *M. campechiensis* in North Carolina in the 1950s (no longer active) with individual clams up to 140 mm shell length and 4 pounds (1.81 kg) total weight. Four specimens were collected off the intracoastal waterway at Redington Beach, Boca Ciega Bay, Florida in December 1964 measuring 149.5, 155.5, 160 and 168 mm SL. The last of these weighed 6.5 pounds (2.95 kg). No age data is reported. The last three all exceeded the largest specimen know at that time; a 153 mm SL in the U.S. national Museum. More recently an "old clam", nicknamed "Lincoln", was reported in the popular press (https://www.usatoday.com/story/news/nation/2023/03/03/aber-clam-lincoln-214-years-old-florida/11389421002/) at 6 inches (152 mm) SL and 2.6 pounds (1.18 kg). This animal was described as *Arctica islandica*, but based on images in that article, we believe it was in fact *M. campechiensis*. An age estimate of 214 years was made for "Lincoln" based on external shell banding sculpture. The specimen was returned to the sea. Despite these records of large individuals of *M. campechiensis* we can find no records for age at length and terminal values of both, although an extraordinary thin section image published by Moss et al. in Paleobiology (2021) provides intrigue.

Herein we focus on six sets of articulated valves of *M. campechiensis* collected in June 2023 at a site 43.5 km offshore of Virginia Beach, USA, in sandy substrate in 18-42 m depth. Individual clams ranged from 80.2 to 111.6 mm SL. For each individual animal we estimate age at length based on annuli in both the chondrophore and exposed edge of valves sectioned from the growing edge to the hinge and compare age at length estimators from annuli signatures to external shell banding. Finally, we discuss the possible history of these individuals in relation to their collection location north of Cape Hatteras.

This work was supported by Dominion Virginia Energy and an NSF Research Experience Award to Carpenter as the presenting author.

PHYSIOLOGICAL RESPONSES OF CHRONIC THERMAL STRESS OF ARCTIC GRAYLING (*Thymallus arcticus*) JUVENILES

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Arctic Grayling are Holarctic species, with only one native population in the contiguous United States, located in the Big Hole River, Montana. Habitat changes and climate effects have contributed to a gradual rise in mean water temperatures over the last 20 years. This study aimed to assess how different water temperatures will affect Arctic Grayling survival, growth, as well as to estimate a thermal performance curve (TPC) and the enzyme activity and gene expression related to stress responses.

Over a 144-day trial, juvenile Arctic Grayling were exposed to temperatures ranging from 8°C to 26°C. Growth, TPC, survival, gene expression, and antioxidant enzyme activities were estimated across these temperature conditions. Fish exhibited increased growth rates as temperatures rose to 18°C, after which growth sharply declined. No fish survived at temperatures above 22°C. The growth and survival results corresponded with the TPC where the optimal temperature of growth was estimated to be 16.96 °C. The minimum temperature was estimated at 4.87°C and the maximum temperature where growth is projected to cease was estimated at 23.18 °C. Moreover, temperatures above 16°C led to a 3.0-fold increase in superoxide dismutase (*SOD*) gene expression and a 1.5-fold increase in glutathione peroxidase (*GPx*) expression, reflecting increased oxidative stress. However, no significant changes were observed in the ratio of heat shock protein 70 (*HSP70*) to heat shock protein 90 (*HSP90*) gene expression. And no clear pattern was observed in the expression of catalase (*CAT*) Enzyme activities for SOD and GPx also increased at higher temperatures, corresponding with the gene expression patterns. Thiobarbituric acid reactive substances (TBARS) increased correspondingly with rising temperatures.

These results highlight the sensitivity of Arctic Grayling to even small increases in water temperature, suggesting that further warming could critically endanger their survival in the Big Hole River.

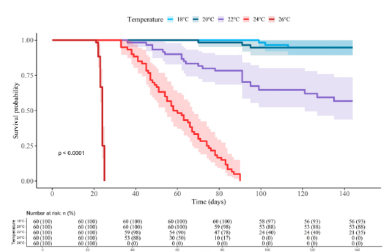

FIGURE 1. survival curves (with 95% confidence interval).

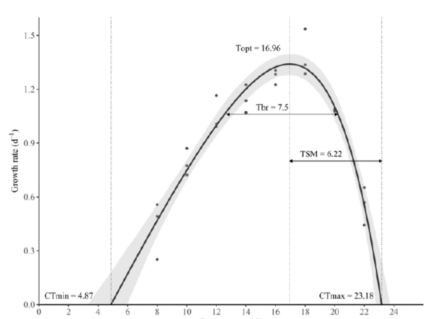

FIGURE 2. Thermal performance curve for Arctic Grayling juveniles.

ENHANCING SPAWNING SUCCESS OF TOPEKA SHINERS *Notropis topeka* THROUGH HORMONAL INDUCTION WITH CARP PITUITARY EXTRACT VS HUMAN CHORIONIC GONADOTROPIN

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The Topeka shiner (*Notropis topeka*) is an endangered freshwater fish species that is native to shallow, clear streams and rivers in the central United States. Due to declining populations, there is an urgent need for innovative conservation strategies. This study explores hormonal induction techniques for spawning Topeka shiners to improve controlled breeding programs and aid in population recovery efforts.

The research aimed to characterize latency periods, test egg deadhesion methods, measure fertilization rates, and optimize dosing protocols for spawning success. Using intraperitoneal injections, we administered 50 µl of carp pituitary extract (CPE) at a dosage of 2 mg/ml or human chorionic gonadotropin (hCG) at a dosage of 500 IU/kg to mature 13-month-old male and female broodstock (Fig. 1). Fish were from a captive population maintained at the USGS Columbia Environmental Research Center, Missouri. Eggs get adhesive quickly, so Fullers earth (15 g/L), tannic acid (400 mg/L) and sodium sulfite (15 g/L) were tested for deadhesion effectiveness.

A dosage of 50 µl of 2 mg/ml CPE per fish was effective at inducing ovulation and spermiation. Latency periods for CPE varied from 8 to 10.5 hours after injection. All deadhesion methods were effective, but sodium sulfite was fast acting and allowed visibility of egg development. Findings for hCG and fertilization rates will also be presented. This research underscores the potential of artificial spawning to bolster Topeka shiner populations, support habitat restoration efforts, and enhance genetic diversity within threatened populations. Future research could prioritize the long-term survival rates of hatchlings and the incorporation of artificial spawning practices into comprehensive conservation plans.

Figure 1: Intraperitoneal injection of adult female (A) and male (B) Topeka shiners to stimulate spawning.

SPATIAL AND TEMPORAL ANALYSIS OF BIODIVERSITY IMPACT BY CHILEAN SALMON FARMS: A STUDY USING GEOSTATISTICAL INTERPOLATION

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Numerous studies have pointed out the environmental effects derived from organic wastes from aquaculture production (Burridge, *et al*., 2010; Wang, *et al*., 2012, Chou *et al* 2002). Quiñones *et al* (2019), find that the organic wastes increase in load of organic matter on the seafloor. Negative effects on the ecosystem have been detected through the degradation of the sediment around the farms (Karakassis, *et al*., 2000; Kalantzi & Karakassis, 2006; Tovar, *et al.,* 2000. Eutrophication of Patagonia channels and fjords due to salmon culture in Chile has been recognized as an environmental risk of salmon production (Buschmann and Pizarro, 2002; Soto y Noranbuena 2004; Buschmann *et al*, 2006; Quiñones *et al*, 2019).

In our study, we reviewed the environmental reports of salmon farming companies in Chile, which are legally required to monitor pollution caused by organic waste. We analyzed data from 2013 to 2019 for farms located in the Salmon Concession Group (SCG) number two in the region of "Los Lagos" in the south of Chile. We focused on those that assessed the impact on biodiversity using ecological indicators such as Dominance (D), Diversity (H), and Evenness (J). To determine the spatial and temporal distribution of pollution we used different geostatistical interpolation methods such as Ordinary Kriging and Inverse Distance Weighted (IDW). Our results (Figure 1) indicate that from 2013 to 2019, most seafloor in SCG number two experienced medium to high pollution levels, with a significant loss in biodiversity during periods of intense salmon farming. This was particularly evident in 2013, 2016, and 2018, when a larger portion of the study area exhibited severe contamination compared to sectors with moderate or mild pollution.

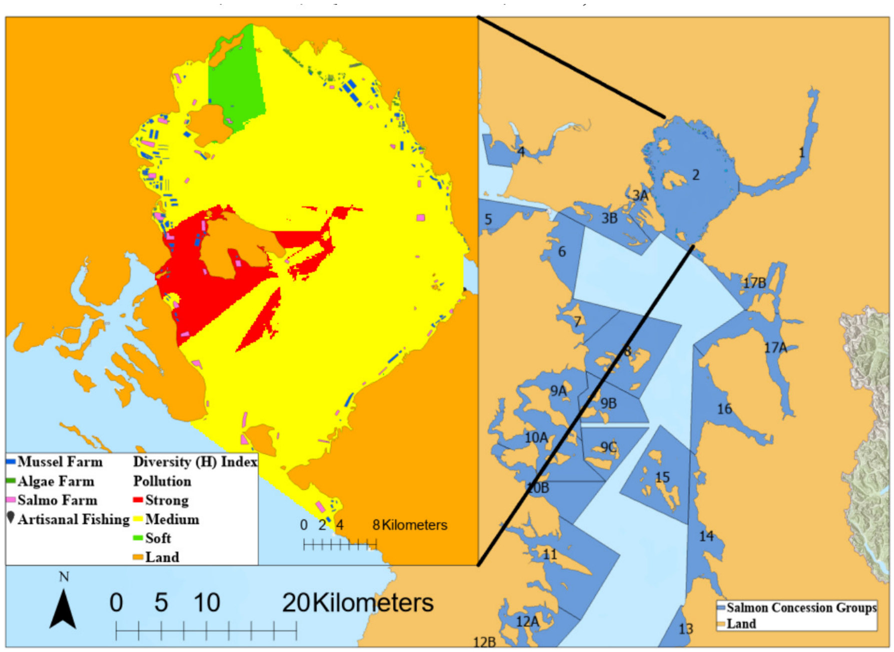

Figure 1: Levels of pollution according to the Diversity (H) Index

NAVIGATING CONTROVERSY: CHILEAN MEDIA COVERAGE OF THE SALMON AQUACULTURE INDUSTRY

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The media serves as a crucial intermediary, playing a pivotal role in shaping public perceptions, particularly in the context of the food sector (Froehlich et al., 2017)offshore production is nascent and distinctions between the types of aquatic farming may not be fully understood by the public–important for collaboration, research, and development. Here we evaluate and report, to our knowledge, the first multinational quantification of the relative sentiments and opinions of the public around distinct forms of aquaculture. Using thousands of newspaper headlines (Ntotal = 1,596. In the case of aquaculture, media coverage not only informs but also directs public attention to specific aspects of the industry, contributing to the formation of public opinion (Kraly et al., 2022; Olsen & Osmundsen, 2017)Atlantic salmon (S. salar. Our study examines the social perceptions of the salmon aquaculture industry in Chile from 2018 to 2023, as portrayed in national newspapers. Our Research involved a systematic review of articles published in major Chilean newspapers from January 1, 2018, to December 31, 2023. A total of 327 articles were analyzed from the Nexis Uni database using the search terms "aquaculture" and "salmon farm" in Spanish. Each article was coded for tone (positive, neutral, negative) and categorized according to PESTE (Political, Economic, Social, Technological, and Environmental) attributes.

The findings reveal a predominant negative perception of the salmon industry, with the highest negative perception recorded in 2022 and 2023 (Figure 1A). Despite a brief period of predominantly positive coverage in 2021, negative perceptions generally outweighed positive ones throughout the study period. Economic and environmental issues were the most frequently discussed (Figure 1B), with significant increases in articles focusing on these topics in recent years. Political articles also saw a substantial rise, especially by 2023. The Chi-square test of independence confirmed a significant association between PESTE attributes and temporal trends ($X^2 = 50.064$, $p < 0.001$). These results highlight the critical role of media in shaping public opinion and regulatory landscapes, impacting the sustainable development of the salmon aquaculture industry in Chile.

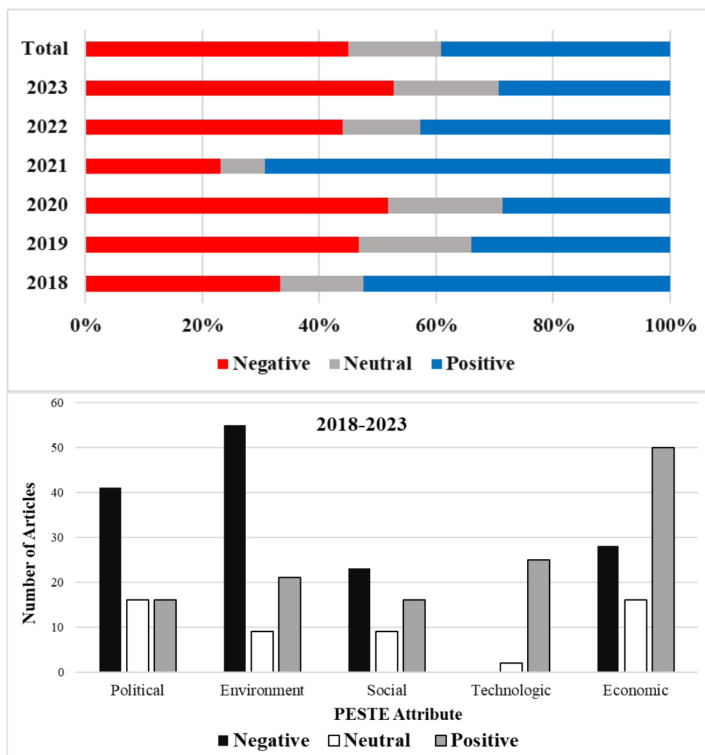

Figure 1A: Proportion of perception of the salmon industry in newspapers; **Figure 1B**: Social perception between the PESTE attribute.

USING BLACK SOLDIER FLY LARVAE TO EFFICIENTLY CONVERT SEAFOOD PROCESSING BYPRODUCTS INTO VALUE-ADDED MARINE FEEDSTUFFS

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This project has produced fishmeal analogs from black soldier fly (*Hermetia illucens*) larvae (BSFL) by rearing them on seafood processing byproducts that are currently being discarded at the expense of the seafood processor. A comprehensive set of evaluations have been conducted in which BSFL were reared in two pilot-scale trials, two benchtop trials and one industrial-scale trial on substrates consisting of different percentages of Gainesville commercial fly diet and three different seafood processing byproducts. The processing byproducts consisted primarily of head and skeleton (racks) of yellowfin tuna (*Thunnus albacares*), Atlantic salmon (*Salmo salar*) and red drum (*Sciaenops ocellatus)* after fillet removal. The various BSFL rearing trials compared substrates consisting of either a 50/50 or a 75/25 ratio of seafood processing byproducts to Gainesville diet as well as the 100% Gainesville substrate. The various substrates resulted in different compositions of the BSFL with those reared on tuna byproduct having the highest crude protein and lowest lipid relative to the other substrates (Table 1). Significant changes also were observed for both saturated and unsaturated fatty acids in the BSFL (Table 2).

The BSFL reared on the various substrates were evaluated in terms of nutrient and energy digestibility with sub-adult red drum. Apparent digestibility coefficients (ACDs) for energy, protein, lipid and organic matter were considerably higher for the BSFL reared on processing byproducts compared to the Gainesville substrate (Table 3).

Results from an ongoing comparative feeding trial with red drum also will be presented. The nutritional value of BSFL is favorably altered by seafood processing byproducts and allows the recovery of valuable marine nutrients.

Table 1. Proximate composition values (g $100g^{-1}$ dry weight) of BSF **larvae** reared with various seafood waste substrates.

Sample	DM	CP	Lipid	Ash
Initial (pre-trial)	29.6	55.9	7.3	12.1
Gainesville substrate 100%	35.3^{C}	48.9^{A}	20.7^{D}	9.9^{A}
Salmon 75/25	49.8^{A}	37.4^{C}	43.9^{A}	5.2^{C}
Tuna 75/25	41.8^{B}	43.5^{B}	27.9^{C}	7.2^{B}
Red Drum 75/25	48.2^{A}	39.1^{C}	35.9^{B}	6.7^{B}
PSE	0.013	1.923	3.903	0.505
One-way ANOVA				
Prob > F	**<0.001**	**<0.001**	**<0.001**	**0.004**

*Values represent means of four replicates.

Table 2. Fatty acid profile (% of total fatty acids) of black soldier fly (*Hermetia illucens*) **larvae** reared in substrates supplemented with various fish processing waste products.

Fatty Acids	Fish Processing Waste - Larvae				P.S.E	P-value
	Gainesville	Salmon	Tuna	Red Drum		
C12:0	47.67**a**	20.74**c**	37.61**b**	36.35**b**	2.98	0.001
C14:0	7.85**a**	3.70**c**	5.18**b**	4.88**b**	0.376	0.001
C16:0	12.30**c**	12.40**c**	14.68**b**	16.67**a**	0.674	0.001
C18:3 n-3	0.86**b**	1.57**a**	0.37**c**	0.32**c**	0.142	0.001
C20:5 n-3	0.04**d**	2.33**b**	2.80**a**	1.48**c**	0.15	0.001
C22:6 n-3	ND	1.27**b**	3.41**a**	0.34**c**	0.17	0.001

ND: not detected

Table 3. Percent ADC values of **BSFL meal ingredients** from insects reared in substrates containing various fish processing waste products.

Diet	CE	CP	Lipid	OM
FPW - Gainesville	58.4	57.9	15.6	64.4
FPW - Red Drum	73.2	77.4	54.8	86.7
FPW - Tuna	80.7	75.0	43.8	92.1
PSE	9.4	10.3	12.9	10.8
One-way ANOVA				
Prob > F	0.200	0.265	0.114	0.154

PUT YOUR EGGS IN ONE BASKET: COLLECTING MARINE ORNAMENTAL FISH EGGS AT THE TEXAS STATE AQUARIUM

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Public aquariums provide access to invaluable information about ocean conservation for guests of all ages. However, many visitors are unaware that most marine ornamental species are collected from wild stocks. Little is known about the early life histories, and human impact on population dynamics, of many popular aquarium species. The Texas State Aquarium is one of many institutions working towards the collective goal of creating a comprehensive guide to egg identification. The Open-Source Marine Fish Egg Catalog (larvalcultureproject.org) is making it easier than ever to identify and sort mixed-species eggs for future larval rearing research. The present study aims to answer the questions: 1) what is spawning in our exhibits and when? and 2) what can aquarium spawning events and the eggs themselves tell us about the overall health of the animals in the collection?

A floating egg collector design will be utilized in the Living Coral Reef exhibit at the Texas State Aquarium to collect marine fish eggs once per month for 12 months. The number of viable and unviable eggs will be estimated by measuring the volume of eggs for each spawning event. Unviable eggs will be photographed and examined for morphological quality estimators including opacity, fragmenting of oil droplets, malformations in the early blastula stages, and the presence or absence of cytonemes assisting in cellular development. Samples of fertilized eggs will be individually photographed and preserved for genetic identification. Morphological data on viable eggs will include minimum and maximum egg, yolk, and oil droplet diameters, number and color of oil droplets, estimated age, or number of cells in the blastula, and the presence or absence of pigmentation and cytonemes within the developing egg. It is predicted that the number, quality, and species of eggs collected will vary seasonally throughout the year.

The results of this study will be applied towards the goals of understanding and prioritizing species vulnerable to overfishing, developing replicable techniques for egg collection and identification, and creating an animal health metric based on the quality and abundance of collected eggs. Subsequent research and development of larval rearing in public aquariums can further promote aquaculture and sustainable fisheries through new exhibits, public programming, and marketing to reduce man-made pressures on fisheries.

EFFECT OF FERMENTATION CONDITIONS ON PROTEIN CONTENT AND ANTIOXIDANT CAPACITY OF MARINE MICROALGAE *Porphyridium cruentum* FERMENTED WITH THE YEAST *Kluyveromyces marxianus*

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Marine rhodophyte *Porphyridium cruentum* is a known source of polysaccharides, proteins, and bioactive compounds. Microalgae biomass fermentation with yeast, such as *Kluyveromyces marxianus*, can enhance nutritional and health properties, like protein content and antioxidant activity. Adding fermented *P. cruentum* biomass to fish diets could improve feed efficiency, boost immunity, and reduce mortality rates, promoting sustainable fish farming. In livestock, antioxidant ingredients help protect against oxidative stress. For human nutrition, fermented *P. cruentum* biomass could act as a functional component that provides a rich source of plant-based protein and antioxidants.

A three-factorial experimental design was used to investigate the effects of incubation time (24h, 48h, and 72h), glucose (0%, 1%, and 2% w/v), and *K. marxianus* inoculum concentration (0%, 1%, and 2% v/v) on the fermentation of 4% (w/v) aqueous solution of lyophilized *P.* cruentum biomass. The fermentation was conducted in Erlenmeyer flasks with a 40 mL working volume, incubated at 30°C, with orbital agitation (150 rpm) and anaerobic conditions. This study investigated the fermentation effect on protein content and antioxidant activity in *P. cruentum* biomass to elucidate its potential as a nutrient ingredient for aquaculture, animal feed, and human food industry.

The highest protein content (50.99±0.99 %) was observed with 48h of incubation, 1% (w/v) glucose, and 6% (v/v) *K marxianus* inoculum (Figure 1; P = 0.002). All treated samples showed visually higher protein content compared to untreated biomass. Antioxidant capacity also increased, positioning fermented *P. cruentum* as a versatile, eco-friendly ingredient that meets the nutritional demands of various industries.

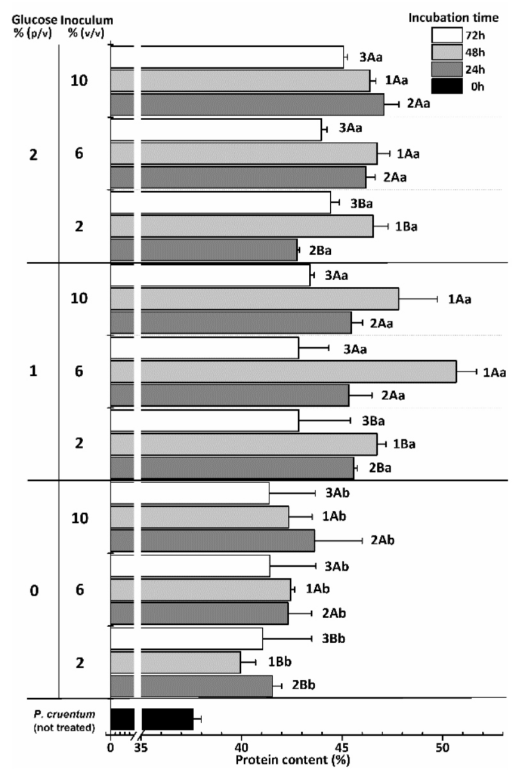

FIGURE 1. Mean values of protein content (%) of fermented *P. cruentum* by effect of incubation time, glucose, and *K. marxianus* inoculum concentration (Factorial ANOVA (α = 0.05); Tukey *a posteriori* test: incubation time 1>2>3, glucose (a>b>c), and inoculum concentration (A>B>C)

NMR-BASED METABOLOMICS AND MACHINE LEARNING FOR REPRODUCTIVE BIOMARKER DISCOVERY IN ATLANTIC SALMON *Salmo salar*

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Atlantic salmon (*Salmo salar*), a key species in marine aquaculture, has experienced a production drop of over 35% since 2000, partly due to decreased embryo survival rates. Currently, selective breeding programs use metrics that are mostly based on growth related traits, but these traits do not correlate well with reproductive performance. We are developing measurement techniques to assess broodstock quality through the analysis of biofluids (plasma, ovarian fluid, and skin mucus) collected from Atlantic salmon female broodstock with the objective of identifying a suite of metabolic markers that correlate with reproductive success by comparing metabolite profiles in high performers (>70% eye-up rate eggs) and low performers (<70% eye-up rate eggs) using NMR metabolomics in combination with machine learning.

Plasma, ovarian fluid and skin mucus were collected in collaboration with USDA and the University of Maine. Two cohorts were sampled during spawning in 2021 and 2022. Plasma and ovarian fluid were filtered using centrifugal filters (3 kDa MWCO). Mucus samples collected on filter paper, were extracted with 70% methanol. NMR spectra (Fig. 1) were acquired at 298K on a Bruker Avance II 700 MHz spectrometer using both 1D and 2D NMR experiments (^{1}H NOESY, and ^{1}H,^{13}C-HSQC). Spectra were binned, normalized to total spectral area, and scaled (Pareto) prior to multivariate analysis (PCA and PLS-DA). Metabolite identification was performed by comparison of experimental chemical shifts to reference values in available metabolomics databases (Chenomx, HMDB and BMRB), and an in-house library.

Our preliminary results from metabolomics analysis of 2022 data show that among the three different biofluids analyzed in this study, ovarian fluid NMR metabolite profiles showed better clustering according to egg eye-up rates. Since ovarian fluid is the obvious reproductive matrix, machine learning approaches will be used to identify correlating metabolic features in other biofluids like plasma and mucus to evaluate the fish metabolome for reproductive fitness. Skin mucus is of particular interest since it constitutes a more readily accessible and non-invasive biological matrix. Results from this study will guide the development of robust molecular tools for efficient broodstock selection, with the potential for early culling of low-yield broodstock, thereby enhancing reproductive success, and improving environmental sustainability.

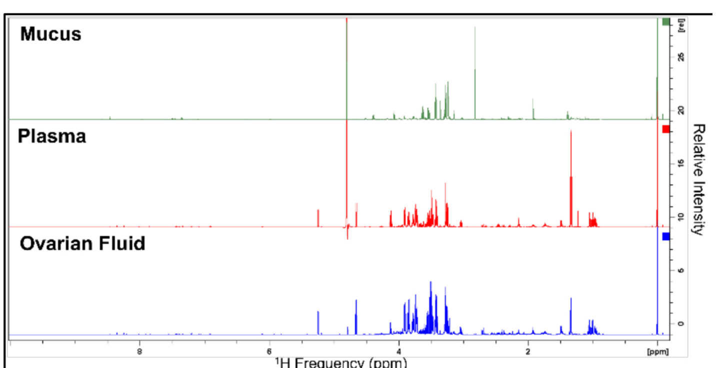

Fig. 1. Representative 1D ^{1}H NMR spectra of the three Atlantic salmon biofluids (mucus, plasma, and ovarian fluid) evaluated in this study.

PATHOGENECITY OF GULF *Vibrio spp*. AND THEIR LD50 IN LARVAL EASTERN OYSTERS *Crassostrea virginica*

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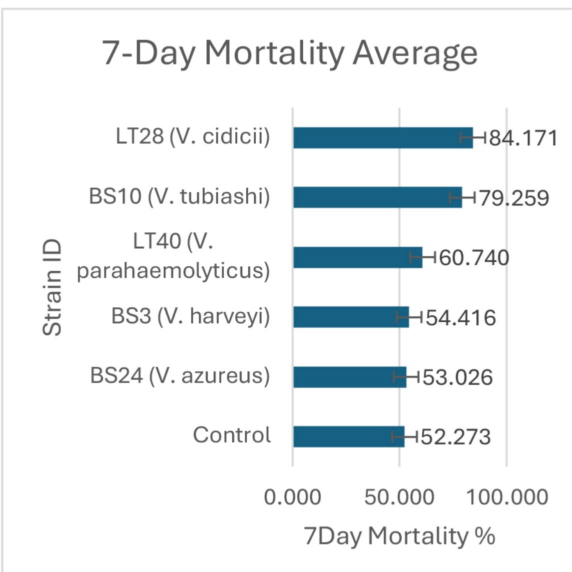
The *Vibrio* genus of bacteria includes several species naturally occurring in Gulf waters that can be pathogenic to larval oysters. Aquaculture facilities and hatcheries handling larval oysters are at risk of mass mortality events if these *Vibrio* species infect their larval stock, leading to significant economic challenges. *Vibrio* strains were isolated from water samples collected at two hatcheries in 2022 & 2024 following mass mortality events. After isolation and purification, selected strains were tested for pathogenicity in a 7–day experiment. Approximately 30 oyster larvae (*Crassostrea virginica*), which were spawned at the Louisiana Sea Grant Oyster Research Lab between April and September 2024, were exposed to about $4*10^4$ CFU/ml of *Vibrio* species in 2 ml of sea water. The larvae were fed 25 µl of algae daily, also collected at the Oyster Research Lab. The graph below compares the 7-day mortality rates of several *Vibrio* strains to a control group (larvae suspended in sea water fed algae without *Vibrio* exposure).



These Vibrio strains were further tested for their LD50. The results are presented in the following table.

train	24hour LD50 ($*10^4$ cfu/ml)
S10 (*V. tubiashi*)	2.13+/-0.30
T40 (*V. arahaemolyticus*)	3.88+/- 0.44
S24 (*V. azureus*)	4.621+/-2.75
T28 (*V. cidicii*)	5.46+/-2.39
S3 (*V. harveyi*)	6.072+/-1.05

is research will help hatchery erations gain a better understanding of e *Vibrio* pathogens present in the Gulf d their potential to disrupt hatchery tivities.

MATCHING PROPERTY RIGHTS AND TRANSBOUNDARY ECOLOGICAL PROCESSES: THE CASE OF THE NORWEGIAN SALMON INDUSTRY

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This study examines the relationship between ownership concentration and the management of transboundary ecological processes in the Norwegian salmon industry, focusing on the spread of sea lice, a costly and environmentally damaging parasite. Utilizing the Biomass Herfindahl-Hirschman index as an ownership concentration measure, we assess the extent to which concentrated ownership within production zones (PZs) mitigates the impact of sea lice. Our empirical analysis demonstrates that higher BHHI values are associated with significantly lower sea lice levels. Specifically, a 100-point increase in BHHI reduces the sea lice load per ten fish by an average of 13.56%. These findings suggest that concentrated ownership enhances the coordination of management practices, thereby addressing scale mismatches between property rights and ecological processes. We employ an instrumental variable approach to address potential endogeneity, using variation in ownership structures across PZs. Our results are robust to alternative specifications and highlight the importance of ownership concentration as a governance strategy for managing transboundary externalities in aquaculture. This study contributes to the literature on resource management and provides empirical evidence for aligning property rights with ecological dynamics to achieve sustainable aquaculture practices.

EXPLORING THE IMPACT OF DOMESTICATION AND BREEDING ON THE GUT MICROBIOTA OF THE PACIFIC WHITELEG SHRIMP *Litopenaeus vannamei*

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The aquaculture of Pacific whiteleg shrimp (*Litopenaeus vannamei*) is a significant economic activity in Mexico. The gut microbiota plays a crucial role in shrimp health by contributing to digestion, metabolism, and immunity. Furthermore, gut microbiota composition is influenced by factors such as diet and environment. However, limited studies have been conducted on how aquaculture practices affect the composition and function of microbiota compared to wild-type specimens. Analyzing these differences is essential for improving shrimp health and optimizing aquaculture practices, but it also highlights the integral role of industry experts in shaping the future of the shrimp aquaculture.

The gut microbiota of Pacific whiteleg shrimp from both Aquaculture and Wild-Type groups (Fig. 1A) was characterized using taxonomic and ecological approaches. The Wild-Type group exhibited 161 bacterial genera, while the Aquaculture Group presented 186 genera. Both groups showed the presence of *Vibrio* in all samples, with similar relative abundances (Wild-Type: 28.16%, Aquaculture: 27.66%). Additionally, the Aquaculture group had a lower relative abundance of *Photobacterium* but a higher abundance of *Candidatus Bacilloplasma* (Fig. 1B), suggesting a different abundance dynamic of taxa influenced by the environment.

The Shannon index showed that the Aquaculture Group had higher diversity, indicating a greater variety and more homogeneous distribution of species. The observed ASVs median also indicated higher taxonomic richness in the Aquaculture group (Fig. 1C). Beta diversity showed that both groups were significantly different in bacterial composition (p=0.002) (Fig 1D).

Our analysis revealed that the Aquaculture group showed greater microbial diversity and taxonomic richness, highlighting distinct microbial behaviors compared to the wild-type group. Exploring the dynamics of microbiota that promote beneficial changes in shrimp health can enhance One Health approaches, holding the potential to generate significant improvements in shrimp farming and offering hope for the future of the industry.

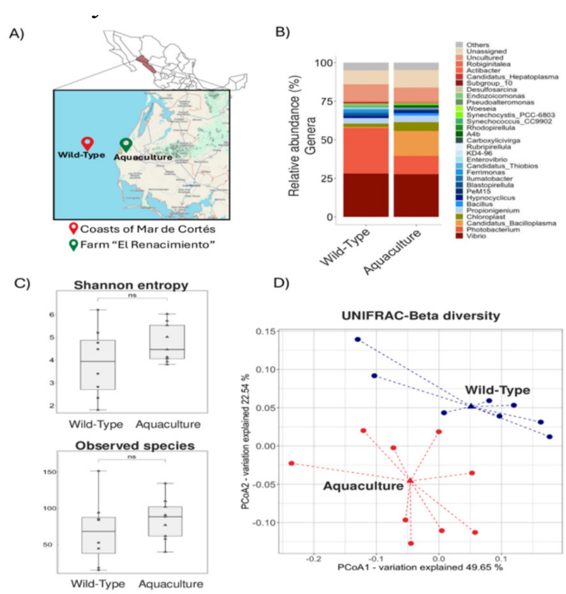

Fig. 1. A) Geographic location of the samples. B) Relative abundance of the 30 most abundant bacterial genera. C) Alpha diversity indexes. D) Beta diversity analysis.

IMPROVING COLD STORAGE OF LIVE MICROALGAE: REDUCING RE NUMBERS WITH COMMERCIAL THICKENERS TO ENHANCE CELL VIABILITY AND STORAGE TIME

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Microalgae culture is crucial for marine hatcheries, serving as feed for shellfish and greenwater for larval finfish. Unlike commercial freeze-dried or non-viable algae products, many hatchery operations require live microalgae. Maintaining these cultures is resource-intensive and prone to crashes. To address this, an open-source microalgae ultrafiltration method is being developed to provide a reliable backup of live cells. Proper storage of these concentrates is essential for preserving cell viability.

When placed in cold storage, microalgae cells tend to settle over time, with some species settling more rapidly than others. Preliminary observations indicate that flagellates settle faster than diatoms and may not actively swim at low temperatures. However, they show increased motility as they warm to ambient air, suggesting the potential to "re-animate" live microalgae concentrates, a critical characteristic for some plankton that rely on consuming live algae.

In culture, microalgae cells are typically kept in suspension through aeration or swirling of the vessels. However, introducing aeration into cold storage, such as refrigeration, is often impractical and can introduce contamination risks. Therefore, alternative methods to maintain cell suspension are needed. This study investigates the use of sodium alginate and xanthan gum, two cost-effective food-grade thickeners, to prolong the suspension of concentrated microalgae during cold storage. By increasing the viscosity of the storage medium, these thickeners aim to reduce the sedimentation rate of algae cells, reflected in decreased Reynolds numbers within the fluid, thereby improving storage time without aeration.

The study's goal is to identify the optimal thickening agent, concentration, and preparation method to maximize cell viability and ensure easy resuspension for feeding in hatcheries. Preliminary data indicate that increasing water viscosity with thickeners reduces microalgae settlement, potentially extending their usable storage time. By understanding the dynamics of cell suspension and settling through Reynolds numbers and fluid dynamics, this research aims to develop practical solutions for the long-term storage of live microalgae in marine hatcheries and laboratories, where viable algae are essential.

COMMUNITY-SCALE STEELHEAD TROUT OPERATIONS IN NEW HAMPSHIRE PART II: FROM HARVEST TO THE MARKET PLACE

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Part II will follow the Part I presentation "From Hatchery to Harvest" that discusses the University of New Hampshire's (UNH) community-scaled, integrated multitrophic aquaculture (IMTA) system called the AquaFort [1, 2]. In Part I, information was provided from the latest growout (2023-2024) of 4000 juvenile steelhead trout (*Oncorhynchus mykiss*) at the UNH permitted site. Part II will focus on the processing of trout, sales and distribution to markets in Portsmouth, NH and Boston, MA.

Weekly harvests were conducted from June through September 2024 onboard a 35' lobster vessel called the *R/V Sugar Daddy*. Trout averaged 2.5 kg with some close to 5.5 kg. A seine net was used to capture and corral trout close to the surface for dip netting and placement into an Xactic with an ice brine slurry. After 10-15 min, fish would succumb and then be spiked in the brain cavity to instantaneously euthanize the fish. This in known as Ike jime, a traditional Japanese slaughter technique. Fish are then thoroughly bled and undergoes spinal cord destruction (shinkei jime). This preserves the flesh at a high quality that's ready for sushi grade markets. Trout were then gutted, rinsed and packed in coolers with ice. Current and previous markets mostly focused on a gutted, heads on product that's delivered to local restaurants and seafood outlets at prices ranging from $6.00-9.00/lb. This year, new markets were explored to increase the shelf life and value of the uniquely grown, IMTA fish. Seafood processors were sourced in Boston to fillet, debone, smoke and vacuum pack 4-6 oz packets that were frozen. This presentation will share insight into the costs of fish processing, packaging and movement of trout to the marketplace.

[1] Chambers, M., Coogan, M., Doherty, M, Howell, H. 2024. Integrated multi-trophic aquaculture of steelhead trout, blue mussel and sugar kelp from a floating ocean platform. J. Aqua. 582:740540. https://doi.org/10.1016/j.aquaculture.2024.740540.
[2] Chambers, M.D., Coogan, M., Doherty, M., Berghahn, E., Fredriksson D.W. 2024. AquaFort, a floating, integrated multi-trophic aquaculture system. W. Aqua. Mag. 55(3): 34-36. https://www.was.org/Magazine/2024/03/34/#zoom=z.

THE RED SEAWEED LEARNING COLLABORATIVE: EXTENSION ENHANCING RESEARCH IMPACT

Samuel Chan[1], Gregg Rorrer[2], Hamzah Alzanbaki[2], Arthur Veramchuk[2], Rainey Campbell[3], Emily Hicks[1], Yuri Sokolov[1]

Sea Grant Extension[1]
Chemical, Biological and Environmental Engineering[2]
Oregon State University[1,2]
University of North Carolina, Chapel Hill[3]

The Red Seaweed Learning Collaborative (RSLC) demonstrates how Extension approaches can transform traditional academic research and enhance its relevance for real-world application. At its core are seven students bringing together transdisciplinary perspectives – chemical and biological engineering PhD candidates work alongside undergraduates in fisheries and wildlife, business and marketing, environmental sciences, and marine resources management. Through this unique collaboration, fresh insights emerge: fisheries students observe and interpret how seaweeds evolve different morphologies in response to the flow and circulation systems being studied by engineering students, while environmental science and marine resource management students open new perspectives about red seaweeds' role in carbon sequestration and its broader implications for marine ecosystems.

The RSLC's distinctive contribution within the Aquaculture Hub network lies in its integration of Extension methodologies with traditional academic research. When engineering students design cultivation systems, their work is enriched by their colleagues' observations about seaweed biology and ecosystem dynamics. When chemical engineering students plan experiments, they now consider not just scientific variables but also practical implementation challenges identified through industry engagement. These interactions, guided by Extension approaches like applied research engagement, outcome-based Logic Models, and strategic science communication, fundamentally shape how research questions are formed and investigated.

The collaborative brings together diverse expertise through Extension. Students develop skills in engaging stakeholders early in the research process, designing experiments that address real-world needs, and creating visual tools that make research findings accessible. Marketing students help researchers effectively communicate about complex topics like carbon sequestration, while marine resource management students connect technical innovations to broader environmental and social impacts. Early results show how this approach enhances research impact: students develop broader skill sets combining disciplinary expertise with practical application, research projects better integrate technical innovation with ecological understanding, and communication materials make complex concepts accessible to diverse audiences.

Looking ahead, this model offers insights for both Extension and research communities. For Extension professionals, it illustrates how their approaches can transform traditional academic research. For researchers, it shows how Extension methodologies can enhance the relevance and impact of their work. Through this ongoing work, the collaborative demonstrates that Extension approaches have value beyond their traditional applications, offering proven methodologies for making transdisciplinary research more relevant and impactful for real-world implementation.

AqPathDx: DEVELOPMENT OF A LAMP BASED DIAGNOSTIC KIT FOR RAPID DETECTION OF FISH PATHOGENS

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Rapid and accurate detection of pathogenic species and their differentiation from commensal species is essential for disease management and the implementation of strategies to control the spread of infection. AqPathDx diagnostic kit enables rapid, pondside detection of the most common catfish and tilapia pathogens in two separate panels in less than 30 minutes. One panel is designed to target three common catfish pathogens: *Aeromonas* sp., *Edwardsiella* sp., and *Flavobacterium* sp. and another panel targets three common tilapia pathogens: *Aeromonas* sp., *Streptococcus* sp. and Tilapia lake virus (TiLV). This kit is based on a novel multiplex loop-mediated isothermal amplification (mLAMP) method, which allows real-time detection of up to 4 targets in a single reaction in 30 minutes.

LAMP primers were designed for specific amplification of targeted pathogens in under 30 minutes. For LAMP assay development and optimization, genomic DNA extracts of targets normalized to 1 ng/μL were used. Results presented in Table 1 shows high sensitivity of LAMP assay for each pathogen.

Table 1: Sensitivity of LAMP assay for *Aeromonas* sp., *Edwardsiella* sp., *Flavobacterium* sp. and *Streptococcus* sp.

Target conc (pg/rxn)	***A. hydrophila***		***A. salmoncida***	***A. veronii***		***F. covae***	***F. columnare***	***E. ictaluri***	***S. agalactiae***	***S. iniae***
	09-119	**ATCC 7966**	**(GA97-30**	**ALP 17-18**	**ALP 17-22**	**(C2)**	**(ATCC 23463)**			
	Time to Results (minutes)									
4.00E+02	8.2	10.5	7.2	6.3	7.1	3.0	3.1	7.2	8.9	11.5
4.00E+01	10.7	11.3	8.3	7.5	7.5	3.2	3.5	7.8	9.2	12.8
4.00E+00	11.6	13.2	9.1	9.1	9.1	3.6	4.0	9.0	10.1	13.4
4.00E-01	13.9	15.2	13.0	9.6	13.1	4.1	4.8	10.5	12.8	16.6
4.00E-02	no amp	25.6	17.5	11.7	no amp	4.3	13.0	9.2	13.5	18.6
4.00E-03	no amp	no amp	16.4	no amp	no amp	4.7	no amp	9.3	14.9	no amp
4.00E-04	no amp	no amp	no amp	no amp	no amp	4.4	no amp	8.9	no amp	no amp
Negative control	no amp	no amp	no amp	no amp	no amp	no amp	no amp	no amp	no amp	no amp

EXPLORING GENETIC DIVERSITY AND HYBRIDISATION PATTERNS OF MUSSELS IN NORTHERN SCOTLAND

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Understanding genetic diversity and gene flow within and between mussel populations informs population fitness and contributes towards aquaculture sustainability. Shetland, in the north of Scotland, is responsible for >60% of UK blue mussel production. As such, we aimed to investigate the genetic diversity and population structure of mussels grown in this important location compared with populations elsewhere in the north of Scotland. DNA was isolated from samples taken from four populations, each containing 30 animals: two from northeast mainland Scotland (Cromarty Firth); one from northwest of Scotland (Western Isles); and two populations of locally adapted Shetland mussels. Single Nucleotide Polymorphisms (SNPs) analysis was performed using a medium density multi-species *Mytilus* array. To analyse subspecies hybridisation, we also included reference samples of *Mytilus* subspecies: *M. galloprovincialis, M. edulis* and *M. trossulus*. The initial analysis reveals that Cromarty populations appear to predominantly have *M. edulis* genetics. On the other hand, mussels from Shetland and the Western Isles display levels of introgression with *M. galloprovincialis*. Higher levels of genetic conservation were seen in the Western Isles compared with the relatively diverse genetics observed in both Shetland populations. Further analysis of each population using Nanopore sequencing was carried out to explore the levels of large structural variation (SV) and Presence/Absence Variations (PAV) in stress-response genes. Taken together, these data demonstrate that this SNP array provided a robust platform for consistent genotyping of individuals and may be used to further investigate appropriate growing environments for background genetics to enhance mussel health and productivity.

PROJECTING THE FUTURE POTENTIAL FOR PRODUCTION OF SAFER ROHU, TILAPIA AND PANGAS IN BANGLADESH

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Bangladesh's aquaculture production contributes substantially to the local food security, health, economic benefits and livelihood opportunities. Sustainable seafood production relies on the adoption of Good Aquaculture Practices (GAqPs). However, the success of the adoption of GAPs depends on at least two key factors: changes in production costs and consumer's willingness to pay. In this study, we developed a dynamic, multi-market and multi-species partial equilibrium model of the fish sector of Bangladesh (BDFish) for assessing the potential impact of safer rohu, tilapia and pangas initiatives on fish production in Bangladesh. The modeling of safer rohu, tilapia, and pangas scenarios was based on the results generated from separate production and consumption studies of this project. Applying these into the BDFish, we projected increased safer production for all the 3 species. Results also illustrate that other socio-economic scenario (i.e., changes in seafood spending/income) also play a significant role in projected sustainability of safer fish initiative in Bangladesh. The findings highlight the potential markets for safer rohu, tilapia and pangas in Bangladesh and the importance of integrating policies that create an enabling environment into the aquaculture development pathways.

UTILIZING UNCREWED AERIAL SYSTEMS TO FACILTATE THE RECOVERY OF OFF-BOTTOM OYSTER AQUCULTURE GEAR IN COASTAL MISSISSIPPI, USA

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Marine debris is defined as any persistent solid material that is manufactured or processed and directly or indirectly, intentionally or unintentionally, disposed of or abandoned into the marine environment or the Great Lakes (NOAA). Within coastal Mississippi (MS) USA, marine debris continues to economically negatively impact commercial fishing industries. Mississippi State University and Mississippi Commercial Fisheries United (MSCFU) established the Derelict Trap Reward Program was implemented in 2019 to remove entangled derelict fishing gear while providing stewardship opportunities and monetary incentives to commercial shrimpers to help reduce marine debris. In 2023, the partnership expanded the program and began to focus on off-bottom oyster aquaculture and including farmers in stewardship efforts.

Off-bottom oyster aquaculture farms in MS are leased from the state approximately 1 km south of Deer Island, MS. Gear type varies, but the gear is primarily comprised of large floats attached to cages that can be suspended in the water column with rows of cages attached via line. The farm grounds are protected by the island in the north and by Katrina Reef Breakwater 1 km south. However, wind and wave action from the east and west leave aquaculture gear exposed, and as a result, gear often becomes derelict. Starting in late 2022, monthly aerial mapping missions over Deer Island have been conducted with a UAV. The primary goal of these flights is to identify off-bottom aquaculture equipment and return the gear back to farmers. Through a streamlined workflow, farmers are contracted by MSCFU to retrieve lost farm gear identified. Current efforts have led to the recovery of off-bottom aquaculture floats, cages, or a combination of both. Aquaculture equipment is collected by MSCFU and given back to respective farmers, when identifiable. Additionally, derelict crab traps and fish accumulator devices are identified for removal by the state.

A 3-D PRINTABLE OPEN-SOURCE SOLUTION TO FIELD-BASED CRYOPRESERVATION WITH AN AFFORDABLE LIGHTWEIGHT 2.1 L NITROGEN VAPOR SIPPING DEWAR

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Traditional sperm cryopreservation for aquatic species heavily depends on expensive computer controlled programmable freezers, which are often unaffordable for resource-limited communities. In recent decades, open soured hardware has been developed for cryopreservation processing in aquatic species to assist global communities in establishing germplasm repositories. For example, open source 3-D printed hardware developed by the Aquatic Germplasm and Genetic Research Center (AGGRC, www.aggrc.com) can be shared by use of digital files around the world.

The lack of commercially available products for field-friendly cryopreservation solutions can cause a pervasive lack of standardization and reproducibility. To address this, the Shipping Dewar Position Cooling Device, also known as the "Cajun Ejector" was developed as a 3-D printed, affordable, open-source solution to use with large nitrogen vapor shipping dewars to process French straws at various cooling rates to meet the needs of users. Large nitrogen vapor shipping dewars are typically expensive and bulky, making them less than ideal for wide adoption by the community. To overcome this, the "Universal Cajun Ejector" was developed to be used with various sizes of shipping dewars, increasing accessibility and for global communities.

The Universal Cajun Ejector (Figure1) consists of five 3-D printed components and non-3-D printed part (i.e. a spring) The 3D-printed elements include the upright support, positioning rod, ejection cap, and dewar collar. To assemble the Universal Cajun Ejector, the positioning rod fits into the upright support, securely holding 22 French straws between the two pieces. The spring is placed around the positioning rod with the ejection cap screwed on to fasten the spring in place. This device is being tested for reproducing target ranges of cooling rates.

These efforts are standardizable through community efforts and improve the reproducibility of field-based cryopreservation. The development of open-source hardware can potentially revolutionize the community-level progress in establishment of germplasm repositories for aquatic species to support biomedical research, aquaculture, and conservation.

Figure 1. BL-7 Shipping Dewar (left) and Universal version of the Cajun Ejector (right)

HUMIC ACID: A PROMISING APPROACH TO MANAGING *Microcystis aeruginosa* BLOOMS

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Harmful algal blooms (HABs) are increasingly reported across the southern United States, leading to fish, livestock, and companion animal deaths, losses in agricultural productivity, and interruptions in domestic water supplies. This rise is attributed to improved identification and monitoring but is also likely exacerbated by environmental changes, particularly the high heat and prolonged droughts that concentrate nutrients and diminish water availability for flushing. HABs, including those caused by *Microcystis aeruginosa*, thrive in hot, nutrient-rich, stagnant conditions.

Traditional management strategies, such as nutrient control, flushing, algaecides, and toxin oxidation, face significant limitations. Nutrient management is costly and impractical for large water bodies, flushing is unfeasible under drought conditions, and algaecides are expensive and must be applied precisely before toxin production escalates. Similarly, oxidizing toxins with potassium permanganate is both costly and potentially harmful to aquatic life. These challenges have highlighted the need for cost-effective, sustainable alternatives, such as humic acid.

Humic acid, an organic compound derived from natural decomposition, offers promising potential for HAB management. Previous studies on *Prymnesium parvum* demonstrate it can be used prophylactically to prevent blooms, disrupt algal cell processes, and bind nutrients. By providing a carbon source for beneficial bacteria, humic acid may enhance natural nutrient cycling and reduce conditions conducive to algal proliferation.

This presentation will explore the evaluation of humic acid as a management tool during blooms of *M. aeruginosa*. Results on humic acid use as a treatment, prophylactic preventative, and nutrient binder when used during M. aeruginosa blooms will be shared, focusing on its practical applications and potential to serve as a low-cost, environmentally sustainable alternative to traditional HAB management strategies.

EFFECTS OF EXPOSURE TO ELEVATED TEMPERATURE ON THE OUTER MANTLE OF THE FLUTED GIANT CLAM *Tridacna squamosa*

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Giant clams are found living in nutrient-poor seawaters where light is adequately available due to their mutualistic association with phototrophic dinoflagellates of the family Symbiodiniaceae. These symbiotic dinoflagellates also known as zooxanthellae are found inside a zooxanthellal-tubule system mainly in the colorful and extensible outer mantle. The outer mantle uniquely possesses iridocytes that absorb harmful ultraviolet radiation and deflect light of wavelength conducive for photosynthesis to the symbionts. The iridocytes can also back-reflect light of other wavelengths engendering multiple color patterns to the outer mantle. They possess a mechanism which involves vacuolar-type H^+-ATPase (ATP6V1), to facilitate the transfer of carbon dioxide from the host to the symbionts for photosynthesis. They are therefore strongly ATP6V1 subunit A (ATP6V1A)-immunopositive. Global warming leads to rising seawater temperatures can result in the loss of symbionts, depletion of symbiont's pigments and/or host's pigments in the outer mantle of giant clams or bleaching. The outer mantle of *Tridacna squamosa*, showed a reduction in its brownish hue when exposed to an elevated temperature of 31 °C for 57 days as compared to that of the control kept at 26 °C. There was a drastic loss of 34% of symbionts quantities from the outer mantle after *T. squamosa* were exposed to 31 °C for 15 days followed by another 27% loss after exposure to 31 °C for 30 days. Thereafter, the symbionts quantities levelled off at 1.7×10^7 with no further loss observed on 57 days at 31 °C. Interestingly, the total chlorophyll content (expressed as per g outer mantle) did not decrease after *T. squamosa* were exposed to 31 °C for 15 days. It only decreased after *T. squamosa* were exposed to 31 °C for 30 days and 57 days. However, when the chlorophyll content was expressed as per symbiont, there was an increase in synthesis of chlorophyll by the symbionts when *T. squamosa* were exposed to 31 °C. The transcript level and protein abundance of symbiont-derived form II ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) decreased significantly in the outer mantle after exposure to 31 °C for 15, 30 and 57 days. The decrease in phototrophic potential could probably be due to the instability of RuBisCo at 31 °C. The ATP6V1A protein abundance in the outer mantle decreased significantly as compared to that of the control after *T. squamosa* were exposed to 31 °C for 57 days. Additionally, immunofluorescence microscopy confirmed the reduction in both iridocyte and symbiont populations. Thus, there was a reduction in the capacity of the iridocyte to transfer carbon dioxide to the symbionts for photosynthesis. The transcript levels and protein abundance of host's copper-zinc- (CuZn) and manganese- (Mn) superoxide dismutase (SOD) in the outer mantle were also measured to assess oxidative stress occurring at 31 °C. There was a significant increase in the protein abundance of CuZnSOD but not MnSOD after *T. squamosa* were exposed to 31 °C for 15 days. Taken together, there could have been an increase in reactive oxygen species production, leading to the drastic loss of symbionts after *T. squamosa* were exposed to 31 °C for 15 days. The remaining symbionts were able to adapt to 31 °C after 30 days of exposure. These findings signify the importance of elucidating the effects of elevated temperature on both the symbionts and the host in order to fully understand the process of temperature-induced bleaching in giant clams.

INFLUENCE OF CROWDING STRESS ON THE GROWTH PERFORMANCE AND HEMATOLOGICAL PARAMETERS OF OLIVE FLOUNDER *Paralichthys olivaceus* IN LAND-BASED INTENSIVE RAS

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Olive flounder (*Paralichthys olivaceus*) sometimes referred to as Japanese flounder is a highly valued fish in East Asia due to its rapid growth, excellent aquaculture performance, and high market value. This species has been domesticated in Korea since 1980's and has potential to be a lucrative aquaculture candidate in other countries as well. Recirculating aquaculture systems (RAS) reduce the amount of water and space required to intensively produce seafood products. In landlocked states such as Kentucky where shrimp production in RAS systems has been growing, olive flounder can be reared in the same flat-bottom culture tanks used for shrimp.. Stocking density is one of the major factors affecting animal welfare and system productivity. The level of stress resulting from high stocking density may also affect energy and metabolism, potentially affecting growth rates and suppressing the immune response. This project focused on assessing the effect of stocking density in a RAS on production dynamics and stress response of olive flounder in nursery-level RAS.

In this study, fingerling (~55 gm) olive flounder were stocked at three densities: 2.7, 5.8 and 7.2 kg/m^2 (low-density: LD, medium density: MD, and high-density: HD) in 1.2 m^2 round fiberglass tanks. Each of these three treatments were replicated in three randomly assigned tanks connected to a common sump and shared filtration system. To maintain clear water, water from the tanks passed through a drum filter with 40 μm screen, foam fractionator, and a moving bed bioreactor (MBBR) aerobic bio filter. Ozone (O_3) gas was injected into the fractionator reaction chamber and water then passed through a UV radiation lamp to destroy ozone and further sterilize the water. In order to prevent the accumulation of nitrate, an anaerobic MBBR denitrification chamber was also used. To assess the health of olive flounder, stress indicators including blood glucose, cortisol, growth hormone and Insulin like growth factor (IGF-1) concentrations were analyzed using a Dynex DS2 System (Chantilly, Virginia, USA), an automated ELISA (Enzyme-linked immunosorbent assay) processor.

At the end of the 90 days long experiment, we observed significantly higher average weight in LD (452.7 g) tanks followed by MD (399.3 g), & HD (347.1 g). Survival in all treatments were very high (>95%) with no significant differences. Similarly, significantly higher biomass was found in HD (54.5) followed by MD (40.4) and LD (23.2) kg/m^2. In terms of stress response, no significant difference was observed in cortisol, GH and IGF-1 across all treatments. Based on this trial, we can say that with appropriate culture practices, fingerlings of olive flounder can be grown to a final stocking density of ~55 kg/m^2 without any negative impact on their health. However, like many animals, there is a tradeoff between density and individual growth rate. Producers must therefore balance production goals and decide what factors are most important. Nonetheless, this species seems well-suited for RAS and the levels of production in this project are encouraging for an industry that must balance productivity with the relatively high cost of equipment

PHB/STARCH AND PHB/CELLULOSE BLENDS FOR AMMONIA ASSIMILATION IN RECIRCULATING AQUACULTURAL SYSTEMS (RAS)

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The process of assimilation utilizes carbon-enriched beads to promote the growth of heterotrophic bacteria, which have a higher specific growth rate than nitrifiers. This makes them ideal for faster biofilter start-ups and for absorbing ammonia during sudden increases in organic loading rates in RAS systems, mitigating the negative impacts of ammonia on cultured animals. Recently, carbon beads, such as poly(3-hydroxybutyrate) (PHB), have been introduced as passive assimilation beads, serving as both biofilm carriers and electron donors for heterotrophic bacteria, which allows farmers the opportunity to separate the cultured animals from the filtration unit. While PHB eliminates the need for external control systems, its application as a carbon substrate is limited by high cost. To reduce the cost of PHB while maintaining biodegradability, it has been blended with cheaper materials like cellulose and starch. This present study extruded PHB/cellulose and PHB/starch blends with compositional ratios (100:0, 80:20, 70:30, and 60:40), and tested their effectiveness in ammonia removal in an aerobic assimilation process to reduce toxic ammonia buildup in RAS facilities. The COD release of these materials was evaluated to assess their solubility.

A single-screw extruder was used to produce PHB/cellulose and PHB/starch beads. A 5-day experiment was conducted using these blends as carbon substrates in an up-flow fixed-bed bioreactor to promote bacterial growth and remove ammonia (~20 mg/L ammonia-N) from synthetic aquacultural wastewater. Water quality parameters, including ammonia-N, nitrite-N, COD, and nitrate-N, were measured every 24 hours, with oxygen, pH, and temperature in the reservoir maintained at 7.85 mg/L, 8.1, and 25°C, respectively.

After 5 days of experimentation PHB/starch beads had lower cost, and higher ammonia-N removal rates but with high COD accumulation, which eliminates their applications in RAS facilities. PHB/cellulose had good assimilation rates and reduced the cost of PHB while maintaining low solubility. PHB showed the lowest solubility, but its application is hampered by high cost.

Beads	Overall TAN removal ($mg\ l^{-1}$)	Apparent VTR ($Kg\ d^{-1}\ m^{-3}$)	Accumulated Tank COD ($mg\ l^{-1}$)
P=100:0	7.53 ± 0.61^{a}	0.83 ± 0.07^{a}	11 ± 1.39^{a}
P: C=80:20	7.7 ± 0.83^{a}	0.84 ± 0.08^{a}	29 ± 3.85^{b}
P: C=70:30	7.83 ± 0.9^{a}	0.86 ± 0.1^{a}	41 ± 1.41^{c}
P: C=60:40	8.0 ± 0.7^{b}	1.03 ± 0.09^{b}	56 ± 8.64^{d}
P: S=80:20	9.0 ± 1.28^{b}	0.99 ± 0.14^{b}	109 ± 8.04^{e}
P: S=70:30	11.63 ± 2.53^{c}	1.3 ± 0.28^{c}	220 ± 21.6^{f}
P: S=60:40	11.7 ± 2.44^{c}	1.45 ± 0.23^{c}	301 ± 6.48^{g}

Table 1. TAN removal and apparent VTR of beads with different compositions of PHB (P), cellulose (C), and starch (S) showed a positive trend with the accumulated COD level in the tanks, with different superscript letters attached to values in the column indicating significant differences (p<0.05).

POLYHYDROXYBUTYRATE/CELLULOSE BLEND (PHBC) AS A CARBON SUBSTRATE FOR DENITRIFICATION IN RECIRCULATING AQUACULTURE SYSTEMS

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Nitrate contamination in recirculating aquaculture systems (RAS) can harm aquatic species, affecting reproduction, growth, and survival. Biological denitrification is considered the most cost-effective method for nitrate removal in RAS. Traditional methods rely on liquid carbon sources like methanol or acetate, which require constant monitoring with sophisticated computer systems to avoid overdosing, increasing operational costs. Solid-phase denitrification (SPD) using biodegradable polymers, such as polyhydroxybutyrate (PHB), offers a promising alternative but is not cost-effective. Cellulose, being renewable, cost-effective, and biodegradable, is a suitable candidate for blending with PHB to reduce costs. This study investigates the impact of cellulose on the biodegradability and denitrification performance of PHB.

PHB/microcrystalline cellulose blends (PHBC) containing 20, 30, and 40% cellulose were prepared using a benchtop single screw extruder. In a laboratory-scale setup, pure PHB bio-pellets were compared to PHBC blend bio-pellets in terms of nitrate reduction rates, COD accumulation, consumption rates, and denitrification costs. A 7-day experiment was conducted in triplicate in a temperature-controlled dark room, using four identical 12” up-flow PVC bioreactor columns, each filled with 200 mL of different bio-pellets, along with four 109 L source water reservoirs and four 20” degassing columns for stripping dissolved oxygen with pure nitrogen gas. The bioreactor units were backwashed at 12 h intervals using pure nitrogen.

The study found that PHBC60:40 and PHBC70:30 achieved average apparent peak nitrate reduction rates of 3.9±0.80 kg NO_3–N/m^3-d and 3.9±0.24 kg NO_3–N/m^3-d, respectively, on day 2. PHBC80:20 reached 3.7±0.017 kg NO_3–N/m^3-d on day 3, and PHB100% attained 3.4±0.03 kg NO_3–N/m^3-d on day 4, with statistically significant differences between treatments. COD accumulation on day 7 increased with cellulose content, ranging from 19±2.9, 29±4.2 in PHB100% to 58±11.5 in PHBC60:40, partly due to solid release during backwashing. The blends were more cost-effective than PHB. The results show that the blend is effective for SPD.

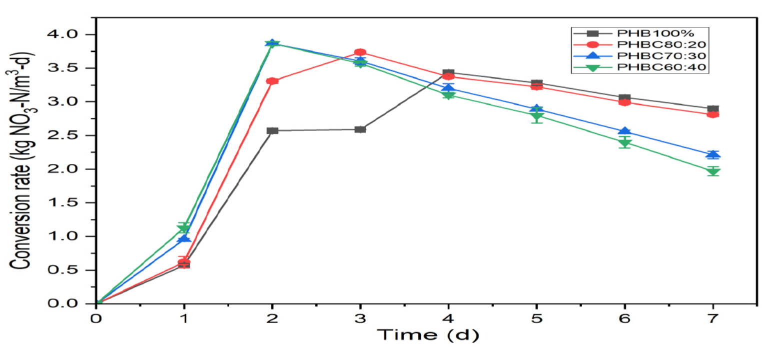

Fig.1. Nitrate reduction rate with PHB and PHBC of different compositions. Some error bars do not appear because the standard deviation values were small.

EFFECTS OF SOYBEAN MEAL, ENZYME TREATED SOYBEAN MEAL AND CORN-FERMENTED PROTEIN MEAL IN THE DIETS ON GROW-OUT STAGE OF CHANNEL CATFISH *Ictalurus punctatus* CULTURING IN POND RACEWAYS SYSTEM

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This study was conducted to investigate the effects of solvent-extracted- soybean meal (SBM), enzyme-treated soybean meal (ESBM) and corn-fermented protein (CFP) meal in the diets on the growth performance, body composition and blood chemistry of grow-out stage channel catfish (*Ictalurus punctatus*) culturing in pond raceways system. Three ingredients were formulated into three isonitrogenous (32%) and isolipidic (6%) test diets. The basal diet contained 49% SBM as the primary protein source. The second diet (ESBM) utilized enzyme-treated soybean meal as a replacement for SBM, and the third diet (CFP) was supplemented with 10% corn-fermented protein as a partial replacement for SBM. Channel catfish (102.9± 1.8g mean weight) were randomly assigned to 12 raceways (11ft x 4 ft x 4.5ft each) housed in a 1-acre pond. The fish were hand-fed to near satiation once a day. After the feeding trial for four months, no significant differences were found in weight gain(g), weight gain (%), feed conversion ratio (FCR), apparent net protein retention (ANPR), condition factor and survival rate between the channel catfish fed the three diets, but there did seem to be a trend towards an increase in weight gain of fish fed with ESBM diet and CFP diet. Channel catfish fed the ESBM diet have the lowest FCR and highest ANPR than those fed basal and CFP diets. However, fish fed ESBM diet were found to have significantly higher hepatosomatic index (HSI) value and intraperitoneal fat (IPF) value than those fed the basal and CFP diets. Blood chemistry, data will be discussed in the presentation. The results suggest that using enzyme-treated soybean meal as a primary source or including 10% corn-fermented protein to the diet, are both acceptable ingredients that can be utilized in production diets for channel catfish.

Table1. Growth performance and body indices of channel catfish growth out (initial 102.9± 1.8g) stocked at 400 fish per raceway feed with three treatment diets for four months.

	Weight gain (g)	Weight gain (%)	FCR	ANPR	HSI	IPF	Condition Factor (K)
Basal	462.89	445.73	1.63	27.48	1.296^{b}	37.159^{ab}	1.091
ESBM	476.98	462.38	1.58	28.20	1.467^{a}	38.335^{a}	1.119
CFP	468.72	465.51	1.63	25.44	1.290^{b}	34.365^{b}	1.114
PSE	18.54	33.78	0.05	2.15	0.087	1.774	0.025
P-value	0.579	0.684	0.487	0.225	0.028	0.030	0.292

EFFECTS OF REPLACING SOYBEAN MEAL PROTEIN WITH VARIOUS PROCESSING SOY PROTEIN SOURCES IN THE DIETS ON THE GROWTH OF JUVENILE NORTHERN LARGEMOUTH BASS *Micropterus nigricans*

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This study was conducted to investigate the effects of replacing dietary solvent extract soybean meal (SBM) with low oligosaccharide soybean meal (L-SBM) or enzyme treated soybean meal (E-SBM) on the growth performance and body composition of juvenile largemouth bass (*Micropterus nigricans*). Nine isonitrogenous (40%) and isolipid (8%) treatment diets were designed for juvenile largemouth bass. The basal diet contained 50% SBM as the primary protein source. The SBM was incrementally replaced on an isonitrogenous basis with 40%, 60%, 80% and 100% using L-SBM or E-SBM. Each aquarium was stocked with fifteen 8.55±0.18g juvenile largemouth bass and the fish were randomly assigned to the above nine treatment diets (six replicates of Basal, LSBM100% and ESBM100%; four replicates of the other six treatments). The fish were fed twice daily for six weeks. The mean weight and weight gain (g) of the fish offered the diet containing 60% and 80% of E-SBM were significantly higher than those of fish fed the other treatment diets. The mean weight and weight gain (g) of the fish fed with LSBM40%, LSBM60%, LSBM80%, LSBM100%, ESBM40% and ESBM100% diets were not significantly different. The fish fed with the basal diet had significantly lower mean weight and weight gain(g) than those fish fed the other diets. No significant differences were found in weight gain (%), PER and survival between the fish fed the nine diets. Results demonstrate that L-SBM and E-SBM are two potential ingredients which could be used in juvenile northern largemouth bass diets as a primary protein source without negative effect on growth performance.

Table 1. Growth performance of juvenile (initial mean weight 8.55±0.18g) *M. nigricans* for six weeks growth trial.

Treatments	Mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	PER	Survival (%)
Basal	22.05[b]	13.61[b]	161.52	1.53[b]	1.61	98.8
LSBM40%	26.20[ab]	17.76[ab]	211.50	1.26[ab]	1.97	98.3
LSBM60%	25.92[ab]	17.68[ab]	214.89	1.24[ab]	1.98	98.3
LSBM80%	27.16[ab]	18.32[ab]	208.48	1.27[ab]	2.07	93.3
LSBM100%	24.87[ab]	16.37[ab]	192.77	1.32[ab]	1.83	94.4
ESBM40%	26.23[ab]	17.78[ab]	212.12	1.28[ab]	1.95	96.6
ESBM60%	28.31[a]	19.64[a]	226.77	1.14[a]	2.15	93.3
ESBM80%	26.83[ab]	18.13[ab]	208.40	1.27[ab]	1.93	98.3
ESBM100%	28.01[a]	19.31[a]	221.53	1.18[ab]	2.10	93.3
PSE	2.6213	2.6098	32.7362	0.1644	0.2711	5.0479
p-value	0.0168	0.0258	0.0885	0.0350	0.0899	0.3667

REPLACEMENT OF LIVE FEEDS WITH A COMMERCIAL DIET IN FLORIDA POMPANO *Trachinotus carolinus*

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The larviculture of carnivorous marine finfish is heavily dependent on live feeds. However, the production of live feeds requires specialized labor and are prone to population crashes due to their sensitivity to environmental factors. Replacing live feeds with commercially available pelleted diets will significantly benefit producers by reducing both feed and labor costs. The objective of this study was to evaluate the potential of a commercial formulated diet (INVE Natura pRo and ExL; INVE Aquaculture, Belgium) to replace the use of rotifers and/or *Artemia* in the larviculture of Florida Pompano (*Trachinotus carolinus*). This feed has previously demonstrated promising results with seabream (*Sparus aurata*) larvae, achieving over 50% substitution of rotifers, with satisfactory growth rates, larval quality, and survival. The present study was designed to determine whether similar success could be achieved with Florida Pompano.

Our experimental design included a control group using standard rotifer and *Artemia* live feed protocols, along with three treatment groups that were each tested in four replicate tanks (n = 16). Treatment 1 (T1) received an 80% pelleted feed replacement, Treatment 2 (T2) received a 100% pelleted feed replacement along with an additional probiotic slurry, and Treatment 3 (T3) switched from live feeds to 100% pelleted feed replacement after 4DPH. Larvae were sampled at 0, 7, 11, 20, and 25 days post-hatch for fatty acid, biometric, and microbiome analyses. Preliminary biometric results revealed that larvae fed T2 and T3 underwent total mortality before reaching the weaning stage, indicating that the commercial diet is unsuitable for complete replacement of rotifers and *Artemia* in Florida pompano. In contrast, T1 larvae survived past the weaning stage, though they exhibited lower survival rates and slower growth compared to the control group (Figures 1 & 2). Although a total live feed replacement was not achieved in this study, a high replacement percentage of 80% still demonstrates its potential to significantly reduce live feed reliance. Our results show that this commercial diet can serve as a viable alternative, especially in circumstances where producers face challenges in consistently sourcing rotifers and *Artemia* for larviculture.

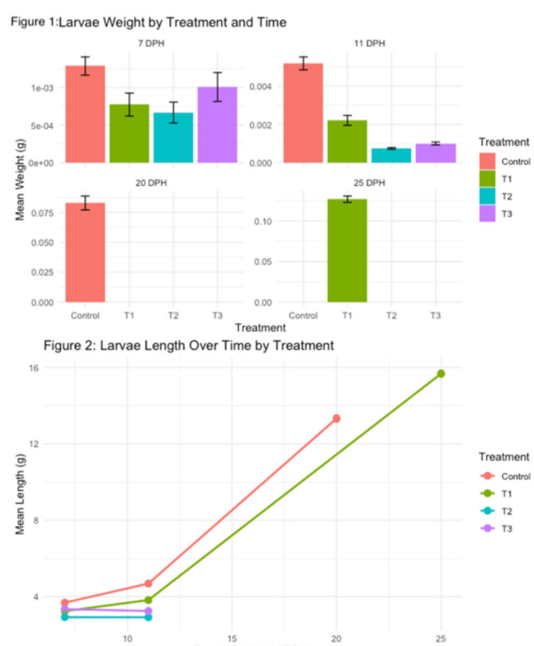

Figure 1 & 2: Weight and length of larvae across treatments and time.

VOLATILIZATION OF AMMONIA BY PADDLEWHEEL AREATION

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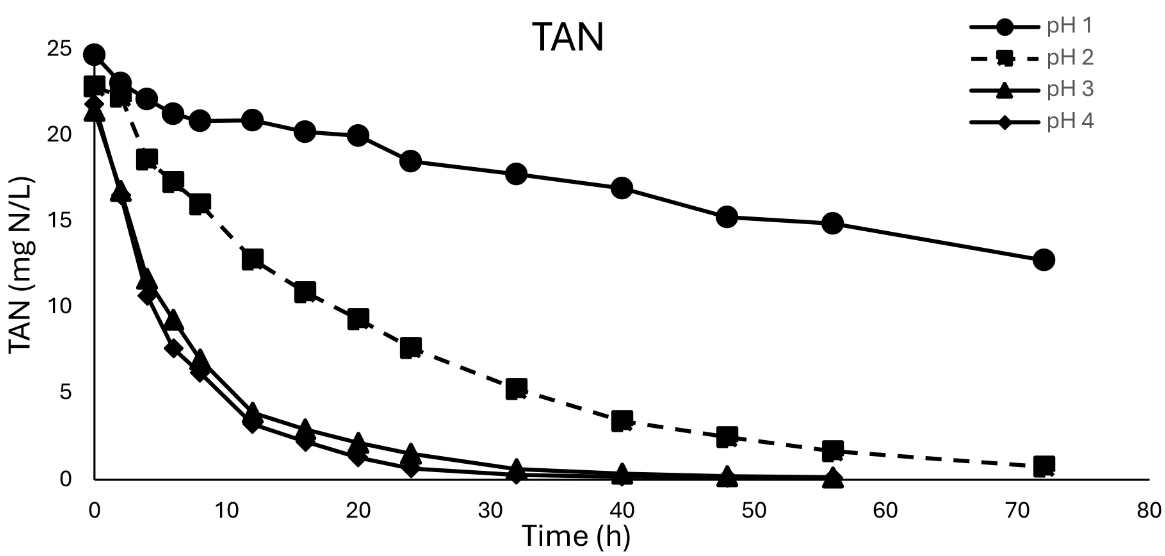
Volatilization of ammonia is the process where dissolved ammonia gas escapes from the liquid phase and releases into the atmosphere. Rates of natural volatilization are influenced by pH, concentration of ammonia, wind, and temperature, however, the impact of paddlewheel aeration on volatilization has not been measured. This experiment was conducted to determine if volatilization of ammonia by paddlewheel aeration is an effective management technique for aquaculture producers. For this experiment, four 14,000 -L raceways were filled with water at 26.6 ˚C and 22 mg of N/L total ammonia and aerated with 1-hp paddlewheel aerators. Four different pH values were tested: 8, 8.8, 9.5, 10.5 and total ammonia, pH, and temperature were measured every 2, 4, or 12 hours. Higher concentrations of total ammonia lead to faster rates of volatilizations, and water with a higher pH volatilizes ammonia faster than water with a lower pH. As pH rises, a greater proportion of total ammonia concentration exits as ammonia gas (NH_3), whereas in lower pH the ammonium ion (NH_4+) becomes the dominant form which cannot be volatilized. Although 20-25 mg N/L of total ammonia can be volatilized within 24 hours at pH values above 9.5, the concentration of un-ionized ammonia would be lethal for catfish and given the diel swings in pond pH, the duration at this pH would be limited (1-3 hours). Unfortunately, using paddlewheels as an ammonia removal tool is not feasible for most catfish aquaculture ponds.



FATIGUE LOADS ON AN OFFSHORE MUSSEL FARM: A GUIDE TO SPECIFYING ROPE

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Offshore mussel farming critically depends on the structural integrity of rope components that endure wave-induced cyclic loading. Accurate assessment of rope fatigue is essential for predicting failures, optimizing design, and enhancing maintenance strategies to ensure safety and longevity. This study evaluates the fatigue performance of mooring lines, main lines, floater lines, and straps under both extreme and typical weather conditions. Extreme and typical loading conditions in the Northeast region were modeled using historical data. Finite element analysis-based numerical simulations were conducted to estimate the cyclical load response levels in terms of stress. The numerical simulation results were then fed into an empirical model [1,2] to predict the total number of cycles to failure from fatigue for each rope component, considering the duration of an extreme event or typical loading conditions during periods of larger biomass. Optimal replacement periods were calculated separately for extreme events like 50-year storms and for typical operational conditions. This offers initial practical guidance for specifying rope dimensions and materials in offshore mussel farms. Implementing these recommendations can improve structural integrity, optimize maintenance schedules, and enhance the overall safety and longevity of farming operations. Future work includes validating the models with field measurements and conducting breaking tension tests comparing used and new ropes to refine fatigue predictions.

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ANAEROBIC DIGESTION OF RAS WASTE: CURRENT STATUS AND RECENT DEVELOPMENTS

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Anaerobic digestion (AD) is a naturally occurring biological process where microorganisms degrade organic matter to produce methane (CH_4), carbon dioxide (CO_2), and trace gases like hydrogen sulfide (H_2S). An anaerobic digester is an engineered system where this process is controlled and optimized, and the gas mixture, also known as biogas, can be captured to generate renewable energy. While AD can be a suitable technique for waste solids treatment from a recirculating aquaculture system (RAS), the components of the waste may lead to several challenges that inhibit the stable functioning of biological processes within a digester. These challenges include low solids content, high salinity, low carbon-to-nitrogen (C:N) ratio, high fat content, and high sulfur content. Typically, all these issues affect the biogas quality and quantity, leading to reduced energy recovery from the waste, increased downtime, and longer return on investment.

In the last few years, researchers at The Conservation Fund Freshwater Institute and around the world have conducted more in-depth studies on AD to understand and overcome some of these challenges. Studies have investigated thickening to concentrate the waste solids as a pretreatment, co-digestion to alleviate inhibition due to the low C:N ratio and high fat content, acclimated microbes to reduce the negative effects of salinity, different reactor setups to enhance biogas production, and biochar addition to minimize H_2S production. In this presentation, the results of these studies will be discussed. Future research directions for further resource recovery will also be presented.

A REPETETIVE *Acipenser gueldenstaedtii* GENOMIC REGION ALIGNING WITH THE *Acipenser baerii* IGLV GENE CLUSTER SUGGESTS A ROLE AS A TRANSCRIPTION TERMINATION ELEMENT ACROSS SEVERAL STURGEON SPECIES

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DNA was isolated from Russian sturgeon (*Acipenser gueldenstaedii*) tissue samples or individual caviar grains of Russian and Siberian sturgeon (*A. baerii*) from farms in Israel, North Carolina, and Florida. A repetitive 675 bp VAC-2M sequence identified in Russian sturgeon DNA aligns with the Siberian sturgeon IGLV gene cluster. A specific 218 bp long portion of the sequence was found to be identical between *A. gueldenstaedtii*, *A. baerii* and *A. stellatus* species and NCBI blast analysis confirmed the presence of the respective DNA segment in the *A. ruthenus* genome. Multiple mutated copies of the same genomic region were also detected by PCR analysis, indicating that different versions of this highly repetitive sequence exist simultaneously within the same organism. The process of selection toward specific mutation appears to be not random and is ongoing based on the sequence variations within DNA samples that derived from different individual caviar grains but originated from the same fish. The homologous between Russian and Siberian sturgeons 376 bp DNA fragment of the repetitive sequence was cloned either ahead or after the human cytomegalovirus immediate early promoter (HCMV-IE) into a pBV-Luc reporter vector expressing the luciferase gene. The DNA segment significantly reduced luciferase expression with maximum inhibition achieved when it was cloned in both orientations immediately after the HCMV-IE promoter (Figure 1). Thus, this genomic region functions as a transcription termination element. These results provide some new information about the role of repetitive sequences within eucaryotic organisms in general and indicate that they may play an important role in sturgeon immune system regulation.

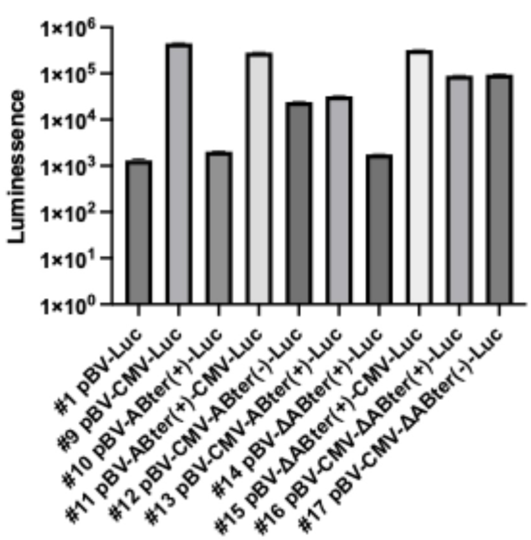

Figure 1. Luciferase expression levels in Vero cells using constructs containing ABter (*A. baerii* nts 3202-3558) and ΔABter (*A. baerii* nts 3361-3558) cloned under control of the HCMV-IE promoter into pBV-Luc plasmid. (+) denotes positive DNA strand orientation, while (-) is an indication that the same sequence was cloned in opposite orientation.

FINANCING INDUSTRY IMPROVEMENTS THROUGH JURISDICTIONAL INITIATIVES

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Shrimp aquaculture production has increased by more than 1,000% over the last three decades, contributing to the loss of over 40% of the world's mangroves and 70% of mangroves in Indonesia through deforestation and pollution. Aquaculture Improvement Programs (AIPs) aim to reduce the environmental and social damage caused by shrimp farming by moving individual farms towards certification under internationally recognized environmental and social responsibility standards. Despite notable improvements, only 13% of global shrimp farms are certified. Jurisdictional AIPs, a novel approach to aquaculture improvement borrowed from agriculture, seek to improve production across entire landscapes through coordination among diverse stakeholders and utilization of market, policy, and finance incentives for responsible production. Jurisdictional AIPs appear to have enormous potential to scale improvement, but they remain experimental. Consequently, most jurisdictional AIPs are financed by philanthropy and long-term non-philanthropic financing models are needed.

To investigate the potential financial benefits of implementing jurisdictional AIPs, we developed a cost-benefit analysis model (CBA) for the shrimp industry in Banyuwangi Regency (the Regency), East Java, Indonesia. The goal of the CBA is twofold - to demonstrate the likely financial value of the initiative to stakeholder groups (e.g., farmers, farm groups, government, supply chain companies, financial institutions) and to seek their long-term financial support for the jurisdictional AIP based on their potential return on investment.

The CBA model incorporates assumptions on how the Regency is expected to perform under the jurisdictional AIP's best management practices. As the project in Banyuwangi progresses, these assumptions will be replaced by empirical data. These assumptions have been informed by expert practitioners with extensive experience in the Regency's shrimp farming sector.

The CBA model will be a valuable tool to assess the likely financial beneficiaries of the jurisdictional AIP, and the benefits accrued to the Regency throughout the project. If the data show that the Regency meaningfully benefits in an environmentally positive and financially profitable manner from the practices outlined in the jurisdictional AIP plan, we will subsequently encourage NGOs and project developers to create jurisdictional AIPs that are financed with capital from non-philanthropic sources.

UNITED STATES CONSUMERS' ATTITUDES TOWARDS CELL-BASED SEAFOOD: EFFECTIVE MARKETING STRATEGIES AND POLICY RECOMMENDATIONS

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As global seafood demand intensifies with population growth and nutritional needs, developing sustainable production methods is essential for securing future food systems. In the U.S., seafood consumption is projected to double by 2050, making cell-based seafood a promising, sustainable alternative. While advancements in scalable cell-based seafood technologies are progressing, significant challenges remain, particularly in regulatory, socio-political, and consumer acceptance areas. The success of cell-based seafood as a sustainable food source heavily depends on consumer acceptance, yet public attitudes toward this innovation are not well understood. To address this gap, a survey was conducted via Qualtrics with a nationally representative sample of 2,121 U.S. respondents. This study provides critical insights into consumer attitudes toward cell-based seafood by examining socio-demographic influences and adoption barriers. Results indicate a complex landscape where willingness to try these cell-based seafood varies significantly across demographics, with younger consumers, urban residents, and environmentally-conscious consumers displaying a higher acceptance for cell-based seafood. Additionally, consumers are already inclined to try innovative foods and current seafood eaters are more open to cell-based options. However, cell-based seafood adoption barriers are artificialness, unfamiliarity, and lack of exposure. Findings from this study emphasize that fostering consumer trust in cell-based seafood requires concerted efforts in regulatory clarity, and educational initiatives that enhance familiarity. On the other hand, Total Unduplicated Reach and Frequency (TURF) analysis shows that food safety is the primary factor driving consumer acceptance, highlighting the need for transparent, safety-focused communication. These insights inform actionable strategies for industry stakeholders and policymakers, advancing a market integration framework essential to positioning cell-based seafood as a viable and sustainable component of the U.S. food system.

STRUCTURAL AND TEXTURAL CONSIDERATIONS FOR DEVELOPING ALTERNATIVE SEAFOOD ANALOGS

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The global surge in seafood demand has led to significant overexploitation of wild fish stocks, intensifying the need for sustainable seafood alternatives. Both plant-based and cell-based seafood analogs have emerged as promising solutions, aiming to meet consumers' needs while addressing environmental and food security challenges. Texture, a critical sensory attribute for seafood, plays a pivotal role in consumer acceptance of these alternatives. However, replicating the diverse textures of commercial fish species remains a significant obstacle due to limited baseline knowledge of fish muscle architecture and its relationship to texture. This study provides a comprehensive, species-specific analysis of the structural and textural properties of four commercially significant fish species: salmon (*Salmo salar*), tilapia (*Oreochromis niloticus*), tuna (*Thunnus spp*.), and grouper (*Epinephelus spp*.). Using Texture Profile Analyzer (TPA), colorimetry, collagen assays, and collagen imaging, this study established critical baselines for muscle fiber size, collagen density, and their correlations to textural attributes which are hardness, adhesiveness, springiness, gumminess, cohesiveness, and chewiness. Notably, hydroxyproline content, a proxy for collagen levels, varied significantly across species, with tilapia exhibiting the highest levels, followed by grouper, salmon, and tuna. Collagen content positively correlated with hardness springiness, gumminess, and chewiness ($p<0.05$), while muscle fiber size was inversely correlated with hardness ($p<0.05$). These findings offer valuable insights into the structural underpinnings of fish texture, informing material selection, scaffold design, and muscle alignment considerations for both scaffold-based and scaffold-free seafood analog production. By bridging this critical knowledge gap, this study supports the development of alternative seafood products that align with consumer expectations and advance sustainability goals in cellular and plant-based aquaculture.

SEAWEED FOOD SAFETY: DRAFTING A HAZARDS AND CONTROLS GUIDE

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Interest in seaweed production and consumption is growing across the United States. With increasing production comes a greater need for processing and enhancing processing infrastructure for seaweed and seaweed products. As the industry continues to develop new products and works towards growing the consumer market, it is important to provide guidance on food safety hazards and their controls to protect public health.

For nearly two years, a global team of food safety experts has been actively discussing the challenges surrounding seaweed food safety. Within this group, a core team has successfully secured funding through the USDA Food Safety Outreach Program to develop a *Seaweed and Seaweed Products Hazards and Controls Guide*, like the FDA's "*Fish and Fisheries Products Hazards and Controls Guide"*. The guidance document, under development, will support the growing industry by providing the resources necessary to develop and implement effective controls for significant food safety hazards. Drawing on the available scientific knowledge, the guidance will cover all potential food safety hazards associated with seaweed species and processing methods. In addition to outlining the hazards, it will provide some insights on strategies for controlling such hazards within seaweed processing operations.

The project team is in the final stages of completing first draft of the guidance document, which will go under review by experts both nationally and internationally. Once published, it will support safe processing and marketing of seaweed products globally and serve as the foundation for future discussions on developing a seaweed specific food safety training.

Table 1: Table of Contents for Seaweed Hazards Guide.

CHP	Title
1	Introduction and Background
2	Seaweed Regulations and Guidance
3	Prerequisite Programs
4	Seaweed Food Safety Hazards
5	Pathogens in Harvest Area
6	Parasites
7	Environmental Chemical Hazards
8	Iodine
9	Natural Toxins
10	Pathogen Growth due to Temperature Abuse
11	Pathogen Contamination
12	Clostridium botulinum Toxin Formation
13	Pathogen Survival through Cooking or Pasteurization
14	Allergens and Food Intolerance Substances
15	Physical Hazards
16	Knowledge Gaps and Summary

HIGH RESOLUTION MONITORING REVEALS SENTINEL SIGNAL IN THE WATER COLUMN PRECLUDING MORTALITY IN FARMED OYSTERS IN NORTH CAROLINA

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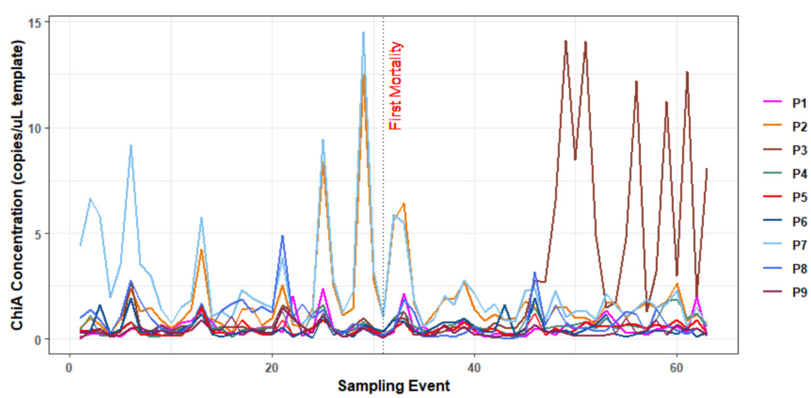
Mortality events in farmed oysters throughout the coastal landscape of North Carolina (NC) have stymied growth in the oyster aquaculture industry. Histological analyses of oysters harvested during these mortality events indicate that disease is playing a role in the loss of valuable product. To better understand etiology and onset of disease, longitudinal studies have been conducted to characterize both oyster condition and surrounding environmental conditions during periods of stress. However, previous efforts have not always succeeded in capturing critical moments in the progression of disease development. To combat these shortcomings and increase resolution, a daily sampling campaign was undertaken at a lease that has consistently experienced severe mortality over the past decade.

For 63 consecutive days, a subset of triploid oysters (*Crassostrea virginica*), water samples, and environmental parameters were collected from a single site. Histology sections were taken from each oyster censored and water samples were subjected to analysis via ddPCR (droplet digital PCR) to quantify pathogen presence within the water column. Using novel molecular assays designed to quantify multiple *Vibrio spp*. by targeting the GH18 domain of the *chiA* gene, our analyses revealed a strong microbial signal in the water column prior to the first observations of mortality. The peaks in concentration of two distinct *Vibrio* clades occurred 6 days before the first observed death, revealing a small window of time between initial challenge and eventual onset of mortality. On any given day, no more than 5 oysters were found dead, suggesting that the general perception of mortality is often colored by a lack of resolution with weekly assessments often appearing more jarring. In contrast, daily observations demonstrate a slower progression of disease. Given that the increase in concentration of subpopulations of *Vibrio* bacteria in the water column occur prior to the first instance of mortality, this signal could offer predictive capacity and indicate when mitigation or preventative measures would be most effective.



DIFFERENCE BETWEEN NUTRIENT DENSITY AND GROWTH OF THAI BASIL GROWN IN SOIL AND IN AQUAPONICS

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Currently the world's population is nearing 8 billion people and approximately 1 billion of those people are food insecure. There are many reasons for food insecurity but one of the main reasons is the lack of resources. Aquaponics has the ability to produce 10x more than agriculture and 5x more than aquaculture. It also uses 2-10% the amount of water that traditional agriculture requires. These attributes alone make aquaponics a promising solution to the world's increasing food insecurity. To determine if aquaponics can produce a product that is nutritionally adjacent to traditional agricultural, there were four treatments. Two of the treatments were soil type (topsoil vs Miracle Gro) and the other two were fish feed type in aquaponics (Purina Aquamax Fingerling Starter 300 vs Optimal Fish Food's Optimal Aquaponic feed). Topsoil and Miracle Gro potting mix allows for comparison of growth and nutrient density from plants grown in normal soil vs fertilized soil. The Optimal Aquaponic feed fortifies potassium, phosphorus, and iron that will enter the water column via fish excrement. These nutrients are often lacking in aquaponics and will elicit optimal plant growth. To determine if this feed is better, it will be compared to a non-aquaponic formulated feed (Purina). The plants grown in the two soil types will also be compared to those grown in the two aquaponic treatments. Basil from all four treatments will be harvested after 48 days of growth. Leaf samples will be sent to A&L Great Lakes Laboratories for nutrient testing. Total harvest wet weight and sample dry weight will be used to evaluate plant growth. It is hypothesized that basil grown in aquaponics with fish fed an aquaponic formulated diet will have better nutrient richness, higher total harvest weight and higher dry weight. Results will be presented at the conference.

HISTORICAL LANDINGS, SALES TRENDS, AND THE ECONOMIC OUTLOOK OF THE VIRGINIA SHELLFISH AQUACULTURE INDUSTRY

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Virginia's shellfish aquaculture industry has a rich history, playing a vital role in the state's coastal economy. However, unlike traditional agriculture and commodity markets, historical time series data is rarely utilized to document the industry's evolution. This review seeks to bridge that gap by utilizing the limited data available to provide historical context on landings and sales. Key economic and environmental events that have shaped the industry over time will be analyzed, highlighting their impact on production and market trends. A central focus will be the comparison of nominal versus real sales values, offering insights into price shifts and the implications for market resilience and growth. The primary emphasis is on the broader trends that define the industry's past and inform its future. This review will conclude with an outlook on potential challenges and opportunities that lie ahead for Virginia's shellfish aquaculture industry.

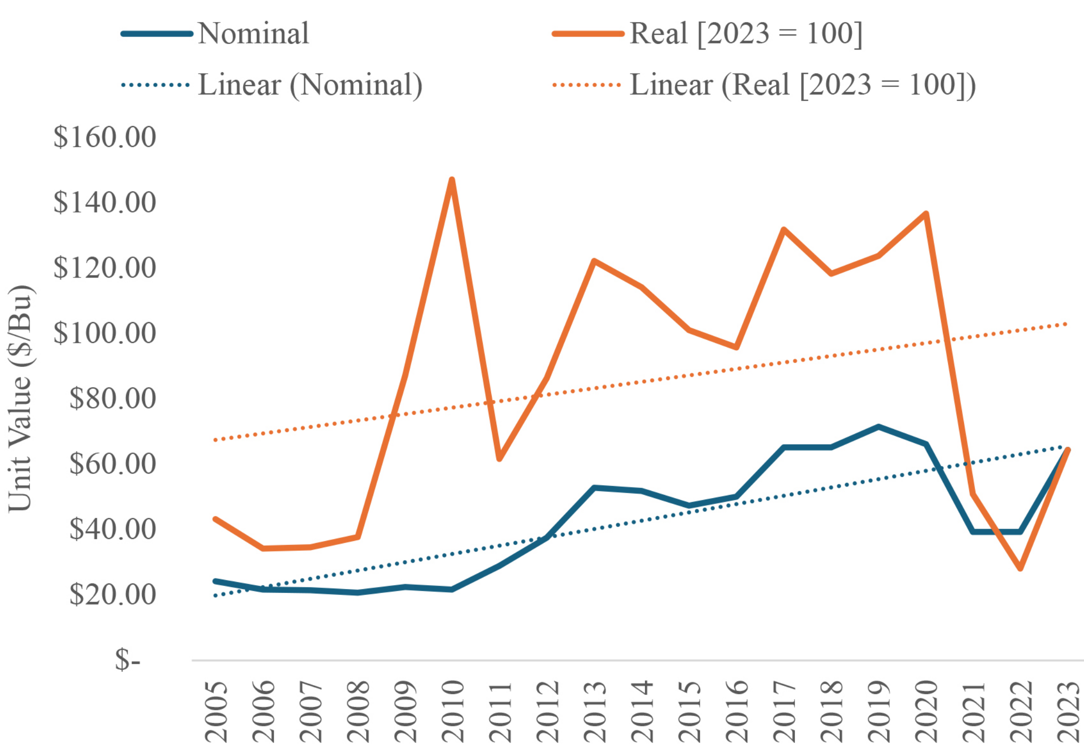

Figure 1. Unit price of Virginia-farmed Oysters, 2005 – 2023. Landings and sales data provided by the Virginia Marine Resource Commission (VMRC). Real prices calculated using the Median Consumer Price Index (2023 = 100) provided by the Federal Reserve Bank of Cleveland.

ANALYSIS OF ATLANTIC SEA SCALLOP *Placopecten magellanicus* LARVAL DEVELOPMENT UTILIZING ELECTRON MICROSCOPY

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Hatcheries have the potential to reliably produce Atlantic sea scallop *Placopecten magellanicus* spat, but challenges remain in rearing larvae through critical stages of early development. Current larval husbandry methods rely on light microscopy to observe growth and anatomy, which can often obscure key characteristics of complex and often translucent features of larvae at these stages. This ongoing project explores the use of scanning electron microscopy (SEM) to capture high-resolution images of sea scallop larvae at various developmental stages. Currently images of p1 shell development, early p2 development, and the pediveliger stage sourced from the Downeast Institute in Beals, Maine have been captured. The aim of these micrographs is to provide insights into the shell and organ development of these animals grown in a hatchery setting. Alongside promoting hatchery innovation, these images are intended to have pedagogical value as they can replace illustrated life cycle diagrams currently used in university course curriculums.

AUTOMATING WHITE BLOOD CELL IDENTIFICATION IN LARGEMOUTH BASS *Micropterus salmoides* BLOOD SMEARS

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Immune response is a critical indicator to assess fish health. The profile of white blood cells (WBCs) is a valuable metric for evaluating immune status, as variations in WBC abundance can reflect underlying health issues. In this project, we employed machine learning (ML) to automate image recognition of WBCs in blood smears from juvenile largemouth bass. The goal is to replace manual WBC counting, which is time-consuming, requires technical training, and is potentially inconsistent among readers.

Blood smear slides were created from 38 fish. Three independent readers manually counted and classified 100 WBCs per slide. These slides were scanned at 83x, digitized using an Aperio ScanScope CS, and uploaded for labeling using SageMaker Ground Truth software. "Regions of interest" (ROIs) were selected and divided into 48 "tiles". WBCs were labeled to create a labeled image dataset to train the model (Fig. 1). Labeled cells included 1242 lymphocytes, 493 monocytes and 297 granulocytes; 90% of these were used to train the model and 10% were used to test the model.

To validate the ML model, we compared manual cell counts among the 3 human readers, manual cell counts to automated cell counts by the model, and automated cell counts of novel tiles taken from the training slides. Manual counts of lymphocytes were most similar among the three readers, with each reader being within 95-106% of the average count for all three. Manual counts of granulocytes and monocytes were more variable, with each reader within 73-149% of the average. These manual cell counts will be compared to automated counts to evaluate effectiveness of the model. At present, the model is approximately 80% accurate in its ability to correctly recognize all three cell types in blood smears from largemouth bass. Additional training tiles can be labeled to increase model accuracy. The potential benefits of this automated tool include increased accuracy and efficiency in WBC analysis, less reliance on specialized training, and enhanced accessibility.

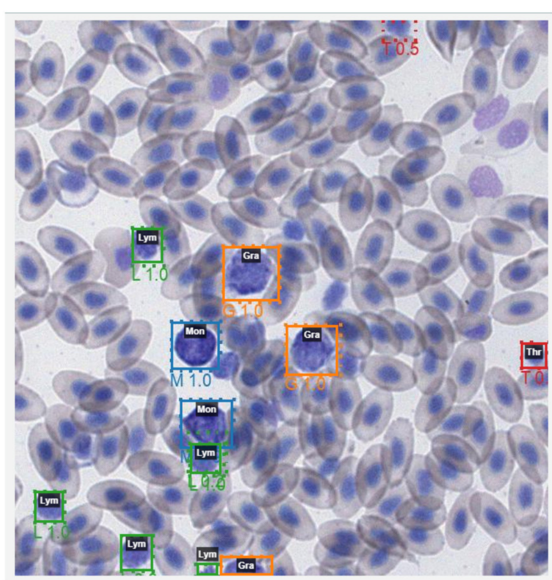

Fig 1. WBCs in a training tile: granulocytes (orange), monocytes (blue), lymphocytes (green), and thrombocytes (red).

FACTORS INFLUENCING LOBSTER FISHERS' PERCEPTIONS OF AQUACULTURE AND THEIR WILLINGNESS TO ADOPT

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Aquaculture is often seen as a way for commercial fishers to diversify their livelihoods. However, the adoption process is complex. Maine (USA) provides an opportunity to explore the potential for commercial fishers to adopt aquaculture, given the state's growing aquaculture industry and a coastal economy highly dependent on the commercial fishery for the American lobster (*Homarus americanus*). The lobster fishery faces challenges such as declining landings, market uncertainty, and complex regulations. In response to these changes, there is interest in helping fishers adopt aquaculture, and several aquaculture training programs targeted at commercial fishers have been launched. Given the changes occurring in fisheries and aquaculture and the potential for aquaculture to be a diversification strategy for commercial fishers, we aimed to explore the implications of a growing aquaculture sector within the context of environmental and social change in coastal communities. We applied the adoption of innovation theoretical framework to determine individual characteristics and perceptions that would predispose commercial lobster license holders to consider adopting marine aquaculture.

In 2018, we distributed a structured mail survey to Maine lobster license holders to determine their willingness to consider employment in aquaculture. Survey questions focused on demographic background, fishing history and experience, and perceptions of the future lobster fishery and aquaculture. Individuals were asked to rate their level of agreement with a statement, "I will be employed in the marine aquaculture industry within the next 5 years." Our initial analysis shows that most respondents did not think they would work in aquaculture, and their views on aquaculture were generally neutral.

The study will provide a summary of the demographic and fishing history, experience, and perceptions of aquaculture. This research is important as it establishes a baseline before the COVID-19 pandemic for lobster fishery participants and their attitudes towards aquaculture. The results of this study are particularly relevant to Maine, as it will help practitioners understand the potential of aquaculture as a diversification strategy for Maine's coastal communities and working waterfronts. In a broader context, this study adds to the literature on livelihood diversification and the adoption of innovation, specifically focusing on the integration of aquaculture and commercial fishing in a developed nation.

EXTREME SUMMER HEATWAVES DRAMATICALLY INCREASE INDIRECT SOFT-SHELL CLAM FISHING MORTALITY VIA SHIFTS IN PREDATOR-PREY DYNAMICS

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The increasing frequency and severity of heatwaves driven by global climate change are projected to have multifaceted ecological effects. While studies have assessed the impacts of heatwaves at the organism and population level, field-based studies documenting the effects of heatwaves on *in situ* ecological interactions are rare. Furthermore, studies assessing the biological and ecological implications of direct anthropogenic disturbance during heatwaves are absent. Here, we leverage mesocosm field experiments to document the effects of clam fishing during an extreme heatwave on the behaviour and survival of sub-legal sized soft-shell clams, *Mya arenaria*, in eastern Canada. In monthly field experiments from May to September, we simulated clam fishing at low tide and subsequently returned marked, sub-legal sized clams to the sediment surface at three different tide levels; predator exclusion containers were used to protect half of the clams from crab predation. Reburrowing and mortality rates were recorded immediately after fishing and after 24- and 48 hours. Results showed that, typically, indirect fishing mortality was low and the vast majority of clams reborrowed within 24 hours. However, during an extreme heatwave in June, the clams appeared visually unhealthy during fishing and failed to reborrow, leading to near complete mortality during the heatwave. Estimates of predator activity were ≈5× higher during the heatwave compared to other months, and 87% of indirect fishing mortality was attributable to predation. When put into the context of air temperature thresholds, there was a clear shift after 30ºC, whereby clam reborrowing plummeted, and predator activity and mortality increased dramatically. Ultimately, our study provides strong field-based evidence that ecological shifts during heatwaves, facilitated by human activity, can drive substantial indirect fishing mortality in a nearshore fishery where such mortality is otherwise low. These results not only provide support for climate driven alterations to predator-prey dynamics and ecosystem function, but generate pertinent information for an ecosystem approach to fisheries management.

DYNAMIC SHIFTS AND A DRASTIC DECLINE IN REPORTED LANDINGS FOR THE SOUTHERN GULF OF ST. LAWRENCE COMMERCIAL CLAM FISHERY OVER THE PAST TWO DECADES

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Clams constitute an important socioeconomic and Indigenous resource in the southern Gulf of St. Lawrence (sGSL); however, detailed analyses of the commercial fishery are outdated. Using landings data, I provide a synthesis of the sGSL clam fishery from 2003-2022. Three species comprised >99% of total landings: *Mya arenaria*, *Mercenaria mercenaria*, and *Spisula solidissima*. Annual landings mostly came from Prince Edward Island (75 ± 7%), followed by New Brunswick (23 ± 6%) and Nova Scotia (2 ± 2%). For the sGSL as a whole, the three species contributed equally to landings from 2003-2020, but *M. arenaria* dominated landings from 2021-2022. This trend was not consistent when provinces were considered individually: province-specific fluctuations in species composition and a contemporary shift from multi-species to single-species harvests were apparent. Overall, landings and their associated value have sharply declined by 80% and 66%, respectively, over the time series. The number of catch records also declined by 80%, suggesting progressively fewer people engaging in the fishery. Annual catch records were a strong predictor of annual landings, and declines in landings per catch record (proxy of catch per unit effort) were evident. This analysis ultimately suggests a dying Canadian fishery. Understanding the proximate causes of fishery declines, how to address them, and determining whether such declines reflect population trends, should be prioritized to revive and save the sGSL clam fishery.

PROFILING GROWTH PERFORMANCE, INSULIN-LIKE GROWTH FACTORS (IGF) AND IGF-BINDING PROTEINS (IGFBP) IN RAINBOW TROUT LACKING IGFBP-2B

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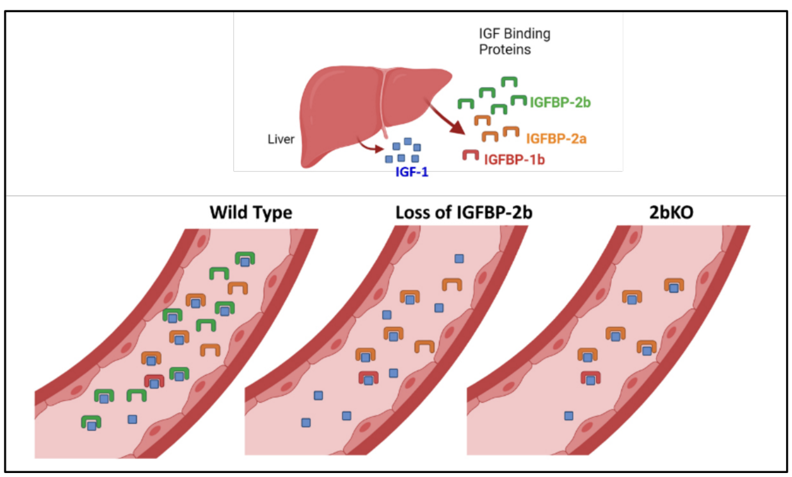
Insulin-like growth factor binding proteins (IGFBP) regulate insulin-like growth factor (IGF) signaling, but IGFBP-specific functions are not well characterized in fish. A line of rainbow trout (*Oncorhynchus mykiss*) lacking a functional IGFBP-2b was produced using gene editing and subsequent breeding to an F2 generation. This loss-of-function model (2bKO) was subjected to either continuous feeding or feed deprivation (4 wk) followed by refeeding (2 wk). During continuous feeding, the 2bKO line displayed faster specific growth rate for both biomass and fork length, higher feed intake, and reduced feed conversion ratio compared to a wild type (WT) line. However, loss of IGFBP-2b did not affect the feed deprivation or refeeding response in terms of weight loss or weight gain, respectively. Several components of the IGF/IGFBP system were affected by loss of IGFBP2b. Total serum IGF-1 in the 2bKO line was reduced to 0.5 – 0.8-fold of the WT line although the concentration of free serum IGF-1 was not affected. These findings suggest that IGFBP-2b might only carry 20 – 50% of circulating IGF-1 while the 32 kDa IGFBP (putative IGFBP-2a) serves as an additional stabilizer of serum IGF-1 (Figure 1). Gene expression differences include reduced abundance of *igfbp1a1*, *igfbp1b2*, *igfbp5b2*, and *igfbp6b1* transcripts, and elevated *igf2* and *igfbp6b2* transcripts in liver of the 2bKO line. Collectively, these findings suggest that although IGFBP-2b is a carrier of circulating IGF-1 in salmonids, the presence of IGFBP-2a and compensatory responses of other IGF/IGFBP system components likely contributed to improved growth performance and nutrient utilization, supporting the significance of hepatic-derived IGFs and IGFBPs as regulators of IGF signaling in peripheral tissue. Collectively, these findings advance understanding of the role of IGFBPs as carriers of circulating IGF-1 in fish and warrant further investigation into the functional role of the putative IGFBP-2a.

Figure 1. Proposed model for the role of the putative IGFBP-2a as a major carrier of IGF-1. The hepatic production of IGF-1 and circulating IGFBPs is shown at the top. The bottom represents the relative abundance of IGF-1 and IGFBPs in circulation in the WT line (left), along with the proposed phenotype of these proteins in the 2bKO line (right). The center represents the transient increase in free IGF-1 due to loss of IGFBP-2b.

Figure 1. Proposed model for the role of the putative IGFBP-2a as a major carrier of IGF-1. The hepatic production of IGF-1 and circulating IGFBPs is shown at the top. The bottom represents the relative abundance of IGF-1 and IGFBPs in circulation in the WT line (left), along with the proposed phenotype of these proteins in the 2bKO line (right). The center represents the transient increase in free IGF-1 due to loss of IGFBP-2b.



SCIENCE-DRIVEN SOLUTIONS: NOAA SCIENCE TO INFORM AQUACULTURE TOOLS, MANAGEMENT, AND DECISION-MAKING

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NOAA's Office of Aquaculture (OAQ) strives to develop, support, and share the best available science for decision-making at local, regional, and national scales. Along with critical partners in other federal and state agencies, Tribes, NGOs, non-profits, industry groups, and more, OAQ uses science to inform management and decision-making for sustainable aquaculture development. Leveraging the best available science from NOAA and our partners is critical for fulfilling the strategic vision, mission, and goals of the NOAA Aquaculture Program. This presentation will outline the Aquaculture Program's science vision and current aquaculture science portfolio, and highlight examples of how NOAA-supported science has been applied to decision-making tools.

AN UNUSUALLY LARGE INDIVIDUAL OCEAN QUAHOG *Arctica islandica L.*

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The ocean quahog, *Arctica islandica*, is the longest-lived metazoan with a single individual, nicknamed Ming, having an estimated age at collection of 508y. Despite this extraordinary age, Ming was comparatively small with a shell length (SL) of 86.9 mm from a live collection off Iceland in 2006. Small but old specimens of this species are not rare. Large individuals (90-120 mm SL) are regularly retained in fishery surveys in the U.S. Mid-Atlantic and Georges Bank regions. Large size is not always associated with older age. Ropes & Murawski (ICES report 1983) describe 3 large individuals from NOAA-NMFS surveys: a 132 mm SL at 157 y from Central New Jersey; a 130 mm SL at 53y also from Central New Jersey; and a 130 mm SL at 93 y from Georges Bank. These animals represent over 100 years variation in age but were comparable in size. A perusal of NEFSC and other industry supported surveys over the past 40 years indicate that individuals exceeding 130mm SL are rare. We report herein on a recent collection and examination of the largest individual ocean quahog for which we can find a record.

A single left valve from a live specimen of 141 mm SL was collected in a commercial hydraulic dredge on August 14, 2023 at 40.18.60°N and 73.19.20°W. This location is on the north side of the Hudson Canyon at ~50 m depth, in a region where the inshore limit of the bottom seasonal cold pool abuts warmer water above the seasonal thermocline. This inshore limit is, in successive years, gradually retreating to deeper water, and an ecotone of mixed surf clam (*Spisula solidissima*) and ocean quahog benthic dominants is developing in this transition region. The collection depth of this large specimen is in this expanding ecotone.

The valve was embedded in clear polyester resin, sectioned through the axis from hinge to growing edge, and the exposed edge polished prior to acquiring reflected light images of the hinge region and entire section from hinge to the growing edge. Initial low-definition images suggest an age at collection of between 62-65 years (to be updated as higher definition images are acquired after the abstract submission date). We discuss the growth history of this specimen in relation to both the local environment of the collection site and the general long term warming of the Mid Atlantic with climate change.

This work was supported by the National Science Foundation (NSF) Science Center for Marine Fisheries (SCEMFIS, www.scemfis.org) and an NSF Veteran Research Experience Award to Cohn as the presenting author.

TISSUE-SPECIFIC RESIDENT AND TRANSIENT MICROBIAL COMMUNITIES OF THE FRESHWATER UNIONID *Elliptio complanata*

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Research into bivalve microbiomes has identified distinct microbial communities across tissue types, including the hemolymph, gills, and gut complex. The consistent presence of these microbial communities likely indicates that bivalve microbiomes play crucial roles in host physiology and have a functional role similar to those in vertebrates. The bivalve suspension-feeding strategy exposes these animals to a number of microbes, both free-living and particle associated. Some of these microbes are unlikely to become established as members of a particular community. Most research in this field has focused on marine bivalves, with little focus on freshwater species. The purpose of this study was to characterize the resident gill and gut microbial communities of the freshwater eastern elliptio, *Elliptio complanata*. Mussels were collected from natural populations in the Delaware River. One subset of mussels was dissected immediately to isolate both resident and transient microbes associated with the gill and gut tissues. A second subset of mussels was placed into individual depuration chambers for 24 hours to allow animals to egest feces and transient microbes. After 24 hours, egested feces were collected, and gill and gut tissues isolated to characterize the resident microbial communities. Approximately 2/3 of mussels collected were gravid, therefore one demibranch containing eggs was also collected from a subset of mussels. Water samples were taken from the Delaware River to examine the pool of microbes from the natural water. All samples underwent DNA extraction, PCR, and 16S rRNA sequencing to identify the associated microbial communities. Data from this experiment will provide essential baseline knowledge of Elliptio microbial communities and inform future work investigating the dynamics of freshwater bivalve host-microbe interactions.

DEVELOPMENT OF AUTONOMOUS VEHICLE FOR EDNA COLLECTION

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The oyster pathogens *Perkinsus marinus* and *Haplosporidia* spp. are responsible for major outbreaks of the diseases known as dermo, multinucleate sphere unknown (MSX) and seaside organism (SSO). These diseases are common to the Eastern oyster and have been known to proliferate in Rhode Island waters causing mortalities. Oysters in RI are routinely monitored for these diseases to prevent the movement of pathogens and subsequent outbreaks. However, there is a need for an alternate detection system, one that allows for screening before detrimental effects take hold in the organisms. Environmental DNA (eDNA) screening through water sampling could help provide aquaculturists with information on potential pathogens before outbreak occurs on the farm.

Traditional water sampling methods limit the volume of water, and therefore the DNA, that can be collected from a site. Jaia Robotics provides innovative methods of aquatic data collection using their autonomous vehicles known as JaiaBots. These vehicles have been able to help record data for a multitude of water quality parameters and eDNA collection methods are now being explored as a potential addition to these JaiaBots. This project furthers the developmental process by comparing the water sample collection and filtration used by the JaiaBot with traditional collection and vacuum filtration methods.

JaiaBots equipped with Sylphium eDNA dual filter capsules were deployed among areas with high levels of shellfish pathogens. At the same time of collection, 1L water samples were also collected in triplicate using sterile glass bottles and concentrated onto 0.8μm filters for comparison. DNA extractions were performed using the Sylphium water DNA extraction kit for the JaiaBot samples, and the Qiagen PowerSoil Kit for the 1L water samples. Quantity and quality of the DNA was evaluated, and diagnostic quantitative polymerase chain reaction assays (qPCRs) for Dermo, MSX, SSO were carried out to determine their prevalence in the water column.

Preliminary results show that DNA collected via autonomous methods had higher average concentrations of DNA (865.1 ng/uL) than those collected using traditional methods (478.1 ng/uL). The JaiaBots with Sylphium eDNA capsules filtered 17.75L of water, more than 5 times what was collected by hand. The completion of this research will both determine the current prevalence of shellfish pathogens surrounding major aquaculture sites in Rhode Island, as well as explore a new method of water collection that could alter the way marine research is conducted. Use of these JaiaBots can help researchers collect much larger sample sizes, inform upon potential risks, and allow for the deterrence of disease outbreaks.

USING VIDEO-BASED APPROACHES TO CENSUS THE AQUATIC COMMUNITY SURROUNDING OYSTER AQUACULTURE AND NATURAL REEF STRUCTURES IN DELAWARE BAY, DE

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Gear associated with off-bottom oyster aquaculture adds artificial structure to areas otherwise devoid of structural complexity and heterogeneity. As the commercial development of aquaculture grows, assessing the influence of culture gear on local marine communities becomes imperative. Similarly, shellfish-based living shorelines and oyster reef restoration projects require monitoring to assess the added habitat value of these landscape modifications. Camera-based approaches have been used to monitor and assess these habitats previously in other areas. But low visibility, like what is observed in Delaware Bay, poses a significant challenge to estimating species assemblages in estuarine environments. In this study, we developed an effective means of monitoring fish assemblages in low-visibility locations: comparing the biodiversity, including species composition, and abundance, as well as behavioral responses of organisms found near oyster rack-and-bag systems to those in natural oyster reefs.

We deployed stationary cameras to record video at a natural reef site, a rack-and-bag oyster cage, and a flat, sandy bottom in Lewes, DE from June to August 2024 for 1.5 hours (n=20). Animals were identified to the lowest possible taxonomic classification. The time the animal entered and exited the view was noted along with any behavioral responses. Preliminary results suggest that oyster structures enhance local abundance ($p = 0.045$, $n = 8$, Kruskal-Wallis test, Fig. 1). The type of oyster-derived structure may minimally influence abundance ($p = 0.070$, $n = 8$, Dunn test) while not affecting species richness ($p = 0.143$, $n = 8$, Kruskal-Wallis test); however, each structure supported communities that forage in different zones within the water column.

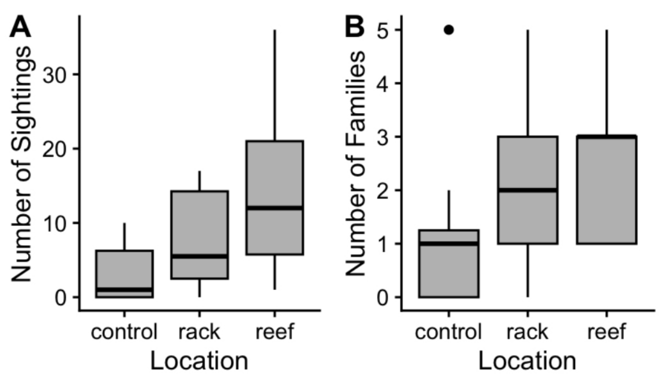

FIGURE 1: Distribution of (A) sightings (standardized number of observations) and (B) family richness at each site. Sightings across locations varied, while richness did not.

GROWTH AND SEXUAL MATURITY OF CRAYFISH *Faxonius immunis* EXPOSED TO LOW PH

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Environmental contamination of ecosystems, leading to their eventual degradation, can result from various sources, including agricultural, industrial, and municipal runoff. This poses a significant threat to Mexico's rich biodiversity. Pollutants from runoff alter water chemistry, notably by disrupting pH balance, which can impact the ornamental fish trade. One species particularly vulnerable to such environmental changes is the Mexican orange dwarf crayfish (*Cambarellus patzcuarensis*), which is endemic to Michoacán. Though currently listed as endangered, it has become a popular ornamental species. Gaining a better understanding of how environmental stressors affect ornamental species is crucial to improve production practices.

Due to the precarious status of *C. patzcuarensis*, we utilized calico crayfish (*Faxonius immunis)* as a proxy species for our experiments. Both species live in comparable water conditions (pH of 6.5 to 8.0 and temperatures between 20 and 29 °C) inhabiting sandy or muddy environments, and unlike *C. patzcuarensis, F. immunis* is not threatened or endangered. To test the effect of lowered pH on ornamental crayfish, we exposed 48 calico crayfish to one of four pH treatment ranges (4.5-5.0, 5.0-5.5, 5.5-6.0, or 6.5-7.0). Individual crayfish were stocked into 18-liter tanks in one of four small RAS (12 tanks/RAS). Each RAS had a total volume of ~379 liters and was fitted with mechanical and biological filtration as well as UV-sterilization to maintain water quality. RAS were randomly assigned to one of the four pH treatment ranges, and pH was adjusted by adding muriatic acid (31.45%) every other day until target pH levels were reached. Crayfish are being maintained in the RAS for six months. During this time, they are being fed a diet of sinking algae pellets. Animals will be measured every four weeks for total length, carapace length, and weight. Additional observations will include post-molt malformations and ability to molt into a sexually mature form (males = Form 1: females = mature annulus ventralis & glair activity).

Prolonged exposure to acidic or alkaline pH can significantly impact the availability of essential ions, such as calcium, which are vital for *F. immunis* to absorb. Calcium is crucial for healthy exoskeleton formation, and disruption in its availability can lead to deformations. It is hypothesized that *F. immunis* exposed to low pH levels (below 7.0) will exhibit abnormal exoskeleton development, potentially impeding growth and the ability to molt into a reproductive state. Conversely, *F. immunis* maintained at a pH closer to neutral (7.0) are expected to display normal growth and better survival. Therefore, maintaining an optimal pH range within aquaculture and natural systems may be essential for species' health and reproductive success.

MODELING LARVAL PERFORMANCE OF THE EASTERN OYSTER (*Crassostrea virginica*) UNDER FIELD CONDITIONS: *IN SITU* SIZE PROFILES OF THE FOOD ASSEMBLAGE IN THE WESTERN MISSISSIPPI SOUND

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Eastern oyster, *Crassostrea virginica*, larval performance is dependent on ambient endogenous (i.e., physiological variation) and exogenous (i.e., environmental and food conditions) factors. In 2019, a mass mortality event decimated oyster populations in the western Mississippi Sound, ultimately causing the closure of the oyster fishery in the state of Mississippi. Slow recovery suggests impacted larval performance post-2019. Little is known about larval performance in relation to food particle size under field conditions. Monitoring of oyster food supply size composition in 2024 provides a basis for such an evaluation through the use of a biochemically based larval performance model. In this study, Mississippi Sound food composition data acquired in 2024 are evaluated by a larval performance model to investigate how the food assemblage interacts with larval performance, which may ultimately influence local oyster population recovery. The goal of this study is to relate real-time observations of local larval development with the food assemblage with respect to food size. Simulations with varying biochemical compositions within food assemblages and particle size compositions corroborate that food quality and quantity are important for larval growth, and further suggests that the size of the food available throughout larval development is impactful in the larva's ability to undergo successful metamorphosis. Assessments of the food assemblage requires distinguishing these size classes are consistent with well-known size classes of phytoplankton (i.e. picoplankton, <5 μm; nanoplankton, 5-20 μm; and macroplankton, >20 μm).

OSMORESPIRATORY COMPROMISE IN TRIPLOID RAINBOW TROUT (*Oncorhynchus mykiss*)

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This study is examining the effects of family and ploidy on salinity tolerance of all-female populations of rainbow trout for net-pen aquaculture in Atlantic Canada. Triploidy renders fish sterile and therefore ensures that escaped fish cannot successfully breed in the wild, but triploids often have lower thermal tolerance than diploids. Conducted at the Huntsman Marine Science Centre (Saint Andrews, New Brunswick), this experiment used similarly sized sibling diploid and triploid females from the same eight families. After a two-month freshwater acclimation period at 13±1°C, fish were transitioned to seawater. Temperature was then gradually increased to 21°C at 1°C per day, decreased and maintained at 18°C for 10 days, and finally decreased at 1°C per day back to ambient temperature (7±1°C).

There was no effect of ploidy on plasma osmolality, but there was evidence of osmoregulatory difficulties (higher and more variable values) for both ploidies at 18°C in seawater (Figure 1). Additional data will be presented on plasma ion levels and interlamellar cell mass size (an indicator of osmorespiratory compromise) in these same fish. This study provides valuable insight into the physiological responses of sterile triploids to temperature and salinity challenges that can be encountered in aquaculture.

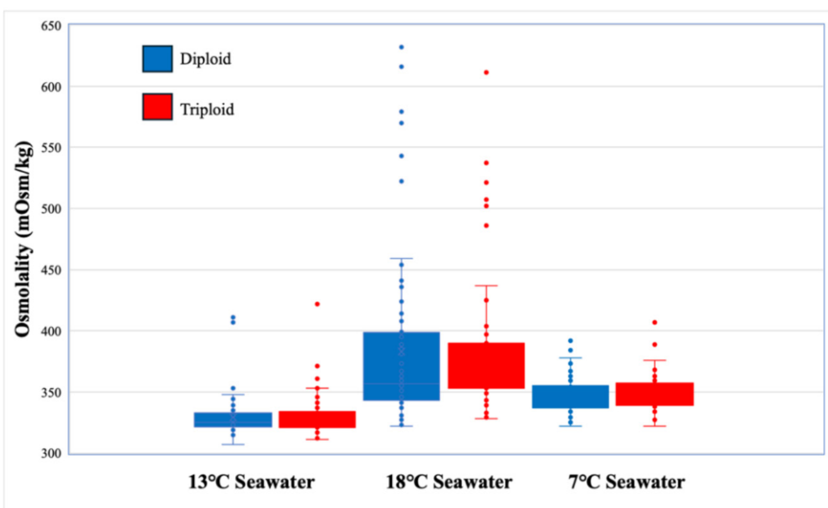

FIGURE 1: Blood plasma osmolality of diploid and triploid rainbow trout at various sampling points.

GROWTH AND SURVIVAL OF MIXED-SEX DIPLOID AND ALL-FEMALE DIPLOID AND TRIPLOID RAINBOW TROUT (*Oncorhynchus mykiss*) IN VARIABLE-TEMPERATURE SEAWATER

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This study is examining the effects of family, sex ratio, and ploidy on tolerance of rainbow trout to full strength seawater at variable temperature. Triploidy, characterized by three complete chromosome sets, renders fish sterile and offers advantages in net-pen production in Atlantic Canada, but triploids often have lower thermal tolerance than diploids. Conducted at the Huntsman Marine Science Centre (Saint Andrews, New Brunswick), this experiment used similarly sized fish from 48 mixed-sex diploid families, 15 all-female diploid families, and 12 all-female triploid families, with each fish individually PIT-tagged for tracking purposes. After a two-month freshwater acclimation period, fish were transitioned to seawater at ambient temperature (13±1°C). Temperature was then gradually increased to 21°C at 1°C per day, decreased and held at 18°C for 10 days, and finally decreased at 1°C per day back to ambient temperature (7±1°C).

Growth was assessed at three time points: PIT-tagging, 30 days after seawater entry, and at mortality or the end of the experiment. Initial mortality among all sizes was low after seawater entry but then increased as temperature rose (Figure 1). Statistical analysis of growth rates and survival by ploidy and family will be discussed.

This experiment seeks to evaluate the performance of sterile triploids under variable temperature conditions that may occur within marine net-pens, while also considering the potential to breed for improved performance based on family-level variations in response.

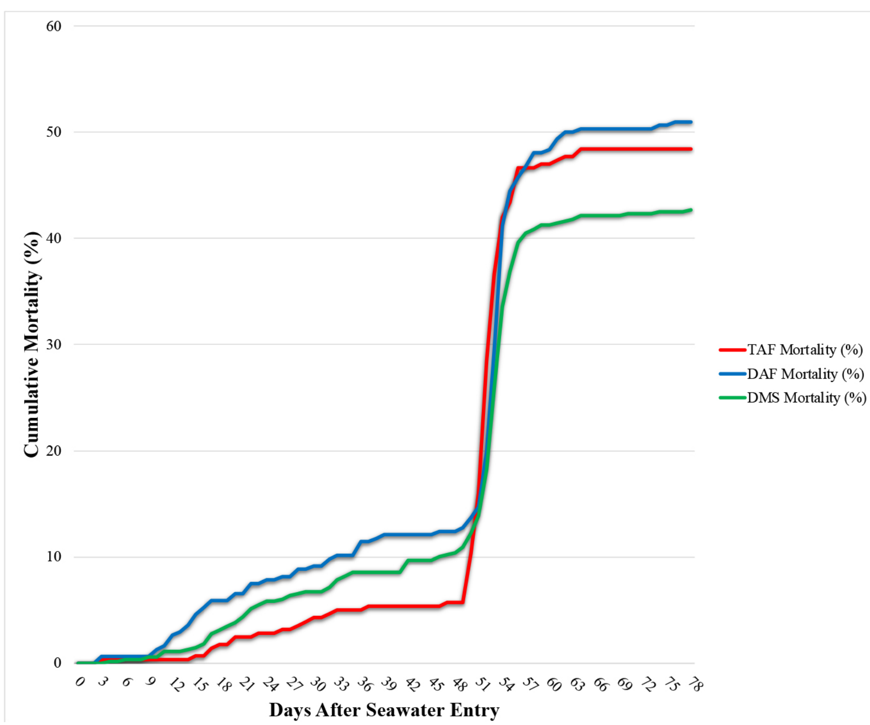

FIGURE 1: Cumulative mortality of diploid mixed sex, all-female diploid and all-female triploid rainbow trout after transfer to seawater.

NATIONAL SEAWEED COLLABORATIVE HUB: NURTURING THE SUCCESSFUL GROWTH AND MATURATION OF THE DOMESTIC SEAWEED AQUACULTURE SECTOR

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According to NOAA, seaweed farming is the fastest growing aquaculture sector in the United States. Despite growing interest and the rise in number of applications to farm annually, barriers preventing the expansion of the sector exist. While advocacy-driven messaging touts the potential environmental benefits and uses of seaweed aquaculture, established pathways into many of those outlets are either developing or non-existent. Individuals seeking practical information (i.e. accessible markets) are left feeling frustrated. A more complete picture of the seaweed aquaculture sector was needed. Led by Connecticut Sea Grant, the collaboration of 11 Sea Grant programs created the Sea Grant National Seaweed Hub (https://seaweedhub.extension.uconn.edu/) to serve as a central clearinghouse for available science-based, non-advocate, and practical resources related to the emerging domestic seaweed aquaculture industry. This goal was achieved in two phases: Phase I – to identify and addresses emerging challenges through the development of responsive, non-advocate resources in a publicly accessible format; and Phase II - to continue addressing emerging needs through the development of responsive resources and create more professional networking opportunities for stakeholder engagement.

Phase I activities included performing a comprehensive national needs assessment of all relevant groups involved with the sector, hosting the first National Seaweed Symposium, establishing a dedicated website to serve as a centralized repository for seaweed resources developed through this effort, creating diverse stakeholder-driven work groups to refine and devise work plans to address challenges and opportunities identified from the Symposium and results from the needs assessment, and create publicly accessible resources that directly meet the sector's needs.

Phase II activities included the hosting of the second National Seaweed Symposium, hosting of a webinar series based on topics requested by stakeholders in Phase I, virtual meet-ups for specific sector groups, and the development of resources to support culinary uses of seaweeds and the needs of the regulatory sector.

Since the launch of the National Seaweed Hub in 2019, the seaweed aquaculture sector has doubled, making the need for this effort more relevant than ever. As a result of the National Seaweed Hub activities, collaborations between various stakeholder groups have increased. Industry members have formed cooperatives and connected with buyers, regulatory agencies are sharing knowledge and expertise to support industry expansion in their states, and general audiences are using the hub's resources to gain more knowledge about the emerging industry.

PRIMARY PROCESSING BOTTLENECKS: ADDRESSING BARRIERS FOR SMALL TO MEDIUM-SCALE SEAWEED PRODUCTION IN THE UNITED STATES

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Seaweed farming continues to be an emerging aquaculture sector in the United States. Results from a national needs assessment conducted by the Sea Grant National Seaweed Hub shows that infrastructure to stabilize and process cultivated seaweed is lacking, preventing the seaweed sector from expanding. Sugar kelp (a type of seaweed) has a water content of approximately 90%, making it highly perishable. In addition, the harvest season is limited to approximately 2 to 3 months, depending on region. Therefore, processing or stabilization methods need to be employed to transition freshly harvested kelp into a more shelf-stable form, while ensuring food safety and product quality is maintained in a cost-effective manner. As processing technology and equipment for small to medium sized operations are limited or non-existent in the US, the Sea Grant programs from Connecticut and Alaska reached out to colleagues overseas for potential solutions.

Scotland has been utilizing various kelp species as fertilizer, animal feed, and as a seasoning in food products since the 1800s. While historically harvested from the wild, there is a shift toward increasing cultivation of their species. The Scottish Association for Marine Science (SAMS) is one of Europe's leading marine science research organizations and one of the oldest oceanographic organizations in the world. SAMS seaweed-related research focuses on the farming of seaweed and the interaction between aquaculture and the environment from both perspectives, including the services and impacts the environment provides to the aquaculture industry and a sea farm's impacts on the environment. Specifically, SAMS hosts a commercial seaweed nursery and has a mid-scale seaweed farm that supports national and European R&D projects. They also have numerous collaborations with academics and seaweed-related industries across the supply chain. The Scottish Seaweed Industry Association (SSIA) is an organization dedicated to the growth and innovation of the seaweed industry. They serve as a liaison between industry stakeholders, research organizations, and government bodies to foster the sustainable and responsible development of the sector.

SAMS and the SSIA hosted seaweed industry members from Connecticut and Alaska in May 2023 for a 5 day industry-to-industry knowledge exchange tour of stabilization facilities in Scotland. Supported by funding from the National Sea Grant Program's Aquaculture Workforce Technologies and Education Travel Grants Projects, participating US seaweed industry members met with Scottish kelp farmers, nursery technicians, seaweed processors, food manufacturers, and start-up companies focused on product development. A webinar providing an overview of the tour and industry experiences is available on the National Seaweed Hub's website.

EVALUATION OF COMMERCIAL PREBIOTICS FOR FINFISH AQUACULTURE

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Carp and tilapia contribute to more than 60% of aquaculture production worldwide. High demand of these species has led to the shift from extensive to intensive aquaculture practices. However, intensification of aquaculture systems is linked to risks of disease occurrence, which causes massive mortalities. Antibiotics have been used as growth promoters and to prevent disease in fish farming. However, these components pose a threat to public health and have a negative effect on the environment. The use of prebiotics is a strategy being studied to prevent these issues. The focus of the present study was to determine the impacts of prebiotic supplemented diets on growth and the microbial community composition of the intestinal tract of Nile tilapia *Oreochromis niloticus* and goldfish *Carassius auratus,* used as model cichlid and cyprinid species respectively.

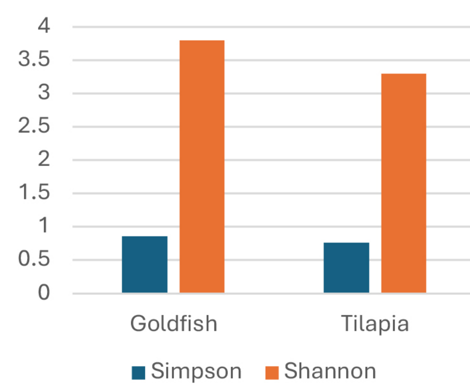
Fingerlings of goldfish and Nile tilapia (1.4 g average individual weight) were cultured in tanks within a common recirculating system. Experimental diets consisted of fish feed supplemented with commercially available prebiotics (2%). Fructo- (diet One, FOS), isomalto- (diet Two, IMO), xylo- (diet Three, XOS), and galactooligosaccharides (diet Four, GOS). Two control diets were added. After completing the feeding trials, fish were euthanized for growth measurements and dissected for intestinal tissue sampling. Intestinal bacterial communities were determined using next generation sequencing.

Goldfish samples showed a higher microbiome diversity than that of Nile tilapia (Figure 1) and appeared to have a higher acceptance of the supplemented diets, which was exhibited in their feeding behavior and their final weight and length ($P < 0.05$). Results in this study suggest that the use of commercial prebiotics in intensive aquaculture systems may enhance fish growth and shape their intestinal microbiome. Different prebiotic concentrations, prebiotics combinations, and changes in the duration of feeding trials might yield different results.

TABLE 1. Experimental diets fed to goldfish and Nile tilapia.

	Diet			
	One	Two	Three	Four
Protein	45%	45%	45%	45%
Lipid	12%	12%	12%	12%
Prebiotic	FOS	IMO	XOS	GOS
Oil	10%	10%	10%	10%

FIGURE 1. Diversity indexes indicating differences in richness and evenness between species.



EFFECT OF PREBIOTIC SUPPLEMENTED-DIETS ON GROWTH AND SURVIVAL RATE OF CHANNEL CATFISH *Ictalurus punctatus* AFTER EXPERIMENTAL INFECTION WITH *Edwardsiella ictaluri*

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Channel catfish *Ictalurus punctatus* is one of the most cultured fish in the United States, and enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri,* is the most prevalent disease affecting this species. Romet®, Terramycin®, and Aquaflor® are approved antibiotics for the treatment of bacterial infections in catfish. Effectiveness of these drugs can be limited since fish can show anorexia at early stages of infection, intestinal microbial flora imbalance, and the emergence of antibiotic-resistant *E. ictaluri* strains can potentially occur. The use of prebiotics has been considered due to the low cost of these components in relation to antibiotics and the effectiveness of supplemented diets that can potentially modify the gut-associated microbiota, enhancing disease resistance and survival of farmed fish. In this study, we evaluated four different commercial prebiotics and their effect on growth, gut microbiome, and the survival of channel catfish after experimental infection with *E. ictaluri*.

Specific pathogen free channel catfish fingerlings were cultured in a flow-through system. Experimental diets consisted of fish feed supplemented with commercially available prebiotics fructo- (diet One, FOS), isomalto- (diet Two, IMO), xylo- (diet Three, XOS), and galactooligosaccharides (diet Four, GOS) (Table 1). Two control diets were added. After completing the feeding period, fish were challenged with *E. ictaluri,* euthanized after a two-week period, and dissected for intestinal tissue sampling. Intestinal bacterial communities were determined using next generation sequencing. *Edwardsiella ictaluri* infection was confirmed by tissue culture, and MALDI-TOF.

Results indicated significant differences among the diets and fish weight ($P < 0.05$). Survival of fish challenged with *E. ictaluri* was also significantly different ($P < 0.05$) (Table 2). Different prebiotic concentration, combinations and microbial manipulation might yield different results.

TABLE 1. Experimental diets fed to channel catfish fingerlings.

	Diet			
	One	Two	Three	Four
Protein	45%	45%	45%	45%
Lipid	12%	12%	12%	12%
Prebiotic	FOS	IMO	XOS	GOS
Oil	10%	10%	10%	10%

TABLE 2. Survival rate (SR) of channel catfish after experimental infection with *E. ictaluri* and as a function of diet.

	Diet			
	One	Two	Three	Four
SR (%)	30	63.3	46.6	43.3

QUANTIFYING THE ENVIRONMENTAL IMPACT OF OFFSHORE INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA)

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Offshore finfish aquaculture in the United States faces several challenges for expansion including difficulty of permitting, pushback from environmentalists and stakeholders, and high startup costs. Many of the challenges stem from the common misconception that offshore aquaculture is inherently harmful to the environment. However, well-run operations typically have minimal effect on surrounding water quality and benthic environment and can substantially enhance surrounding biodiversity. Moreover, the substitution of wild caught fish with farm raised fish alleviates pressure on wild stocks. While these positives are often understood within the industry, there has been little comprehensive analysis of the potential benefits of offshore finfish aquaculture. This study attempts to quantify the environmental effects of offshore finfish aquaculture on the surrounding environment. A suite of tools, including water quality and hydrodynamic monitoring, sediment sampling, environmental DNA (eDNA), FlowCam Imaging Microscopy, and benthic/bathometric surveying were used. A baseline was generated for three months prior to stocking the net pens and sampling continued through the entire 10-month growout. The results are summarized in this presentation.

ASSESSING THE ENVIRONMENTAL IMPACT OF AN OPEN OCEAN FINFISH FARM IN HAWAII: A 12-YEAR CASE STUDY

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The results and underlying trends of 12-years of environmental data collected from Blue Ocean Mariculture in Hawaii will be presented.

A PANGENOME FOR THE MARINE BACTERIUM *Phaeobacter inhibens* REVEALS CONSERVED GENES FOR HOST PROTECTION IN BIVALVE LARVICULTURE SYSTEMS

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Phaeobacter inhibens S4, a promising marine probiotic, protects larval *Crassostrea virginica* from vibriosis through several mechanisms when administered prophylactically. S4 produces an antimicrobial compound, tropodithietic acid (TDA), which is lethal to the pathogen *Vibrio coralliilyticus* RE22 and helps exclude it from biofilm formation. S4 also forms strong biofilms, reinforcing its role in niche exclusion. Additionally, it prevents RE22 cell-to-cell communication via quorum quenching, thereby inhibiting the production of virulence factors. Transcriptomic studies reveal that S4 disrupts RE22's ability to cause larval mortality by downregulating flagellar genes, affecting motility, and impeding the formation of disulfide bonds necessary for proper flagellar protein development. Probiont S4 also primes *C. virginica* immune genes and restores healthy microbial diversity after UV treatment of hatchery tank water. Given the broad range of probiotic attributes of S4, and the known probiotic potential of a handful other *P. inhibens* strains in aquaculture, a pangenome of *P. inhibens* was constructed using 37 unique strains, along with an antiSMASH analysis to explore the distribution of probiotic traits. This analysis also sought to identify other probiotic capabilities within the species.

The pangenome revealed over 11,500 genes, with the core genome containing 3,118 genes, representing 72% of the average genome size of the strains studied. Key genes within the core genome include those responsible for cobalamin (vitamin B12) biosynthesis, biofilm formation, and TDA production. All but one strain possessed a non-ribosomal peptide synthetase (NRPS)-independent siderophore gene cluster, typically located on a plasmid. All strains had at least two homoserine lactone (AHL) clusters potentially involved in quorum signaling and quorum quenching. These findings suggest that all *Phaeobacter inhibens* strains hold significant potential for probiotic activity, but strain-specific genes may lead to differences in functionality or host specificity.

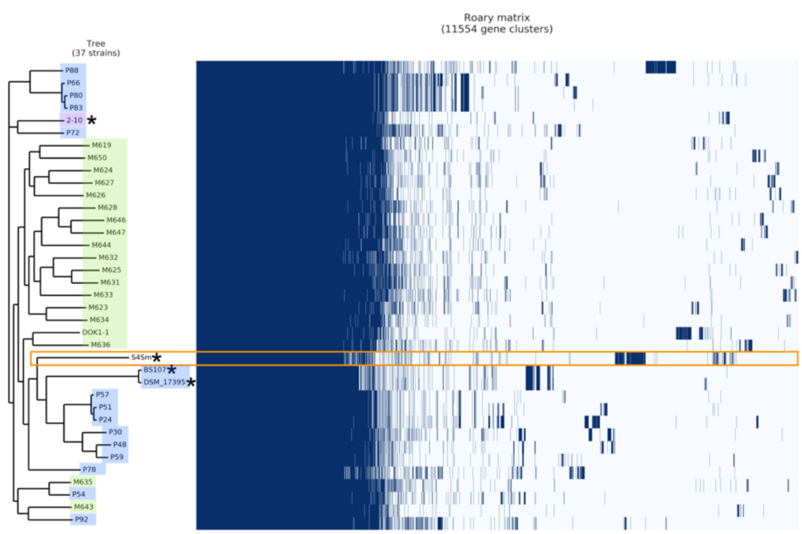

Figure 1: Gene presence and absence phylogenetic tree. Dark blue indicates gene presence. S4 is highlighted by the orange box, it is the only strain with a western Atlantic origin (RI, USA), the rest are isolated from Europe (blue), Australia (purple), and Asia (green). * Indicates known probiont strains.

DIETARY METHIONINE REQUIREMENTS FOR JUVENILE FLORIDA POMPANO (*Trachinotus carolinus*)

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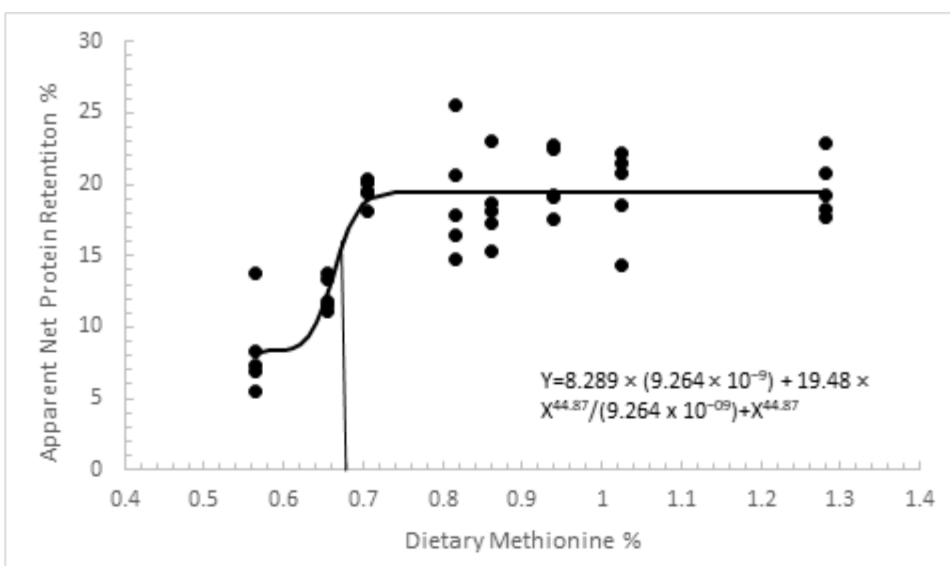
A 56-day feeding trial was conducted to evaluate the quantitative methionine requirements in the diets of Florida pompano (*Trachinotus carolinus*). Eight practical diets using soybean meal, poultry meal, and red lentil meal as the primary protein sources were formulated using graded levels of methionine supplement (0 to 0.70 g/100 g diet). Groups of 15 juvenile Florida pompano (4.04 ± 0.05 g) were size-sorted and placed into one of 40 glass aquaria (132 L) with five replicates per diet. Significant differences ($p \leq 0.05$) were observed in overall biomass, mean weight, weight gain, thermal growth coefficient (TGC), and feed conversion ratio (FCR). To estimate the dietary methionine requirement, a series of statistical models, including the one-slope broken line model (BLM1), two-slope broken line model (BLM2), broken quadratic model (BQM), and four-parameter saturation kinetic model (SKM-4) were used to assess mean weight, weight gain, TGC, apparent net protein retention (ANPR), and methionine retention (MR). The model selection showed that BLM1 fit the data best for MW and TGC, SKM-4 for PWG and ANPR, and BQM for MR. Based on these results, a minimum dietary methionine requirement of 0.68% of the diet or 1.70 g/100 g protein is recommended.



GROWTH AND SURVIVAL OF CLAM *Chione cortezi*, CULTURED IN RECIRCULATION AQUACULTURE SYSTEM (RAS) AT DIFFERENT TEMPERATURES AND CULTURE DENSITIES

Juan Gabriel Correa-Reyes*; Cesar Omar Rodríguez-Arana; Beatriz Cordero-Esquivel; Tatiana N. Olivares-Bañuelos and Ramiro Hernandez-Garcia

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Seed spat production of bivalve mollusks, specifically the size 1.0 to 10.0 mm, represents a limitation for aquaculture production. These stages usually develop in the ocean or continuous water-flux systems, leaving the organisms exposed to environmental conditions which cause irregular production throughout time. Recirculation systems might be a practical option and economically viable to culture larvae, post-larvae, and juveniles of bivalve mollusks. The objective of this work was to culture clam seed of the genus *Chione cortezi* at two different temperatures (24 and 28 °C) and three different stocking densities (11,000, 16,000 and 21,000 organisms by 2.3 liters experimental unit) in recirculation aquaculture systems (RAS). After 28 days of carrying out experiments, it was observed that the water quality in the systems (DO, pH, TAN, NO_2, NO_3, alkalinity, CO_2, CO_3^{-2}, HCO_3^- and salinity) remained in values and concentrations reasonably adequate for the growing of clam seeds. Moreover, the organisms cultured at 24°C and density of 21,000 organisms by 2.3 liters per experimental unit, were the ones that showed the best specific growth rates regarding size and weight. Despite that, a high survival of the organisms maintained at 28°C was observed. The use of microalgae concentrates resulted in a practical option and economically viable for the feeding of *Chione cortezi* juveniles cultured in RAS. It is important to consider that this research is a pioneer in the study of the growth and survival of *C. cortezi* juveniles so future studies should be carried out to allow contrast in different culture scenarios that could better its production. It is also essential to evaluate the degree of tolerance of this species at different concentrations of CO_2.

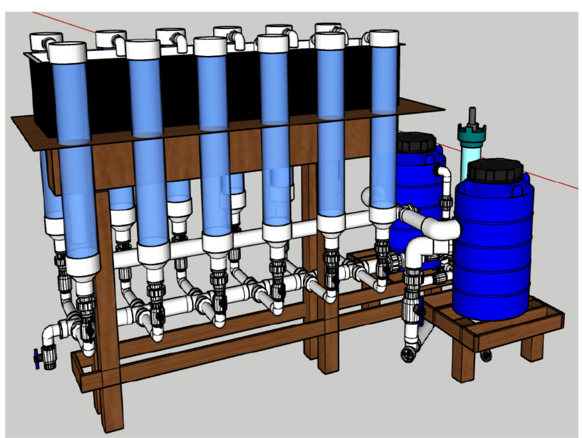

Figure 1. Image of RAS system used to evaluate the growth and survival of *Chione cortezi* at two temperatures (24 and 28 °C) and three culture densities (11,000; 16,000 and 21,000 organisms by experimental unit).

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AQUACULTURE OF *Sardinhas*: SMALL, NATIVE FISH AS LOW COST, HIGHLY NUTRITIOUS, DELICIOUS FOODS

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Sardinhas is the Portuguese word that describes a cacophony of smaller sized aquatic animals eaten in complex cuisines, not only as sardines in "tinned fish". We are engaged in applied, small-scale aquaculture R&D of native fish as *sardinhas* and promoting these as alternatives to the overuse/spread of invasive, non-native species being chosen and used in the development of freshwater aquaculture and aquaponics systems, especially in educational settings. The many benefits of small, native fish are well known internationally among development agencies, led by the pioneering works of Dr. Shakuntala Haraksingh Thilsted who received the 2021 World Food Prize (the "Nobel Prize for food and agriculture") for the development of small, native fish aquaculture and fishery systems for nutritional benefits (enhancing intakes of vitamin A, omega3's, micronutrients, etc.). Small, native fish are more accessible, affordable, less wasteful, and have considerable potential to enhance seafood consumption. In many places, consumers who want to consume fish are being priced out, resulting in the selection of poorer quality, less nutritious terrestrial meats, or avoiding seafoods altogether. For example, prices for US seafood were 120% higher in 2024 vs. 1997, exceeding the overall inflation rate (US Bureau of Labor Statistics). The "tinned fish" movement in North America/Europe is gaining in popularity; an example is "Fishwife" who source from healthy fisheries and certified sustainable aquaculture farms, with the aim to "make ethically sourced, premium, and delicious tinned seafood a staple in every cupboard". Our works complement this "tinning movement" as we are developing aquaculture for a variety of native, small fish aquatic products, targeting small farmers.

North American native fish had a central place in the diets of Indigenous First Nations until usurped by colonization and racism. The destructive, wasteful practice in the 1800's of intensive river netting and fish processing on riverboats led to the labelling of many native North American fish as "rough fish". Biologists used this to develop the concept that native fish limited the maximum sizes of preferred gamefish populations. Biologists then led large scale attempts to destroy native fish by promoting intensive netting and whole ecosystem poisoning. This outrageous, unscientific "rough fish" concept persists in some parts of society today.

The family Cyprinidae is the most diverse grouping of native freshwater fish in North America. Our first choice for *sardinhas* aquaculture given our hyperlocal farm settings was to use the widely abundant and tolerant golden shiner (*Notemigonus crysoleucas*) as food. We have successfully moved fish from bait shops to ~2.5 m^3 algal tanks, created dense blooms with recycled fertilizer (our program in urine diversion), and acclimatized them to these solar algal pond ecosystems, first using commercial feeds. Since these are lower trophic level fish, we are experimenting with bioflocs from various low-cost agricultural meals to replace commercial feeds. All tanks have aeration powered by solar energy. Are we wanting more people to consider eating bait? Yes.

CHALLENGES AND OPPORTUNITIES OF CO-LOCATION OF OFFSHORE AQUACULTURE AND WIND FARMS IN THE UNITED STATES

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As human populations and per capita demands continue to grow, industries are increasingly moving into offshore ocean spaces. The U.S. has outlined goals to accelerate the development of healthy and sustainable energy and food industries in ocean spaces. Although they can appear vast and unexploited, these areas are often busy with fishing, shipping and other activities, creating conflicts to additional uses or privatization. Despite continued enthusiasm and ever growing need for these industries, expansion into ocean spaces has proven difficult due to public opposition, conflicts with existing ocean uses, and high costs of working offshore.

Meanwhile, research and development into the co-location of aquaculture and offshore energy production is moving forward in some of the top ocean use countries in Europe and Asia, for example, to increase efficiencies in ocean space use and improve public opinions about additional marine industries.

We investigated the existing state of knowledge on the co-location of offshore wind and aquaculture from a multi-disciplinary perspective to understand challenges and opportunities. Given the novelty of this approach, we also reached out to early movers to understand the motivations, impacts, and experiences of different industry players, governing agencies, and third party interests. We distill these lessons down and apply them to the U.S. context to assess the potential for co-location in the U.S. EEZ. To address the diversity of environmental and social conditions throughout the US waters, we conduct assessments of each region to discuss the opportunities and challenges to co-location in each.

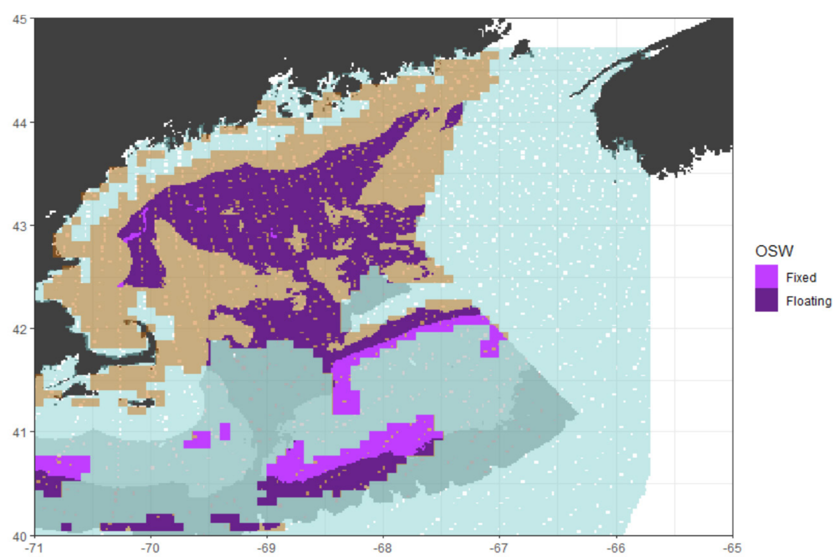

Figure 1: Macro-suitability of co-located OSW and aquaculture in the Gulf of Maine, USA. Light purple areas are where aquaculture can co-located with fixed infrastructure OSW and dark purple represents areas of co-location with floating infrastructure.

GLOBAL INSIGHTS, LOCAL IMPACT: TECHNOLOGY TRANSFER REVOLUTIONIZING MAINE'S AQUACULTURE INDUSTRY

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Maine's wild fisheries and its flourishing aquaculture sector are at a pivotal moment, grappling with the unprecedented challenges posed by climate change. To ensure the survival and prosperity of Maine's waterfront communities, adapting and evolving these industries is not just an option—it's a necessity. One of the most effective strategies to drive industry growth is through "technology transfer." For Maine aquaculture, this means learning about innovative species, resources, and cultural practices from around the globe and integrating these insights into local practices.

Maine has leveraged technology transfers with great success in recent years to introduce new (native) species, technologies, culture techniques, equipment, market development and culinary uses for emerging species of shellfish and kelp. Technology transfer allows participants to fully immerse in a local culture and meet with chefs, fishermen, growers, processors, retailers, restaurateurs, equipment manufacturers, government officials and scientists.

During this presentation several examples will be highlighted including recent trips studying the kelp industry in South Korea, culinary uses of scallops in Northern France, the kelp and farmed scallop industries of Hokkaido and Aomori, Japan and farmed scallops in Atlantic Canada. As our nation's fisheries and aquaculture sector navigate these transformative times, we must actively seek out and implement strategies that will sustain and support the hardworking communities at the heart of our waterfronts.

EFFECT OF HUMIC SUBSTANCE INCORPORATED DIET ON RAINBOW TROUT IMMUNE RESPONSE TO *Flavobacterium psychrophium*

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As sustainability becomes a pressing concern in aquaculture, innovative approaches to enhancing production without depleting natural resources are essential. One such solution is the use of humic acids, which are proposed as additives to promote fish growth and bolster immune defense. Humic acids, naturally occurring in water sources, are organic substances that derived from decomposition of animal or plant material. These substances may be antioxidants with antimicrobial, anti-inflammatory, and immunostimulatory properties. Depending on their structure, humic substances may provide benefits as well as the promotion of growth.

In this current study, 750 juvenile rainbow trout are used in an 8-week feeding trial testing 3 experimental diets formulated with humic substances, as well as control diet. Throughout this period, growth among groups is recorded biweekly through biomass, as well as the individual weights and lengths of 10 fish per tank. At the end of the 8-week period, blood, mucus, kidney, spleen, and intestine are sampled before being challenged with the causative agent of Coldwater Disease: *Flavobacterium psychrophilum*. These samples are taken again 24 hours, 7 days, and 28 days post-challenge.

Growth metrics, including relative growth, feed conversion ratio, specific growth rate, and weight gain are calculated at the end of the diet trial to determine growth potential of each humic substance. Mortality, and innate/adaptive immune responses are evaluated through challenge survival, antibody assays, and lysozyme activity to determine the impact of the experimental diets on immune function.

OPEN CAPABILITY CRYOPRESERVATION KITS FOR COMMUNITY-DRIVEN REPOSITORY DEVELOPMENT IN AQUATIC SPECIES: AN EXAMPLE FROM THE AXOLOTL *Ambystoma mexicanum*

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Cryopreservation is an essential tool in the protection of genetic diversity of valuable species, but the current capabilities of cryopreservation are highly limited due to lack of standardization and poor accessibility to equipment. Using the axolotl (*Ambystoma mexicanum*) as a model, we show how 3-D printed open-hardware devices, which are standardizable and inexpensive, can be used to cryopreserve sperm for *in-vitro* fertilization. Using a previously developed protocol, sperm from multiple axolotls were collected using a 3-D printed axolotl positioning device (i.e., AxoLounge), cooled at a rate of 15-20 °C/min using a positional cooling platform device (i.e., CryoKit), thawed, and applied to eggs to produce offspring. In the coming work, these devices and other essential supplies will be incorporated into a comprehensive cryopreservation capability kit to collect and cryopreserve axolotl sperm. This kit can be used in laboratories possessing only basic supplies and equipment to work with valuable biomedical or aquaculture species. In addition, such kits would allow multiple groups to contribute to the protection of imperiled species. A broad range of capability kits can be developed for aquatic species to increase accessibility to cryopreservation for new users interested in preserving genetic resources and provide much-needed standardization.

Figure 1. A preliminary kit used to collect (yellow, left) and cryopreserve (blue, right) axolotl sperm in a location possessing only basic supplies and equipment. 3-D printed open-hardware devices and a small nitrogen vapor shipper are included.

ATTEMPTS TO INDUCE POLYPLOIDY IN HARD CLAM *Mercenaria mercenaria*

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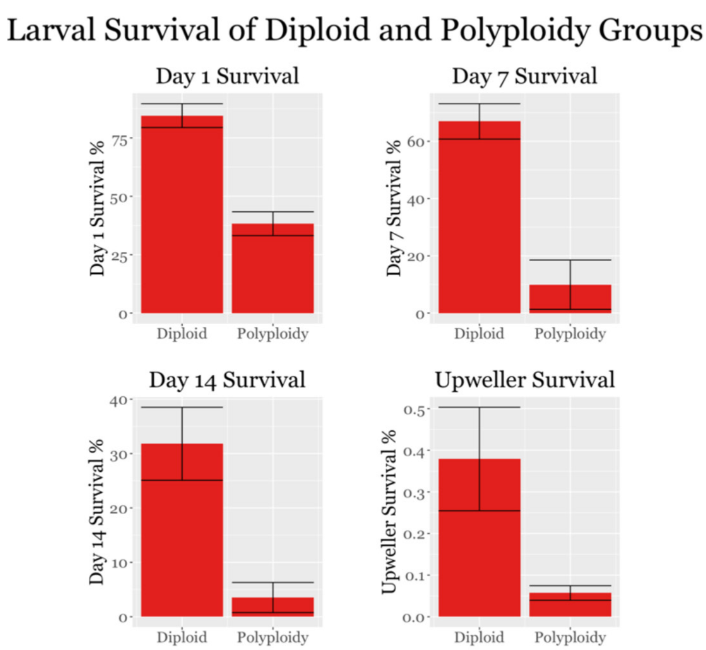
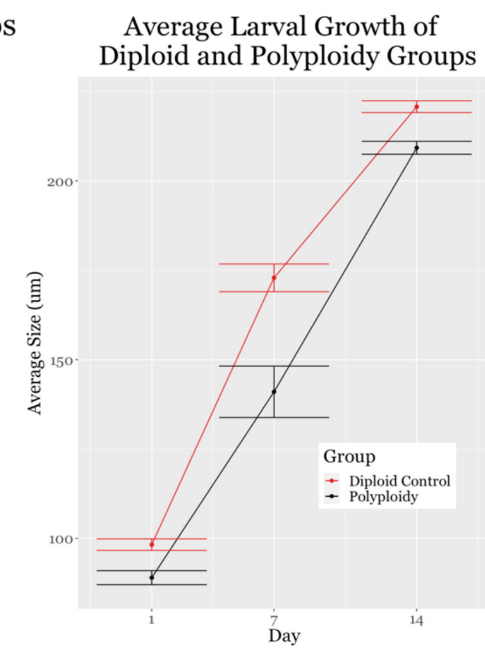
Hard clam aquaculture is largely inhibited by the time investment required for animals to reach market size compared to other cultured bivalves. *M. mercenaria* juveniles are held minimally in nursery systems for 3 months prior to field deployment, where it will take an additional 2-3 years to reach the earliest market size. For species such as the eastern oyster (*Crassostrea virginica*), juveniles can be field deployed from nursery settings as early as 6 weeks of age and reach market size at 2 years of age. Triploid *C. virginica* produced from tetraploids reach market size up to half a year earlier due to faster growth. In order to explore if tetraploid clams can be produced and if triploid clams would receive the same growth benefit, an experiment was performed to inhibit the release of polar body I in fertilized eggs, which is known to produce tetraploids and triploids in other organisms. Approximately 6 minutes post-fertilization, cytochalasin B was added at 1 mg/L to the fertilized eggs for 15 min. This process was repeated across 4 separate spawns. Additionally, 8 spawns were conducted separately as untreated diploid controls. Modified groups exhibited significantly reduced larval survival and growth when compared to controls. Across the polyploidy spawns, juvenile triploid induction ranged from 10-68%, determined via flow cytometry; no tetraploids were observed among the clams per group sampled. Additional sampling and measurements will be conducted to determine relative nursery and field performance of triploids and if any induction of tetraploids was achieved.





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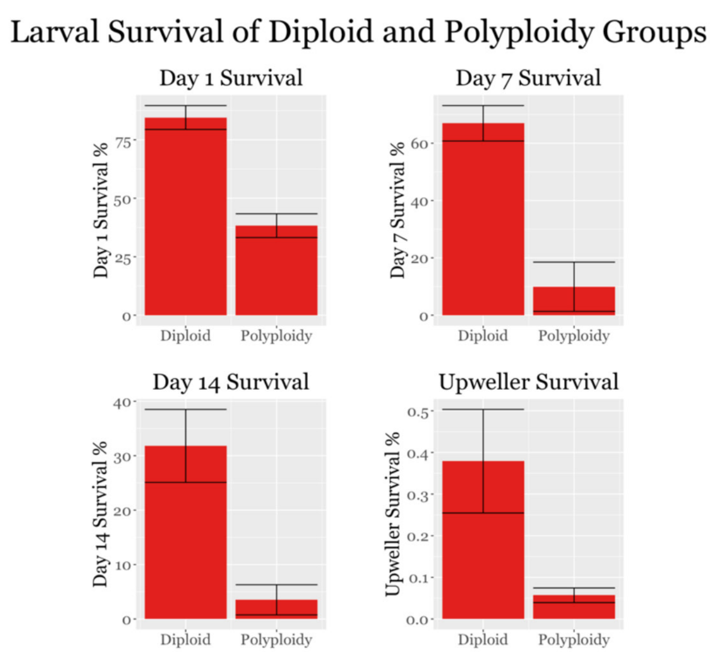

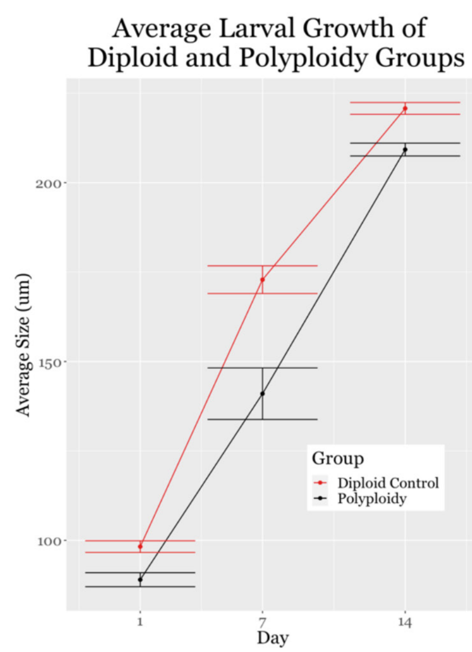

MICROORGANISMS LINKED TO HATCHERY DIE-OFFS AND THEIR ENVIRONMENTAL DISTRIBUTIONS

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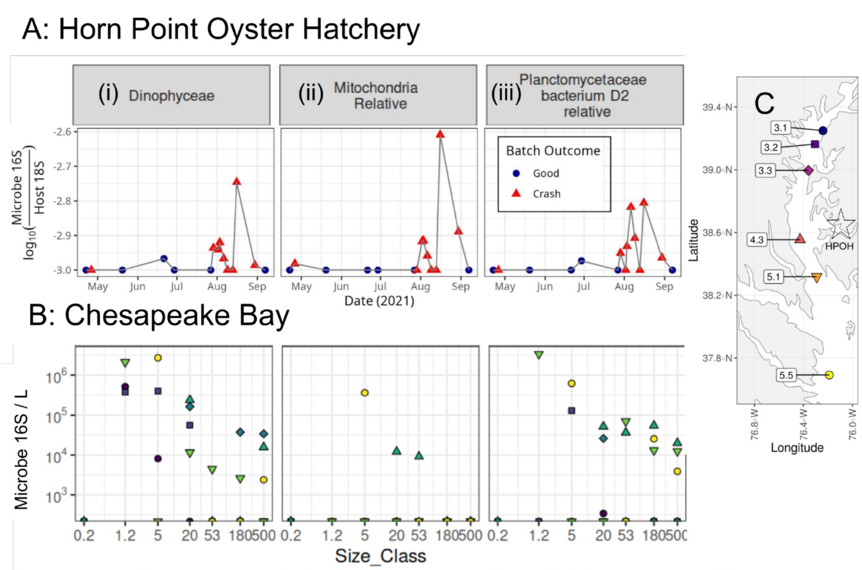
Bivalve hatcheries across the U.S. frequently face unexplained production failures, or "crashes." At the Horn Point Oyster Hatchery, we identified three microbial taxa— (i) Dinophyceae (dominated by *Gyrodinium jinhaense*), (ii) an unidentified mitochondria-like sequence, (iii) and a sequence related to *Planctomycetaceae bacterium D2*—that were more abundant in 3- to 5-day-old oyster larvae that later crashed than in successful batches (Figure 1A). The mitochondria-like sequence also preceded a crash in 2024.

To understand the ecology of these taxa in the surrounding environment, we analyzed their distribution across the Chesapeake Bay using existing data. The mitochondria-like ASV was linked to particles ≥5 microns and was found at only some locations, while the *Planctomycetaceae* relative and Dinophyceae were widespread (Figure 1B).

The size association of the mitochondria-like bacteria suggests it may be part of a toxin-producing algae or an endoparasite. Its limited distribution in the Bay suggests some hatchery crashes may result from its seasonal occurrence in nearby waters.



Abundance of taxa associated with hatchery crashes: (i) Dinophyceae, (ii) mitochondrion-like ASV, and (iii) *Planctomycetaceae bacterium D2*. (A) Taxon-to-host gene copy ratios in 3- to 5-day-old larvae, showing distinctions between batches that crashed (Crash ▲) and those that grew normally (Good ●). (B) Gene copies per liter across Chesapeake Bay stations, grouped by particle size class (x-axis), with station locations shown in panel (C).

EXAMINING ENVIRONMENTAL DRIVERS AND IMPACTS OF MARICULTURE ACROSS THE GULF OF ALASKA

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The Mariculture Research and Restoration Consortium is a collaborative initiative focused on understanding environmental drivers and impacts of seaweed and shellfish farming in diverse ecosystems across the Gulf of Alaska. Key objectives are to understand variation in crop productivity to inform future site selection and to understand impacts, positive or negative, that farms have on the surrounding ecosystem. We are using production arrays at nine commercial kelp and oyster farms throughout Kodiak, Kachemak Bay and Prince William Sound. Each array includes a standardized cultivation system for oysters and kelp and environmental sensors that measure salinity, temperature, dissolved oxygen, turbidity, and chlorophyll at fixed depths and throughout the water column. This multi-year project includes active and funded participation of farmers working with an interdisciplinary team of scientists. This research will inform economically and environmentally sustainable growth of the mariculture industry in Alaska.

DEVELOPING A HIGHER EDUCATION AQUACULTURE PATHWAY IN CONNECTICUT TO INCREASE AQUACULTURE WORKFORCE DEVELOPMENT

Emma Cross[1*], Alysa Mullen[2*], Tessa Getchis

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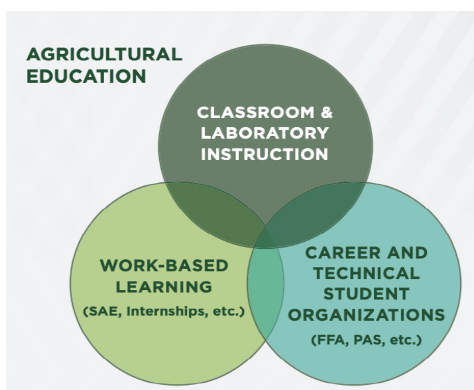
In the state of Connecticut, the aquaculture industry has been growing for the past several decades and has become very diverse. The industry includes cultivation of seaweed, shellfish, and finfish for food consumption, the production of ornamentals, salmonid hatcheries for recreational stock enhancement, as well as mitigation strategies to help combat climate change. To help support this growing industry, workers who have post-secondary training and specialized skill sets are needed. The state of Connecticut's Vocational Agricultural High School Programs are deeply invested in career and technical education as well as preparing students for competitiveness in post-secondary education. Those students who follow the aquaculture strand have the opportunity to learn hands-on aquaculture education in fully functioning fish production laboratories. Despite Connecticut having these impressive high school programs, there is a lack of post-secondary education pathways in aquaculture offered and in turn students with this training are leaving to seek established pathways in other states.

As part of a CT SeaGrant award, we discuss an aquaculture pathway being developed at Southern Connecticut State University (SCSU). This includes the state's first Early College Experience (ECE) courses in aquaculture (Aquaculture I and Aquaculture II) that have been collaboratively developed by The Sound School and SCSU. These classes are currently being taught in Fall 2024 and Spring 2025 to senior high school students at The Sound School and to freshman and sophomores at SCSU. Through this initiative, high school students will gain 8 college credits whilst at high school. The goal of this aquaculture pathway is to increase recruitment of highly skilled students from Connecticut's Vocational Agricultural High School Programs at a CT higher education institution to further prepare the next generation for a diverse, highly skilled aquaculture workforce.



Three circle model of agricultural education.

BIODIVERSITY ASSOCIATED IMPACTS OF SHELLFISH AQUACULTURE IN MARTHA'S VINEYARD, MA

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Shellfish aquaculture has carbon-sequestering properties, provides nutrient stabilization, and aids in coastal protection and resilience. All these aspects are becoming increasingly important as our oceans are experiencing rising temperatures, a decrease in pH and a reduction in biodiversity. The goal of this research is to quantify the impact of shellfish aquaculture on local biodiversity and water quality at Cottage City Oysters' commercial eastern oyster (*Crassostrea virginica*) farm located off the coast of in Martha's Vineyard MA. Biodiversity was measured via environmental DNA (eDNA) metabarcoding complemented by visual surveys including GoPro video footage of larger organisms and zooplankton tows of smaller organisms. Seawater samples for eDNA metabarcoding, GoPro video footage and zooplankton tows were collected monthly from March 2022 through November 2023 from the commercial shellfish farm as well as a reference site, which contained no aquaculture equipment and had no known history of aquaculture. Water quality parameters, including seawater temperature, dissolved oxygen, pH, salinity and turbidity, were measured in the commercial farm and the reference site using a continuously monitoring Eureka multi-probe from March 2022 to November 2023. Biodiversity of organisms identified through eDNA metabarcoding, video footage and zooplankton images was quantified using the Shannon-Wiener Diversity Index. Biodiversity was highest in early autumn and lowest in early spring, demonstrating a seasonal trend following productivity trends expected for the region. The shellfish aquaculture farm also had consistently greater biodiversity than the reference site, indicating the increase of local biodiversity with the presence of shellfish farming equipment. Through the utilization of environmental DNA metabarcoding, visual surveys and long-term water quality data, this research can be used to inform resource management and policy decisions surrounding the placement of shellfish farms as a coastal resilience strategy.

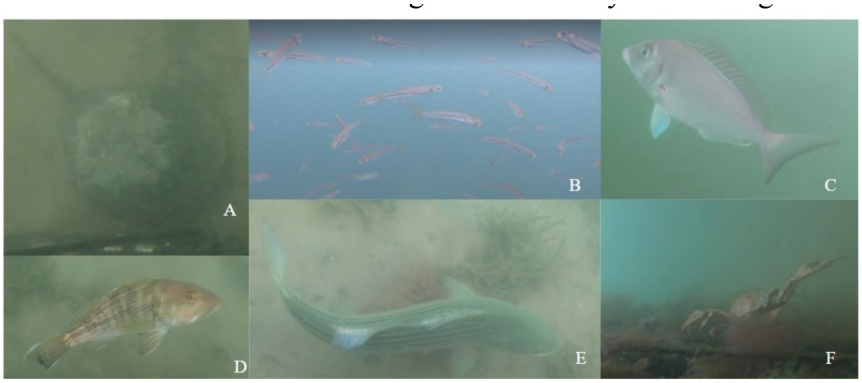

Figure 1 - Examples of species captured in GoPro footage. A: Atlantic horseshoe crab (*Limulus polyphemus*), B: Atlantic silversides (*Menidia menidia*), C: Scup (*Stenotomus chrysops*), D: Black sea bass (*Centropristis striata*), E: Striped bass (*Morone saxatilis*), F: Common spider crab (*Libinia emarginata*).

IDENTIFICATION OF A NOVEL ANTIMICROBIAL RESISTANCE-ASSOCIATED MEGAPLASMID IN *Pseudomonas* SP. FROM DISEASED TILAPIA (*Oreochromis niloticus*)

Jocelyne García Armenta[1], Roberto Cruz-Flores[1*], Olivia Cabanillas-Bernal[2], Jorge Cáceres-Martínez[1]

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This study identifies and characterizes a novel antimicrobial resistance-associated megaplasmid in *Pseudomonas* sp. isolated from diseased Nile tilapia (*Oreochromis niloticus*) in Mexico. Aquaculture's growth has heightened concerns over antimicrobial resistance (AMR) due to the widespread prophylactic use of antibiotics to manage bacterial infections, which contributes to AMR in pathogens like *Pseudomonas* spp., an opportunistic agent of fish disease. A bacterial isolate from symptomatic tilapia was identified as closely related to *Pseudomonas soli* through 16S rRNA gene sequencing. Whole-genome sequencing revealed a 362,580 bp megaplasmid encoding 984 genes, including multidrug resistance elements, confirmed by PCR targeting key genes (repA and virB4/traC) (Figure 1). The megaplasmid exhibited high homology to known resistance plasmids, underscoring the potential for horizontal gene transfer in aquaculture environments. This first identification of such a megaplasmid in tilapia pathogens highlights the urgent need for sustainable antibiotic practices in aquaculture to mitigate AMR's spread. Our findings contribute to understanding the genomic basis of AMR in *Pseudomonas* spp. and offer insights for developing management strategies to safeguard fish health and food security.

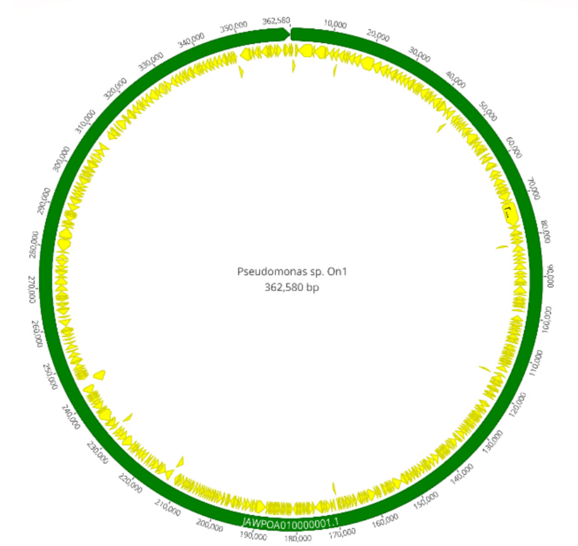

Figure 1. Circular representation of the antimicrobial resistance-associated megaplasmid in *Pseudomonas* sp. isolated from diseased tilapia. The plasmid contains a total of 984 annotated genes, including 972 protein-coding sequences, 1 rRNA gene (5S rRNA), 7 tRNA genes, and 4 pseudogenes. This plasmid has been deposited in GenBank under the accession number NZ_JAWPOA010000001.

DETECTION OF PATHOGENS IN THE CULTURE ENVIRONMENT OF PACIFIC WHITE SHRIMP (*Penaeus vannamei*) THROUGH NEXT-GENERATION SEQUENCING

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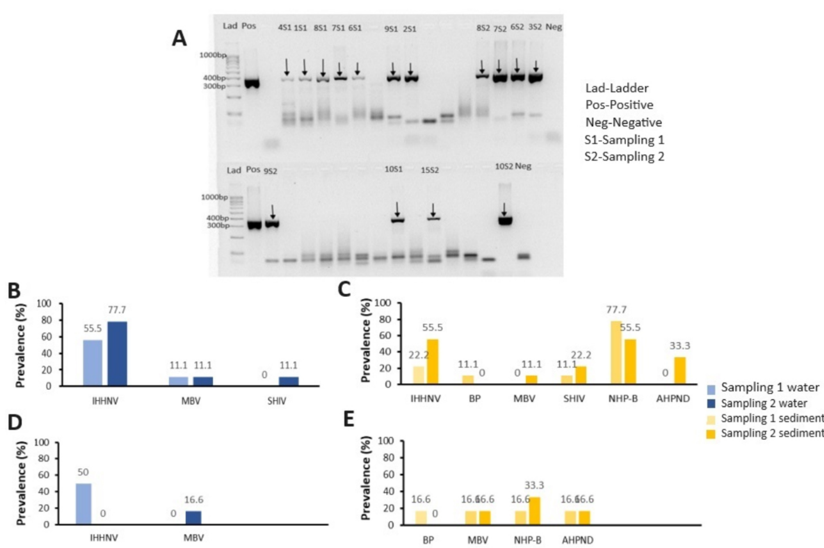
The Pacific white shrimp (*Penaeus vannamei*) holds significant economic value in Mexican and global aquaculture. However, high production rates, suboptimal farming practices, and environmental factors have increased the prevalence of diseases affecting shrimp populations. Conventional molecular diagnostics, such as PCR and qPCR, facilitate pathogen detection by targeting specific nucleic acids. Despite their utility, these methods cannot simultaneously identify multiple pathogens present in a single sample. Next-generation sequencing (NGS) has emerged as a promising tool for detecting entire microbial communities in environmental samples (e.g., water, sediment), offering enhanced pathogen identification capabilities. This study evaluated the sensitivity of NGS in detecting DNA-based pathogens in *P. vannamei* from two shrimp farms in Northwest Mexico. Conventional PCR identified several pathogens, including infectious hypodermal and hematopoietic necrosis virus (IHHNV), shrimp hemocyte iridescent *virus (SHIV), Baculovirus penaei (BP), Candidatus Hepatobacter penaei (NHP-B), and Vibrio parahaemolyticus* (AHPND agent) (Figure 1). Sanger sequencing confirmed these findings. In contrast, NGS only detected AHPND, likely due to low DNA concentrations in water and sediment samples and the limitations of whole-genome amplification reagents. This is the first study to apply a universal PCR protocol for multi-pathogen detection in shrimp aquaculture.

Figure 1. Detection and prevalence of shrimp pathogens utilizing eDNA. (A) Detection of an ~420bp amplicon of the NHP-B genome from water and sediment samples. (B-C) Prevalence of IHHNV, MBV, SHIV, NHP-B and AHPND in water and sediment from Farm 1. (D-E) Prevalence of IHHNV, MBV, NHP-B and AHPND in water and sediment from Farm 2.



SELECTING THE RIGHT GAS MANAGEMENT SOLUTION TO COMBAT NITROGEN SUPERSATURATION

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The competition for quality water sources has and will continue to force aquaculture facilities to contend with less favorable water quality (WQ) conditions on selected sites. A common, but often poorly addressed water quality issue is nitrogen supersaturation, which can lead to negative health conditions in livestock, such as gas bubble disease. Continuous nitrogen removal can prove costly not just to implement, but also to operate if not chosen properly. Determining the optimal degassing solution for your system is essential to keeping a productive farm. There are six major design criteria to consider when selecting the most efficient and effective technology for each application. These six design criteria are dissolved nitrogen level (DN), dissolved oxygen level (DO), system water flow, reuse percentage, salinity, and power availability. Selecting the best solution using the design matrix of all six factors is sometimes difficult.

Case studies for five Innovasea-designed nitrogen removal systems will be presented, providing a strong basis for selecting one of three industry-standard systems for nitrogen gas management: Vacuum Degassers, Gas Management Towers (GMTs), and Low Head Oxygenators (LHOs). In the first study, LHOs were supplied to a flow-through trout hatchery system with an incoming DN of approximately 105%. The second case study evaluates LHOs for nitrogen removal in a large-scale post-smolt P-RAS salmon system in Iceland. Similarly, the third study assess another large-scale post-smolt P-RAS salmon farm, however, it required GMTs as an alternative to the LHOs supplied in the Icelandic farm case. The final two case studies both assess vacuum degassers supplied as a pre-treatment for a small-scale hatchery in Utah and a saline broodstock system in Mexico. The experience gained from these five case studies has led Innovasea to create a design matrix for selecting nitrogen mitigation technologies.

Figure 1: *Innovasea's Gas Management Tower*

FACES AND VOICES OF FLORIDA AQUACULTURE

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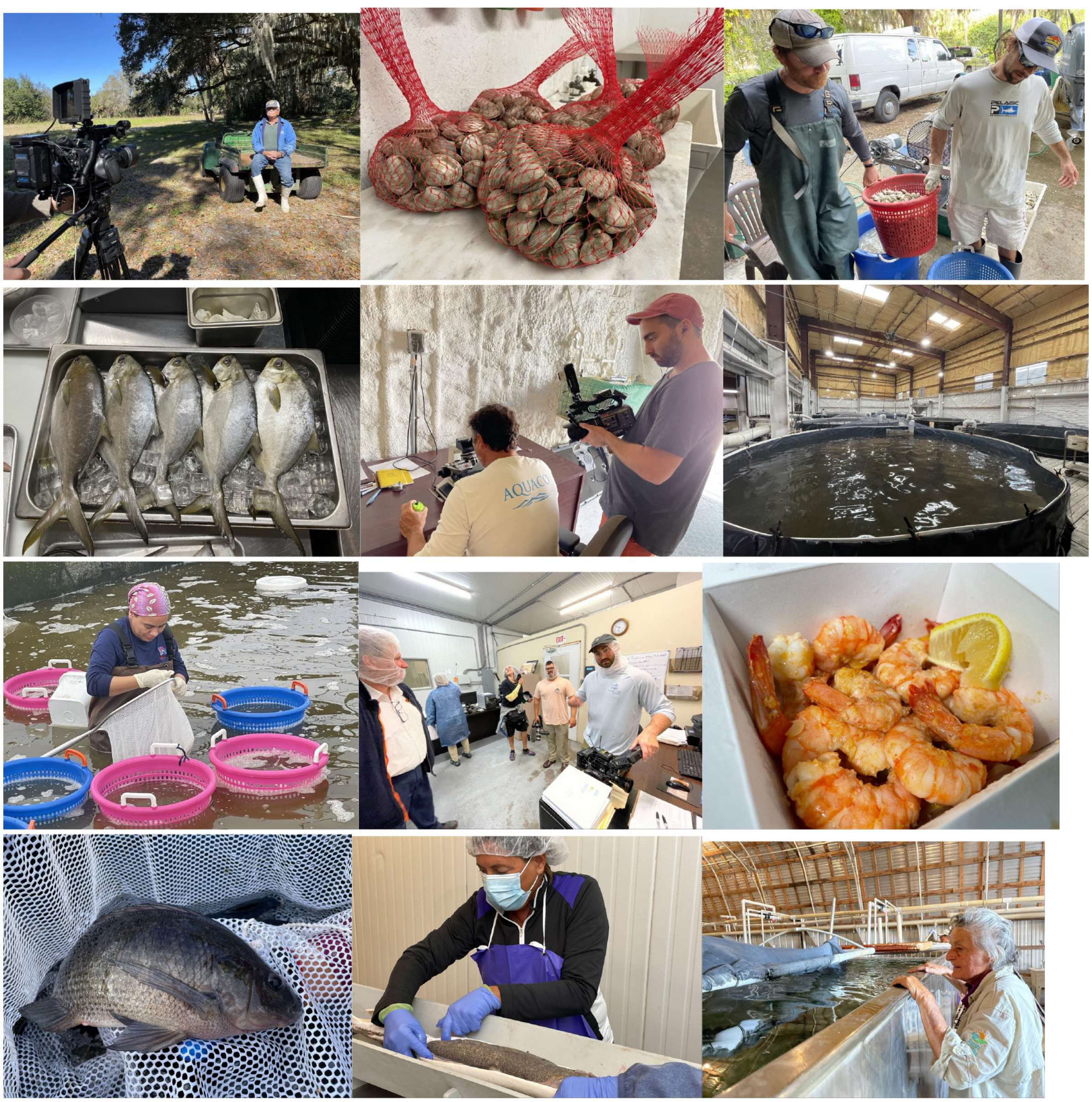
Giving aquaculture farmers an outlet to share their stories is critical to a broader acceptance and understanding of aquaculture. Since 2021, Sereia Films has been capturing the stories of seafood stories in Florida. We've featured a clam farm, a land-based pompano farm, a shrimp farm, a sturgeon farm, an oyster farm, and a tilapia. Not only do we connect consumers with the people directly involved with growing these seafoods and starting these businesses, but also the distributors and chefs.



EFFECTS OF ANTHROPOGENIC NITROGEN EXPOSURE ON REPRODUCTION AND OFFSPRING QUALITY OF THE CRITICALLY ENDANGERED *“Xenotoca” doadrioi*

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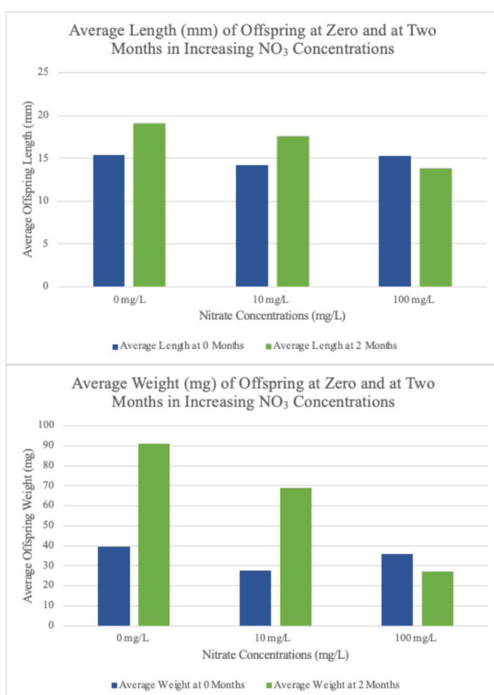
“Xenotoca” doadrioi is a species of viviparous teleost fishes in the Goodeidae family endemic to shallow freshwater ecosystems of the central Mexican plateau and the western Great Basin of the United States. The Goodeidae family is the most diverse and threatened family of teleost fishes in Mexico with 83% considered threatened or endangered. *X. doadrioi* are critically endangered and in danger of extinction, with declining populations in the wild and an estimated range of only 12-88 km^2. According to the IUCN, the main threat *X. doadrioi* faces is water pollution through agricultural sources. *X. doadrioi* is a candidate for stock enhancement projects similar to other closely related endangered and extinct in the wild species such as *Skiffia francesae* and *Zoogoneticus tequila*.

The goal of this project was to determine how an anthropogenic agricultural runoff contaminant impacts the evolutionary unique form of viviparous reproduction and offspring output of *X. doadrioi*. Three concentrations of nitrates were tested: 0 mg/L NO_3 (no nitrate pollution), 10 mg/L NO_3 (low nitrate pollution), and 100 mg/L NO_3 (high nitrate pollution). Reproductive data (estimated length of gestation periods, number of birthing events, number of offspring birthed, survivability, and deformity rates) were measured over a six-month sampling period. Additionally, growth rates of adults and offspring were also sampled monthly.

Impacts on reproduction and offspring output were predicted to be more significant in the higher concentrations of nitrates. After two months, the length (mm) and weight (mg) of offspring were significantly less ($p < 0.01$) in higher concentrations of nitrates, with offspring in the 100 mg/L weighing less than they did at birth. It was also observed that fewer offspring were produced, survival rates were decreased, and both gestation periods and deformity rates increased. These results suggested that offspring exposed to high concentrations of nitrates are being affected most severely. This project provides insights into how wild populations and future generations are affected by nitrates, which can help guide restoration efforts for this critically endangered species.

Figure 1. Average Weight and Lengths of *X. doadrioi* at zero and two months.



ATLANTIC SALMON MATURATION UNDER IMPACT OF TEMPERATURE AND LIGHT REGIMES – PRELIMINARY STUDIES IN SMALL RECIRCULATION SYSTEM

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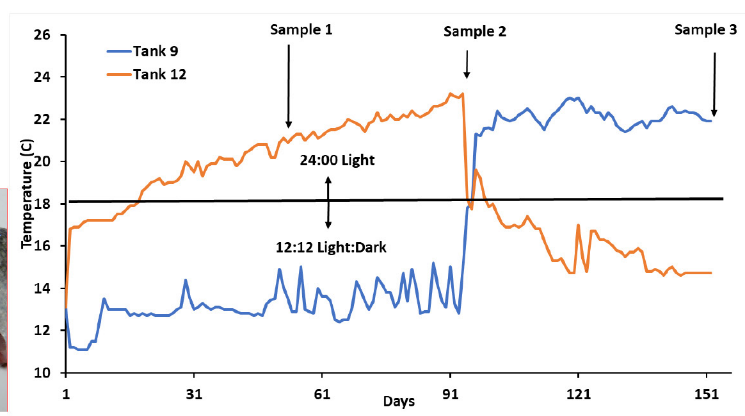
A better understanding and utilization of the life history pattern Atlantic salmon can serve both to improve control of maturation and gamete use as well as serve determining the highest meat quality for human consumption. Earlier studies demonstrated that 82% of Atlantic salmon (AS) males matured under a 24-hour (hr) light (L) regime in comparison to 8% in 12:12 light:dark (LD) simulating natural conditions. Further, higher temperatures (16^{o}C) and 24 hr L resulted in 47% maturation in comparison to no maturation at 5-10^{o}C. Most recent observations have shown maturation taking place at 18^{o}C (Martinez et al. 2024. JWAS). Our study explores the response of pre-smolt AS of mean initial weight 63 ± 21 and 67.5 ± 16.3 g (initial hot and cold water, respectively) kept in a small set up of flow-through or recirculated, side-by-side systems, allowing the predictable maturation of fish throughout the year. The recirculated system allowed adjustable temperature changes between 12 and 23^{o}C within hours (Fig.1). Body weight reached up to 10- fold increase, however mean body weight has not differed between "Hot" and "Cold" regimes after 2 cycles 407 ±117 and 421 ± 58 g, respectively. We present growth rate, maturation process, and fragmentary biochemical characteristics of fish muscle neutral (NL) and phospholipids (PL), fatty acids, blood osmolality, hematocrit) over a period of "shortened" light and temperature annual cycle. The first maturation of male AS releasing sperm was induced after 168 days of rotation of light and temperature cycles (Fig.2). Fish was 258g and gonadosomatic index (GSI) 5.2%. The first phase difference in experimental conditions between fish reared on commercial diet at temperatures 13.6 ± 1.5 and 21.7 ± 2.8^{o}C under 12:12 LD and 24:L regimes, respectively, resulted in significant difference in muscle NL gamma-linolenic fatty acid, 34.1 ± 10.6 and 16.9 ± 1.8%, EPA 1.8 ± 0.24 and 2.33 ± 0.04, and PL DHA 17.6 ± 1.4 and 15.7 ±0.9%. We will present results of fatty acid profiles in the following 160 days of growth and maturation. These studies were supported by North Central Regional Aquaculture Center, USDA, and Ohio Agriculture Research and Development Center, O.S.U.



HOW TO DESIGN VALID SYSTEM OF PRODUCING MONOSEX POPUATION OF WALLEYE AND AVOID POSTZYGOTIC SEX REVERSAL IN GYNOGENETIC INDIVIDUALS DUE TO STRESS – CORTISOL HYPOTHESIS

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This research addresses both conventional pond culture and intensive indoor facility of walleye production that requires preventing the release of domesticated fish. Implementation of fish sterility (improved feed efficiency) and all-female (fast growth) production has resulted in nearly 30% cost reductions in related percids; therefore, it has the potential to enhance the economic value of cultured walleye for recreational stocking and food markets. Specifically, by securing all-female progeny and polyploidy (sterility), both improved growth rate and prevention of introgression of domesticated stocks with wild populations can be achieved. Results of this project will alleviate one of the main concerns of the general public, prevention of invasions of fertile fish from aquaculture. The importance of understanding the effects of environmental conditions (stress; hormonal regulation) on sex determination is magnified by the threat of climate change.

Walleye were spawned in 2021 as part of a NCRAC supported project, underwent experimental treatments for gynogenesis and hormonal sex reversal with methyltestosterone (Fig. 1). Their growth, survival, and sex ratio has been monitored. We validated genetic sex of gynogenetic walleye (produced with UV-irradiated yellow perch sperm, which produces non-viable hybrids) and gynogenetic individuals that were subjected to masculinization. Both groups produced sperm in January 2024 (Table 1). Validation of stress-related hypothesis of phenotypic sex-reversal in walleye by cortisol-induced masculinization and antagonistic effect of estradiol (E2) via dietary source is currently being developed. To test the viability and fertilizing ability of OSU walleye gynogens, sperm will be sent to Iowa DNR and Wisconsin DNR state hatcheries to fertilize wild walleye females and compare with their local walleye stocks in 2025.

One of the objectives of this presentation is to identify Midwestern State DNRs agencies (Wisconsin and Iowa already committed) that would be interested in collaborating in research and contribute financially with expected results in the domain of analysis of walleye ploidy (Farrell, Delomas et al. 2022), sex related sequences allowing to identify genome of individual fish and validate against phenotypical sex (including male sperm ducts functionality). This area of research is connected closely with their own state walleye hatchery management programs and addresses regional food security as well as the importance of walleye to Tribal nations.

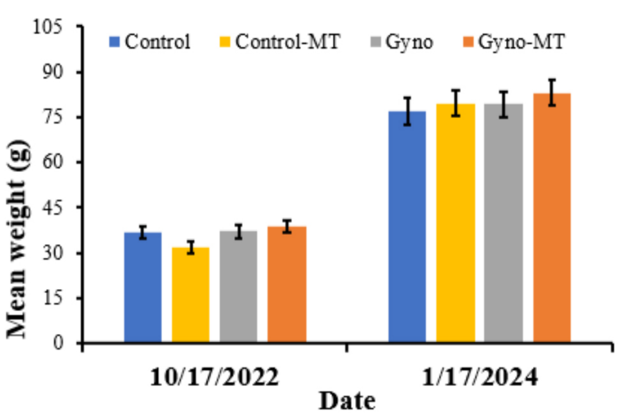

Fig 1 Weight (g) of walleye in the experiment.

Table 1 Sex of walleye from treatment groups dissected on 1/17/2024. (MT – hormone

Treatment	Fish dissected Female	Fish dissected Male	Fish kept
Control	10	2	13
Control-MT	14	7	14
Gynogen	8	5	18
Gyno-MT	4	3	18

THE EFFECTIVENESS OF A HUMIC SUBSTANCE TO CONTROL MOTILE AEROMONAS SEPTICEMIA AND COLUMNARIS DISEASE ON GROWTH, CHEMOTAXIS AND VIRULENCE IN CHANNEL CATFISH *Ictalurus punctatus*

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AC Aqua (MTS Environmental Inc. Exeter, Ontario, Canada) is a natural liquid humic acid product. This substance is a non-toxic, environmentally friendly substance that could be used as a substitute for chemical and antibiotic treatments for diseases in channel catfish *Ictalurus punctatus*. Humic substances have been shown to have many effects, and there is increasing interest in using these products in the aquaculture industry. Exposure to humic acid can be beneficial for fish species. Rainbow trout (*Oncorhynchus mykiss*) eggs infected with the spores of *Saprolegnia* sp. have been shown to have significantly decreased mortality and increased hatching rates in the presence of humic acid. When humic acid is added to the diet of Arctic Char *Salvelinus alpinus*, the survival rates of fish exposed to *Flavobacterium columnare* increased significantly. Studies in channel and hybrid catfish have shown humic acid increases growth and improves the immune response when exposed to various bacterial pathogens. Therefore, the following research aims to assess the safety and efficacy of AC Aqua when used as a water treatment against Motile Aeromonas Septicemia caused by a virulent strain of *Aeromonas hydrophila* (vAh). The following objectives were investigated: 1) the effects of vAh growth on bacterial plates with humic acid added, 2) the effect of humic acid concentrations and treatment time on bacterial flocculation and settling rate, 3) the chemotactic response of vAh to catfish mucous and 4) detection of bacterial adhesion. Results of these objectives will be presented.

A NOVEL MARINE BACTERIUM AS A PROBIOTIC SUPPLEMENT TO IMPROVE HEALTH, FEED DIGESTION, AND RESILIENCE OF THE GILTHEAD SEABREAM *Sparus aurata*

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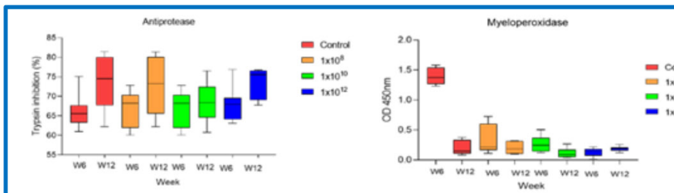
Improving productivity in aquaculture demands maintaining the well-being of the cultured organisms. For this purpose, probiotics have become common in culture setups to enhance fish growth, health, and resilience. The current study examined a novel marine bacterium for its feasibility in improving the growth, feeding, and health performances of gilthead seabream *Sparus aurata*. For this aim, a feeding trial was performed over 12 weeks in which fish were fed commercial aquafeed with different inoculation rates of the probiotic bacterium of $1x10^8$, $1x10^{10}$, and $1x10^{12}$ CFU/Kg compared to a probiotic-free diet control. Fish growth rate and yield, digestibility, digestive enzyme activity, immune response, and microbial community composition were measured throughout the culture period. Sequentially, trials to examine feed digestibility fish resilience when challenged with pathogenic *Streptococcus agalactiae* were conducted using identical diets.

Results indicated no effect of probiotic treatment on fish growth performances, but protein and lipid digestion was improved when the probiotic was supplemented at $1x10^{10}$ CFU/Kg, proposing an improved absorption of these macronutrients. The probiotic supplement at $1x10^{10}$ CFU/Kg also induced an increase in antiprotease activity, while myeloperoxidase activity increased only during the first six weeks. Probiotic supplementation influences microbial community composition, indicating their resilience to varying treatments. Last but not least, the probiotic treatment improved fish survival after challenging them with *Streptococcus agalactiae,* with highest survival rate of 70% in the probiotic supplementation level of $1x10^{12}$ CFU/Kg. Overall, the novel probiotic bacterium efficiently improves feed digestion and fish health. The origin of such bacterium, from the gut of an algivorous sea urchin, proposes further study concerning the contribution to fish in feed digestion and energy gaining under diets with high plant or algal- ingredients.



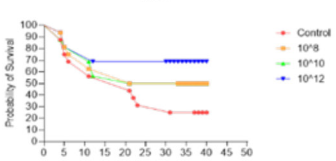


Fig A & B: Immune assay Antiprotease and Myeloperoxidase (weeks 6 & 12).

C: Probability of survival when challenged to *Streptococcus* (4 weeks)

Growth parameter	Control	$1x10^8$	$1x10^{10}$	$1x10^{12}$	P value
SGR (BW d-1)	1.38±0.16	1.30±0.13	1.33±0.16	1.36±0.14	0.942
PWG	22.94±2.2	21.32±2.3	21.97±2.0	22.46±2.2	0.996
FCR	2.01±0.25	2.22±0.28	2.09±0.23	2.07±0.21	0.859
PER	0.61±0.04	0.55±0.03	0.57±0.02	0.62±0.02	0.147
PPV	41.27±0.40[a]	42.19±0.20	43.34±0.53[b]	43.53±0.95[b]	0.016
Survival rate	100	100	100	100	NA

Table 1. Growth performance of *S. aurata* fed with the four experimental diets. SGR, FCR, PER, PPV, weight gain (WG), and survival rate.

SUPPORTING SMALL-SCALE AQUACULTURE BY MAPPING ONLINE HATCHERY RESOURCES

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The U.S. aquaculture industry is diverse in its approach to fish production. Farming techniques vary greatly by geographic location, scale, technology, expenditures, and methods of production for freshwater and marine species. Small-scale aquaculture contributes to economic sectors and supports local food security. Small-scale producers are especially dependent on domestic hatcheries. Present and potential producers undergo several challenges when acquiring fish, due to information gaps and exposure to hatchery resources from poorly integrated digital business platforms. These challenges include issues related to purchasing, species availability, genetics, growth performance, and other commercially valuable factors. This review of U.S. tilapia hatcheries aims to enhance the dissemination of available resources for small-scale producers and to provide insight and guidelines for doing this type of work to support the production of any other species in the U.S. aquaculture industry.

Specific objectives were to: (1) identify publicly accessible hatcheries through online business platforms; (2) establish commercially valuable criteria; (3) assess online user interface and data collection; and (4) outline potential improvements to digital business platforms. A survey was developed for U.S. Tilapia hatcheries to obtain data on commercially valuable parameters. Fourteen tilapia hatcheries were identified (Figure 1), and data was obtained on species availability, hatchery technology, and price analysis (Figure 2). Potential areas for improvements to business platforms were identified after navigating online interfaces, and recommendations were developed based on these findings. The tilapia survey from this review is adaptable for development of a survey template for widespread use in obtaining important information for other aquaculture species. The results are expected to be of value to small-scale producers and hatcheries by improving effectiveness of data access and dissemination of online business platforms for organizing and centralizing data.

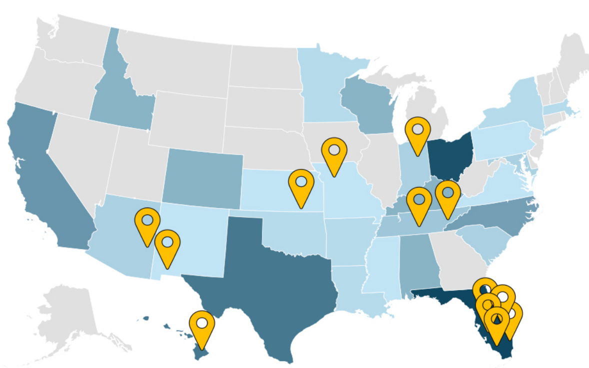

Figure 1. Distribution of 137 registered U. S. Tilapia Farms (blue) and 14 hatcheries (yellow).

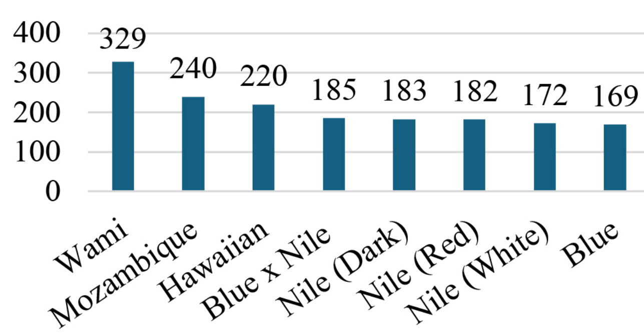

Figure 2. Illustration of generated data obtained on cost. Price in USD (vertical axis) of 100-count fingerlings by species (horizontal axis).

ASSESSING SIZE DYNAMICS AND GRADING TO REDUCE VARIABILITY IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Pacific white shrimp (*Litopenaeus vannamei*) are reared in a variety of RAS systems at high stocking densities. Shrimp tend to arrive from hatcheries with a high rate of size variability, which is compounded by high-density culture conditions. Left uncorrected, the size variation leads to problems with marketing the shrimp later. The purpose of this study was to determine whether a weight to width ratio of shrimp exists and to use that information to reduce size variability by grading and sorting shrimp.

To establish a weight to width ratio, a wide range of shrimp were weighed and measured. A linear regression and polynomial regression were each applied to the data, resulting in R^2= 0.873, P= 0.001 and R^2= 0.909, P= 0.000 values, respectively. The polynomial equation was used to decide the width of a grader in an attempt to separate shrimp that were above and below the mean weight. The mean weights of two shrimp populations were measured and the coefficients of variation (CV) were calculated.

The first population of shrimp (N = 228) had a mean weight of 2.7g and a CV of 4.5%. A simple, wooden frame was made with small dowels fixed to it that were 7.8 mm apart, according to the prediction of the polynomial equation. After grading and separating into two sub-populations of shrimp, the larger shrimp (N = 59) had a mean size of 4.2g and a CV of 1.3%. The smaller shrimp (N = 169) had an average size of 2.9g and a CV of 0.8%. These two sub-populations were grown for two months and sampled again. This later sampling revealed that the larger sup-population having a mean size of 19.3g and a CV of 2.0%, while the smaller sub-population had an average size of 12.9g and a CV of 2.6%. The same process of sampling and grading was repeated on a second population of larger shrimp (N =117) with a mean weight of 9.9g and a CV of 9.3%. After grading the population with a grader width of 11.9 mm, the mean weight of the larger shrimp was 11.4g with a CV of 3.2% and the smaller shrimp had a mean weight of 8.8g with a CV of 3.6%.

These results indicate that there was a predictable relationship between shrimp weight and width, and this relationship could be used to decide how to grade shrimp. Simple, in-house made graders can be an effective way to divide shrimp populations and by doing so, reduce the size variability of each subsequent group. Although size variability remained lower than the original values, in this case variation increased again over the course of a two-month growing period. Furthermore, it seems that grading shrimp earlier was more effective at reducing variability even after shrimp grew larger. Future research should examine how frequently grading should be done in shrimp populations. Also, the question of whether variability is similar among genetically different shrimp populations should be addressed. Ultimately, reducing the variability in shrimp size should help farmers market a more consistent crop and generate more consistent revenue as a result.

OFF-FLAVOR PRODUCTION IN RAS: PROGRESS TOWARDS POTENTIAL SOLUTIONS

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Earthy, musty off-flavor caused by the microbially-derived compounds geosmin (GSM) and 2-methylisoborneol (MIB) is a priority concern for producing lipid-rich fish like salmonids in RAS. Currently, depuration is the only proven method that consistently eliminates objectionable off-flavor from RAS-produced fish before harvest. However, this procedure requires fish transfer to separate systems where substantial water volumes are exchanged, typically while withholding feed for five to ten days. Solutions that limit off-flavor in the primary RAS could reduce or eliminate the need for depuration, thereby lowering water use, energy requirements, fish handling, and weight loss, which could ultimately improve the economics of RAS operations.

Two studies were conducted. Study 1 aimed to improve our understanding of conditions that favor off-flavor production by assessing GSM and MIB levels in water and Atlantic salmon fillets in pre-disinfected- vs. microbially mature freshwater RAS (9.5 m^3 total volume) operated for more than two years without shutdown (n=3 RAS). GSM and MIB levels spiked several weeks after stocking fish in the pre-disinfected RAS. In contrast, levels remained relatively low in the mature RAS (Fig. 1). Similar trends were observed in Atlantic salmon flesh. Off-flavor levels correlated with nitrite-nitrogen concentrations and the establishment of complete nitrification. Microbial analysis indicated that the relative abundance of off-flavor-producing organisms was statistically similar between treatments, suggesting GSM and MIB production was tied to aspects of the fish culture environment such as nutrient concentrations or ratios.

Pre-disinfection of RAS was deemed a procedure that could be repeated for testing off-flavor mitigation strategies due to the consistent spike in GSM and MIB. Thus, all six RAS were disinfected before Study 2. Nitrification was re-established, and the same number of Atlantic salmon were stocked in six identical RAS. Low-dose ozone, which provides significant water quality improvements, was then applied in three RAS along with the periodic addition of peracetic acid (PAA), which has been shown to have biocidal effects against harmful microorganisms. Geosmin and MIB levels were compared in RAS where this advanced oxidation strategy was applied vs. RAS without oxidant use. Off-flavor levels measured during Study 2 will be reported at the meeting.

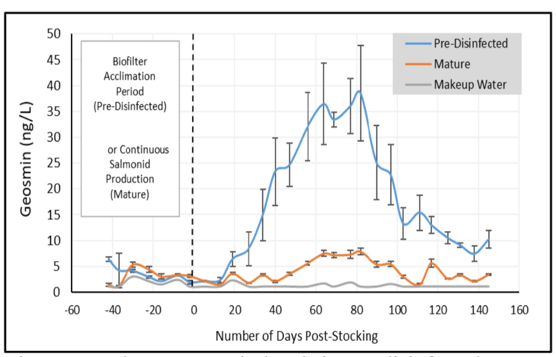

Fig. 1. Waterborne geosmin levels in pre-disinfected RAS vs. mature, continuously operated RAS with Atlantic salmon.

QUANTIFYING ENERGY DEMANDS OF AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM TO INFORM CO-LOCATION WITH MARINE ENERGY

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The co-location of marine energy and aquaculture is a concept of increasing value and interest in the United States, as the desire for sustainably produced seafood and renewable energy continues to grow. The AquaFort is an integrated multi-trophic aquaculture (IMTA) system designed and tested by the University of New Hampshire. Its proven production capabilities in exposed nearshore waters make it a viable candidate for co-location with marine energy. However, the various scales and purposes of aquaculture operations pose a challenge, and dictate a nuanced approach to identifying marine energy devices suitable for co-location. To address this, four unique production scenarios (base, small, medium, and large) were defined based on the total quantity of AquaForts considered. The scale of these scenarios ranged from community-based farms for local food production, to full-scale commercial farms intended for national sale and distribution of product. The operational tasks and components of each scenario were identified, enabling the quantification of daily and peak power and energy demand. The resulting demand estimates and profiles were derived from a collective review of aquaculture industry publications, instrumentation technical data, anecdotal references from farm operators, and Manna Fish Farm's own experience. This quantification of aquaculture energy demands at various scales will assist in the identification of suitable marine energy devices, thus informing future co-location deployments.

Table 1: Farm scales and defining characteristics.

	Base	**Small**	**Medium**	**Large**
Purpose	Local food production	Local and regional food production for sale	Regional food production for sale and distribution	Regional and national sale and distribution
Number of Systems	1-2	3-4	5-6	7-12
Production Scale	Community-based	Small businesses	Medium/large businesses	Commercial
Production Capacity*	45.5 MT	91.0 MT	136.5 MT	272.5 MT

**Assumes 20m (66ft) site depth, 12.2m (40ft) net, 20 kg/m^3 fish density at harvest*
MT = Metric Ton

EVALUATING THE ANTIBACTERIAL ACTIVITIES OF *Allium sativum* AGAINST MAJOR CATFISH PATHOGENS

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Bacterial pathogens such as *Edwardsiella ictaluri, Flavobacterium covae*, and virulent *Aeromonas hydrophila* significantly impact farm-raised catfish in the United States, leading to substantial economic losses. The emergence of antimicrobial resistant strains and the limited availability of licensed vaccines highlights the urgent need to find alternative therapeutics. Garlic (*Allium sativum*) exhibits strong antibacterial activity against a wide range of Gram-positive and Gram-negative pathogens. This study aims to investigate the *in vitro* antibacterial activities of *Allium sativum* and its effects on the cell wall and cell membranes of common fish pathogens. We will also evaluate its potential to protect catfish against enteric septicemia of catfish (ESC) caused by *E. ictaluri*. The *in vitro* antibacterial activities of *Allium sativum* were investigated against *E. ictaluri* 93-146, *A. hydrophila* ML09-119, *F. covae* 94-081, *E. ictaluri* MS-17-156, *E. piscicida* MS-18-199, and *Plesiomonas shigelloides* MS-17-188 using disc diffusion method and broth microdilutions. Secondly, transmission electron microscopy (TEM) was used to examine the effects of *Allium sativum* on major fish pathogens. Furthermore, the ability of *Allium sativum* to protect catfish against *E. ictaluri* 93–146 were assessed using immersion challenge. Four groups were established (minced *Allium sativum* (30g per kg of diet), minced *Allium sativum* combined with trans-cinnamaldehyde (TC) (15 ml/kg), and *Allium sativum* powder combined with TC, and *Allium sativum* water (30g per kg of diet).

The disc diffusion test results demonstrated that *Allium sativum* inhibited bacterial growth, with varying degrees (Fig. 1). The broth microdilution tests further confirmed that *Allium sativum* significantly inhibited bacterial growth at the concentration of 10 mg/ml. TEM of the fish pathogens incubated with *Allium sativum* revealed notable effects, including cell wall lysis, leakage of cytoplasmic contents, pleomorphism, and rupture of the outer membranes (Fig. 2). A significant reduction in mortality rates was observed in the catfish received minced *Allium sativum* with TC (31.7%), *Allium sativum* powder with TC (37.1%), minced *Allium sativum* alone (37.6%), and *Allium sativum* water alone (55.5%) compared to the control group, which had a mortality rate of 90%.

Overall, these results indicated that *Allium sativum* has a strong antibacterial activity against *E. ictaluri, F. covae*, *A. hydrophila*, *E. piscicida,* and *P. shigelloides*. Using *Allium sativum* for treatment of ESC, alone and in combination with TC have showed significant decrease in the mortality rates. These findings collectively highlight the potent antibacterial effects of *Allium sativum* and its potential application in aquaculture for managing fish pathogens.

Fig. 1. Disc diffusion test showing inhibition zones in centimeters for different fish pathogens

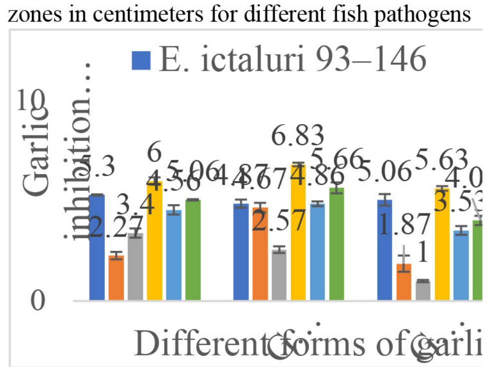
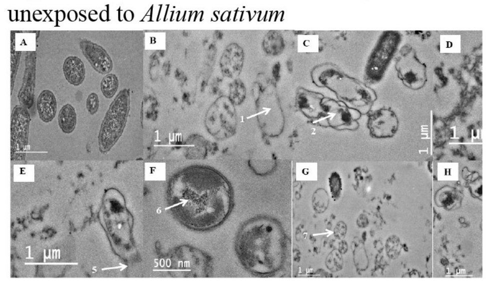


Fig. 2. TEM of *E. ictaluri* 93–146 exposed and unexposed to *Allium sativum*



INVESTIGATING VIRULENCE AND PROTEOMIC DYNAMICS IN ADAPTED STRAINS OF *E. ictaluri*

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Channel catfish is one of the most cost-effective farm-raised food sources, especially in the southern part of the United States. *Edwardsiella ictaluri* is a primary challenge facing catfish farmers which cause enteric septicemia of catfish (ESC). Trans-cinnamaldehyde (TC), a key compound found in cinnamon, has gained significant attention for its antimicrobial activity against various bacteria. Our long-term goal is to support the US aquaculture industry by identifying potential interventional strategies to reduce catfish mortality associated with bacterial infections.

To investigate the effects of continuous exposure to TC on the virulent *E. ictaluri* 93–146 strain, we passaged the bacteria in brain heart infusion (BHI) broth containing a permissive TC concentration of 0.016 µl/ml. *E. ictaluri* was inoculated into BHI broth with TC and incubated overnight at 30ºC, after which samples were transferred to fresh BHI broth with TC daily for 30 days. A control strain was passaged in BHI without TC. The resulting strain after 30 passages in TC was designated D30-TC, while the control strain was labeled D30-BHI. We next explored the pathogenic potential of *E. ictaluri* D30-TC and D30-BHI strains in catfish fingerlings using an immersion challenge model. Catfish fingerlings were placed in 40 L tanks and divided into four groups: one infected with *E. ictaluri* 93-146, one sham-vaccinated with BHI, one infected with the D30-TC strain, and one with the D30-BHI strain. Fish were immersed in water containing 10^7 CFU/ml for 1 hour, and mortality was recorded daily. Notably, no mortality occurred in the D30-TC group, while 88% and 84.37% mortalities were observed in the wild-type and D30-BHI groups, respectively. The D30-TC vaccinated fish demonstrated a survival rate of 63.64%, compared to 20% in the sham-vaccinated group after 21 days.

To investigate the attenuated virulence of the *E. ictaluri* D30-TC strain, we analyzed proteomic differences between the D30-TC and parental strains. The proteomic analysis identified 124 upregulated and 141 downregulated proteins ($p < 0.05$, |fold change| > 2) in the D30-TC strain compared to the wild-type strain. Notably, there was downregulation of proteins associated with the Type III secretion system (T3SS) and Type VI secretion system (T6SS), which are crucial for *E. ictaluri* virulence (Table 1). This downregulation likely contributes to the decreased pathogenicity observed in the D30-TC strain.

Table 1. The most significant downregulated proteins in the *E. ictaluri* D30-TC strain compared to the parental and *E. ictaluri* D30-BHI strains.

Description	Log FC
Type VI secretion system effector, Hcp1 family	-9.96
Type VI secretion system Vgr family protein	-5.43
Type VI secretion protein, VC_A0107 family	-4.27
Type VI secretion protein, VC_A0114 family	-4.26
Uncharacterized protein	-3.64
Type VI secretion ATPase, ClpV1 putative	-3.49
Type VI secretion protein, EvpB	-3.78
Type VI secretion-associated protein, ImpA	-2.51
Type 3 secretion system secretin	-3.74
Pentapeptide repeat family protein	-3.72
Type III secretion apparatus protein, YscD/HrpQ	-3.23
Pathogenicity island 2 effector protein SseE	-7.24
Type VI secretion system EseD	-5.05
Type III secretion system effector protein	-6.97
Type III secretion low calcium response	-4.47
EseB	-3.48
Type III secretion ATPase FliI/YscN family,	-2.85
Type III secretion apparatus protein, YscR/HrcR	-4.08
Type III secretion protein, YscU/HrpY family	-4.41
Uncharacterized protein	-3.23

A ONE HEALTH ANALYSIS OF ESTUARINE POLLUTANTS AFFECTING MANGROVE CRABS AND RELATED CONSUMERS IN ECUADOR

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Mangrove forests provide vital food resources and are an endangered ecosystem worldwide due to pollution, climate change and habitat destruction. Over the last decade, the Guayas estuary in Ecuador has been experiencing degradation due to anthropogenic input of pollutants. Following the One Health principle, the aim of the study was to investigate the effects of a range of (emerging) pollutants on the health of the ecosystem, animals and humans.

As such, we analysed different types of pollutants such as pesticides, metals, perfluoroalkylated substances (PFAS) and polycyclic aromatic hydrocarbons (PAH) in the Guayas estuary in Ecuador, where major shrimp production is taking place. Pesticides and metals were analysed in water, sediment and crab (*Ucides occidentalis*) samples (as a sentinel organism) from the estuary. PFAS and PAHs were analysed in crabmeat samples.

Aquatic and human health risk assessments were conducted to determine potential health risks to the environment and the crab consumer. The findings presented in this research can support the protection of human health and help the management of ecosystems and rivers in Ecuador and globally.

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De Cock, A., De Troyer, N., Eurie, M. A. F., Garcia Arevalo, I., Van Echelpoel, W., Jacxsens, L., ... & Goethals, P. L. (2021). From mangrove to fork: Metal presence in the Guayas estuary (Ecuador) and commercial mangrove crabs. Foods, 10(8), 1880.

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A ONE HEALTH ANALYSIS OF ESTUARINE POLLUTANTS AFFECTING MANGROVE CRABS AND RELATED CONSUMERS IN ECUADOR

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EFFECTS OF SINGLE-CELL DIETARY PROTEIN SOURCES ON GROWTH AND NUTRIENT ALLOCATION IN THE SEA URCHIN *Lytechinus variegatus*

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Sea urchins are economically relevant in fisheries, as the gonads are harvested as a seafood product. Successful aquaculture practices can be enhanced by improving diet formulations, particularly dietary protein sources, which significantly impact growth profiles and gonad quantity and quality (e.g., size, color, texture, flavor). Single-cell protein sources represent a sustainable alternative to current industry standard animal-derived protein sources such as fish and squid meal. In the present study, we evaluated the effects of replacing fish and squid meal with commerically available single-cell dietary protein sources in diets for juvenile *Lytechinus variegatus*.

Juvenile *L. variegatus* (ca. 20 g whole-body wet weight, 22 mm test diameter) were collected from Port St. Joe, FL and transported to UAB, where they were housed in recirculating aquaculture systems. Following a 7-day acclimation period, sea urchins were fed one of 7 randomly assigned diets for 8 weeks. Four diets contained protein from only one source: fishmeal (FP), squid meal (SP), bacteria (BP), or yeast (YP). Three diets contained 50% protein from fishmeal and 50% protein from either squid meal (FP/SP), bacteria (FP/BP), or yeast (FP/YP).

Survival was 100% in all treatments. Terminal whole-body wet weight gain was significantly lower in BP-fed urchins compared to all other treatments (Fig. 1A, $p<0.002$). Terminal gonadosomatic index (GSI) was significantly lower in BP-fed urchins compared to all other treatments (Fig. 1B, $p<0.002$) except YP-fed urchins. GSI was significantly lower in YP-fed urchins than in FP-fed and SP-fed urchins ($p<0.002$); however, there were no other significant differences in GSI among any of the diet treatments excluding BP and YP. Overall, the results indicate that the bacteria and yeast proteins used in this study cannot completely replace fishmeal in diets for *L. variegatus*, but they may be viable as partial replacements for fishmeal. Funded by NSF EDGE.

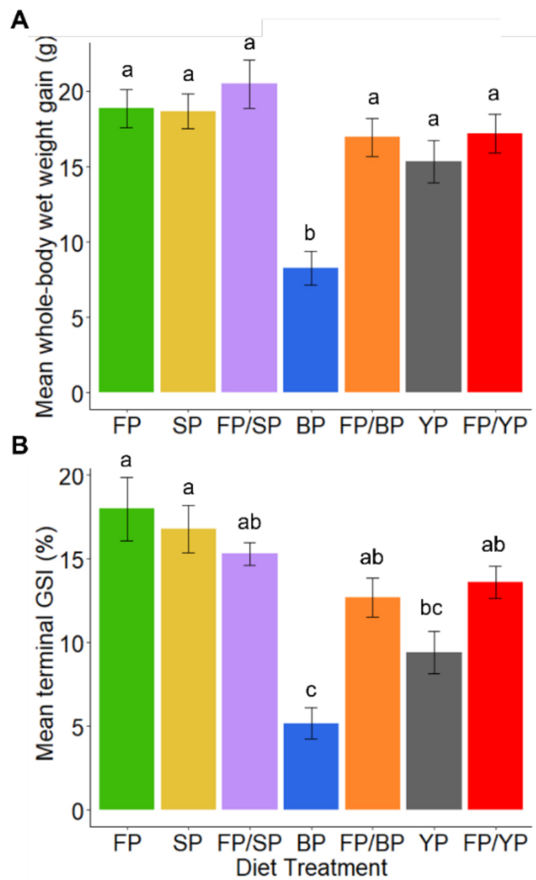

Fig. 1. Mean whole-body wet weight gain over 8 weeks (A) and mean terminal gonadosomatic index (B). Bars represent mean values ± SEM for each diet. Letters indicate significant differences in means among diets ($p<0.05$).

EFFECTS OF INCLUDING *Nannochloropsis oculata* WITHIN A MIXED ALGAL DIET ON GROWTH AND SURVIVAL OF PACIFIC OYSTER *(Crassostrea (Magallana) gigas)* LARVAE AND SPAT

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Pacific oyster, *Crassostrea (Magallana) gigas*, is the most commonly reared shellfish on the United States Pacific coast and a large percentage of the Aquaculture industry is reliant on hatchery production of animals. Pacific oyster rearing techniques use variable microalgal mixtures for different life stages, vary among producers, and over geographic areas. Subsequently, the effects of including *Nannochloropsis oculata* in algal mixtures used in hatchery production of Pacific oysters is not well understood. This study quantified the effects of *N. oculata* introduced into algal mixtures containing *Tisochrysis lutea* and *Chaetoceros muelleri* on Pacific oyster larvae and spat.

There were two algal ration methods used to identify differences amongst methods; a "grazing" technique where larval "grazing" was quantified and used to calculate the amount of feed each day, and a "non-grazing" feeding method analogous to standard hatchery protocols where animal consumption was not quantified. Two algal treatments were used in this study; one including all three algae species (NOC), and a second without *N. oculata* (STD). Larvae were reared in static tanks for 24 hours, then standardized to a density of 40 larvae per milliliter and stocked into the Hatfield Ultra Dense Larval System (HUDLS) at the D-stage. HUDLS is a flow-through seawater system that reduces environmental variance between tanks. Larval samples were collected at 24 hours (immediately pre-stocking into HUDLS), six days, and 12 days post-fertilization. Within the non-grazing ration method, inclusion of *N. oculata* increased growth rate, measured via shell length. No strong effect of ration method was found on larval size. There was no difference in the total number of larvae produced per HUDL between NOC and STD algal diets, however the non-grazing method produced 18,866 more larvae per HUDL, on average, than the grazing feeding method. In the spat experiment no effect on growth was observed between NOC and STD treatments using a non-grazing feeding method.

Our data show that inclusion of *N. oculata* increases larval growth rate but has no effect on larval survival or growth at the spat stage. This result demonstrates that using *N. oculata* during larval production can improve a vital parameter for shellfish hatchery success. Future research should investigate the effect of *N. oculata* across a variety of commercial larval husbandry systems as well as in different feeding methods as our study highlights that different algal diets and feeding methods can influence important shellfish hatchery production parameters.

IMPACT OF INFLATION ON THE COMPETITIVENESS OF U.S. FARM-RAISED CATFISH PRODUCTS

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Inflation has been a significant concern to most Americans and has experienced the highest over the last 40 years. In June 2022, the U.S. inflation rate (12-month percentage change) reached to the highest (9.10% for all items and 10.40% for food) and declined to 2.5% for all items and 2.10% for food in August 2024. Available information suggests that inflation reduced the purchasing power of seafood consumers. The U.S. demand for fish is more price elastic compared to other countries. Existing studies suggest that the demand elasticity of fish among U.S. consumers ranged between 0.50 and 0.79. Imports of fish products are also price sensitive. This study has quantified the impact of inflation on the competitiveness of U.S. farm-raised catfish products and the import of catfish and catfish-like products. The study analyzed catfish production, import, and consumption data from multiple sources. Using a constant market share (CMS) approach, it has examined the changing pattern of competitiveness of the U.S. catfish industry for four periods: (i) Normal Period (January 2015 to February 2020), (ii) Stressed Market Period, affected by the COVID-19 pandemic (March 2020 to June-2021), (iii) Inflationary Period (July 2021 to June 2023), and (iv) Post-Inflationary Period (July 2023 to June 2024). Further, it has decomposed the changes in competitiveness into three effects: (1) structural effect, (2) competitive effect, and (3) second-order effect. Finally, the study has articulated the implications of the research findings for the United States aquaculture and trade policy.

AN OPEN-SOURCE DATABASE INTERFACE TO STORE GENOTYPE, PHENOTYPE, AND PEDIGREE DATA FOR BREEDING PROGRAMS

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Breeding programs typically collect genotype, phenotype, and pedigree data in order to inform breeding and programmatic decisions. As programs progress, the cumulative amount of data collected can become substantial. Storing and organizing this data in a manner that facilitates analysis is often challenging because there are few available options that store these data types at the needed scale. Additionally, products are often not designed for the specific needs of aquaculture species (e.g., polyploid genotypes). Programs with sufficient resources may invest in the design of a custom database and rely on a database administrator to oversee its operation. While this is a feasible solution, it has not been universally adopted due to the cost. Many breeders resort to storing data in a series of flat files (e.g., excel spreadsheets, PLINK files, delimited text files) and attempt to maintain consistency across years in how the files are organized. There are obvious drawbacks to this approach as it does not facilitate quality control, standardization, or data access.

To address this problem, an open-source interface was written for the widely available MySQL database management system. The interface works with a standard, freely available version of MySQL to efficiently store and retrieve genotype, phenotype, and pedigree data. Genotypes for biallelic markers are stored by converting them to bitwise representations. Genotypes for multiallelic markers are stored by either representing genotypes or alleles as integers and automatically interfacing with a table matching integers to text representations. Genotype data can be stored for haploid, diploid, or polyploid individuals. Pedigree and phenotype data are stored in the same database as genotype data, which simplifies the process of exporting data for analysis.

EVALUATING THE IMMUNOMODULATORY EFFECTS OF PHYTOCHEMICALS IN FARMED TILAPIA AND BASS IN FARMED CONDITION

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The world is undergoing global food shortage, a transition of food source from traditional meat sources to more sustainable alternatives like aquacultured fish is pending, which shows great potential to improve food productivity. However, factors like overcrowding, poor husbandry practices are posing chronic stress and challenging farmed fish's immunology, and due to this reason, the fish would not get enough production and even have high mortality. Due to the special characteristic of aquatic systems, natural products are preferred in managing this problem to minimize the impact to aquatic systems. Therefore, we are studying phytochemical's capacity of modulating immunity of fishes. We chose Nile tilapia and bass for their high adaptivity and high reproduction rate. We separate the fishes into four groups, Group 1 completely controlled, Group 2 with controlled feeding and stress factors; Group 3 with enhancer chosen and controlled environment; and Group 4 with stress factors and enhancers. The experiment will last for about 8 weeks and we will assess the immunity by evaluating factors including Hematological Parameters, Lysozyme Activity, Phagocytic Activity, Cortisol Levels. The expected result is Group 1 serving as baseline with normal growth rate, survival rates and immune responses; Group 2 show decreased immune function due to stress, slower growth and higher mortality; Group 3 by contrast, show improved immune responses, higher growth rate and lower mortality; Group 4 improved immune function, lower mortality and higher productivity compared to Group 2, but maybe slightly lower than Group 3. The result will help identify the potential of phytochemicals as natural immunomodulators in aquatic farming, contributing to higher and more sustainable fish productivity.

PHYTASE SUPPLEMENTATION ENHANCES THE POTENTIAL OF ALFALFA NUTRIENT CONCENTRATE AS A FEED INGREDIENT FOR JUVENILE YELLOW PERCH (*perca flavescens)*

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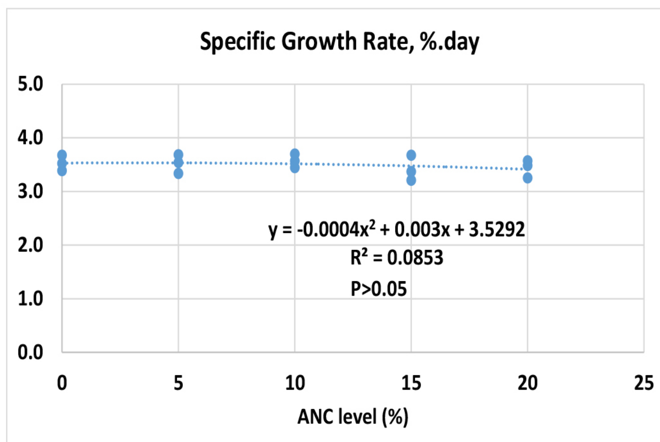
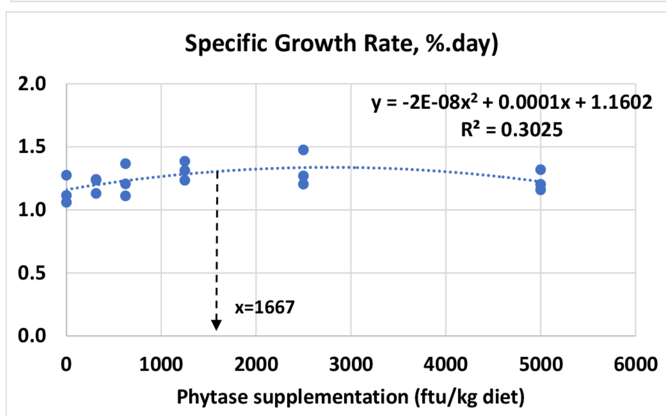
With the growing aquaculture industry, the exploration of alternative ingredients is crucial for the sustainability of aquaculture feed. While alfalfa meal is commonly used in animal feed, its use in aquatic feed remains limited. This study conducted two feeding trials to evaluate the potential of using alfalfa nutrient concentrate (ANC) as a partial replacement for fishmeal in the diet of yellow perch, a cool-water fish native to the Great Lakes region. The aim was to investigate alternative sources of protein for fishmeal and provide a new revenue stream for alfalfa producers. In the first trial, we assessed the growth performance of yellow perch fed diets containing varying levels of ANC (0, 5, 10, 15, and 20 g/100 g diet) to replace fishmeal in a control diet. The trial lasted 9 weeks with three replications for each diet. The growth rate, feed conversion ratio, satiation feed intake, and protein retention were similar ($P > 0.05$) across all diets. However, fish fed the ANC-20 diet had lower levels of ash, phosphorus, calcium, and manganese compared to those fed the ANC-0 diet ($P < 0.05$). A lower apparent digestibility coefficient for phosphorus was observed in the ANC ingredient compared to menhaden fishmeal, which partially explains the reduced phosphorus content in fish fed the ANC-20 diet.

The second 9-week second trial investigated the growth performance of yellow perch fed the ANC-20 diet supplemented with varying levels of phytase (0, 312.5, 625, 1250, 2500, and 5000 FTU/kg diet) compared to a control diet without ANC. Polynomial regression analysis revealed that the optimal growth rate was achieved with phytase supplementation at 1666 FTU/kg diet, highlighting the potential benefits of phytase in ANC-based diets. This study suggests that ANC can serve as a partial protein source to replace fishmeal in perch feed. However, longer-term feeding trials are needed to validate these findings, and the cost-effectiveness of using ANC in aquatic feed should be considered.





MICROBIOME COMPARISON OF SEDIMENTS COLLECTED FROM CATCHMENTS BENEATH OYSTER AQUACULTURE EQUIPMENT WITH INTERTIDAL BOTTOM SEDIMENTS AROUND WILD OYSTER COMMUNITIES IN COASTAL ALABAMA

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The microbiome of seafloor sediments near oyster communities on intertidal shorelines in coastal Alabama were compared to sludge sediments collected on a fiberglass catchment beneath submerged oyster aquaculture equipment. The top 1 cm of shoreline sediments from three coastal Alabama sites (Mobile Bay, Portersville Bay and Bayou LaBatre) spanning different levels of salinity and National Shellfish Sanitation Program classifications (conditionally approved and prohibited) were sampled with a sterile spoon during intertidal exposure on November 8, 2021. Shoreline sediments from these three areas primarily consisted of coarse sand whereas the sediment sample collected from the fiberglass deck of a submersible oyster aquaculture vessel (Shellevator) positioned ~100m offshore from the Portersville Bay shoreline site had a sludge-like consistency likely from the droppings of feces and pseudo-feces that had accumulated on a flat fiberglass deck.

The purified metacommunity DNA from triplicate samples at each location was subjected to high-throughput amplicon sequencing (HTS) targeting the V4 region of the 16S rRNA gene using an Illumina MiSeq platform with 250 bp paired-end kits. The resulting HTS datasets were demultiplexed into FASTQ files, quality-checked, and denoised with DADA2 to generate amplicon sequence variants (ASVs). Data were then rarefied, and taxonomic identifications were assigned using the Silva database. All bioinformatics analyses were conducted using tools and commands available in Quantitative Insights into Microbial Ecology (QIIME2; v2022.2).

The three most prevalent bacterial genera identified in the Portersville Bay sludge sample were 10-fold higher than any of the shoreline sediment samples and >100-fold more abundant than in the Portersville Bay shoreline site (Anaerococcus: 10.86%/0.1%), (Tyzzerella: 7.48%/0.0%) and (Clostridioides: 4.51%/0.07%). Genes associated with carbohydrate, lipid and amino acid metabolism generally occurred at a higher frequency than in the Portersville Bay sludge sample than in the three shoreline sediment samples.

The microbiome of sludge from oyster secretions and excretions is readily distinguishable from than seafloor sediments associated with natural oyster communities. Examination of the sludge microbiome may provide insight into the oyster community health status analogous to early identification of community public health threats like COVID 19 by sampling sewage treatment plant influents. This approach could be implemented to identify the cause and/or provide an early signal for the onset of Sudden Unusual Mortality Syndrome that has plagued oyster in coastal Alabama and other locations in recent years.

RELATING PERFORMANCE AND BEHAVIOR FOR SIX STRAINS OF BROOK TROUT

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We investigate drivers of performance and behavior for six strains of brook trout. Although less studied than commonly stocked and aquacultured rainbow trout, brook trout have a higher per mass protein content and popular among anglers. Therefore, brook trout represent an untapped resource and understanding variability in hatchery performance among strains could optimize culture. We compare growth, feeding styles and catchability for Assinica, Horn Lake, Little Tupper, Temiscamie, Temiscamie x Domestic hybrid and Windfall.

Differences were observed among strains in specific growth rate (Figure 1A), length-to-weight relationships, and dry weight. Although there was not a relationship between surface feeding frequency and specific growth rate, surface feeding frequency was strongly related to order of capture in a lined earthen pond (Figure 1B).

Our study shows among strain variation in traits relevant to hatchery and aquaculture production, with Assinica, Temiscamie x Domestic hybrids and Little Tupper well suited due to strong growth. Interstrain variability in performance underscores the importance of exploring a range of strains to better understand potential under aquaculture conditions. An improved understanding of strain-specific traits will improve our capacity to select ideal strains for different contexts. For example, selecting strains with more aggressive surface feeding to support catchable populations or culturing strains that maintain high growth and fat content.

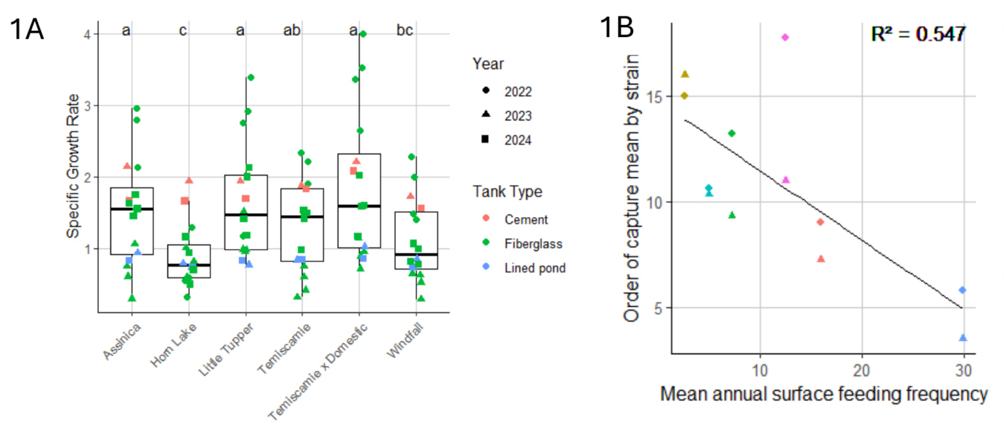

Figure 1A. Mean specific growth rate (SGR) for six strains of brook trout. Points reflect SGR for given experiments (color) and years (symbol). Letters indicate significant differences between strains; strains with the same letter are not significantly different ($p > 0.05$), while those with different letters are significantly different. The model used for these comparisons was a linear mixed-effects model with random effects of year and experimental system. Figure 1B. Scatter plot of the mean annual surface feeding frequency ($n = 22$ for 2023 and $n = 38$ for 2024) for each strain (denoted using color) and the order of capture of brook trout from a lined earthen pond in 2023 and 2024 (year is denoted using symbols).

SEPARATION AND CHARACTERIZATION OF WHITE SHRIMP (*Penaeus vannamei*) HEMOCYTES USING CELL SORTING REVEALED NEW TYPE OF HEMOCYTE SUBPOPULATIONS

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Hemocytes are a key player since they actively participate, either through direct or indirect involvement, in the immunological responses of invertebrates. Numerous studies have been conducted on classifying shrimp hemocytes into different subpopulations. Moreover, several methods have been used to understand the hemocytes in crustaceans: ultrastructural microscopic observation, percoll gradient centrifugation, and single-cell RNA sequencing. As a result of this inconsistent classification of crustaceans, it still needs to be further investigated because this means that the hemocytes of crustaceans have yet to be well classified. Therefore, in this study, we separate the hemocyte subpopulations using flow cytometry cell sorting to understand the immune activity of white shrimp (*Penaeus vannamei*) hemocytes. Flow cytometric analysis (Fig. 1) showed that the presence of four hemocyte subpopulations of white shrimp, differing not only in size (FSC) but also in the degree of granulation (SSC) categorized as the hemocyte 1 (H1), hemocyte 2 (H2), hemocyte 3 (H3), and hemocyte 4 (H4). The FSC and the diameter size showed that there were 4 different sizes of cells that are 9.25 µl (H1), 10 µl (H2), 11 µl (H3), and 12.5 µl (H4). Based on the SSC, two major groups of hemocyte subpopulations could be distinguished: the agranulocyte group, referred to as H1 and H2, and the granulocyte group, referred to as H3 and H4. Moreover, at the transcriptional level, immune-related genes have different functions in each hemocyte subpopulation, such as LGBP, CTL-2, ProPO1, ProPO2, Serine protease, SOD, GPx, CP, Tgase, etc. Further research is needed to explore the immune reaction of each hemocyte subpopulation after injection with a pathogen to better understand the immune response of hemocytes.

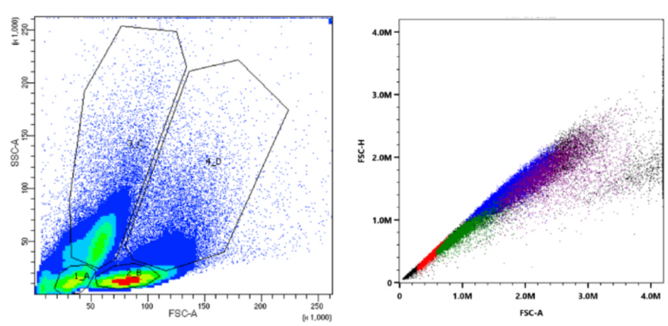

Figure 1. Hemocyte subpopulations of white shrimp (*Penaeus vannamei*) by flow cytometric analysis. Cells were displayed by dot plot, and expressed as cell size (forward scatter, FSC value), versus cell complexity (side scatter, SSC value). Four subpopulations of hemocytes were identified (H1: Hemocyte in gate 1; H2: Hemocyte in gate 2; H3: Hemocyte in gate 3; H4: Hemocyte in gate 4).

THE ENVIRONMENTAL SUSTAINABILITY ROTARY ACTION GROUP (ESRAG): HELPING TO IMPLEMENT SUSTAINABLE PRACTICES ON BIODIVERSITY, CIRCULAR ECONOMY, FOOD SYSTEMS, SUSTAINABILITY, CLIMATE AND POLLUTION

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This presentation explores the ESRAG (Environmental Sustainability Rotary Action Group) framework, emphasizing its role in promoting sustainable practices within the aquaculture sector. The framework focuses on biodiversity preservation, supporting the transition to a circular economy, and improving food systems. These elements are crucial for minimizing environmental impacts, enhancing resource efficiency, and mitigating climate change. By integrating sustainability into aquaculture, the ESRAG framework also addresses the pressing challenges of pollution in aquatic ecosystems, fostering innovation in sustainable production. The presentation will highlight practical strategies to protect biodiversity, reduce waste, and promote responsible aquaculture practices, ensuring long-term resilience against climate change and environmental degradation. Through collaboration and education, this framework paves the way for a sustainable future, aligning with global efforts to promote a healthier and more resilient planet.

A more in-depth response involves the following:

ESRAG has been engaged in various initiatives to conserve fish, shellfish, and mangroves, and to support aquaculture businesses. These efforts align with ESRAG's broader environmental sustainability goals, particularly in promoting healthy ecosystems and sustainable resource management.

ESRAG has been involved in projects that focus on mangrove conservation and restoration. Mangroves are vital ecosystems that protect coastal regions, support biodiversity, and provide nurseries for fish and shellfish. By restoring these areas, ESRAG helps ensure the sustainability of marine life and the livelihoods of communities dependent on fishing and aquaculture. Supporting sustainable aquaculture practices that minimize environmental impacts while boosting local economies. By promoting practices such as responsible fish farming, ESRAG helps reduce pressure on wild fish populations and supports the circular economy by promoting more efficient, sustainable food systems. Promoting the conservation of marine ecosystems by supporting efforts to protect fish and shellfish populations. Through educational initiatives, ESRAG encourages the adoption of best practices in fishing and aquaculture that reduce overfishing, bycatch, and habitat destruction. Projects often focus on community-driven efforts to manage marine resources in ways that support biodiversity while providing economic benefits. Actively advocating for reducing pollution that affects marine ecosystems, including plastic pollution and chemical runoff that harms fish and shellfish habitats. Efforts to clean up waterways and promote sustainable land-use practices contribute to healthier marine ecosystems and more sustainable aquaculture operations. Networking and collaboration on a global scale enable partnerships with local communities, businesses, and governments to advance initiatives that integrate environmental advocacy with practical, on-the-ground conservation efforts.

HOW TO WRITE EFFECTIVE GLOBAL GRANTS TO APPLY FOR FUNDING FOR YOUR PROJECT BY ROTARY INTERNATIONAL

Caroline DeWitt* and Salvador Rico

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Rotary is a global network of 1.4 million neighbors, friends, leaders, and problem-solvers from 36,000 local communities and 46,000+ clubs around the world. Rotarians see a world where people unite and take action to create lasting change – across the globe, in our communities and in ourselves. Our clubs work together to promote peace, fight disease, provide clean water, sanitation, and hygiene, save mothers and children, support education, grow local economies, and protect the environment (https://www.rotary.org/en/about-rotary).

Rotary mission is to provide service to others, promote integrity, and advance world understanding, goodwill, and peace through our fellowship of business, professional, and community leaders. We provide service wherever there is a recognized need with humanitarian and now Environmental Projects.

The Rotary Foundation has spent more than $24 million on Environmental Projects worldwide since 2014 and would like to increase the amount spent on shellfish restoration projects.

To learn more about how to develop projects and write effective global grants for funding by Rotary International go to:

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ENHANCING MARINE ECOSYSTEMS AND PROMOTING SUSTAINABLE AQUACULTURE: ESRAG's COMMITMENT TO A HEALTHIER PLANET

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The Environmental Sustainability Rotary Action Group (ESRAG) is actively engaged in initiatives that protect fish, shellfish, and mangroves, supporting the growth and sustainability of aquaculture businesses and contributing significantly to the broader goals of environmental conservation. These projects reflect ESRAG's dedication to building healthy ecosystems and fostering sustainable resource management.

A key focus for ESRAG is mangrove conservation and restoration. Mangroves are crucial ecosystems that shield coastal areas from erosion, serve as biodiversity hubs, and act as nurseries for fish and shellfish. Through its restoration efforts, ESRAG ensures the sustainability of marine habitats and supports the livelihoods of communities that rely on fishing and aquaculture.

ESRAG also promotes sustainable aquaculture practices that balance economic growth with environmental responsibility. By advocating for responsible fish farming techniques, ESRAG helps reduce the strain on wild fish populations while advancing a circular economy approach that encourages more efficient, sustainable food production systems.

Furthermore, ESRAG actively supports marine ecosystem conservation by educating communities on best practices in fishing and aquaculture. This includes reducing overfishing, bycatch, and habitat destruction. Such community-centered projects empower local stakeholders to sustainably manage marine resources, enhancing biodiversity and offering economic opportunities.

ESRAG's advocacy efforts extend to combating pollution, including reducing plastic waste and chemical runoff that damage fish and shellfish habitats. Through initiatives that clean waterways and encourage sustainable land-use practices, ESRAG contributes to healthier marine ecosystems and more resilient aquaculture industries.

The organization's global network of partnerships with local communities, businesses, and governments amplifies these initiatives. By integrating environmental advocacy with actionable conservation strategies, ESRAG catalyzes impactful, on-the-ground results that align with sustainable development and marine ecosystem preservation.

WORKSHOP: HOW TO WRITE EFFECTIVE GLOBAL GRANTS TO APPLY FOR FUNDING FOR YOUR PROJECT BY ROTARY INTERNATIONAL

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MARKET DOMAINS FOR SAFER FISH PRODUCED THROUGH GOOD AQUACULTURE PRACTICES: EVIDENCE FROM BANGLADESH

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Foodborne illness is a severe global problem. Researchers have been developing good aquaculture practices (GAqPs) for fish production to ensure food safety. The expansion and adoption of GAqPs among fish farmers will depend largely on profitability and market demand for safer fish. With the same production cost per unit, profitability can be increased if farmers receive higher prices. We have defined and measured fish-food safety concerns among auction participants and investigated the relationship between consumers' food safety segments and their WTP for safer fish in Bangladesh. To our knowledge, it is the first study that used experimental auctions to identify consumer segments among fish consumers in Bangladesh based on their level of concern for food safety and quantify their WTP for safer fish. We have conducted a cluster analysis, with 10,000 iterations, to identify the "market domains" or consumer segments.

Based on seafood safety concerns, we have identified two groups of fish consumers: consumers with high food safety (HFS) concerns and consumers with low food safety (LFS) concerns. Our analysis revealed that one-third of the Tilapia consumers were in the HFS group and willing to pay (WTP) 27% higher for safer Tilapia than the LFS group. Thirty-seven percent of the Rohu consumers were in the HFS group, and they were WTP 19% higher for safer Rohu than the LFS group (Figure 1). One-fourth of the Pangasius consumers were in the HFS group, and they were WTP 18% higher for safer Pangasius than the LFS group. Results of the market segmentation analysis imply that until the total supply of safer fish reaches 33% of total Tilapia demand, 37% of Rohu fish demand, and 25% of Pangasius demand in the market, fish farmers and traders can earn higher profits with a price equal to the WTP of the HFS group.

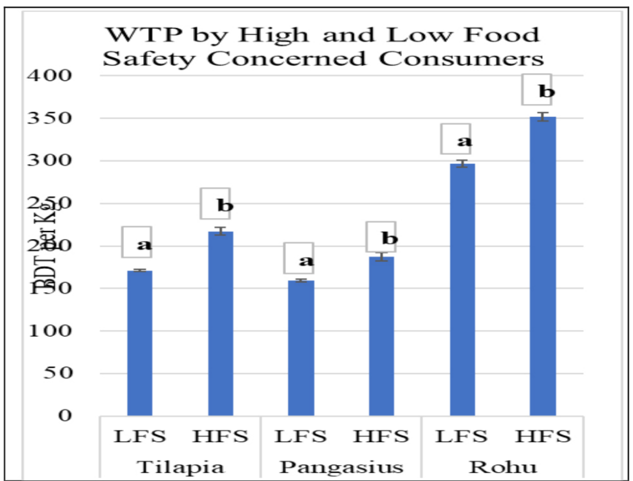

Figure 1. Market Segments: Willingness to Pay (WTP) for Safer Fish Produced through Good Aquaculture Practices (GAqPs). Different letters in the graph indicate significant differences between WTP for safer fish by Low Food Safety (LFS) and High Food Safety (HFS) concerned consumer group at a 1% level of significance.

ASSOCIATION OF NHP AND DHPV WITH REDUCED GROWTH IN FARMED SHRIMP FROM LATIN AMERICA

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Infectious disease remains an important obstacle for shrimp aquaculture in Latin America. While overall production has increased over the past decade, emergence of new diseases, and reemergence of existing ones, is a growing concern. The effect of these diseases is often reflected in growth retardation rather than large scale die-offs. Here, we report a case study from Latin America where severe growth retardation was documented in *Penaeus vannamei*. Clinical signs included high size variation (e.g. 4 gm to 20 gm), soft cuticle, weakness, dark body coloration and red tails and pleopods. Survival rates at harvest were 30-50%. Fecal strands could be seen floating on the water, similar to what is observed in shrimp affected with white feces syndrome. Since hepatopancreatic microsporidiosis had been recently reported in the region, there was concern this facility might had also been affected.

Juvenile shrimp samples were submitted to our laboratory for a complete health assessment by conventional H&E histology. Samples were also submitted for PCR analysis. By H&E, the most significant finding included the presence of lesions diagnostic of infection by *Hepatobacter penaei*, the agent causative of necrotizing hepatopancreatitis or NHP (Fig. 1). Additionally, intranuclear inclusion bodies indicative of infection by Decapod Hepanhamaparvovirus (DHPV) were found in the mucosal epithelium of the anterior midgut caecum. No other known lesions/pathogens of concern to penaeid shrimp were detected in these samples, including *Enterocytozoon hepatopenaei* (EHP). Neither did we find any intranuclear inclusion bodies associated with white spot disease (WSD), which is widely reported in Latin America.

PCR analysis mirrored H&E findings. There were very high levels of NHP and DHPV in samples displaying growth retardation and white feces. EHP were not detected. The DHPV isolate detected in these samples represented the currently circulating DHPV genotype in Latin America, not the isolate reported from Asia in the past. This case study exemplifies why health assessment of farmed shrimp should combine histological and molecular analysis. Had only PCR analysis for EHP been requested for these samples, based on the presumptive clinical signs, NHP would not have been detected.

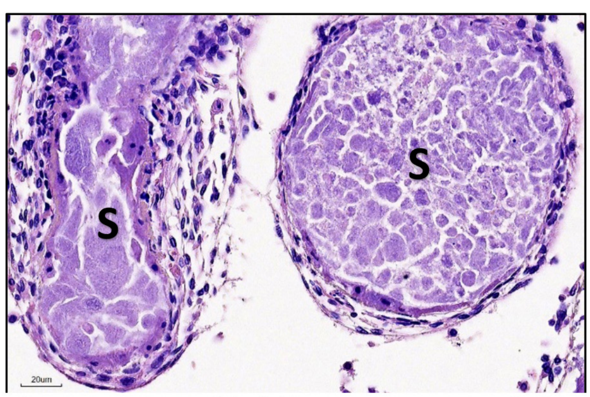

Figure 1 . Atrophied hepatopancreas tubules displaying accumulations of sloughed-off mucosal epithelial cells (S) with intensely stained cytoplasm due to proliferation of the NHP intracellular bacteria. Scale bar = 20 µm

ENGINEERING AN INFECTIOUS FISH VIRAL NERVOUS NECROSIS (VNN) CLONE IN INSECT CELLS: IMPLICATIONS FOR FISH GENOME ENGINEERING AND ORAL VACCINE DEVELOPMENT

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Viral disease pandemics are a major cause of economic losses in fish and crustacean farming worldwide. One of the prerequisites in studying viral pathogenesis and antiviral immunity in fish is to develop an infectious clone of the virus. Availability of an infectious clones enable genomic manipulation feasible and opens avenues to developing viral vaccine.

Recently, we engineered an infectious cDNA clone of a bi-segmented RNA virus of shrimp, *Macrobrachium rosenbergii Nodavirus (*MrNV), using the baculovirus expression system in insect cells, Sf9 (Gangnonngiw *et al.,* 2020. *Virology 2020, 540, 30–37*). Subsequently, we modified the infectious MrNV cDNA clone by replacing RdRp gene with a marker gene, Green Fluorescent Protein (GFP) to generate a replication incompetent viral vector to determine the feasibility of the shrimp viral vector to deliver different payloads (antigen/therapeutic RNA) through diet in crustacean *(*Alenton, Mai & Dhar. 2023. *PNAS-Nexus* 2: 1-9). This opens a new frontier in developing antiviral therapy and gene delivery in crustaceans and opens an opportunity to engineer RNA viruses infecting fish.

To determine the feasibility of generating infectious fish virus in insect cells, we attempted to produce a Viral Nervous Necrosis (VNN) virus, Red-Spotted Grouper Nervous Necrosis Virus (RGNNV), in insect cells, Sf9. VNN causes large-scale mortalities and major economic losses to a wide range of marine and freshwater fish including striped bass, barramundi (also called Asian sea bass), European sea bass, grouper, Atlantic halibut, Atlantic cod, tilapia, and many others.

When full-length genomic RNA1 and RNA2 of RGNNV were cloned using a baculovirus expression system and recombinant baculovirus was used to infect Sf9 cells, mature virus particles of RGNNV were observed by transmission electron microscopy; and the full-length RNA1 and RNA2 of RGNNV were amplified by RT-PCR. This suggests both genomic segments of RGNNV were expressed and packaged within the capsid protein to form mature virion. When Sf9 cell-derived RGNNV was used to infect tilapia cell line Omb, cytopathic effects were observed. Additionally, using a TaqMan real-time RT-PCR assay, the viral copy number was found to increase over time in the study, which indicated successful replication of the insect cell derived RGNNV in fish cells. RGNNV produced in fish cells were then used to inject live hybrid sea bass. The virus was detected in target tissue and histopathological manifestation in brain and eye were recorded. The findings clearly demonstrated that infectious RGNNV can be produced in insect cells. The new ability to produce a fish virus in insect cells opens the possibility of using this RGNNV-based viral vector for gene delivery or editing and accelerates the development of antiviral vaccines.

HEMATOLOGICAL AND BIOCHEMICAL ANALYSIS OF ICTALURID HERPESVIRUS I INFECTION IN CHANNEL CATFISH *Ictalurus punctatus*

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Disease progression is better understood by revealing the pathophysiological mechanisms underlying the clinical infections. Blood indices are highly correlated to the health status of the fish as the organism tries to maintain a steady physiological response to stimuli and disrupts homeostasis. Cell lysis, following tissue damage associated with the disease condition, can result in the release of enzymes into the bloodstream leading to elevated serum enzyme activity. Evaluation of haemato-biochemical indices can be useful in predicting metabolic alterations and timely prognosis. Viral infections are accompanied by extensive degeneration and necrosis of hematopoietic tissues such as the kidney and spleen. Blood-biochemical anomalies in diseased fish have been evaluated and provide insight into specific responses in the pathophysiology of many viral infections. *Ictavirus ictaluridallo1* (Ictalurid herpesvirus 1, IcHV1 commonly called channel catfish virus, CCV) is a significant pathogen infecting fry and fingerling stages of channel catfish that poses a serious threat to commercial hatchery operations. A better understanding of IcHV1-associated pathophysiological alteration in ictalurid catfish is warranted to unravel why infected fish fail to maintain homeostasis and how the physiologically relevant tissues contribute towards mass mortality.

Naïve channel catfish (average weight ~40g) were exposed to IcHV1 at a dose $<LD_{50}$ via immersion. An equivalent amount of serum-free media was added to control tanks. Six tanks, each containing twenty fish were allocated for treatment or control groups. Three fish per tank were arbitrarily sampled at days 1, 2, 3, 5, 7, and 14 post-infection, euthanized with excess MS-222, and the blood was collected from the caudal vein using lithium-heparinized syringes. Blood gas values (pH, pCO_2, pO_2), oximetry values (Hb, osmolality, Hct), electrolytes (K^+, Na^+, Ca^{2+}, Cl^-), and metabolites (glucose, lactate) of the whole blood were tested using a blood-gas analyzer (ABL-90, Radiometer America Inc). Plasma was separated by centrifuging the whole blood at 5000 x g for 3 min. Fifteen plasma biochemical markers were also examined using a clinical chemistry analyzer. The results were statistically analyzed and compared between the IcHV1-infected and non-infected control groups.

Hematological analysis showed significant alterations in circulating metabolites of diseased fish. These changes may represent underlying mechanisms leading to the severe gross clinical signs associated with IcHV1 infection, such as lethargy, ascites, and abnormal swimming patterns. The findings from this study are useful to provide detailed insight into the pathophysiological changes associated with IcHV1 infection, which may possibly cause damage to any off-target organs such as brain, liver, or heart, critically involved in major physiological functions.

RESEARCH AND EXTENSION PROGRAMMING UPDATE: THE UNIVERSITY OF FLORIDA TROPICAL AQUACULTURE LABORATORY

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The Tropical Aquaculture Laboratory (TAL), located in Ruskin, Florida, is a leading research and extension center operated by the University of Florida. Established in 1996, the TAL was created to support the growth of the ornamental aquaculture industry through collaboration with key partners, including the Hillsborough County Board of County Commissioners, the Florida Tropical Fish Farms Association, and the Florida Department of Agriculture and Consumer Services. Over the years, however, its focus has broadened from fish health and applied aquaculture production to include topics such as reproduction, aquatic ecology, physiology, management of non-native species, youth education, and restoration aquaculture. The TAL also operates a fully-equipped diagnostic laboratory that provides services to address various aquatic animal health issues. With a long-standing commitment to advancing Florida's aquaculture industry, the TAL works to address challenges and foster new opportunities. More than 25 University of Florida faculty, staff, and students are actively involved in its operations, and the TAL is positioning itself for future growth. The laboratory's strategic plan aims to expand its programs, reach new aquaculture sectors, and enhance its facilities and resources to better support Florida's diverse and evolving aquaculture industry.

SUSTAINING LONG-TERM YIELD OF THE PUGET SOUND DUNGENESS CRAB FISHERY BY ACCOUNTING FOR POTENTIAL MIXED-STOCK POPULATION STRUCTURE

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The Dungeness crab is one of the most iconic and intensively harvested marine species on the west coast of North America. In Washington state, the Puget Sound Dungeness crab fishery is managed independently from the open coast fishery and accounts for ~45% of the statewide fishery. Vulnerabilities in the current management framework have been exposed by recent fishery closures, yet despite the considerable value of the fishery, the development of adaptive management strategies has been inhibited by a lack of fundamental biological knowledge. Several lines of evidence suggest that Puget Sound may represent a mixed-stock fishery, in which fishery yields vary according to the relative supply, survival, and growth of genetically distinct populations. If this is the case, management under the current system risks overexploitation of vulnerable stocks, as well as underutilization of more robust stocks.

We are working to determine if current spatial management areas align with the true population structure of Puget Sound Dungeness crab. High-throughput genomic techniques are being used to characterize crab population structure, assessing both population connectivity and potential local adaptation among populations. Estimates of effective population size will be used in lieu of census population size to compare stock robustness. The project leverages established regional collaborative efforts of the Pacific Northwest Crab Research Group (PCRG), composed of federal, state and tribal biologists, NGOs, industry members, community scientists and students. Sampling of adult crabs throughout the region was coordinated with commercial fishers and biologists during pre-season test fisheries in 2023. Crab larvae were also sampled by the PCRG larval crab monitoring network to provide the clearest possible picture of sources and sinks of crab cohorts, and their relative strengths in each region. A low coverage whole genome sequencing approach was used to genotype all specimens at a depth of approximately 2x. Results will be discussed in the context of prior genetic research on Dungeness crab and the potential implications for fishery management.

YELLOWTAIL KINGFISH *Seriola lalandi* LARVAL IMMUNE DEVELOPMENT

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Yellowtail kingfish (*Seriola lalandi*) is a promising candidate for aquaculture in the Northeastern United States, where commercial production facilities are under development. However, high mortality rates during the larval rearing stages in hatcheries present a significant challenge, likely in part due to the undeveloped immune system of the larvae. This study aims to investigate species-specific immune function development in yellowtail kingfish larvae to build an immunological development timeline to determine when the immune system becomes fully functional, thereby optimizing vaccination timing to improve survival rates.

Samples from unfertilized eggs to 55 days post-hatching (dph) will be collected. Functional assays will quantify immune proteins, including lysozyme, α2-macroglobulin, complement components, and antibodies at each developmental stage. Immune organ development, such as the thymus, will be assessed using immunohistochemistry with inducible nitric oxide synthase (iNOS) as a marker. qPCR and *in situ* hybridization will be employed to probe larval tissues for the expression of important immune genes, such as RAG-1, IgM, C3, TCRα, MHCII, and lysozyme. Practical vaccination trials will complement these analyses. To date, yellowtail kingfish blood cell types have been characterized, and 17 immune-related genes expression has been analyzed using qPCR in samples collected at 0, 2, 4, 6, 12, 15, 19, 21, and 25 dph.

Preliminary results reveal that adaptive immune genes such as RAG-1, IgM, CD8, TCRα, MHCI, MHCII, and Ikaros were detected as early as 0 dph, suggesting early adaptive immune system development or maternal transfer. IgM, CD4, CD8, TCRα, and MHCI were expressed at baseline levels during the first 25 dph, significantly lower than in adult yellowtail tissues, indicating an underdeveloped adaptive immune system. In contrast, the expression of innate humoral factors, such as C3 and lysozyme, increased significantly during the same period, highlighting larvae's reliance on innate immunity in early life stages. TLR21 expressed low but showed a notable increase at 6 dph (2 days after mouth opening). CRP, SOD, and TNFα maintained consistent expression during the first 25 dph, while catalase expression increased, potentially correlating with high larval mortality. Notably, IgT expression was not detected during the first 25 dph.

This study provides valuable insights into the specific patterns of immune function development in yellowtail kingfish larvae, informing hatchery management strategies and potentially improving larval survival rates.

A COMPARATIVE STUDY ON THE CHARACTERISTICS AND PATHOGENICITY OF *Vibrio* spp. IN POST-LARVAL *Penaeus vannamei* SHRIMP

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Translucent post-larvae disease (TPD) has emerged as a significant new disease in shrimp since it was first identified in Pacific white shrimp (*Penaeus vannamei*) hatcheries in China in 2020. Two *Vibrio* spp. with distinct phenotypes, AG1 (green colonies in TCBS agar) and AY7 (yellow colonies in TCBS agar), were isolated from shrimp experiencing sudden death in an anonymous hatchery suspected to be caused by TPD. Both isolates tested PCR-negative for *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease based on the absence of the *PirA* and *PirB* toxin genes. Moreover, initial PCR screening detected three candidate virulence genes, *Vibrio* high virulent protein (*vhvp*)-1, *vhvp*-2, and *vhvp*-3 in AG1 but not in AY7 isolate, which are believed to be key virulence factors contributing to TPD.

The characterization of two bacterial isolates, AG1 and AY7, were performed using a combination of the Analytical Profile Index (API) 20E and 20NE systems and whole genome sequencing, which identified AG1 as *V. parahaemolyticus* and AY7 as *V. harveyi*. Immersion challenge using *P. vannamei* post-larvae (~0.2g ± 0.05) with doses rangeing from 10^2 to 10^6 CFU/mL showed AG1 isolate is highly virulent with an LD_{50} of 8.51×10^2 CFU/mL compared to AY7 isolate which which had a predicted LD_{50} of 1.95×10^8 CFU/mL at 96 hr post-challenge. Histological examination of the hepatopancreas from shrimp infected with AG1 isolate revealed the loss of hepatopancreatic tubule structure, with epithelial cells detaching and sloughing off. Additionally, these shrimp exhibited reduced intestinal contents, damaged intestinal walls, and some individuals showed signs of hemocytic enteritis. In contrast, shrimp infected with the AY7 isolate typically displayed abnormal cytoplasmic vacuolization in E-type cells within the hepatopancreatic tubules.

An additional bioassay using larger *P. vannamei* juveniles (~0.5g ± 0.1) revealed the virulence of these bacterial isolates is dose-dependent and significantly influenced by the size of the shrimp. Based on the LD_{50} from the first experiment, bioassay via immersion was conducted at doses of 2.42×10^2, 10^3, and 10^4 CFU/mL for AG1 isolate, and 1.89×10^6, 10^7, and 10^8 CFU/mL for AY7 isolate over a 7-day period. No LD_{50} was determined for the AG1 strain since even the highest dose resulted in only 40% mortality. For the AY7 strain, there was no mortality, even at the highest dose of 10^8 CFU/mL. These results confirm that V. *parahaemolyticus* causing TPD is highly lethal to post-larval shrimp, particularly when harboring the *vhvp*-1, *vhvp*-2, and *vhvp*-3 virulence genes. This study provides valuable insights into the etiologic agent of TPD and open avenues for exploring the pathogenic mechanisms of *Vibrio* strains associated with TPD in *P. vannamei*.

DO LONELY PARENTS PRODUCE BIGGER OFFSPRING?

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Social experience impacts a variety of behaviors in fish as individuals learn from and are influenced by the actions of conspecifics. While isolation is often thought to be stressful for social animals, several studies have found that social isolation can reduce stress, anxiety-related behaviors and aggression. Additionally, developmental environment may not only influence the behavior of the individual, but also that of its offspring. Studies surrounding the influence of social context on the epigenetic factors of behavior in fish are increasing, but little is known on how parental social experience affects nutrition-related behavior in the subsequent generation.

The objective of this study was to investigate the effects of parental social experience (social housing or chronic isolation) on offspring feeding behavior, growth performance, and intestinal health when fed traditional and alternative diets. Zebrafish *Danio rerio* was used as a model species. At 21 days-post-fertilization (dpf), offspring from zebrafish raised in either chronic isolation (CIO) or in social housing (SHO) were randomly assigned to 3.0 L tanks with 25 fish per tank. Each tank was randomly selected to be fed either a fishmeal-based (FM) or soybean meal-based (SBM) diet. There were four treatment groups: SHO fish fed a FM (SHOF), SHO fish fed a SBM (SHOS), CIO fish fed a FM (CIOF), and CIO fish fed a SBM (CIOS). There were three replicates for each of the four treatment groups with a total of 12 tanks.

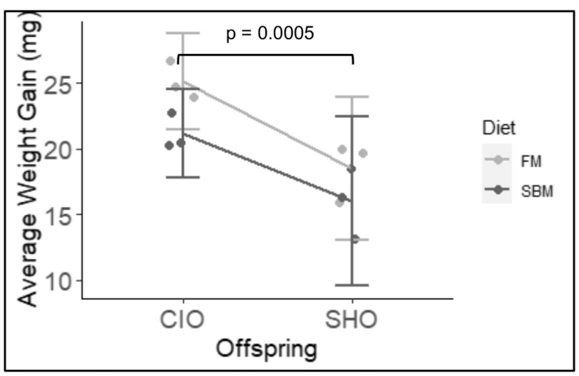

Figure. Average weight gain of treatment groups. Brackets indicate significant difference.

SWITCHING IT UP: MIXED FEEDING PLAN MAY LEAD TO IMPROVED UTILIZATION OF DIETARY PLANT PROTEIN

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In order to combat the increasing cost and environmental detriment of fishmeal (FM), plant protein sources have grown in popularity as a protein replacement in aquafeeds. Soybean meal (SBM) is one of the more notable protein alternatives due to its high protein content and relatively well-balanced amino acid profile. However, at high dietary inclusions, SBM has been found to have negative impacts on feed ingestion, digestion, and growth rates. This is primarily attributed to the presence of anti-nutritional factors that can lead to intestinal inflammation. Thus, the objective of this study was to investigate how alternating feeding regimen influences feed utilization and intestinal health of fish fed a high-inclusion SBM based diet.

Zebrafish *Danio rerio* was used as a model species. At 23 days-post-fertilization, fish were randomly stocked into 3.0 L tanks with 21 fish per tank. Each tank was randomly assigned to one of four treatment groups: fish fed with fishmeal-based diet (FM) every day (FM-FM), fish fed with soybean meal-based diet (SBM) every day (SBM-SBM), alternating FM and SBM daily (FM-SBM), and alternating FM and SBM weekly (FM/SBM). There were three replicate tanks per treatment group with a total of 12 tanks. Fish were fed to apparent satiation three times daily for four weeks.

There were no significant effects of feeding regimen treatment on survival, final average weight per fish, or FCR ($p > 0.05$). However, numerically, the FM/SBM group tended to have the highest average weight per fish (Table). The SBM-SBM group, presenting with the numerically lowest average weight, had higher relative expression of inflammatory gene *tnfa* ($p < 0.05$) compared to the FM-FM group (Figure).

Based on the minimal differences between the FM-FM and FM/SBM groups, alternating FM- and SBM-based diets weekly could represent a practical and cost-effective feeding regimen for fish with potential for applications in other species.

Table. Final weight and FCR measured as average ± standard deviation

Treatment	Avg Weight (mg)	FCR
FM-FM	42.28 ± 2.76	1.15 ± 0.03
SBM-SBM	36.15 ± 2.50	1.27 ± 0.07
FM-SBM	40.58 ± 5.62	1.28 ± 0.11
FM/SBM	42.77 ± 2.47	1.21 ± 0.02

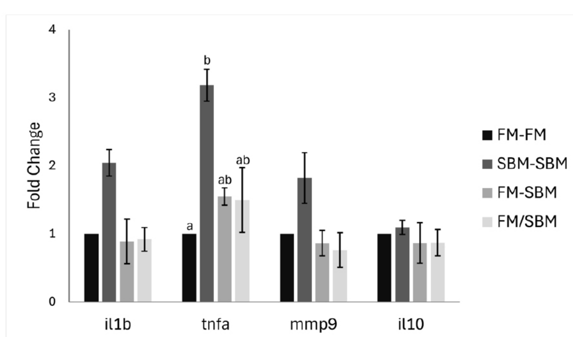

Figure. Relative expression of inflammation-related genes represented as fold change ± standard error of mean

EVALUATING STOCK ENHANCEMENT METHODS FOR HARD CLAMS *Mercenaria mercenaria* AND EASTERN OYSTERS *Crassostrea virginica* IN A RECRUITMENT-LIMITED TEMPERATE LAGOON

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Bivalve restoration in recruitment-limited systems necessitates stock enhancement to boost fertilization efficiency and larvae production. Over ten years, the Shinnecock Bay Restoration Program (ShiRP) has helped rebuild hard clam (*Mercenaria mercenaria*) populations in Shinnecock Bay, NY, through the stocking of spawner sanctuaries. Hydrodynamic models showed that larvae spawned in these sanctuaries were transported to areas where clam densities increased over 50-fold from 2012 to 2022. Increases in hard clam abundance resulted in an over 1,500% increase in commercial landings, with annual hard clam landings reaching levels not seen in 40 years in Shinnecock Bay (Figure 1). ShiRP focused on stocking sanctuaries with adult, market-size clams that were wild-harvested from surrounding coastal waters rather than less-expensive hatchery-produced clam seed, as monitoring and field experiments demonstrated that outplanted seed clams experienced near complete mortality from predation. Adult clams, on the other hand, exhibited high survivorship for over a decade after planting. ShiRP is now using this system-level approach to rebuild an oyster (*Crassostrea virginica*) metapopulation in Shinnecock Bay, establishing sub-populations in zones with maximal larval retention to enhance recruitment and population connectivity. Since 2018, ShiRP has annually deployed bags of oyster spat-on-shell (SOS), creating small-scale experimental reefs to evaluate oyster survivorship, growth, reproduction, disease, and recruitment. Repeatedly, oyster SOS has exhibited about 50% survival in the first-year post-deployment, substantially higher than hard clam seed of similar size. As adults, however, oyster longevity is substantially lower than that of hard clams, primarily due to the oyster disease Dermo. Recently, ShiRP partnered with the Supporting Oyster Aquaculture and Restoration (SOAR) program to deploy adult farm-raised oysters in Shinnecock Bay. The restoration value of this additional stock enhancement strategy in combination with SOS will be discussed in light of the monitoring results.

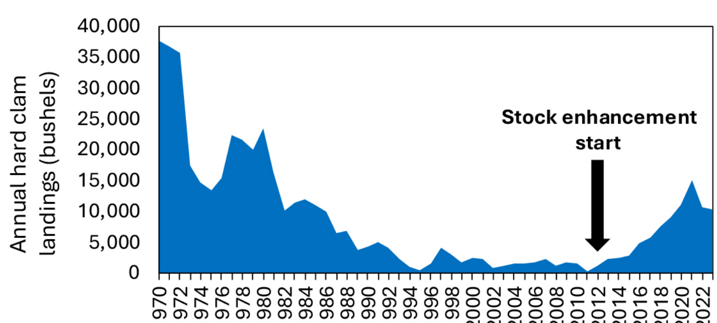

Figure 1. Annual hard clam landings from Shinnecock Bay from 1970-2023.

PRODUCTION AND NUTRITIONAL COMPOSITION OF *Chlorella vulgaris* AND LETTUCE AS A FUNCTION OF TILAPIA STOCKING DENSITY IN A DECOUPLED AQUAPONIC SYSTEM

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Aquaponics offers a sustainable approach to food production by integrating aquaculture and hydroponics. It allows food production on non-agricultural land, increases resource efficiency, and reduces waste compared to traditional farming practices. However, increasing aquaponics productivity poses a significant challenge, as it requires maintaining a balance between fish and plant populations. Stocking density is one of the most critical factors influencing nutrient balance and water quality which directly affect system productivity. Aside from improving productivity, diversifying aquaponics products offers a promising avenue to contribute to the industry's growth. Microalgae is widely considered a high-value culture species in aquaculture because of its diverse applications, including serving as a functional food, source of nutraceuticals, potential for biofuel and biogas production and as a highly nutritious feed ingredient for fish and livestock. The objective of the study was to incorporate algae as a component of aquaponics system and evaluate the impact of varying stocking densities of fish on the overall system productivity, including algal biomass, fish growth and plant yield. Additionally, the experiment also assessed the nutritional profile of the algae to provide insights on its potential application to various industries and examine how different stocking densities affect its nutrient composition.

Four fish densities were tested; 10 kg/m^3, 20 kg/m^3, 30 kg/m^3, and, 40 kg/m^3. Growth parameters from fish, lettuce and algae and water quality parameters were measured after 35 days of culture. The results indicated that after four batches of algae culture, the highest stocking density (40 kg/m^3) produced significantly higher algae biomass yield compared to the lower stocking densities (10 kg/m^3 and 20 kg/m^3). An increasing trend in biomass yield was observed as stocking density increased. Increasing trend with stocking density was also observed in fish total biomass as well as in wet and dry weights of lettuce. The increased algae biomass and lettuce yield can be attributed to the elevated levels of important nutrients crucial for their growth such as nitrate, phosphate and potassium. Lettuce micronutrient analysis revealed that higher fish densities generally led to reduced nitrogen, nitrate and magnesium content, while manganese content increased significantly. The findings demonstrated that increasing fish densities to 30 and 40 kg/m^3 significantly improved the productivity of algae, fish and lettuce. The study also highlights the potential of incorporating algae into aquaponics systems as an additional component to enhance the value of production.

Parameters	Treatments			
	10 kg/m^3	**20 kg/m^3**	**30 kg/m^3**	**40 kg/m^3**
Total fish biomass (g)	994.88 ^{a}	2207.48 ^{b}	4152.63 ^{c}	5027.01 ^{c}
Total wet lettuce yield (g)	312.93 ^{a}	578.40 ^{ab}	959.03 ^{b}	1067.17 ^{b}
Total dry lettuce weight (g)	31.70 ^{a}	34.93 ^{ab}	42.40 ^{bc}	43.10 ^{c}
Total algae biomass (g)	6.70 ^{a}	7.01 ^{a}	7.36 ± ^{ab}	7.88 ^{b}
Lettuce micronutrients				
Nitrogen (%)	5.11 ^{b}	4.78 ^{ab}	4.39 ^{a}	4.44 ^{a}
Nitrate (%)	2.17 ^{b}	1.45 ^{ab}	1.02 ^{a}	0.76 ^{a}
Magnesium (%)	1.36 ^{b}	1.18 ^{b}	0.88 ^{a}	0.85 ^{a}
Manganese (mg/kg)	87.00 ^{a}	143.33 ^{ab}	199.00 ^{bc}	255.67 ^{c}

AQUACULTURE EXTENSION AT OREGON SEA GRANT: BUILDING CAPACITY, BUILDING CONNECTIONS

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Oregon Sea Grant's (OSG) current focus on aquaculture reflects a growing recognition within the U.S. Pacific region that there is significant opportunity for expansion of the aquaculture industry, in a manner that complements other coastal and marine enterprises and supports local communities. In the last several years, OSG has made significant progress in assessing the needs of current growers, opportunities for industry development, and barriers to further growth. Commonly cited barriers include challenges with permits and policies, an uncertain regulatory framework for novel species, limited leasing space within Oregon estuaries, a lack of workforce, and potential climate/ecological limitations. OSG has partnered with regional Sea Grant programs, non-governmental organizations, research groups, and agencies to help address these issues.

Currently, OSG is working closely with local and regional partners to assess policy and permitting barriers and identify new regulatory pathways. Through this Mariculture Collaborative, OSG has been able to engage with a larger community of practice, and support pilot projects advancing new-to-Oregon aquaculture species, production methods, and products – with an emphasis on native shellfish and seaweeds. OSG is also an active partner in the development of an Oregon Aquaculture State Strategic Plan, which is working to identify ways to support both marine and freshwater aquaculture throughout the state. Over the next year, in close coordination with other West Coast and Pacific Sea Grant programs, OSG will increase its focus on strengthening aquaculture literacy and social license.

During this presentation, we will highlight both the successes and challenges faced by Oregon Sea Grant and Oregon's aquaculture industry, and our current and future goals for supporting aquaculture across various species and sectors.

THE AQUACULTURE COLLECTIVE: VIMS USAS STUDENT SUBUNIT

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The Aquaculture Collective (AquaCo) is the Student Subunit of the United States Aquaculture Society at the Virginia Institute of Marine Science (VIMS). Established in the fall of 2023, the purpose of AquaCo is to engage in diverse initiatives that offer educational opportunities, professional development, and social activities for enhancing aquaculture. This Student Subunit is open to graduate and undergraduate students at VIMS and William & Mary. Throughout the academic year AquaCo hosts member meetings that include activities such as trivia and cookie decorating. Additionally, AquaCo collaborates with other chapter organizations at VIMS such as the Society of Women in Marine Science to host events that bridge various sectors of marine science to educate broader audiences. An area of improvement for our chapter is holding meetings and activities that entice both graduate and undergraduate students to attend. AquaCo strives to help connect the W&M undergraduate community to opportunities for learning about and becoming involved with aquaculture. In the future, AquaCo plans to host aquaculture professionals to speak at VIMS and creating more aquaculture fieldwork opportunities for undergraduates at W&M, such as touring the shellfish research farm at VIMS.

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AN ECONOMIC AND PRODUCTION ANALYSIS OF SEAWEED FARMERS IN CALIFORNIA AND THE PACIFIC NORTHWEST

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Since the early 2010s, the U.S. seaweed aquaculture industry has steadily expanded. Notable states such as Alaska, Maine, Hawaii, and Connecticut have led the way in the development of this domestic industry. Unfortunately, information on certain sectors of the domestic seaweed industry is lacking. The goal of this study was to examine the status of the seaweed aquaculture industry along the West Coast, specifically California, Oregon, and Washington State. Seaweed farmers in these states were asked economic and production questions regarding their operations.

Fourteen seaweed farms were identified in this region, 11 of which responded to the survey (79% response rate). Data from nine for-profit seaweed farms was analyzed. The average age of a West Coast seaweed farm was 10 years. In 2023, California produced approximately 144,242 kg of farmed seaweed, making it the fourth-largest producer of farmed seaweed in the U.S. Oregon and Washington were combined into one region, the Pacific Northwest, which produced around 10,432 kg of seaweed. The top four farmed seaweed species on the West Coast were Pacific Dulse (*Devaleraea mollis*), Bull Kelp (*Nereocystis luetkeana*), Sea Lettuce (*Ulva*), and Giant Kelp (*Macrocystis pyrifera*); with Pacific Dulse being the most profitable. The West Coast has six land-based (tank tumble culture) and five longline farms. Our analysis found that only the land-based systems were profitable. In 2023, 37 full-time and 14 part-time folks were employed with the West Coast seaweed aquaculture industry. Many farmers reported selling directly to customers. Key challenges for farmers included obtaining permits, navigating regulations, and limited market demand (Figure 1). Despite these challenges, 67% of farmers felt very optimistic or optimistic about the future of the West Coast seaweed aquaculture industry. Top recommendations for this industry include streamlining the regulatory process, subsidizing seaweed crops, and expanding into new markets, including the pharmaceutical, nutraceutical, and cosmeceutical industries.

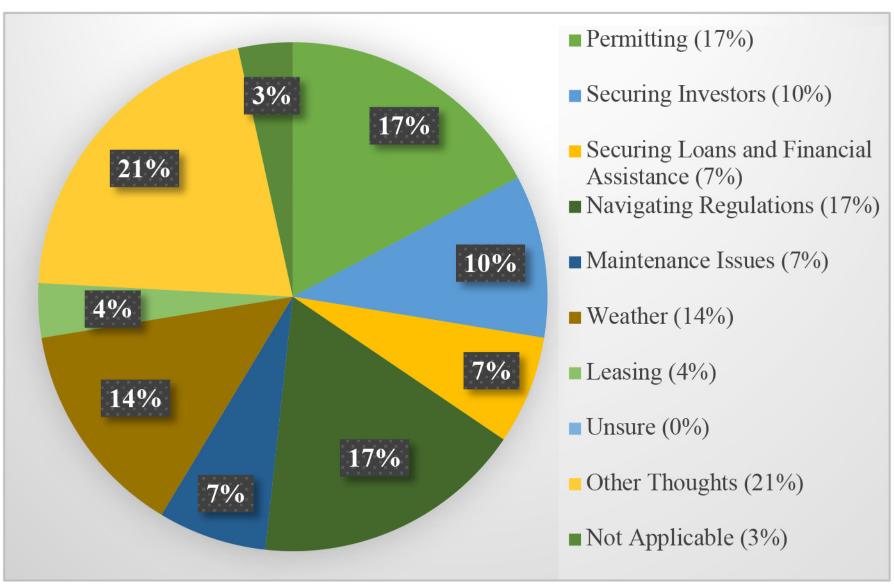

Figure 1. Top challenges for West Coast seaweed farms in 2023

MICROBIOME IN A RECIRCULATING, ARTIFICIAL SEA WATER HATCHERY FOR LARVAL PRODUCTION OF THE EASTERN OYSTER *Crassostrea virginica*

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The oyster fishery in the Gulf of Mexico has been a global leader in oyster production. Declining water quality, storm events, and human impacts (e.g., oil spills, flood control) have diminished wild oyster populations. Aquaculture can support restoration efforts, commercial oyster aquaculture, and wild harvests. The commercial oyster aquaculture industry relies on hatchery production of single-set oyster seed, the supply of which can be unreliable due to variability in the natural seawater (NSW) used for hatchery production. The use of artificial seawater (ASW) has the potential to enhance aquaculture production for the Eastern Oyster, *Crassostrea virginica*, by expanding production capability into inland areas, avoiding fluctuating environmental conditions, and reducing risks from anthropogenic pollutants. Seawater, whether artificial or natural, contains complex communities of microorganisms that play critical roles in maintaining conditions that affect oyster larvae development, growth, and survival. Therefore, understanding the dynamics of microbiomes in recirculating systems may provide information about the state of the system that connects to larval production outcomes.

Water samples were collected from recirculating ASW production systems at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center, in Ocean Springs, Mississippi, USA during the 2020-2023 hatchery seasons. Water samples were filtered through 0.22uM filters, genomic DNA was extracted, and 16S rRNA gene amplification and sequencing targeting the V6–V8 variable regions was performed on the Illumina MiSeq platform. Bioinformatics analyses were performed using Quantitative Insights into Microbial Ecology with DADA2. Water quality parameters were collected simultaneously with water samples. Larval survival data, including assessments of fecundity, percent of larvae reaching day two (D-stage), and percent of larvae reaching pediveliger stage also were collected to calculate brood outcome indices.

Preliminary data show that the location within the system and transition between brood introductions and removals (stocking and harvesting) were key factors shaping microbiome composition. Across the four-year study period, water quality became more stable, concomitant with a more uniform microbiome in different system compartments. Brood outcome indices improved across the study period. This work may provide information that can support improved larval survival and tools to monitor whole system (biotic and abiotic) conditions.

A FARM COLLECTIVE FIELD STUDY OF 4 LINEAGES OF PACIFIC OYSTERS: MIDORI, MIYAGI, TRIPLOID, HYBRID, ACROSS CALIFORNIA COASTAL ESTUARINE ENVIRONMENTS

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As with all sectors of food production, changing environmental conditions pose a significant threat to Pacific oyster cultivation. The combined impacts of ocean warming (OW), acidification and hypoxia (OAH), and Harmful Algal Blooms (HABs), present emerging challenges for Pacific oyster aquaculture Globally. These changing environmental conditions along with persistent disease pressures continue to have large impacts on survivorship and are driving selective breeding programs to minimize impacts to both survivorship and growth rates. These efforts require both on-farm and lab-based evaluations of seed performance across environments and abiotic conditions to both evaluate the quality of seed being produced and reliably select for traits of interest (growth, survivorship, and meat yield). To better understand the biotic and abiotic factors affecting lineage specific survivorship, growth rates, condition index, and shell shape of oysters in estuarine environments we have conducted a multi-estuary farm study comparing commercially produced cohorts of the Midori, Miyagi, hybrid Midori and Miyagi, and triploid Pacific oyster lineages in 3 oyster producing estuaries in California (Humboldt Bay, Tomales Bay, and Morro Bay). We have addressed these goals through a combination of field monitoring and lab-based measurements of condition index, clearance rate, and total antioxidant activity. These findings are providing information to our farmer partners that will contribute to the maximization of sustainable food production by identifying growth and survivorship traits of each lineage.

OVERVIEW OF THE AQUACULTURE OPPORTUNITY AREA PROCESS

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NOAA is continuing to encourage U.S. aquaculture development through the identification of Aquaculture Opportunity Areas (AOAs). NOAA is working with its federal and state partners to identify geographic areas containing locations that may be suitable for commercial aquaculture and complete a programmatic National Environmental Policy Act (NEPA) analysis for each area to assess the impact of siting aquaculture facilities there.

The AOA process will result in the identification of geographic areas that, through scientific analysis and public engagement, may be environmentally, socially, and economically suitable for multiple aquaculture farm sites of varying types. NOAA will combine input received through consultation and coordination with Federal and non-Federal stakeholders, public comments, and spatial modeling by NOAA's National Centers for Coastal Ocean Science (NCCOS) that is based on the best available science.

NOAA began the process to identify AOAs in Federal waters within the Gulf of Mexico and off Southern California in 2020 and within state waters of Alaska in 2023. NOAA will provide an overview of the process and updates on our progress, including continued opportunities for input.

SHUCKING THE SHELL: PIECING TOGETHER THE PHYSIOLOGICAL RESPONSE OF RIBBED MUSSELS *Geukensia demissa* TO TEMPERATURE AND FOOD

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Ribbed mussels act as ecosystem engineers protecting shorelines through their settlement pattern and acting as nutrient buffers through active water filtration. However, as climate change continues to alter marine systems, including changes in water temperature and timing of seasonal food availability, ribbed mussels may be impacted. Ribbed mussels may look simple, but under that shell they exhibit a complex interconnected physiological web. The salt marsh, home of the ribbed mussel, exhibits extreme environmental conditions due to tidal fluctuations and seasonal variability. The physiological network of this species can not only withstand these conditions but can allow ribbed mussels to thrive in them. But we do not yet know how the effects of climate change, especially temperature and food availability, will affect physiological and behavioral responses. In this study, larval ribbed mussels were exposed to a range of temperatures and food levels to determine the effects on growth, survival, and developmental timing. The larval stage is expected to be the most vulnerable life stage to external variation in conditions, and early developmental stages can have long-term effects on population dynamics. From our data, we investigate these complex physiological systems to understand their response to multivariable temperature-food conditions. From these data, we aim to model the complex physiological response of the ribbed mussel and project the species outcome in response to continued climate change.

BIODEGRADABLE PLASTICS FOR MARINE APPLICATIONS – THE CASE FOR INDONESIA

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Marine litter is estimated to have large and wide-ranging impacts on the marine environment. However, few studies have attempted to address the economic costs of marine litter. The few examples that are available tend to address impacts and costs either globally or by economic region. While global commitments are needed to address the marine litter problem, and progress is being made in this regard, mitigation measures will be delivered at the national level.

Abandoned, lost or otherwise discarded fishing gear (ALDFG) is a global problem. Ghost fishing represents one of the main impacts of ALDFG. In addition, ALDFG creates a myriad of environmental and socioeconomic impacts that affects fisheries and other commercial sectors operating in the marine environment, e.g. aquaculture as well as recreational users and land-based sectors e.g. tourism.

In a previous study, we addressed the role of biodegradable fishing gear (BFG) as a mitigation measure to address ALDFG and ghost fishing by developing an economic model to estimate the cost of ghost fishing and the costs and benefits of BFG as a mitigation measure – which was found to be as high as £90,000 at the vessel level in the English Channel Fishery.

In the current study, we adopt the same approach to address the role of BFG in aquaculture, culture based fisheries and the fishing sector in Indonesia, which is estimated to have a large potential to drive the blue economy, but also represents sectors that already generate nearly a million tonnes of plastic waste every year.

AQUACULTURE RESEARCH UPDATE FOR HUBBS-SEAWORLD RESEARCH INSTITUTE

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Hubbs-SeaWorld Research Institute (HSWRI) is a 501-(c)(3) nonprofit research organization that was established in 1963 to "Return to the sea some measure of the benefits derived from it". Marine aquaculture has been a core area of research at HSWRI since the late '70s and it is currently facilitated by a Marine Research Laboratory in San Diego that has a flow-through seawater capacity of 80 m^3 per hour (350 gpm) and a Fish Hatchery in Carlsbad that has a seawater capacity of 273 m^3 per hour (1200 gpm). Within each facility are specialized systems (flow through and reuse) from experimental to commercial scale.

White seabass (*Atractoscion nobilis*) and California halibut (*Paralichthys californicus*) are being cultured at HSWRI for stock enhancement research with potential for future commercialization if a finfish industry develops. California yellowtail (*Seriola dorsalis*) is cultured for research as a promising food fish species in the USA. Larval survival for each of these species is high, so research is mainly focused on maximizing fingerling quality and production efficiencies. In recent years, HSWRI has diversified its portfolio of aquaculture species to include seaweeds and invertebrates to support growing interest in low trophic level aquaculture, including integration with fed species. Current research is focused on *Ulva lactuca*, *Gracilaria spp*., and *Devaleraea mollis* as nutrient scrubbers in the effluent of tanks of fed fish (i.e. integrated multi-trophic aquaculture, IMTA), both in flow through and reuse configurations, as well as using the seaweeds as food for fattening starved sea urchins collected off barrens. Invertebrate research has focused on using wild-caught warty sea cucumbers (*Apostichopus parvimensis*) as a detritivore in IMTA applications and as broodstock for propagation and future commercialization. Additionally, HSWRI has successfully spawned Pacific Calico Scallops (*Argopecten ventricosus*) as a potential new shellfish species for California.

FLOCCULATION OF OLEAGINOUS GREEN ALGAE WITH *Mortierella alpina* FUNGI

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Microalgae are promising sources of valuable bioproducts such as biofuels, food, and nutraceuticals. However, harvesting microalgae is challenging due to their small size and low biomass concentrations. To address this challenge, bio-flocculation of starchless mutants of *Chlamydomonas reinhardtii* (*sta6*/*sta7*) was investigated with *Mortierella alpina*, an oleaginous fungus with high concentrations of arachidonic acid (ARA). Triacylglycerides (TAG) reached 85% of total lipids in *sta6* and *sta7* through a nitrogen regime. Scanning electron microscopy determined cell-wall attachment and extra polymeric substances (EPS) to be responsible for flocculation. An algal-fungal biomass ratio around 1:1 (three membranes) was optimal for bio-flocculation (80-85% flocculation efficiency in 24h). Nitrogen-deprived *sta6*/*sta7* were flocculated with strains of *M. alpina* (NVP17b, NVP47, and NVP153) with aggregates exhibiting fatty acid profiles similar to *C. reinhardtii*, with ARA (3-10% of total fatty acids). This study showcases *M. alpina* as a strong bio-flocculation candidate for microalgae and advances a mechanistic understanding of algal-fungal interaction.

METABOLIC AND METABOLOMIC RESPONSE OF TRIPLOID *Mytilus edulis* FAMILIES TO HEATWAVES

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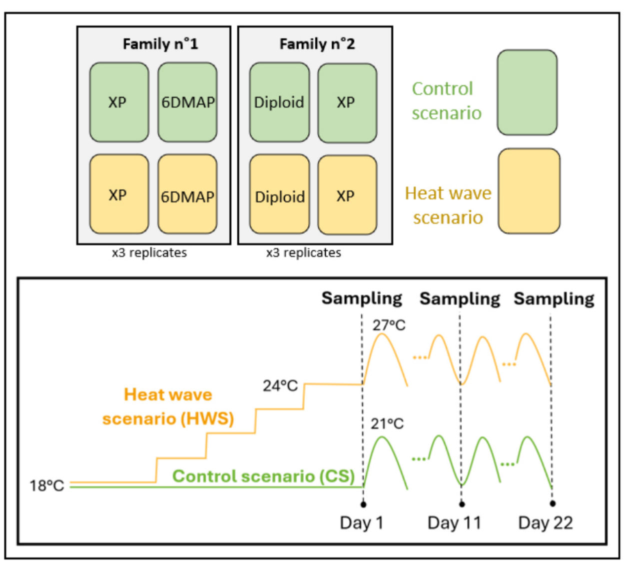
The aquaculture industry increasingly investigates the use of triploid mussels due to their potential for enhanced growth efficiency. Triploidy induction disrupts at least partially gonadal development, potentially redirecting energy towards somatic growth. However, triploid tolerance to environmental stressors like heatwaves remains unclear. Understanding their response to such events is crucial, as heatwaves are becoming longer and more frequent with climate change. The present study compares the metabolic and metabolomic responses of diploid and triploid *Mytilus edulis* families to heatwave conditions.

Two mussel families were produced. The first family containing triploids from both chemical (6DMAP) and pressure shock (XP) induction, and the second family including triploids obtained by pressure shock alongside its diploid control. Mussels were exposed for three weeks to two temperature scenarios reflecting control and heatwave conditions experienced at Prince Edward Island mussel farms. Oxygen consumption rates were measured in each group and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze metabolite profiles for each temperature scenario. The study focuses on two aspects: (1) comparing the effect of heatwave between diploids vs. pressure-shock triploids (family 1), and (2) comparing this effect between triploids obtained from pressure shock vs. chemical shock treatments (family 2).

No significant mortality was observed in any of the treatments during the exposure period. Respirometry recordings indicate higher oxygen consumption rates under heatwave conditions, without differences between triploids and diploids or pressure and chemical shock treatments. By combining these results with metabolomic analyses, we aim to compare metabolic pathways and energy allocations between groups under thermal stress.

FIGURE 1. Experimental design



ESTUARINE LANDSCAPE SCALE ASSESSMENTS OF EELGRASS AND OYSTER AQUACULTURE AS HABITAT FOR NEKTON IN A U.S. PACIFIC COAST ESTUARY

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Remote sensing has become a valuable tool for assessing interactions between shellfish aquaculture and submerged aquatic vegetation at the estuarine landscape scale. A geographic information system (GIS) with data layers for intertidal eelgrass (*Zostera* spp.) and active Pacific oyster (*Crassostrea gigas*) aquaculture distributions was first developed using orthoimagery in Willapa Bay, Washington in the mid-2000's. These historical data served as a baseline for comparisons with updated GIS layers of eelgrass and active aquaculture distribution based on contemporary (2020) high resolution 4-band orthoimagery captured from fixed-wing aircraft during ideal low tide conditions. Contemporary orthoimagery and methodologies allowed for improved classification of eelgrass and delineation of aquaculture (via interpretation of visible evidence of culture, equipment, and physical use of the culture beds) which was then cross-checked and verified with the aquaculture industry (Figure 1). Overall, eelgrass coverage estimates in Willapa Bay declined from 5,938 ha in 2009 to 5,551 ha in 2020 (Table 1). Estimates of total oyster aquaculture were similar, while estimates of active oyster aquaculture increased from 2009 to 2020. This can largely be attributed to improved resolution of the delineation method and better definition of "active" culture boundaries. We present an example of how these new estimates can be used alongside a survey of habitat use by juvenile Dungeness crab and English sole to compare estuary-wide provision of low intertidal channel fringing habitat (<25m from channels) with that at higher tidal elevations.

Table 1. Comparison of landscape scale assessments of habitat in Willapa Bay, WA, USA in 2009 and 2020.

	2009	2020
Total intertidal eelgrass	5,938 ha	5,551 ha
Proportion of intertidal with eelgrass	27.6%	25.7%
Total intertidal oyster culture	3,474 ha	3,137 ha
Proportion of intertidal with oyster culture	16.1%	14.6%
Eelgrass within oyster culture	784 ha	996 ha

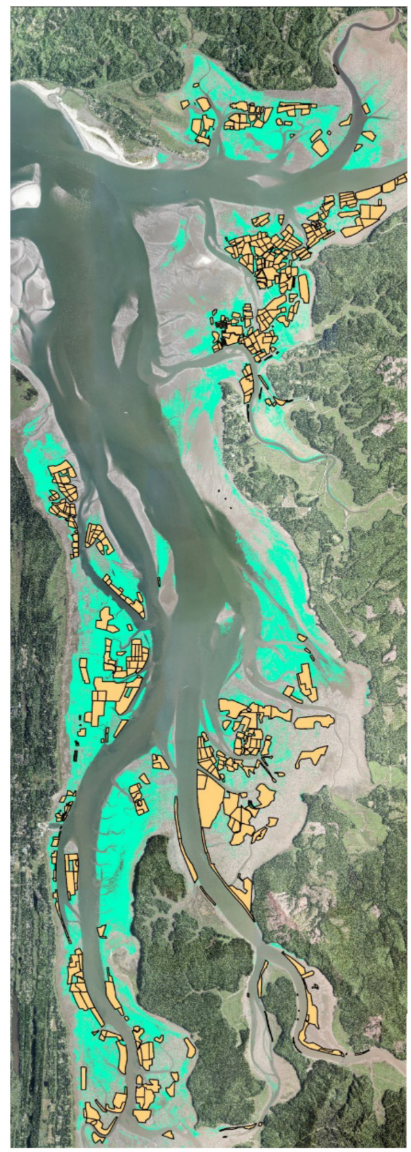

Figure 1. Eelgrass (green) and active oyster aquaculture beds (orange) in Willapa Bay, WA in 2020.

XENOGENESIS FOR EFFICIENT PRODUCTION OF HYBRID CHANNEL CATFISH, *Ictalurus punctatus,* ♀ BLUE CATFISH, *I. furcatus,* ♂ EMBRYOS AND SPAWNING OF DIFFICULT TO BREED AQUACULTURE SPECIES

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Hybrid catfish (♀ channel catfish, *Ictalurus punctatus* × ♂ blue catfish, *I. furcatus*) account for ~60-70% of the catfish production due to superior performance compared to the parent species for several traits. Production of hybrid embryos is labor intensive and requires sacrifice of slow maturing blue catfish males. Xenogenesis has been utilized to produce hybrid catfish embryos more efficiently by transplanting unsorted gonadal cells from donor diploid blue catfish into triploid channel catfish fry. Then xenogenic channel catfish males are mated with normal female channel catfish to produce 100% hybrid progeny.

Normal channel catfish females were paired with normal males and putative xenogenic channel catfish males producing blue catfish sperm and induced to spawn in spawning enclosures in 2024. The reciprocal xenogenic female × normal male was also attempted. No reproductive differences were detected. Spawning percentage for normal ♀ × normal male ♂ (producing 100% channel catfish fry), normal ♀ × xenogenic ♂ (producing 100% hybrid fry), and xenogenic ♀ × normal ♂ (producing 100% reciprocal hybrid fry) was 60.0, 62.5 and 50.0%, respectively. Hatching percentage was 64.6, 62.8 and 69.3% for normal ♀ × normal male ♂, normal ♀ × xenogenic ♂, and xenogenic ♀ × normal ♂, respectively. Fry/kg ♀ BW was 3511, 2965, and 3338 for normal ♀ × normal male ♂, normal ♀ × xenogenic ♂, and xenogenic ♀ × normal ♂, respectively. In 2023 and 2024, spawning xenogenic males were given a second female to mate with and 0.0 and 62.5% were able to spawn twice in 2023 and 2024, respectively.

Xenogenic pairing to produce hybrids is as successful as channel catfish pairings, so this approach appears to be a viable option to make hybrid catfish embryos. We have also had success in developing a system that utilizes white catfish (*Ameiurus catus*) to produce channel catfish, blue catfish and hybrid catfish. To commercialize this approach, a step in the value chain, a company specializing in producing xenogenic males, would be needed. Economic analysis is needed to compare the cost/benefit of the traditional approach to making hybrids versus the xenogenesis approach. The xenogenesis approach can also be optimized further.

KNOCKIN/KNOCKOUT IN CHANNEL CATFISH TO IMPROVE GROWTH DISEASE RESISTANCE AND OMEGA-3 FATTY ACIDS COUPLED WITH STERILITY

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The *mstn*, *mc*4*r* and *lh* loci were knocked out for channel catfish, *Ictalurus punctatus* and replaced with alligator cathelicidin, cecropin, masu salmon Δ5-desaturase like gene (D5D), masu salmon elongase (*elovl2*), *fat1* and *fat2* from *C. elegans* transgenes in various combinations. This resulted in fish that had as much as a 4X increase in bacterial disease resistance, increased viral resistance, a 50% increase in growth rate, upwards to a doubling of various omega-3 fatty acids and an inability to spawn. The omega-3:omega-6 fatty acid ratio was increased. Fertility was restored by using luteinizing hormone releasing hormone analogue (LHRHa) or LHRHa with human chorionic gonadotropin depending upon the genotype of the fish.

Fatty acid biosynthesis transgenic channel catfish demonstrated pleiotropic increases in growth and disease resistance for some genotypes. Other such genotypes had poor disease resistance that could be corrected by mating with cathelicidin and cecropin transgenic lines.

There were significant effects due to insertion site. Insertion of two copies of an antimicrobial protein (AMP) transgene or two different AMP transgenes increase bacterial disease resistance more than a single AMP insert. Off-target effects occurred in one case causing a high frequency of deformed individuals. Suites of traits can be improved with this approach. Future research should address combining this integrated approach with traditional genetic enhancement programs.

TRANSGENIC FISH: PAST, PRESENT AND FUTURE

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The initial thrust into genetic engineering of fish focused primarily on growth hormone transgenesis. The vast majority of experiments resulted in faster growing fish ranging from 10% to an amazing 30X increase in growth rate, typically a doubling. If triploidy was used to sterilize these fish, about half of the growth enhancement was lost, although unpublished commercial data indicated that this could be corrected with family selection. Different transgenic families had a wide range of performance; thus this technology always needs to be coupled with selection to maximize performance regardless of the transgene. Growth hormone gene transgenesis results in many pleiotropic effects including increased feed consumption and feed conversion efficiency, variable changes in disease resistance, changes in body composition, primarily increased protein percentage and decreased fat percentage coupled with changes in muscle ultrastructure. Additionally, behavioral changes can occur and most fitness traits are adversely but variably affected such as reproduction, foraging ability, predator avoidance and swimming ability.

Antimicrobial peptide transgene genetic engineering has consistently increased disease resistance of transgenic fish. Transfer of omega-3 fatty acid biosynthesis genes increased omega-3 fatty acid levels and the omega-3/omega-fatty acid ratio. Negative and positive pleiotropic effects have resulted from the transfer of omega-3 fatty acid transgenes. Random transfer of shrimp DNA affected flavor and nutritional values. Creation of Glo-fish having florescent jelly fish genes has resulted in a variety of new ornamental phenotypes and was the first commercialization of transgenic fish. Use of such florescent reporter genes in experiments should be questioned as the performance of these transgenic fish is damaged. Strain, family, genetic background and insertion site are among factors that affect transgenic performance.

Although environmental risk data indicated transgenic fish would likely be selected against in the natural environment, society requires strict confinement of transgenic fish. Transgenic sterilization or gene editing coupled with hormone therapy are the best options to ensure no long-term ecological effects of transgenic fish. Gene knockin/knockout has arrived. By targeting growth regulating and reproductive loci, reversibly sterile transgenic fish can be created that have increased growth, disease resistance and omega-3 fatty acid levels among others in a single fish. The best aquaculture genotypes today and in the future will be developed by using multiple genetic enhancement programs simultaneously or in sequence.

Public education is a major impediment to the acceptance of transgenic fish as food. There is no logical food safety issues with transgenic meat except for allergenicity in specific cases. The basics will always be important as the phenotype is a result of the genotype, environment, genotype-environment interaction and epigenetics. These principles are related to the commercialization of the first commercially approved transgenic fish, growth hormone gene transgenic Atlantic salmon, on the brink of failure. The best genotype in the world cannot perform well without the proper environmental input and application.

INCORPORATING MACHINE LEARNING INTO A FISH HEALTH DATABASE

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Since 1990, Kentucky State University has had a fish disease diagnostic laboratory, examining about 50 fish disease cases a year, including fish health inspections done to allow fish growers to transport and sell specific pathogen free fish into other states. This service to our stakeholders is regarded as an Extension function and has benefited fish owners including aquaculturists, federal and state fish & wildlife agencies, aquaculture researchers at universities and private businesses, ornamental fish hobbyists, and aquarium owners.

In 2022, KSU completed the development of a mobile responsive clinical fish health database that was used to upload over 1,400 fish disease cases including photographs and video clips. Having 35 years of fish disease cases in a database has enabled KSU Extension specialists, associates and students to search (with the use of a filter) a wide range of disease cases by year, farm owner, type of disease, and other parameters that have been entered into the database from 1990 through 2024.

KSU is now in the process of incorporating machine learning technology into the database to aid in disease recognition (for identification as well as predicting occurrences of diseases). Photographs and videos of diseased fish are being uploaded to the database for use in machine learning, enabling the database to identify these diseases and the likelihood of their occurrence.

SEAWEED AND ITS MULTIFACETED IMPACT ON WOMEN, THROUGH LENSES OF FOOD SECURITY, ENVIRONMENTAL SUSTAINABILITY, TRADE, AND ECONOMIC DEVELOPMENT

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Today's seaweed industry is characterized by strong growth, large regional differences, and high species concentration. Seaweed can be instrumental in achieving many of the SDGs and women play leading roles in the seaweed sector, throughout the value chain. However, despite the significant presence of women in seaweed production and trade, especially in developing countries, there is little to no official reporting on women's labor force participation in the seaweed industry, and policy measures are typically not gender inclusive.

This paper analyzes the multifaceted relationship between seaweed and women, through lenses of food security, environmental sustainability, trade, and economic development. Using recent data, peer-reviewed literature, and findings from semi-structured interviews with selected seaweed stakeholders, it reviews numerous challenges that affect women's effective participation in the sector and identifies multiple ways in which seaweed can support and enhance women's economic empowerment, especially in developing countries.

HEAVY METALS IN MANGROVE SEDIMENT AND NATIVE SPECIES FROM THE GENUS ANADARA sp. AND PENAEUS sp.

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In the northern region of Peru (Tumbes), mangrove forests develop in Puerto Pizarro and in Santuario Nacional Manglares de Tumbes.[1] The first site is a highly impacted area due to its high urban, commercial and touristic unregulated development, while the second one corresponds to a better-preserved area due to the regulation of anthropogenic activities. In both, extractive activities of native species like bivalves, fishes and crustaceans for human consumption take place daily.[1]

Previous research has shown that, thanks to the high retention capacity of fine sediments and organic matter that these ecosystems have, they can accumulate large quantities of heavy metals in such sediments. This accumulation could come from anthropogenic activities (e.g. urban expansion, extractive activities, etc.) and can be influenced by the natural conditions of the system (e.g. geomorphology, stationary hydric regime, etc.). Moreover, this accumulation can be harmful to native bivalve species that inhabit and depend on these sedimentary environments, we consider that metal accumulation capacity can vary depending on the species and habitat.[1]

In this presentation we review the results of the project[1] aimed at determining if the sedimentary environment and seasonal hydric regime are factors controlling heavy metal accumulation in the soft tissue of Anadara commercial species (A*nadara tuberculosa, A. similis, A. grandis)* collected in ranges of comparable sizes to each other. The muscle and sediment samples are taken from the main "conchales" (high bivalve density extraction areas) located in Puerto Pizarro and Santuario Nacional Manglares de Tumbes during dry and wet seasons. The project is determining whether the hydric regime and sedimentary environment are controlling heavy metal accumulation of these bivalve species. Furthermore, it will provide useful information to acknowledge the "conchales" that present 5x higher metal accumulation inside the mangrove ecosystems. The results could be used to develop management, administration, and conservation plans of hydrobiological resources. In addition, information about total mercury in Penaeus sp., fish, and mangrove sediment from the same areas will be presented.[2]

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1. García R., Álvaro Sebastian; Ramos Burga, Gabriel Ernesto. 2022. Acumulación de metales pesados en especies nativas del género Anadara sp. y en sedimentos de los manglares de Tumbes. Repositorio Institucional Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, Urb. Ingeniería, S.M.P. Lima - Perú. https://hdl.handle.net/20.500.12866/11450.
2. Total mercury in bass (*Centropomus sp*), black conch (*Anadara tuberculosa*), mangrove crab (*Ucides occidentales*) and sediment in mangroves of Tumbes, Peru. 2024. Manuscript in preparation.

HEAVY METALS IN MANGROVE SEDIMENT AND NATIVE SPECIES FROM THE GENUS ANADARA sp. AND PENAEUS sp.

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References

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DIVERSITY AND DISTRIBUTION PATTERN OF AQUATIC MACROPYTES IN TWO WATER BODIES IN ADO EKITI METROPOLIS, EKITI STATE, NIGERIA

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This study was aimed at investigating the macrophytes composition and species diversity of Rivers Osun and Elemi, located at Ikere and Ado- Ekiti, respectively. Macrophyte samples were collected from both rivers between between the months of May to October, 2023. Nineteen (19) species of macrophytes were collected from each of the two rivers. Osun River had a total number of 730 macrophytes with a percentage relative abundance of 48% while Elemi River had a total number of 794 macrophytes with a percentage relative abundance of 52%. In River Osun, *Solenastemon monostachyus* was the most abundant (145) and had the highest percentage relative abundance of 19.86% while *Coix larcryma-jobi* was the least abundant (8) species with a percentage relative abundance of 1.01%. In Elemi River, *Alternanthera brasiliana* was the most abundant (212) macrophytes species with a percentage abundance of 26.70% while *Lepistemon owarianse* was the least abundant (7) species with a percentage relative abundance of 0.88%. The diversity indices showed that macrophytes at River Elemi had the highest diversity index (H'= 3.48) and species richness (d = 2.73). It was also noted that macrophyte at River Elemi had the highest evenness index (E'= 1.18). This study also revealed that macrophytes at River Osun had the highest Menhinick index (MH = 0.70) and Simpson index (1-D = 0.91). The dominant macrophytes in both study areas are pollution tolerant species, and the rivers had been impacted by nutrient enrichment.

Table 1. Abundance and Percentage Relative Abundance of Macrophytes in Osun River Ikere -Ekiti, Ekiti- State.

Species	Abundance	Relative Abundance	% Relative Abundance
Pennisetum purpureum	19	0.03	**2.60**
Corchorus aestuans	81	0.11	**11.10**
Solenastemon monostachyus	145	0.20	**19.86**
Adenostemon perrottetii	77	0.11	**10.55**
Laportea aestuans	14	0.02	**1.92**
Asyrtaria gangetica	24	0.03	**3.29**
Ageratum conyzoides	31	0.04	**4.25**
Coix lacryma-jobi	8	0.01	**1.10**
Ludwigia octovalvis	32	0.04	**4.38**
Acacia ataxacantha	25	0.03	**3.42**
Melanthera scandens	11	0.02	**1.51**
Lepistemon owariense	57	0.08	**7.81**
Ipomoea hederifolia	9	0.01	**1.23**
Dactyloctenium aegyptium	30	0.04	**4.11**
Sporobolus pyramidalis	15	0.02	**2.05**
Desmodium aescendes	67	0.09	**9.18**
Tridax procumbens	53	0.07	**7.26**
Trema orientalis	19	0.03	**2.60**
Setaria megaphylla	13	0.02	**1.78**
Total	**730**		

IMPACTS OF TANK DIMENSION AND STOCKING DENSITY ON GROWTH AND NUTRIENT ALLOCATION IN THE SEA URCHIN *Lytechinus variegatus* IN A COMMERCIAL RECIRCULATING AQUACULTURE SYSTEM

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With increased prominence of sea urchins as models for biomedical research comes a variability of husbandry practices that should be standardized and optimized for lab-scale culture of sea urchins. Housing should be efficient to maximize the use of limited space, which can be accomplished through usage of commercially-available recirculating aquaculture systems (RAS), already common in the culture of other aquatic animal models like the zebrafish, *Danio rerio*. These commercial RAS provide tanks that are variable in sizes and volumes; however, their value in housing a demersal species has not been determined. It is important to quantify the impacts of tank design on growth and reproductive outcomes of sea urchins.

We evaluated the impacts of tank size (estimated by available surface area and volume) and stocking density on somatic weight gain and gonad development in sea urchins of *Lytechinus variegatus*. Eighty adult *L. variegatus* (ca. 34 g, 40 mm diameter) were collected from St. Joseph Bay in T.H. Stone Memorial Park. The sea urchins were proffered a formulated diet suspended in agar gel over a nine-week period. The use of an agar gel feed enabled quantification of feed intake to best ascertain growth demographics across the different treatments. Statistic tests performed were ANOVAs and ANCOVAs, with post-hoc Tukey and Dunnett's tests, in RStudio.

The smaller tank dimensions were correlated with a reduction in feed intake and less efficient dry matter production ($p < 0.05$), but no preferential resource allocation was seen among the test, lantern, gut, or gonad at the tank sizes tested. Increased stocking densities resulted in a similar reduction in feed intake as well as decreased dry matter production ($p < 0.005$). Competitive behaviors were witnessed within the higher density tanks in which a single sea urchin would "hoard" all proffered feed, preventing the others from eating. Additionally, increased densities resulted in preferential allocation of growth away from the gonad ($p < 0.05$) in favor of increasing somatic skeleton mass, specifically of the test ($p < 0.05$). There was also a higher percentage of broken spines seen on sea urchins held in higher densities tanks, suggesting intraspecific interactions led to spine breakage ($p < 0.005$). Interestingly, spine regeneration also exhibited a much higher degree of variability in the highest density treatment, with males and pre-gametic sea urchins showing much greater rates of regeneration compared to females ($p < 0.05$), again suggesting a preferential allocation of resources to growth. Overall, this study showed that limited space and increased organismal interactions potentially act as environmental stressors and can reduce growth outcomes in laboratory-scale culture.

REPRODUCTIVE OUTCOMES WHEN USING REGIMENTED SPAWN-INDUCING INJECTION PROTOCOL IN THE SEA URCHIN *Lytechinus variegatus*

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Refinement of sea urchin husbandry is crucial for maintaining a healthy population for both commercial aquaculture and research. It is well established that wild populations of *Lytechinus variegatus* undergo multiple spawning events from early spring through late summer. The purpose of this experiment is to emulate a repetitive spawning cycle to determine if regimented spawning in culture is associated with a significant change in reproductive outcomes.

Seventy-two adult *L. variegatus* (ca. 50-g) were collected from St. Joseph Bay, FL. The sea urchins were chemically induced to spawn upon return to the University of Alabama at Birmingham to determine sex as well as to void the gonad and establish a baseline. Following identification, sea urchins were assigned to either the monthly or bimonthly spawning treatment. The sea urchins were kept on a recirculating aquaculture system (RAS) to acclimate prior to beginning a formulated feed diet. Spawning was induced to sea urchins according to treatment each month on or around the new moon.

Data collected during each spawn included sea urchin diameter, height, weight, mass of gametes produced, and density of eggs produced for female sea urchins. Larvae potential was also recorded through tracking the development of larvae over 3-5 days. Preliminary results indicate that more frequent spawning regimens (once a month) may lead to increased gamete output for some female sea urchins (Fig. 1.) while males may benefit more from every other month spawning regimes (Fig. 2).

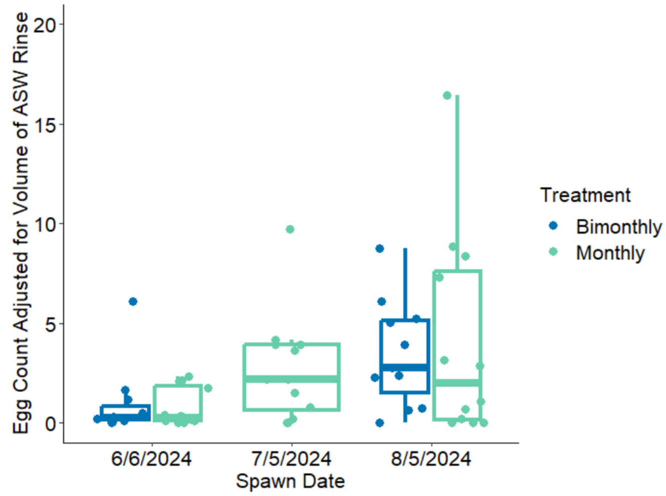

Fig. 1. Egg counts from each spawn. Counts are from homogenized subsample and adjusted for the volume of artificial saltwater (ASW) used to rinse.

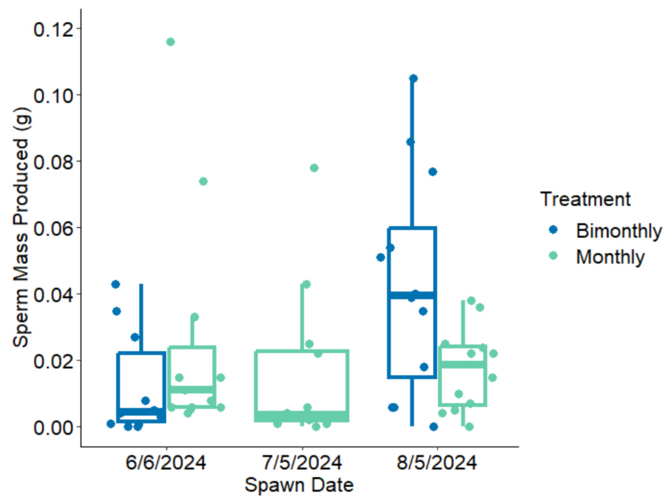

Fig. 2. Dry sperm mass produced each spawn.

EXPLORING ADAPTIVE CAPACITY IN THE NORTH CAROLINA OYSTER FARMING INDUSTRY

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Shellfish farming, specifically oyster farming, is a rapidly growing industry in North Carolina. Similar to finfish aquaculture and commercial harvest industries, oyster farming is subjected to a wide range of economic, regulatory, environmental, and anthropogenic stressors that layer upon the industry. Specifically, the North Carolina shellfish industry has withstood immense pressures in the past five years including increased tropical events and regulatory pressures, a changing business climate, and a drastic increase in catastrophic mortality events. Understanding these pressures can improve both industry and farm level resilience through behavior modification, collaboration, improved site selection, and grow-out practices.

The purpose of this study is to establish an industry framework that identifies and defines the most important stressors affecting oyster farming in North Carolina. The framework will contextualize these stressors, largely associated with mortality events, over different temporal scales at the individual and industry level through qualitative interviews with individual growers, industry stakeholders, and regulatory groups. Lastly, the authors will contextualize and define *adaptive capacity* for the North Carolina oyster farming industry and identify the key drivers of adaptive capacity at both the individual and industry levels. This goal will be accomplished through quantitative surveys developed from analyzing the preceding interviews and identifying commonalities. As the oyster farming industry continues to grow, this work is essential for assessing and identifying strategies to improve adaptive capacity and industry resilience, especially in the face of climate change. Commercial fisheries are often top heavy and favor capitol, but the oyster farming industry is currently flush with small-scale operations. Through collaboration across stakeholders, regulatory bodies, and farmers, the industry has the potential to increase its value while creating enabling industries.

ASSESSMENT OF ENVIRONMENTAL AND CLIMATIC FACTORS FOR SPATIAL PLANNING IN AQUACULTURE PRODUCTION

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Geography information System (GIS) play a crucial role in environmental analysis and can be systematically applied in spatial planning for aquaculture studies. This study analyzes spatial data such as bathymetry, slope, curvature, chlorophyll, temperature, humidity, windspeed, and precipitation to determine the best locations for aquaculture farms in Apalachicola Bay, which is part of the Gulf of Mexico. Data redundancy among variables was examined using the Pearson correlation matrix analysis, while the predictive power of the evidentiary data was evaluated using prediction plot analysis. The Additive Ratio Assessment (ARAS) and Evaluation Based on Distance from Average (EDAS) statistical models were employed for data integration. The prediction accuracy of these spatial models was evaluated using Area Under the Curve and Receiver Operating Characteristics curve (ROC/AUC) analysis. Pearson correlation analysis results indicated a generally low negative correlation and varying positive correlations. Prediction plot analysis revealed a strong correlation (≥ 0.7) between oyster beds and bathymetry (0.83), humidity (0.7), and chlorophyll (0.7). Spatial data integration using the ARAS and EDAS models identified the most ideal zones for aquaculture in the eastern, middle, and western sectors of the study region, accounting for 30.6 percent (ARAS model) and 22.97 percent (EDAS model) of the study site. Validation of prediction accuracy revealed high efficiency for both the ARAS and EDAS models in mapping natural oyster beds, with accuracy scores of 77.0 percent and 77.0 percent, respectively. Basically, the study serves as a valuable environmental and climatic proxy, facilitating integration into oyster aquaculture management programs, promoting informed decision-making, and encouraging sustainable practices. The application of GIS models, integrating climatic, environmental, and geomorphological variables, proves highly effective in identifying optimal sites for aquaculture practices. We recommend implementing these models in other coastal zones with similar geospatial parameters and models.

EVALUATING THE EFFICACY OF THE GREEN SEA URCHIN *Strongylocentrotus droebachiensis* TO MITIGATE BIOFOULING WHEN CO-CULTRED WITH ATLANTIC SEA SCALLOPS *Placopecten magellanicus*

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The emerging sector of scallop aquaculture production, much like other shellfish sectors, experiences problematic biofouling on gear (e.g. lantern nets, on bottom cages), which can oculate gear mesh, restrict water flow and potentially reduce shellfish growth by 30% or more. Farmers need to commit a large amount of labor to removing this fouling to maximize shellfish growth, with many seeking solutions to alleviate this issue. One solution is to integrate sea urchins, whose grazing activity has been shown to reduce fouling on aquaculture gear by as much as 40-50%. Previous studies have used differing shellfish and sea urchin species combinations, but the integration of the Northeastern Green Sea urchin (GSU; *Strongylocentrotus droebachiensis*) with Atlantic Sea Scallops requires investigation. GSUs are also an emerging sector of aquaculture, in response to sustaining a declining fishing industry. Their grow out techniques and environmental tolerances are extremely compatible with scallops, meaning that integration of these species has strong potential. This study investigated this integration in collaboration with the scallop producing farm, Casco Bay Mooring (ME), by focusing on optimizing animal size and stocking densities when cultured in lantern nets towards reducing biofouling and effort and maximizing production. We will overview the promising results of this study and incorporate the farmer's perspective on effort and labor reduction, and likelihood of uptake.

CREATING AN INNOVATIVE SMART FISH SMOKING TECHNOLOGY FOR CATFISH AQUACULTURE FARMERS IN NIGERIA

Elekwachi

As demand for fish continues to rise and traditional marine capture fisheries decline, aquaculture is becoming increasingly vital, particularly in Nigeria. This study presents a newly developed smart fish smoking technology designed to enhance catfish aquaculture in the Niger Delta. Traditional smoking methods, while culturally significant, pose health risks due to harmful chemical residues and are environmentally unsustainable. Our innovative technology addresses these issues by providing a safer, more efficient method of fish preservation that meets international food safety standards.

This advanced smoking technology not only prolongs shelf life and enhances flavor but also reduces waste and lowers the environmental impact associated with traditional practices. By improving the quality of smoked fish, we aim to open new markets both locally and internationally, thus supporting the livelihoods of catfish farmers in the region.

This presentation will detail the development and testing of the technology, its benefits for local producers, and its potential to improve food security in Nigeria. Engaging with stakeholders, we will evaluate the technology’s acceptance and discuss its implications for the future of aquaculture in Nigeria. Ultimately, this research seeks to transform fish processing practices, promoting economic growth while ensuring safer, healthier food options for communities reliant on this critical industry.

TOWARDS NOVEL SOLUTIONS FOR INDUCING OVARIAN MATURATION IN THE BLACK TIGER PRAWN, *Penaeus nonodon*

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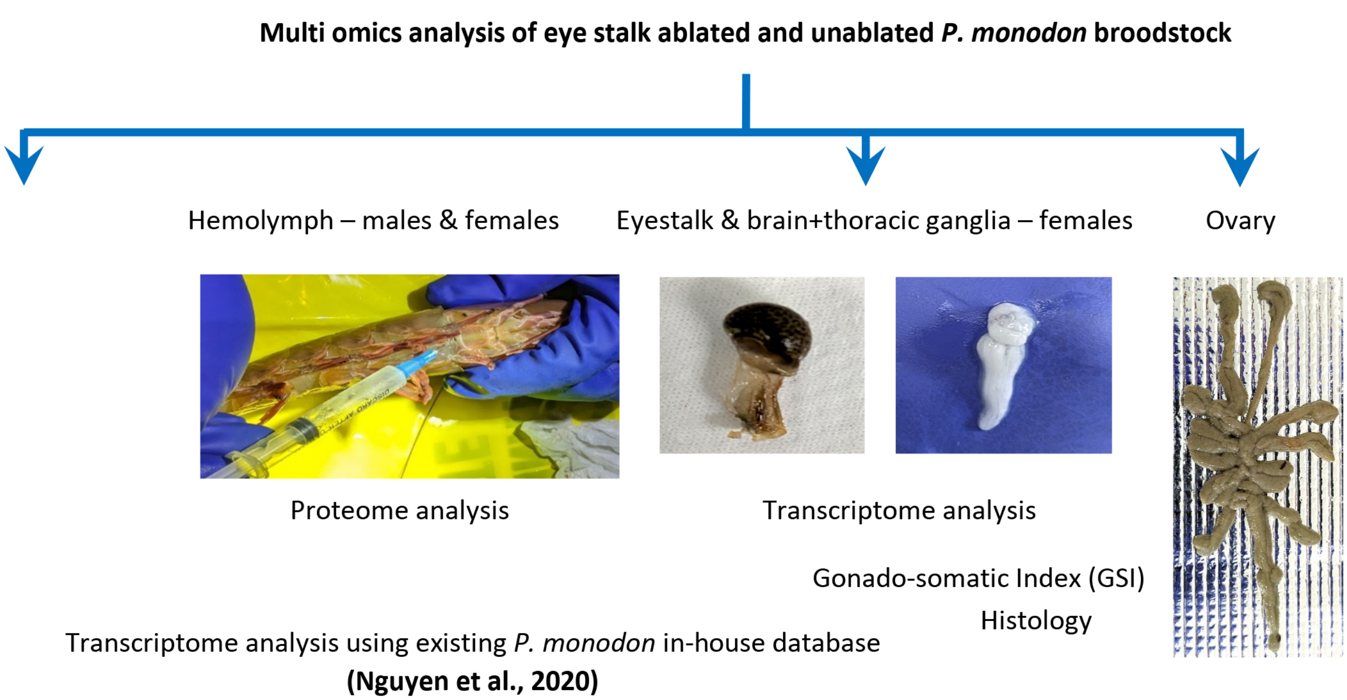
The black tiger prawn, *Penaeus monodon*, is considered one of the world's most commercially important decapod crustaceans. Despite the extensive progress in expanding *P. monodon* aquaculture, the species' reproductive biology is far from understood; control of ovarian development and spawning is essential for a sustainable domestication program. One key challenge is the failure of ovarian maturation and spawning in most captive-bred female *P. monodon*, which requires compromising the eyestalk to secure seed supply. Since eyestalk ablation is deemed unfavorable due to production and animal welfare aspects, exploring cost-effective and efficient alternative(s) to replace eyestalk ablation requires a better understanding of female penaeid reproduction. An in-depth understanding of the intrinsic factors regulating ovarian development in *P. monodon* is a pre-requisite towards the development of a farm-applicable method to overcome ovarian developmental arrest in domesticated broodstock, a long-term goal for sustainable *P. monodon* aquaculture. To address the above challenge, we have set to characterize key molecular factors governing ovarian maturation in *P. monodon* using a multi-omics study, to develop and validate non-invasive tools for assessing ovarian stages in captive-bred *P. monodon* broodstock, and to develop *in vitro* assays for assessing factors impacting reproductive development in *P. monodon* broodstock without sacrificing them.



ENHANCEMENT OF GONAD SIZE AND QUALITY IN PURPLE SEA URCHIN (*Strongylocentotus purpuratus*) FED A FORMULATED DIET, *Macrocystis pyrifera,* AND NUTRIENT-ENRICHED *Ulva australis* AND *Devalerae mollis*

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Urchin barrens along the California coast disrupt the natural, productive kelp forests, displacing important habitats and native species. Uni ranching, which involves collecting and fattening the gonads (uni) of starved urchins from barrens, offers a potentially profitable, new opportunity to produce uni. We measured uni growth and quality in purple sea urchins (*Strongylocentrotus purpuratus*, PSU) over 12 weeks using four different diets: (1) an experimental formulated diet (EFD), (2) nutrient-enriched *Ulva australis* and (3) *Devaleraea mollis*, and (4) wild collected *Macrocystis pyrifera*. *U. australis* and *D. mollis* were considered enriched because they were cultured in the effluent stream of white seabass (*Atractoscion nobilis*). PSUs were fed 2% bodyweight/ind/day, and feces were collected weekly to calculate ingestion rate (*IR*), fecal rate (*FR*), and absorption efficiency (*AE*). PSUs were sacrificed every three weeks to measure gonadosomatic index (*GSI*) and quality. Quality was graded by color (CIELAB color space, where *L** (lightness), *a** (green-red), and *b** (blue-yellow) values were used to calculate ΔE* for quantifying color difference), texture (granulation size), and firmness.

GSI from PSUs fed the EFD (15.7 ± 3.9%) and *M. pyrifera* diet (13.7 ± 2.7%) were not significantly different, but were significantly higher than *U. australis* (8.7 ± 3.1%,) and *D. mollis* (6.3 ± 2.1%) (Figure 1). Quality improved from grade 'D' to grade 'B' after 12 weeks in all treatments. Granulation size was significantly smaller for gonads fed *D. mollis* (0.64 ± 0.2mm) compared to the EFD, *M. pyrifera*, and *U. australis* (0.82 ± 0.24, 0.76 ± 0.26, 0.93 ± 0.26 mm). There was no significant difference in firmness of gonads between any diets. Based on values of *L*, *a* and *b*, ΔE was highest in gonads from EFD (17.54), followed by *U. australis* (16.75), *M. pyrifera* (15.87), and *D. mollis* (8.21). The EFD, which had the highest lipid content (6.42%) resulted in the greatest PSU *IR* (2.55 ± 1.14 DWg/ind/day) and *AE* (97.71 ± 1.52%). In contrast, PSUs fed *M. pyrifera* exhibited lower *AE* (68.78 ± 19.45%). *D. mollis* and *U. australis* had highest protein content (35.4 and 27.6%), but yielded the lowest PSU *IR* (0.17 ± 0.04 and 0.25 ±0.12 DWg/ind/day). Gonads of PSUs reached market size (*GSI* >10%) when fed with EFD and *M. pyrifera* for 12 weeks. *D. mollis* and *U. australis* require more time or feed supplementation to reach market size. Future research will extend the feeding period when using nutrient-enriched *U. australis* as the sole diet, and will also focus on mixed diets (EFD and seaweeds) in enhancing PSU gonad production and quality.

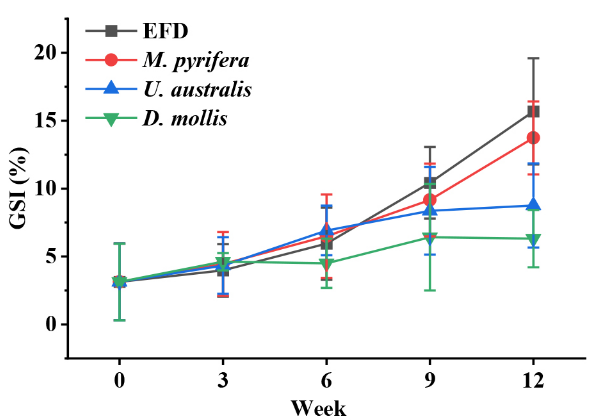

Figure 1. Change in *GSI* (%) for PSUs fed four diets over 12 weeks.

GROWTH AND WASTE REMOVAL CAPACITY OF THE WARTY SEA CUCUMBER (*Apostichopus parvimensis*) FED WITH WASTE FROM WHITE SEABASS (*Atractoscion nobilis*)

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The accumulation of nutrient-rich waste in aquaculture systems poses environmental challenges, particularly in monoculture setups. The warty sea cucumber (*Apostichopus parvimensis*, WSC) has shown potential for managing solid waste through integrated recycling. This study aimed to evaluate the growth, performance, and waste removal capabilities of WSCs when fed waste from white seabass (*Atractoscion nobilis*, WSB). The objectives were to determine the optimal feeding ration of WSB waste for WSCs and assess how WSC size influences growth, performance, and waste removal.

Three trials were conducted to address our objectives. In Trial 1, WSB waste nutritional quality was measured for changes over five days in seawater without WSCs. In Trial 2, 45 WSCs (100-150 g) were fed 1%, 2%, 3%, 4%, or 5% of body weight to determine the effects of daily ration on growth and waste removal capacities. In Trial 3, 45 WSCs were divided into three size classes (<100, 100-150, >150 g) and fed 4% of body weight to assess the impact of size on performance. Environmental parameters were monitored throughout the trials, with temperature ranging from 14.8°C to 23.4°C, pH from 7.82 to 8.30, dissolved oxygen from 7.76 to 11.16 mg/L, salinity from 30 to 35, and total ammonia nitrogen from 0.013 to 0.06 mg/L. All trials were conducted in 175 L tanks, run in triplicate, with regular measurements of waste, feces, and growth.

In Trial 1, the nutritional quality of WSB waste remained stable, with nitrogen content ranging from 2.96% to 3.5% and carbon content from 33.27% to 40.88%. In Trial 2, WSCs fed at 4% of body weight had a significantly higher specific growth rate (*SGR*) of 1.06% per day compared to other rations. Ingestion rate (*IR*) increased with higher feeding rations, ranging from 0.11 g DW/ind/day at 1% body weight to 0.58 g DW/ind/day at 5%. Fecal rate (*FR*) followed a similar trend, peaking at 0.30 g DW/ind/day in the 5% group. Apparent digestibility ratio (*ADR*) varied between 44.78% and 77.84%, with no significant differences between treatments. Waste removal efficiency (*WRE*) was highest at 4%, ranging from 28.75% to 44.05%. Standard length and width (*SLW*) measurements showed that WSCs fed at 4% had the highest *SLW* increase rate of 2.31% per day. In Trial 3, small WSCs demonstrated significantly higher nitrogen removal efficiencies, ranging from 42.67% to 48.71%, and carbon removal efficiencies from 34.95% to 48.16%, compared to large WSCs. The *IR* for large WSCs was 0.70 g DW/ind/day, significantly higher than the 0.30 g DW/ind/day for small WSCs. *FR* ranged from 0.12 g DW/ind/day for small WSCs to 0.29 g DW/ind/day for large WSCs, with *ADR* ranging from 57.10% to 59.84% across size classes. This study confirms WSCs as efficient waste removers, particularly when fed at 4% body weight. Smaller WSCs showed superior waste removal and growth, highlighting their role in optimizing bioremediation. Future research should explore long-term trials and scalability in larger commercial aquaculture systems.

SHRIMP PRODUCTION IN ALGAE-BASED RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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The United States shrimp market is currently dominated by imports, with much of the value captured outside the country. However, consumers are increasingly seeking local, fresh, and high-quality shrimp products. Land-based Recirculating Aquaculture Systems (RAS) offer a promising approach to producing shrimp closer to major markets. Despite this, several challenges remain, including waste discharge management, the high cost of artificial seawater, and production expenses.

Midland Co. has developed an innovative algae-based moving bed bioreactor (MBBR) that addresses these challenges by treating and recovering nitrogen, phosphate, carbon dioxide, and heavy metals. A pilot-scale production system utilizing this technology was successfully operated in Iowa, demonstrating its feasibility.

Preliminary results indicate that shrimp production in algae-based RAS holds significant potential. The system achieved low levels of ammonia and nitrite, prevented the accumulation of nitrate, phosphate, and heavy metals, and effectively removed CO2 through biological processes facilitated by algae. Moreover, the system maintained high water quality even under conditions of high stocking density. These findings suggest a new opportunity for sustainable shrimp production.

DIGITALIZATION OF LAND-BASED SHRIMP FARMING: FOCUS ON ANIMAL WELFARE THROUGHOUT PRODUCTION - (SHRIMPWIZ)

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Due to the high level of turbidity in the shrimp farm pond systems that exist worldwide, the detection of animal welfare using artificial intelligence such as automated image recognition is in principle impossible, as stressed or already diseased shrimp cannot be distinguished from healthy animals visually. Unlike in pond production, the water in Oceanloop GmbH's land-based facilities is clear. This advantage in terms of animal welfare detection and sustainability has already been successfully demonstrated in the previous project; Computer vision allowed the length and number of animals to be determined with an accuracy of over 90% and visual stress indicators (also with 90% accuracy) could be recorded - for the first time in real farming conditions. In the project recently funded for a period of two years (until June 2026), existing AI is to be further developed to market maturity. The aim is to use automated image recognition software to record and validate animal welfare and mortality throughout the entire production chain, from juvenile animals to market-ready shrimp. The project is related to a number of agricultural policy objectives such as competitive agriculture or healthy nutrition and safe food. Such policies will ultimately effect Germany's consumer behaviour. Shrimps in German supermarkets currently come almost exclusively from shrimp farms in non-EU countries. Proof of the welfare in clear water production systems will provide consumers with guidance when buying and at the same time highlight the advantages of local land-based shrimp farming. At the WAS 2025 we precent latest data generated up until the conference date.

The project partners are the company Oceanloop GmbH Kiel and the Alfred Wegener Institute for Polar and Marine Research Bremerhaven. The project was funded by the German Innovation Partnership for Agriculture (DIP) funding, Federal Ministry of Food and Agriculture (BMEL) on the basis of a decision by the German Bundestag. The project was sponsored by the Federal Office for Agriculture and Food (BLE) as part of the programme to promote innovation. Funding code 281DT10B23.

TIDE'S OUT: SHELLFISH AQUACULTURE WORKFORCE DEVELOPMENT PROGRAMS

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Washington State is the largest producer of farmed bivalves in the nation and is currently facing a substantial decline in aquaculture workforce recruitment and retention. An informal needs assessment conducted by Washington Sea Grant (WSG) and Oregon Sea Grant revealed several underlying challenges, including a general lack of career awareness, challenging working conditions, limited housing and transportation infrastructure in rural communities, and cultural and generational shifts around workplace expectations and norms. To begin addressing these challenges, WSG is developing Tide's Out – a bilingual workforce development program that includes both crew training and manager training components. Tide's Out is guided by an Advisory Committee composed of experts with shellfish industry and/or diversity, equity and inclusion (DEI) in maritime expertise. In year one, WSG piloted the manager training program, which included managerial leadership skills, employee recruitment and retention best practices, strategies for working with diverse employees, and resources to improve employee safety and well-being. In year two, we revised the manager training curriculum, developed the crew training curriculum and ran both training courses simultaneously, wrapping up the 2025 Tide's Out program with a job fair connecting newly trained employers and potential employees. In 2026, we will implement both training programs again, and revise and adapt them based on lessons learned from this year.

WEST COAST AQUACULTURE COLLABORATIVE

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Washington Sea Grant (WSG), Oregon Sea Grant, California Sea Grant, and Alaska Sea Grant (AKSG) have continued to grow and maintain a collaborative unit to engage science and policy partners, industry and resource management agencies in tackling complex, region-scale barriers to sustainable aquaculture on the West Coast. Throughout the project period, West Coast Sea Grant programs have regularly shared updates and information related to aquaculture in each state. Specifically, this project began in 2019 with the first grant funding opportunity and then was awarded a second grant to continue and implement new projects in 2022. The first grant funding opportunity focused on the Washington Coast Shellfish Aquaculture Study (WCSAS), a three-year program of integrated stakeholder engagement and research guided by stakeholders and scientists, coordinated by WSG, and funded by the Washington State Legislature, the West Coast Hub, and other grants. The end goal of this project was to sustain long-term shellfish aquaculture in the coastal estuaries by establishing an Ecosystem Based Management Collaborative, which then launched in Spring, 2022. The current iteration of the West Coast Aquaculture Collaborative (WC Hub) is focused on biosecurity issues emerging along the West Coast in the shellfish industry, aquaculture farm preparedness for disasters, and the exchange of information between farms in different regions. The WC Hub has given biosecurity presentations, hosted workshops, and disseminated factsheets addressing the Pacific Oyster Mortality Syndrome OsHV-1µvars (POMS) in California and Washington. The WC Hub has also conducted workshops and presentations on disaster preparedness to train growers on the Emergency Livestock Assistance Program (ELAP) process. Lastly, WSG and AKSG have been working together to build a three-day information exchange between shellfish growers where Alaska growers will visit Washington farms and facilities specializing in off-bottom shellfish culture methods, farm to table operations, Tribal shellfish programs, and restoration aquaculture projects.

EXPLORING THE IMPACT OF OSMOTIC STRESS ON VIRULENT *Aeromonas hydrophila*, THE CAUSATIVE AGENT OF MOTILE *AEROMONAS* SEPTICEMİA (MAS) IN U.S. CATFISH AQUACULTURE

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Virulent *Aeromonas hydrophila* (vAh), a Gram-negative, facultative anaerobic pathogen, causes severe outbreaks of motile *Aeromonas* septicemia (MAS) in U.S. catfish production, the largest component of the aquaculture industry in the United States. In 2022, catfish production contributed $447 million to the nation's economy according to estimates by the USDA. Tens of millions of pounds of market-size catfish are lost annually due to this significant catfish pathogen. Typically, *A. hydrophila* causes swift outbreaks characterized by rapid onset and high mortality rates. However, there are no preventive strategies developed for this pathogen and the current control measures are ineffective.

An analysis of farm-level risk factors for MAS outbreaks in farmed catfish in the southeastern U.S. showed that using salt (sodium chloride, NaCl) in catfish farms significantly decreased the incidence of vAh outbreaks. Therefore, the objectives of this study are to explore bacterial growth dynamics, morphological changes, survival, biofilm formation, and virulence of vAh grown in a standard culture medium but with different salt concentrations (0.5%, 1.5%, and 4.5% salt). Our preliminary data suggests a negative correlation between salinity and vAh growth. Moreover, biofilm formation was reduced at higher salinity conditions. Bacterial morphology and survivability were also assessed and the results will be presented. In vivo trials revealed that high salt concentration decreased the virulence of vAh. Overall, the results of our research show that high salt concentration negatively impacts vAh. This research provides a better understanding of how vAh survives in the aquatic environment and includes important information regarding the management of MAS outbreaks in catfish ponds.

CHARACTERIZING THE MECHANISMS UNDERPINNING THERMAL TOLERANCE IN SELECTIVELY BRED GREEN-LIPPED MUSSELS *Perna canaliculus*

Jessica Ericson*, Natali Delorme, Stella Mackrell, Summer Norgate, Rodney Roberts, Alba Cervantes-Loreto, Paul South, Camille Baettig, Leonardo Zamora, Benjamin Knight, Laura Biessy, Olivier Laroche, Xavier Pochon, Jacob Thomson-Laing, Kasey Mollison, Samantha Carter, Tom Corbin, Daniel Cross, Haydn Read, Nick King, Kirsty Smith, Norman Ragg, Megan Scholtens

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New Zealand's aquaculture industry is at increasing risk from intense marine heatwaves which threaten the productivity and sustainability of marine farms, emphasizing the need for targeted genetic solutions. *Perna canaliculus* (Greenshell mussels; GSM) are an endemic New Zealand mussel of cultural and commercial importance that have been selectively bred since 2002. This well-established family-based breeding programme provides an opportunity to breed for thermal tolerance and improve survival and growth during periods of elevated temperature.

In collaboration with industry, we have developed a reliable and repeatable survival challenge to compare thermal tolerance of different mussel families, with survival assessed as the number of days to death at 26°C (maximum sea surface temperature in summer). We have used this method to assess heat tolerance in adult families of different ages (from 1-3 yrs). Results to date show that heat tolerance in GSM is moderately heritable ($h2 = 0.27$-0.54), suggesting that if thermal tolerance is included in a selection index, there is adequate genetic variation to enable genetic improvement of thermal tolerance in this GSM population. We have also found that in juvenile and sub-adult mussels (aged 6-52 weeks post-fertilisation), both genetics and ontogeny strongly influence thermal resilience when these mussels are exposed to an acute heat challenge.

In parallel to GSM survival challenges, we have measured gene expression levels of the 70 kDa heat shock protein (Hsp70), immune responses, the transcriptome and the microbiome of mussel haemolymph or gill samples from selected families. This helps us to understand how the different families respond physiologically to heat stress, and gives us the opportunity to identify and compare biomarkers of heat stress in families with high and low heat tolerance.

These results highlight the complex interactions between genetics, age and size which all determine heat tolerance in GSM. Our findings indicate that selective breeding for heat tolerance is promising in GSM, and is likely to be a key tool used for climate change adaptation into the future.

SHELLPULSE: A CLIMATE CHANGE OUTREACH PROGRAMME THAT CONNECTS COMMUNITIES WITH SHELLFISH AQUACULTURE

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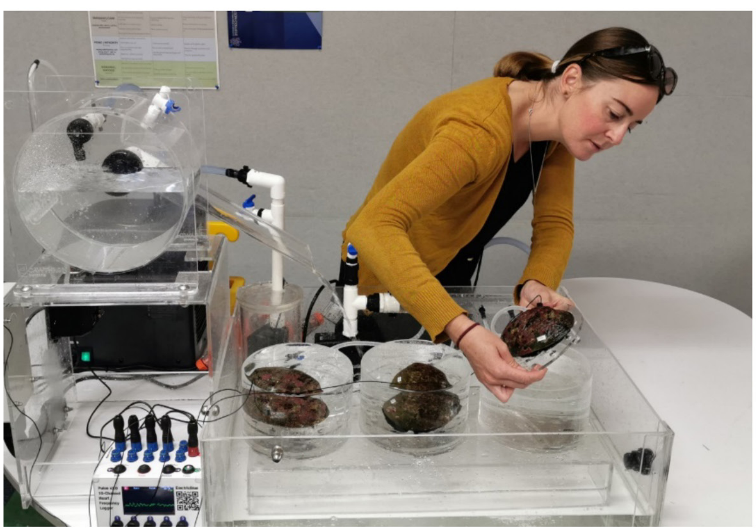
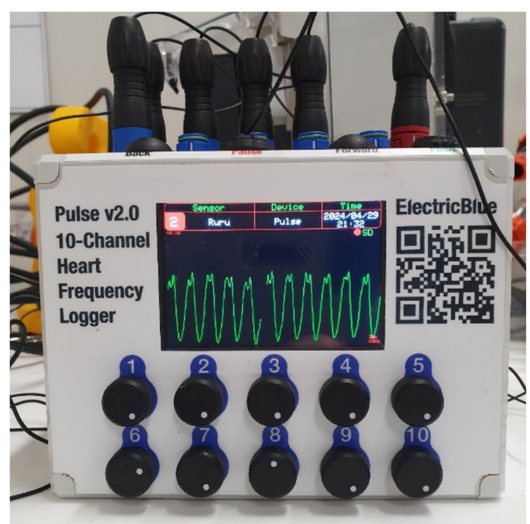
Climate change is altering our marine ecosystems and threatening global aquaculture production. We have a responsibility as aquaculture scientists and marine farmers to communicate our research effectively to the public, and connect communities with our shellfish. It is through these interactions that our science can be most impactful.
The majority of scientific research tools are arguably ‘not that interesting’ to the public. However, biosensor equipment that measures shellfish behaviour (e.g. movement, heart rate, oxygen consumption) can engage audiences in a relatable way (e.g. shellfish have hearts like us!?), and the confinement of these tools to the lab is a missed opportunity.

Cawthron’s Shellfish Aquaculture Research Platform has recently created a portable “marine living lab” to showcase our biosensors to the wider community. This simple recirculating seawater tank (see below) enables shellfish to be transported and used for public outreach. We use mussels and abalone as our case study species, and tailor our hands-on activities to different audiences. For example, our non-invasive heart rate monitors can be attached to the shellfish, and members of the community can compare the shellfish heart rate with their own. A key focus of the activities is to facilitate discussions on how climate change might influence shellfish behaviour, resilience and health. We then discuss what this means for shellfish aquaculture, and how we use our research tools to enable climate change adaptation.

I will highlight some examples of the living lab in action, and discuss the dual powers of using hands-on experience and narrative in science communication.





ELUCIDATING MUSSEL LARVAL DYNAMICS IN CASCO BAY, MAINE WITH USER-FRIENDLY, ERNA-BASED TOOLS

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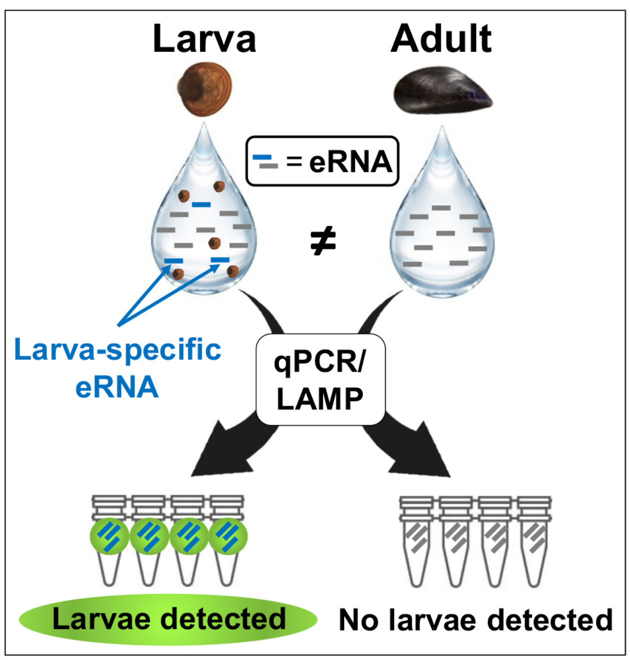
Blue mussel aquaculture's dependence on wild-caught seed has posed considerable complications for farmers in recent years due to increasingly variable seasonal fluctuations in larval availability. Regular monitoring of the plankton community on an aquaculture lease to identify the optimal timing for seed collection can help alleviate potential seed recruitment failures and resulting financial and production losses. Recently, we developed a promising mussel larvae monitoring protocol that combines traditional plankton tow sampling with environmental RNA (eRNA) quantitative PCR (qPCR) assays that allow for the specific detection of blue mussel larvae. Here, we will discuss our monitoring results from a mussel farm in Casco Bay, ME over a nearly two-year period, compare the results of plankton tow counts vs. eRNA qPCR assay quantification, and relate these results to environmental data collected on the farm. In addition, we will describe current efforts to convert our eRNA qPCR assays into more accessible loop-mediated isothermal amplification (LAMP) assays, which do not require specialized equipment and offer a simple colorimetric result for the visual determination of presence/absence of mussel larvae in a water sample. These tools and results will not only provide stakeholders with a user-friendly and rapid method to predict the ideal timing for seed collection, but they will also result in a better understanding of the phenology of mussel larvae and the environmental variables that drive it.

Figure 1: Schematic for detecting mussel larvae in water samples with eRNA tools.



CHARACTERIZING THE DEVELOPMENTAL TRANSCRIPTOME AND METHYLOME OF THE BLUE MUSSEL *Mytilus edulis*

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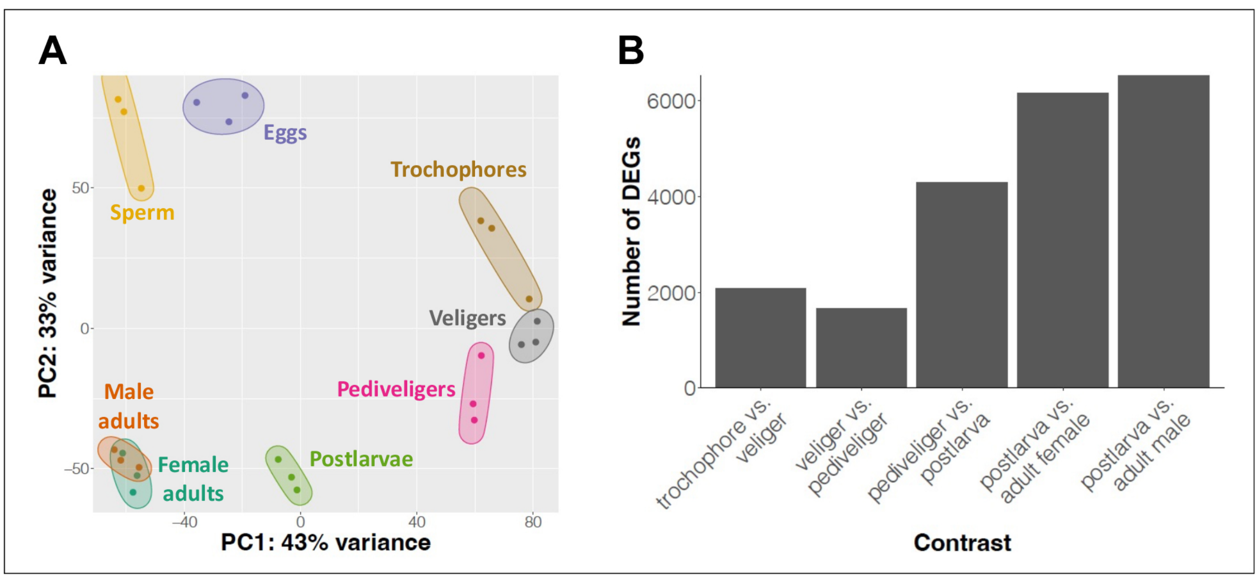
The blue mussel, *Mytilus edulis,* plays important roles as both a keystone and aquaculture species across the globe. Although many studies have focused on understanding mussel development for ecological and hatchery-related purposes, surprisingly little is known about the molecular changes that underlie mussel ontogeny. To obtain a comprehensive view of these molecular patterns, we collected hatchery samples in various stages of development (egg, sperm, trochophore, veliger, pediveliger, postlarva, and adult), co-extracted RNA and DNA, and sequenced each of these samples using RNA-seq and whole genome bisulfite sequencing (WGBS), respectively. Gene expression analyses revealed strong stage-specific clustering patterns (Fig. 1A) and large numbers of differentially expressed genes between developmental transitions (e.g., pediveliger to postlarva; Fig. 1B), indicating extensive differences in gene expression profiles across stages. In addition, a large set of genes exhibiting stage-specific expression were identified. Similarly, differential DNA methylation analyses revealed many differentially methylated loci throughout the genome when contrasting stages spanning developmental transitions, as well as several loci that exhibited stage-specific methylation. These results significantly advance our understanding of blue mussel development and provide valuable novel genomic resources for aquaculturists and scientists alike.

Figure 1: (A) Principal components analysis (PCA) plot showing how mussel developmental stages cluster by gene expression profile. (B) Bar chart of number of differentially expressed genes (DEGs) for each developmental transition.



OPTIMIZING PROCESS PIPING DESIGN FOR RECIRCULATING AQUACULTURE SYSTEMS: FLUID MECHANICS AND PRACTICAL INSIGHTS

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Process piping is a critical component in recirculating aquaculture systems (RAS), where poor design can lead to a range of issues including degraded water quality, suboptimal unit performance, excessive energy consumption, and, in extreme cases, safety hazards for both fish and personnel.

This presentation provides an overview of essential fluid mechanics principles in pipe design, with a focus on the role of water velocity in optimizing RAS unit processes. Practical design and construction tips, grounded in field experience, will also be shared to help overcome common challenges and ensure efficient system performance.

CHARACTERIZING CULTIVABLE BACTERIAL COMMUNITIES IN INVASIVE BLUE CRAB (*Callinectes sapidus*) HAEMOLYMPH: EFFECTS ON MARINE ECOSYSTEMS IN THE NORTHERN ADRIATIC SEA

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This preliminary investigation examined the bacteriological profile of blue crab (*Callinectes sapidus*) haemolymph to identify potential pathogens and evaluate their impact on marine ecosystems. Haemolymph samples were collected from blue crabs in the Northern Adriatic Sea (Italy), with analyses conducted on specimens categorized by carapace length (small: 51-99 mm and large: ≥100 mm). Additionally, seawater samples from the collection site were analysed to assess the bacterial communities present in the surrounding environment. Bacteriological assessments were carried out using various culture media, MALDI-TOF MS, molecular identification based on the *rpoB* gene, and antibiotic susceptibility testing (Kirby-Bauer method).

The results indicated a diverse array of bacterial species, predominantly *Vibrio* spp., some of which displayed resistance to antibiotics. In particular, the *Vibrio* species found in the blue crabs were similar to those identified in the adjacent seawater, emphasizing the direct ecological relationship between the two environments. These findings highlight the necessity of monitoring bacterial pathogens in blue crab populations, particularly given their invasive nature and the considerable ecological and economic impacts they have inflicted in the Adriatic region.

This results are critical for formulating effective management strategies to control the dissemination of pathogens and preserve the health of marine ecosystems. Understanding the bacterial composition of blue crab haemolymph will help address the challenges posed by this invasive species, contributing to the sustainability of blue crab fisheries and the protection of marine biodiversity.

GENETIC DIVERSITY IN SARDINIAN COASTAL ECOSYSTEMS (ITALY; MEDITERRANEAN SEA): PERSPECTIVES ON *Ostrea* spp. (BIVALVIA: OSTREIDAE)

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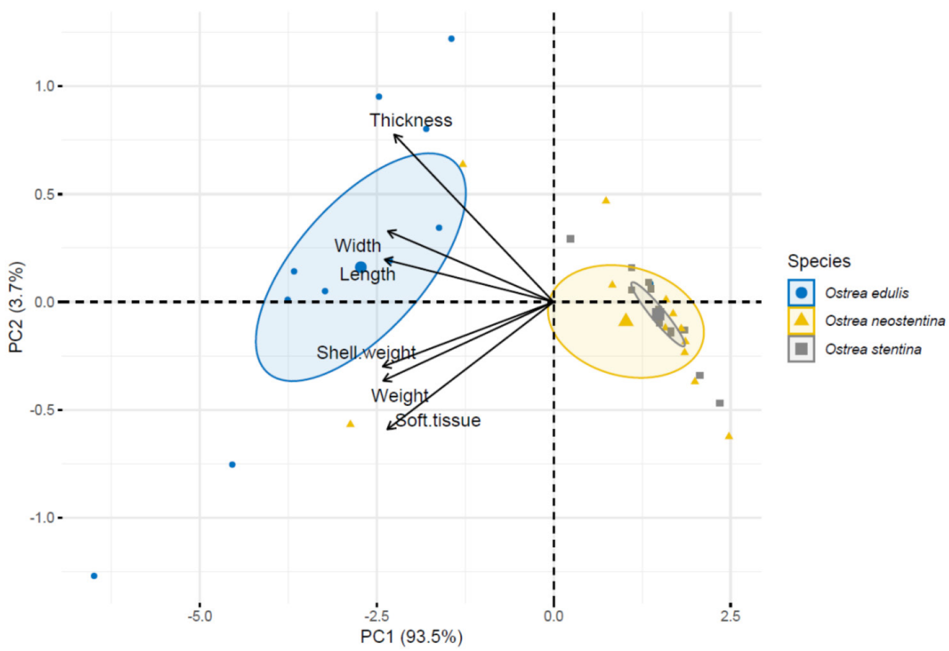
Oysters, as sessile, filter-feeding bivalves, are prevalent in estuarine and coastal ecosystems around the globe. They are crucial for both fisheries and aquaculture, offering significant ecological benefits. However, their genetic diversity and distribution remain largely unexplored. Variability in shell shape adds complexity to species classification, which is affected by both environmental and genetic influences (Fig. 1). While advances in molecular phylogenetics have improved oyster taxonomy and revealed approximately 100 currently recognized species, many taxonomic ambiguities still exist.

This study focused on documenting the presence of small flat oysters belonging to the genus *Ostrea* in the Mediterranean coastal regions of Liguria and Sardinia, Italy. To identify *Ostrea* species, we utilized 16S rRNA sequence data. Our results provide new insights into the *O. stentina* species complex and identify *O. neostentina*, a novel species within Mediterranean coastal areas of Italy. The findings enhance our understanding of the diversity, distribution, and evolutionary dynamics of *Ostrea* species.

Figure 1. Biplot of Principal Component Analysis (PCA) of morphometric data.



MORPHOMETRIC AND ENVIRONMENTAL VARIATIONS OF THE GROOVED CARPET SHELL (*Ruditapes decussatus*) IN DIFFERENT MEDITERRANEAN COASTAL SITES: IMPLICATIONS FOR MARINE ENVIRONMENT AND AQUACULTURE

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The Ramsar Convention (1971) emphasizes the conservation and sustainable use of wetlands, encompassing 1,100 sites across Europe that cover 27.9 million hectares. Coastal Mediterranean lagoons are crucial for fish production and biodiversity but face increasing pressures from human activities. Approximately 400 lagoons exist in this region, covering 641,000 hectares, where traditional aquaculture practices have been implemented. However, the degradation of these ecosystems has prompted investigations into the environmental and health impacts of such activities.

Italy hosts 198 coastal environments, totalling over 167,000 hectares, with 40,000 hectares dedicated to extensive aquaculture. Key species include the Mediterranean mussel (*Mytilus galloprovincialis*), Japanese carpet shell (*Ruditapes philippinarum*), and Pacific cupped oyster (*Crassostrea gigas*), along with significant mullet species such as thinlip grey mullet (*Chelon ramada*) and flathead grey mullet (*Mugil cephalus*).

Sardinia, with its extensive 1,900 km coastline, contains 77 brackish environments, 27 of which are used for aquaculture, notably the lagoons of Cabras and S. Giovanni-Marceddì, which are vital for the production of the grooved carpet shell (*Ruditapes decussatus*). This species is harvested seasonally, contributing significantly to local aquaculture and cultural identity.

This study investigates the morphometric and environmental variations of *R. decussatus* across different coastal sites in Sardinia. Measurements of shell length (cm), width (cm), and thickness (cm) were taken from specimens collected at various sites, revealing substantial morphological variation. Results indicate that shell characteristics correlate with environmental factors. These findings underscore the necessity for continuous environmental monitoring to support sustainable management practices in both wild populations and aquaculture systems.

ENHANCING AQUACULTURE RESILIENCE: INSIGHTS FROM THE AQUAE STRENGTH PROJECT

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Aquaculture has become a rapidly expanding sector in animal production, driven by the increasing demand for sustainable protein sources. Yet, fish diseases pose ongoing challenges, requiring innovative control strategies to optimize aquaculture productivity. The Aquae Strength project was launched to strengthen fish disease management and enhance GIS capabilities for effective disease surveillance and response. The initiative spans multiple beneficiary institutions across regions from North Africa to the Indochinese Peninsula.

The Aquae Strength project, titled "Strengthening Capacity on Aquatic Animal Health and Epidemiological Surveillance", focuses on key objectives aligned with WOAH's Aquatic Animal Health Strategy. Led by a consortium of seven Italian institutes - namely, Istituto Zooprofilattico Sperimentale (IZSVe, IZSSA, IZSPLV, IZSAM, IZSLT, IZSUM, and IZSM) - and three international advisory partners from the UK (Cefas), Denmark (DTU), and Norway (NVI), the project began with online webinars that introduced stakeholders to its core topics. Following this, the project team conducted in-person evaluations of diagnostic capacities for fish pathogens in each beneficiary country. These assessments, comprising tailored questionnaires, farm and laboratory visits, and discussions with local teams, identified critical gaps to guide the project's next steps.

Key intervention areas focus on enhancing diagnostic capabilities, promoting responsible Veterinary Medicinal Product (VMP) use, engaging veterinary services in outbreak management, and developing an official farm registry. Notably, differences in diagnostic capacities and VMP management among the beneficiary countries were observed, as well as a need for clearer roles for veterinary services during outbreaks. The project team also advocated for diagnostic laboratories to implement antimicrobial susceptibility testing (AST). The absence of an official fish farm registry and limited diagnostic facilities pose challenges to epidemiological evaluation and traceability, which are crucial for these countries seeking to establish EU trade compliance in animal health and food safety.

Training was tailored to the unique needs of each stakeholder, taking place both in beneficiary countries and within the consortium's laboratories. By enhancing fish health management and disease response, the project contributes to food safety, economic growth, and environmental sustainability. Supporting these nations' aquaculture sectors unlocks the full potential of aquatic resources, making the Aquae Strength project a key international effort in building a sustainable and resilient aquaculture industry aligned with the One Health approach.

IMPACT OF PISCINE LACTOCOCCOSIS OUTBREAK ON SERUM BLOOD PARAMETERS IN FARMED RAINBOW TROUT (*Oncorhynchus mykiss*)

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The aquaculture sector plays a vital role in global food security, yet it grapples with significant challenges posed by infectious diseases. Piscine lactococcosis represents a major threat in rainbow trout farming, as it can lead to severe economic losses through increased mortality rates, decreased growth, and heightened vulnerability to additional pathogens. This situation complicates disease management approaches, affecting the sustainability and profitability of rainbow trout aquaculture.

The study investigates the changes in serum blood parameters in farmed rainbow trout (*Oncorhynchus mykiss*) during an outbreak of piscine lactococcosis caused by *Lactococcus garvieae*. Blood samples were collected for biochemical analysis, and the fish were screened for parasites and bacteria. DNA from the isolated bacterial colonies was subjected to PCR amplification and sequencing for identification. A total of thirteen biochemical parameters, including proteins, enzymes, lipids, chemicals, and minerals, were assessed in serum samples from both infected and healthy fish.

The findings demonstrate significant variations in the levels of these parameters during the outbreak, underscoring the effects of infections on the blood profiles of rainbow trout. Specifically, urea concentrations were higher in infected fish compared to healthy controls, with creatinine, phosphorus, and magnesium exhibiting similar patterns. Conversely, alanine aminotransferase and total protein levels were elevated in the control group. There were significant differences in chloride levels between the two groups, while iron concentrations were found to be higher in the controls and lower in the diseased fish. Other parameters did not show significant differences.

This study highlights considerable changes in serum blood parameters of rainbow trout during an outbreak of lactococcosis caused by *L. garvieae*. These alterations suggest that these parameters could serve as indicators for monitoring health status, stress levels, and aquaculture management. Ongoing monitoring can provide valuable insights into disease severity and overall fish health, assisting in the formulation of enhanced management strategies. The results contribute to a deeper understanding of the pathophysiology of piscine lactococcosis and the development of effective intervention measures for farmed rainbow trout.

EXPLORING THE ETIOLOGY OF SYSTEMIC GRANULOMATOSIS IN MEAGRE (*Argyrosomus regius*)

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The meagre (*Argyrosomus regius*), a fast-growing species, is increasingly valued for sustainable aquaculture along the Mediterranean and Eastern Atlantic coasts. However, Systemic Granulomatosis (SG), a condition characterized by multifocal granuloma formation in multiple tissues, presents a major challenge for meagre aquaculture.

This study investigates the potential link between *Mycobacterium* spp. and SG in offshore floating cage aquaculture facilities in Sardinia (Italy; Mediterranean Sea).

In June 2022, thirty-four adult, apparently healthy meagre were randomly selected from the facility with a stocking density of 10-20 kg/m³. Environmental conditions included water salinity levels of 37-40, and a surface water temperature of 25±1.0 °C. The fish averaged 34.5±3.5 cm in total length and 389±67 g in weight. Samples were analysed using histological, microbiological, molecular, metagenomic, and *in situ* methods to detect potential pathogens. Ziehl-Neelsen (ZN), periodic acid-Schiff (PAS), and Giemsa stains were applied to identify acid-fast bacteria, common parasites, and fungi within granulomas.

Granulomas were observed in 31 out of 34 fish (91%), affecting organs including the kidney (88%), liver (47%), heart (41%), intestine (17.6%), and brain (5%). Acid-fast staining, alongside *Mycobacterium* spp.-specific quantitative PCR (qPCR), *in situ* hybridization (ISH), and microbiological analyses, returned negative for *Mycobacterium* spp., and other known bacteria involved in granuloma formation.

However, PCR amplification and sequencing of the 65-kDa heat shock protein gene identified *M. chelonae* in 13% of formalin-fixed and frozen liver samples. Despite this, bacterial culture did not yield nontuberculous mycobacteria (NTM) or other bacteria commonly associated with granulomas, and ISH with an *M. chelonae*-specific probe failed to detect this bacterium in granulomas.

Overall, the findings do not support *M. chelonae* as a cause of granulomas and suggest ruling out a link between nontuberculous mycobacteria (NTM) and SG. These results highlight the need for further investigation into the underlying causes of SG in meagres, emphasizing the importance of distinguishing between infectious and non-infectious factors (e.g., nutritional or autoimmune factors). Future studies should aim to identify specific pathogens or conditions contributing to granuloma formation to enhance disease management strategies in aquaculture.

BIO-TUTELA PROJECT: REGIONAL CENTER FOR AQUATIC BIODIVERSITY (BIOAQUA), SUPPORTING CONSERVATION AND PROTECTION OF COMMON BLEAK (*Alburnus arborella*) POPULATIONS

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The common bleak (*Alburnus arborella*) is a small zooplanktivorous cyprinid fish that holds significant importance both for its ecological role in lacustrine ecosystems and its naturalistic value. Historically, it also played an important role in the fishing economy of Italian subalpine lakes.

Several factors have been identified as potential causes for the alarming decline of this freshwater species in prealpine lakes. These include the trophic evolution of water bodies, habitat alterations affecting reproduction, predation (e.g., wels catfish *Silurus glanis*, etc.), and competition with non-native species.

The BIO-TUTELA project, aims to conserve and protect the populations of *A. arborella* in the Piedmont region (Northern Italy). Managed by the Regional Centre for Aquatic Biodiversity (BIOAQUA) of the "*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria, e Valle d'Aosta* (IZSPLV)", the project focuses on key objectives, including:

1. Studying the demographic structure of *A. arborella* populations in the Avigliana Lakes (Turin, Italy) to develop management strategies and targeted restoration interventions;
2. Conducting genetic analyses to understand the genetic diversity of the species and identify potential conservation risks;
3. Implementing controlled breeding and aquaculture programmes to support the conservation and restoration of local fish populations.

Through these integrated actions, the project aims to provide essential data for the conservation and sustainable management of fish resources in the Piedmont region of northern Italy.

STEPWISE INVOLVEMENT OF PEPTIDASES IN THE DIGESTIVE PROCESS OF THE WHITELEG SHRIMP *Penaeus vannamei*

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While significant progress has been made in understanding the digestive process of *Penaeus vannamei*, the specific sequence of enzyme participation in the protein digestion process is still unknown. Since the whiteleg shrimp is a major aquaculture product in Mexico, this knowledge becomes important to improve the culture process. To accomplish this, the digestive peptidases abundance and activity in the shrimp digestive gland were analyzed at three different times, each representing an essential stage in the digestion process. Zymograms and Tandem Mass Tags (TMT) were used for activity and relative quantitative proteomic analysis.

Midgut glands were sampled three times during the digestion process: preprandial, postprandial 1 h, and postprandial 3 h. Samples were examined by activity tests (zymograms) and proteomics. Proteins were digested with MS-grade trypsin, peptides labeled with Amine-reactive TMT-10plex and fractionated by SCX. Protein identification and relative quantitation analysis was performed by SPS-MS3 using an Orbitrap and raw-data were processed with Proteome Discoverer 2.1 using Amanda, Sequest-HT, and Mascot search engines against the *P. vannamei* proteome database (UP000283509).

Results showed that serine peptidases (trypsin and chymotrypsin isoforms) were active overall during the digestion process, but differences in relative abundances were detected in only two trypsins, trypsin 2 which decreased at postprandial 1 h and 3 h, and trypsin 1 which increased at postprandial 1 h and decreased again in postprandial 3 h, while both chymotrypsins increased their abundance at postprandial 3 h. Digestive cathepsin D (A0A423SAG9) and cathepsin L both showed significant changes in activity and abundance at preprandial (cathepsin D) and postprandial 3 h (cathepsin L). Two digestive metallopeptidases showed a similar change with their maximal abundances during postprandial 3 h, and no activity during postprandial (3 h).

Even though trypsin and chymotrypsins are the most important peptidases in the digestive process, regulated peptidases were cathepsins, cathepsin D being most abundant at preprandial, whereas cathepsin L during postprandial. Metallopeptidases had similar abundance in the three times but showed no activity at postprandial.

AQUACULTURE IS AN EDUCATION

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Using aquaculture and especially aquaponics for STEM education has the potential to energize the way we approach learning about food production and sustainability. Our experience with the LSSU aquaculture club demonstrates the multifaceted benefits of this innovative approach to education. Aquaponics serves as an excellent platform for interdisciplinary learning, combining elements of biology, chemistry, physics, and environmental science.

Specifically, in the classroom, students have the opportunity to gain hands-on experience with water chemistry and system monitoring, fish behavior and health management, plant growth and nutrition, insect pest control and integrated pest management (IPM), microbiology and ecosystem dynamics. Aquaponics also reinforces mathematical concepts through calculations of fish and plant densities, determining growth rates and feed rations, and analyzing system parameters such as temperature, pH, TDS or conductivity. Students can also gain a holistic view of ecosystem interactions by understanding fish-microbe-plant relationships, nutrient and energy flows, and environmental factors that limit system boundaries. Aquaponics also provides a foundation for diverse upper level research projects. Some examples of LSSU projects are: multi-trophic mariponics, aquaponic silviculture, microbiome DNA sequencing, indicators of environmental pollutants, nitrogen cycle dynamics.

The LSSU aquaculture club oversees our aquaponics facilities, and their club activities foster essential skills such as teamwork and leadership, responsibility and time management, problem-solving and critical thinking. The club also learns valuable lessons in marketing and sales through campus plant sales, upcycling and repurposing through creative projects and financial management to use revenues for conferences and field trips. The club's initiatives extend beyond the campus as their system are available for K-12 tours that promote STEM education. Beyond academic knowledge, aquaponics teaches a respect for living organisms, sustainable food production practices and resource management and conservation.

In conclusion, aquaculture education through aquaponics offers a unique and engaging approach to learning that transcends traditional disciplinary boundaries. It equips students with practical skills, fosters environmental awareness, and provides a platform for innovative research and entrepreneurship. This hands-on, fun learning environment not only produces food but also cultivates a deep understanding of the interconnectedness of life and the importance of sustainable practices.

PUBLIC ENGAGEMENT IN THE AQUACULTURE OPPORTUNITY AREA PROCESS

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An AOA is a defined geographic area that NOAA has evaluated through both spatial analysis and the programmatic National Environmental Policy Act (NEPA) process and may be environmentally, socially, and economically appropriate to support multiple commercial aquaculture operations. Final suitability will be determined with project-specific details.

This is a multi-year planning process through which we intend to maximize the compatibility of AOAs with other ocean uses while maintaining our commitment to ocean stewardship and our marine resource conservation responsibilities. NOAA has worked extensively with our partners to seek public comment and engage with the public throughout the AOA identification process. This presentation will provide and overview of the engagement process, what feedback was received, and how this shapes NOAA's efforts to identify AOAs.

PARENTAL PRIMING FOR OCEAN ACIDIFICATION RESILIENCE IN MANILA CLAMS: MECHANISTIC INSIGHTS GAINED THROUGH EGG TRANSCRIPTOME ANALYSIS

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Manila clams (*Ruditapes philippinarum*) are an economically valued bivalve species that are the target of a valuable global aquaculture industry. As ocean acidification (OA) increases, bivalves like Manila clams and the calcium carbonate structures they utilize are threatened by ongoing changes in carbonate chemistry. Investigations into specific impacts of acidification on clams and other environmentally and economically important bivalves have revealed that acidic conditions reduced larval growth, settlement rates, shell thickness and adult growth. Parental priming is one method that can be used to combat these impacts and maintain or improve growth in an aquaculture setting. As a first step to evaluating the physiological influence of environmental perturbation on clam offspring it is valuable to understand how parental gametes are impacted.

This study uses transcriptomics to evaluate differences in egg gene expression to investigate the impacts of OA conditions on unfertilized eggs from Manila clam parents reared in acidic (pH 7.4) and ambient (pH ~7.8) conditions. Forty-six differentially expressed genes were identified in a preliminary analysis with a majority of them expressed at a lower level in eggs from clams in low pH conditions. Interestingly, biological processes associated with these genes include protein transport and processing, immune responses, developmental processes, as well as metabolic activities like glycolysis. These results suggest an impact on resource allocation and future studies should be designed to evaluate how this translates into offspring performance. This information will provide insights for other shellfish and deepen our understanding of potential impacts on the success of Manila clam aquaculture practices.

FIGURE 1. Heatmap of differentially expressed genes identified in eggs of clams reared under low pH (pink) or ambient (green) conditions.

REMOTE SETTING AND HOLDING TIME OF OYSTER SPAT IN SETTING TANKS: EFFECT OF SPAT SIZE UPON DEPLOYMENT ON OYSTER SURVIVAL

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Remote setting is a technique developed in the Pacific Northwest in the late 1970s to produce spat-on-shell oyster seed for restoration and aquaculture, consisting of two main phases: (1) larval setting, in which larvae attach to a substrate; (2) nursery period, in which oysters seed are grown until they are ready for planting. This technique was successfully introduced to Chesapeake Bay in the mid-1980s and it is currently used in Maryland since then. However, the duration of nursery period still represents a controversial aspect of remote setting, with knowledge gaps surrounding the adequate holding time for newly settled spat in the setting facilities. While longer holding times of oyster spat in the nursery may promote spat survival, they also require maintenance and resources, like food and space, that can increase costs of production. On the other hand, a premature deployment could affect spat survival after planting, jeopardising the whole production operation.

An experiment was performed at the setting pier of the Horn Point Oyster Hatchery (HPOH) in July 2024 to investigate the effect of holding time on the survival of *Crassostrea virginica* and to determine an optimal holding time that promotes spat survival and minimizes costs and impacts on production. Larvae from two different broodstock mixes (2 treatments) were added to six setting tanks (3 replicates/treatment). Following settlement, spat-on-shell were kept in the tanks for 5, 11, and 17 days (3 holding times) before being deployed at the Cooperative Oxford Lab pier by the Oyster Recovery Partnership (ORP), in accordance with the current planting practices used by ORP. For each holding time, a subset of settled spat were counted and measured before planting, as well as 48 hrs, 1 month, and 4 months after planting, and will be counted again after 1 year.

The results of this study have the potential to provide new information on the optimum holding time of *C. virginica* seed in nursery facilities, which could lead to cost-effective improvements of remote setting practices that support oyster restoration, aquaculture, and commercial fishing activities in the Chesapeake Bay and beyond.

ALTERNATIVE SUBSTRATES USED FOR OYSTER RESTORATION, FISHERIES, AND AQUACULTURE: ASSESSMENT OF SPAT RECRUITMENT, TOXIC LEACHING AND BIOFILM ASSOCIATED WITH SHELL AND NON-SHELL MATERIAL

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Chesapeake Bay is home to one of the largest oyster (*Crassostrea virginica*) restoration efforts in North America, as well as flourishing commercial fishing and aquaculture sectors. The shortage of fresh shell substrate has become a major barrier to all these activities, leading to the consideration of alternative substrates for large-scale use across all sectors. A laboratory experiment was performed at the UMCES Horn Point Laboratory in June 2024 to determine the biological and chemical suitability of alternative substrates for spat recruitment. In this project, alternative substrate was defined as anything other than fresh shells of *C. virginica*, including both natural (shell) and artificial (non-shell) substrates.

Nine different substrates were tested (dredged *C. virginica* shell, clam shell, whelk shell, *C. gigas* shell, recycled concrete, limestone marl, granite, river rock, and amphibolite) with fresh shells of *C. virginica* used as a control. Composition and diversity of the microbial communities associated with each substrate were characterised after 72h of conditioning to assess any differences in biofilm formation. Spat recruitment on each substrate was assessed after 6 weeks from settlement to determine larval preferences. The leaching of toxic chemicals and water quality were measured and monitored throughout the duration of the experiment to evaluate the impact of alternative substrates on water chemistry. The results of this study have the potential to improve public perception and facilitate the design of regulatory permits, both of which are considered crucial hurdles associated with the use of alternative substrates in restoration and harvest areas.

Oyster spat settled on welk shell (top) and granite (bottom).

THE MAINE AQUACULTURE HUB: UPDATES, LEARNINGS AND FUTURE DIRECTIONS

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In 2019, NOAA Sea Grant funded the creation of eleven advanced aquaculture collaborative programs, or Hubs, to "provide broad, non-proprietary support and investment for building and/or enhancing an aquaculture industry". Born out of longstanding partnerships, the Maine Aquaculture Hub (MAH) formed in order to identify barriers to the industry and design activities to address those barriers. Since its inception, a project coordinator has directed MAH's activities in collaboration with Maine Sea Grant, Aquaculture Research Institute, Maine Aquaculture Innovation Center, Maine Aquaculture Association, and University of Maine School of Marine Sciences, and the Maine Department of Marine Resources. Each of these organizations brings unique expertise, capacities, and perspectives to the Hub's shared work.

From 2019-2022, the Maine Aquaculture Hub focused on: 1) improving and delivering Aquaculture in Shared Waters, a community-based aquaculture training program which offers trainings for fishermen and sea farmers based in Maine; 2) funding industry-led research projects through a responsive Request for Proposals; 3) developing and writing a shared statewide vision for the future of Maine's aquaculture industry, the Maine Aquaculture Roadmap. The steering committee used findings from that Roadmap in part to shape its priorities for 2022-2024, focusing on maintaining and strengthening the network, implementing aquaculture and education needs identified by Hub participants, and strengthening and diversifying existing aquaculture training programs.

Maine's aquaculture industry is evolving rapidly, creating a needs landscape in which priorities shift continuously in ways both anticipated and unexpected. Reviewing the interdisciplinary, collaborative work achieved by the Maine Aquaculture Hub over the past five years is an opportunity to reflect on the industry's trajectory in Maine, assess the impact of the Hub's work, and consider potential future directions for the Maine Aquaculture Hub network.

OPTIMIZING PROTEIN AND LIPID LEVELS TO IMPROVE GROWTH PERFORMANCE, NUTRIENT UTILIZATION, AND MESENTERIC FAT DEPOSITS IN ON-GROWING WHITE STURGEON *Acipenser transmontanus*

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Nutritional challenges in white sturgeon aquaculture, particularly during the grow-out phase, have been linked to suboptimal commercial diets, often resulting in excessive visceral fat deposition and fatty ovaries. These issues suggest inefficiencies in nutrient partitioning, prompting the need for diet optimization. A 2×3 factorial experiment was conducted to evaluate the effects of varying dietary protein and lipid levels on growth performance, nutrient utilization, and mesenteric fat deposition in white sturgeon. Six experimental diets were formulated with two levels of dietary protein (40% and 45%) and three levels of dietary lipid (15%, 20%, and 25%).

A total of 264 one-year-old white sturgeon, with an average initial weight of 930.29 g ± 25.58 g, were randomly allocated to 12 tanks (1500L), with two replicates per diet (n=22 fish per tank) for 20 weeks. The fish were fed a 6mm pellet diet at 2% body weight per day, using a combination of hand feeding and automatic feeders, and growth was monitored every four weeks. Growth trends were analyzed using simple linear regression, with weight gain modeled as a function of time and dietary treatment.

In the 45% protein diet, fish fed the 25% lipid diet showed the highest growth rate (83.12 g/week) and the lowest feed conversion ratio but had higher liver fat accumulation (HSI = 2.01). Fish fed the 20% lipid diets showed moderate growth while minimizing liver and visceral fat accumulation (HSI = 1.55, VSI = 6.67). In the 40% protein diet, fish fed the 15% lipid had the slowest growth rate (65.28 g/week) and the highest visceral fat accumulation (VSI = 8.15). These findings suggest that a 45% protein and 20% lipid diet provides the best balance between growth and reduced fat accumulation in on-growing white sturgeon and that higher lipid levels may contribute to excess fat deposition. This study is funded by the USDA Western Regional Aquaculture Center.

ALLEVIATING AMMONIA TOXICITY IN CATFISH FARMING: PROTECTIVE ROLE OF ELEVATED WATER pH ON GROWTH AND PHYSIOLOGICAL PERFORMANCE

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Catfish farming is the largest and most successful segment of the U.S. aquaculture industry, contributing significantly to the country's food supply and rural economies. However, this industry is confronted with major challenges, particularly due to toxic ammonia buildup in aquaculture systems, which reduces fish growth and physiological performance and even causes mortality. Therefore, to maintain and improve the productivity of catfish farming, it is crucial to implement effective strategies to alleviate ammonia-induced toxicity.

This research was, therefore, undertaken to investigate the potential mitigation of ammonia toxicity in channel catfish (*Ictalurus punctatus*) by altering the pH of culture water. First, to provide primary information on the sensitivity of this species to ammonia toxicity under realistic fish culture operation, a 10 day-LC_{50} test was conducted, which was found to be 21.69 mg/L (total) ammonia. Thereafter, to get insight into the protective effect of water pH against chronic ammonia exposure, catfish were cultured under three pH levels (7.1, 7.8, and 8.5) and simultaneously challenged high environmental ammonia (HEA, 5.42 mg/L representing 25% of 10-day- LC_{50}). As such, there were six experimental groups (with three replicated tanks) viz. $pH_{7.1(control)}$, $pH_{7.8}$, $pH_{8.5}$, $pH_{7.1(control)}$+HEA, $pH_{7.8}$+HEA, and $pH_{8.5}$+HEA. Following two months, weight gain (%), specific growth rate, and feed conversion were significantly reduced in $pH_{7.1(control)}$+HEA compared to $pH_{7.1(control)}$ (Fig. 1). Interestingly, the growth parameters in $pH_{8.5}$+HEA groups were significantly higher than $pH_{7.1(control)}$+HEA, signifying that HEA inhibited growth performance at normal rearing pH (7.1), but the toxic effect of HEA was alleviated by rearing the fish at a high pH of 8.5. Ammonia excretion rate (J_{amm}) was strongly inhibited in $pH_{7.1(control)}$+HEA.

In contrast, HEA-exposed fish reared at a high pH level (8.5) were able to increase J_{amm} efficiently, which was associated with upregulated branchial expression of ammonia transporters, Rhesus glycoproteins 'Rhcg'. These responses prevented a build-up of excess ammonia in plasma. A series of histopathological alterations were observed in the gills, with the most severe lesions in $pH_{8.5}$ and $pH_{7.1(control)}$+HEA. Ions (Na^+, K^+) levels were disrupted in $pH_{7.1(control)}$+HEA but remained stable in $pH_{7.8}$+HEA and $pH_{8.5}$+HEA. Overall, our findings suggest that raising catfish at a higher pH level (typically at 8.5) can ameliorate the inhibitory effects of HEA by protecting the gill morphology as well as mitigating HEA-induced ammonia excretory and ion-regulatory disruption. In conclusion, we recommend fish farmers consider raising catfish in higher pH environments when facing elevated ammonia levels in the culture units.

ALLEVIATING AMMONIA TOXICITY IN CATFISH FARMING: PROTECTIVE ROLE OF ELEVATED WATER pH ON GROWTH AND PHYSIOLOGICAL PERFORMANCE

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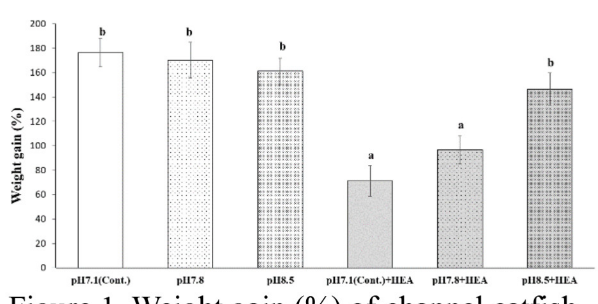

Figure 1. Weight gain (%) of channel catfish

EMPOWERING FUTURE AQUACULTURE FOOD SAFETY EXPERTS: STUDENT FOOD HEALTH CERTIFICATION

Toheeb O. Falakin*, Tanaboon Junlaprom, Cole Daleiden, Andre Rodriguez, Faith Ohwofasa, Sophia Okoh, Sagun Chhetri, Brandon Wright, Noel Novelo, Vincent Teye, Oluwafemi Adebayo, and Sandhya Lamichhane

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Every year, thousands of people die, and many millions fall ill due to the consumption of unsafe food. As seafood consumption is increasing in the United States, the aquaculture industry needs well-trained food safety experts to minimize risks of foodborne diseases and contamination.

This project aims to enhance food safety knowledge and practical skills among aquaculture students at Kentucky State University (KSU) by providing professional food health certification and fish processing training. This project will support the certification of four KSU students as Food Safety Managers and twelve students with Food Handlers Cards through the state-approved platform. Also, the project will focus on the purchase of essential fish processing tools, allowing students to practice their newly acquired skills within KSU's USDA-Certified Food Processing Facility.

This project will build capacity in food safety certification and practical fish processing. The students will have greater skill diversity, which is highly useful in a competitive job market, and also be empowered to process seafood safely, contribute to public health, and participate in educational events like product demonstrations, fostering a safer aquaculture practice and raising more certified experts in aquaculture.

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VIABILITY OF HOMESTEAD FISH FARMING VENTURE IN ADO LOCAL GOVERNMENT AREA OF EKITI STATE, NIGERIA

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This research determined the feasibility and biotechnical factors of homestead fish farming in Ado-Ekiti, Nigeria. Data was collected from 75 homestead fish farmers through structured questionnaires and analyzed to determine biotechnical factors, challenges, and economic viability. The primary challenges faced included financial constraints (41.3%) and lack of quality fish feed (37.3%). The study revealed that homestead fish farming is economically viable with a significant portion of farmers achieving yields of 101-250kg per cycle (46.7%). The research highlighted the importance of continuous training and support, with 57.3% of farmers receiving trainings from farmer associations. The study explored the biotechnical aspects of homestead fish farming in Ado-Ekiti. It revealed that this practice enhances food security, offers economic benefits, and can be adopted by individuals with basic training and resources.

The study concluded that homestead fish farming in Ado-Ekiti is a viable venture. Formulating and implementing supportive government policies that promote homestead fish farming, including tax incentives, grants, and technical support programs, is essential. Homestead fish farming in AdoEkiti can be further optimized, ensuring it remains a sustainable and profitable venture that contributes significantly to the socioeconomic development of the region.

Table 1: Biotechnical Factors of Homestead Fish Farmers in Ado- Ekiti

Item		**Frequency**	**%**
Number of years in Homestead fish Farming	1-5years	20	26.7
	6-10years	27	36
	11-15years	16	21.3
	16-20years	10	13.3
	21-25years	2	2.7
	Total	**75**	**100.0**
Level of practice?	Full time	35	46.7
	Part time	40	53.7
	Total	**75**	**100.0**
What species of fish do you rear?	Catfish	58	77.3
	Tilapia	7	9.4
	Both	10	13.3
	Total	**75**	**100**
What type of fish culture?	Monoculture	42	56
	Polyculture	14	18.7
	Both	19	25.3
	Total	75	100
Type of pond	Concrete pond	33	44
	Earthen pond	17	22.7
	Drum/Plastic	15	20
	Fiber/Tarpaulin	10	13.3
	Total	**75**	**100.0**
Size of the pond	1mby1mby1m	10	10.3
	2mby2mby1m	29	38.7
	3mby2.5mby1.4m	36	48
	Total	**75**	**100**
Source of water	Reservoir	42	56
	Well	19	25.3
	Borehole	14	18.7
	Total	**75**	**100.0**
Current level of production	Active	37	49.3
	Moribund	10	13.3
	Resting	28	37.4
	Total	**75**	**100.0**
Constraints/ challenges faced	Financial	31	41.3
	Technical	6	8
	Environmental	5	6.7
	Fish feed	28	37.3
	Water quality	5	6.7
	Total	**75**	**100**
Training or support	Yes	75	100
	Total	**75**	**100.0**
Training from where	Government	14	18.7
	Colleague	18	24
	Farmers' group	43	57.3
	Total	**75**	**100.0**
How many fingerling do you stock/cycle	500-1000pcs	9	12
	1001-2000pcs	31	41.3
	2001-3500pcs	20	26.7
	3500pcs & above	15	20
	Total	**75**	**100.0**
Average yield/cycle	5kg-50kg	10	13.4
	51-100kg	14	18.6
	101-250kg	35	46.7
	250kg & above	16	21.3
	Total	**75**	**100.0**

EVALUATING BLACK SOLDIER FLY LARVAE *Hermetia illucens* OIL SUPPLEMENTATION ON GROWTH PERFORMANCE AND HEALTH PARAMETERS OF HYBRID CATFISH *Ictalurus punctatus* × *I. furcatus* JUVENILES

Ana Beatriz de S. Farias*, Jing Huang, Crystal L. Conde, J. Grant Reifers, Penelope M. Goodman, Caitlin E. Older, Heather R. Jordan, Delbert M. Gatlin III, Matt J. Griffin, Ligia U. Gonçalves, and Fernando Y. Yamamoto

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Black soldier fly (*Hermetia illucens*) larvae oil (BSFL oil) is characterized by a high concentration of saturated fatty acids (SFAs), with lauric acid as the predominant fatty acid (~21% to 50%), which can destabilize bacterial cell membranes. The present study evaluated the effects of a plant-based diet (negative control), a plant-based diet supplemented with 1% BSFL oil, and an animal-based diet, formulated with 13.9% fish meal inclusion (positive control).

The experimental diets (negative control, 1% BSFL Oil, and positive control) were formulated to be isonitrogenous, isolipidic, and isoenergetic, containing 38% crude protein, 6% crude lipid, and 16.2 MJ gross energy/kg. Three hundred and seventy-five hybrid catfish juveniles (average initial weight ~30 g) were equally distributed into fifteen experimental aquaria (110-L, 25 fish/tank, n=5), operating as a recirculating system. Fish received feed rations twice daily, proportional to the tank biomass, which was adjusted biweekly. After 63 days of feeding, fish were weighed and three fish per tank were anesthetized to collect blood, and then euthanized for tissue sampling. The remaining fish from the feeding trial were moved to a flow-through system, and after the acclimation period, fish were subjected to an immersion challenge (3.65×10^6 CFU/mL) using *Edwardsiella ictaluri* (S97-773), and survival was monitored for 28 days. Hybrid catfish from the positive control group had a higher final weight, biomass gain, feed intake, feed efficiency, and hematocrit percentage (Figure 1). No differences were observed in viscerosomatic index, intraperitoneal fat, hemoglobin, or erythrocyte count. The dietary treatments did not have a significant effect on survival after the bacterial challenge. It can be concluded from the results of this feeding trial that 1% BSFL oil supplementation did not significantly enhance the production performance and health of hybrid catfish fed a plant-based diet. This also highlights the importance of animal proteins in the diets of hybrid catfish juveniles, as the positive control led to significantly better growth parameters.

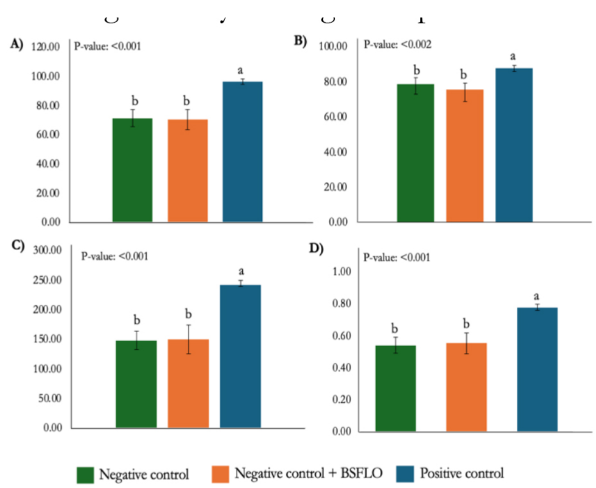

Figure 1. Hybrid catfish fed the positive control diet demonstrated significantly (all $p<0.01$) better growth performance after 63 days based on A. Final weight/fish (g), B. Biomass gain (%), C. Feed intake/fish (g), and D. Feed efficiency.

DIETARY INCLUSION OF BLACK SOLDIER FLY *Hermetia illucens* FRASS IN DIETS FOR CHANNEL CATFISH *Ictalurus punctatus*

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Black soldier fly larvae (*Hermetia illucens*, BSFL) can reduce waste by 50–60% and generate raw feedstuffs such as BSFL meal, oil, or frass. BSFL frass contains relatively high protein and lipid concentrations (21.6% and 6%, respectively), and is typically considered a by-product by the BSFL industry. Frass contains chitin, a polymer present in the exoskeleton of insects that presents antimicrobial properties and can improve intestinal health. Thus, the present study evaluated the potential of BSFL frass as an ingredient in the diet of channel catfish (Ictalurus punctatus) juveniles.

Four experimental diets with 0, 1.5%, 2.5%, and 5.0% inclusion levels of BSFL frass were manufactured to meet the nutrient requirements of channel catfish. Diets were formulated to be isonitrogenous, isolipidic, and isoenergetic, containing 37% crude protein, 6% crude lipid, and 18.2 MJ crude energy /kg. Six hundred channel catfish juveniles (average initial weight of ~1.8 g) were equally distributed into twenty experimental aquaria (110-L, 30 fish/tank, n=5), operating as a recirculating system. Fish received a ration according to the tank biomass, and the amount of feed was adjusted biweekly. After feeding for 63 days, fish were weighed and three fish per tank were anesthetized for blood collection, then euthanized to collect digesta samples for intestinal microbiota. Dietary BSFL frass increased fish liver weight and fish-fed diets containing 5% BSFL frass had a higher hepatosomatic index. No differences were observed for weight gain, feed efficiency, survival, feed intake, viscerosomatic index, intraperitoneal fat, hematocrit, hemoglobin, or erythrocyte count. The posterior intestine of channel catfish fed the control diet was mainly composed of the genera Cetobacterium (67%), Lactococcus (16%), and Plesiomonas (15%) (Figure 1A). Higher relative abundances of some gram-positive bacteria were observed in channel catfish fed the containing frass at 1.25% of diet (Figure 1B). Gut bacterial community structure and diversity were different among dietary treatments. The findings from this study suggest that BSFL frass can modulate the intestinal microbiota, but still be included up to 5% in catfish diets without compromising growth performance or overall health.

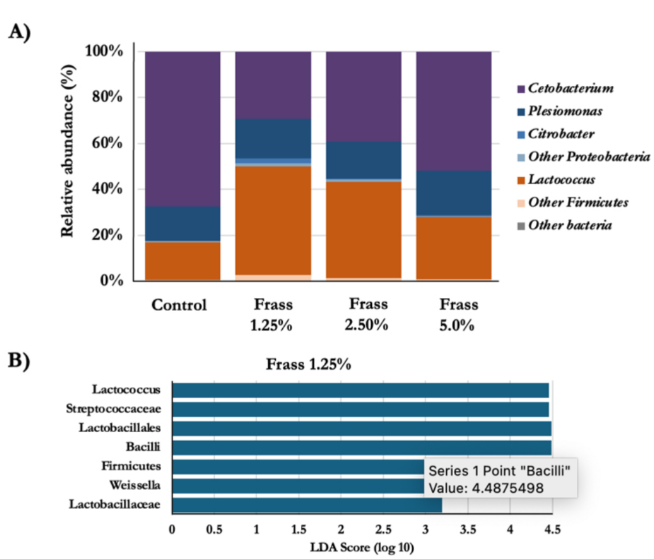

Figure 1. A) Relative abundance of bacteria in digesta of channel catfish fed diets with varying frass inclusion levels for 60 days. B) Bacteria with significantly higher relative abundance in channel catfish fed the diet with frass at 1.25% of diet.

EVALUATION OF FERMENTED SILAGE TECHNOLOGY USING DIFFERENT CATFISH PROCESSING WASTE

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The by-products from processing plants are rich in nutrients and they can be further manufactured into an alternative feedstuff. Fermentation of these by-products increases the degree of protein and lipid hydrolysis, and the production of antioxidant compounds. This technological approach to treat this resource is safe, environmentally friendly, and it has a low-energy demand. This study explored the utilization of the fermented silage on catfish by-products (viscera, head, and frames, as well as the whole by-product). The catfish by-products were frozen, ground, and homogenized. They were further subjected to incremental levels of *Lactobacillus plantarum* (ATCC14917) at 2.5, 5.0, and 10% w/v, and three inclusion levels of carbohydrate (5, 10, and 15% w/v of corn syrup). The samples were incubated at 30°C for 7 days, and temperature and pH of the samples were monitored daily. After the incubation period, the resulting samples were subjected to proximate composition, foaming capacity, emulsifying capacity, and protein solubility. The pH presented a steady decrease until the third day of ensiling and remained stable until the seventh day. This result was consistent for all the other treatments, except for the silages with a 5% carbohydrate inclusion, in which pH was stable and samples spoiled, likely due to the insufficient carbohydrate nitrogen ratio, which his necessary for the proliferation of lactic acid bacteria. The proximate composition varied among the silage manufactured, reflecting the characteristics of the nutrients from the original by-products. The silages had an average dry matter content of ~38%, and the protein and lipid content were similar for silage made from All waste and frames, with an average of 36% crude protein and 34% lipids. The mineral content was lower for silage made from viscera (1.5%), but it had a higher lipid content (60%). The emulsification properties of catfish silage varied across different treatments, in which the whole by-product displayed a superior emulsification stability when incubated with a 10% bacterial inoculum and 10% carbohydrate inclusions. There was little variation in foaming stability in catfish silage, except when considering the interaction between silage and the levels of bacterial inoculum employed. The soluble protein varied between the source of by-product, with the viscera silage showing the highest soluble protein content (3.3 mg/mL). Fermented silage performed best with a 5% inclusion of lactic acid bacteria and a 10% carbohydrate source.

EFFECTIVENESS OF ARTEMIA REPLACEMENT FOR LARVAE OF TWO ORNAMENTAL FRESHWATER FISH SPECIES

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Artemia spp. (brine shrimp) nauplii are widely used in the aquaculture of many larval and juvenile fishes, both marine and freshwater, and provide essential nutrients during the early developmental stages of many aquatic species. Most freshwater ornamental fish larvae are typically fed live *Artemia* as their first food source until they grow large enough to transition to alternative feeds. This initial feeding period typically lasts 1 – 4 weeks, depending on the species' growth and developmental rate. As larvae mature, they are gradually weaned onto other feeds such as crushed flake food, pellets, or other suitable live or frozen foods. Considering the high cost of *Artemia* cysts, variable supply, and the dependence of many cultured species during the larval stage, this study aims to evaluate commercially available liquid diets as cost-effective alternatives for *Artemia* replacement. Specifically, we assessed the inclusion of these liquid diets for larval feeding in two freshwater ornamental fish species: rainbow shark (*Epalzeorhynchos frenatum*) and tiger barb (*Puntigrus tetrazona*).

Three trials are being conducted to evaluate two commercially available liquid diet brands as *Artemia* replacements, and to identify the most suitable diet for optimizing growth and survival of each species. Each trial will be conducted in 25 3L tanks, with five replicates per treatment. During the experimental period, larvae will be fed one of five dietary treatments: 1) 100% *Artemia* nauplii reference diet (200 nauplii/L), 2) 100% Cargill Liqualife M-PL (200 particles/L), 3) 100% Zeigler EZ Artemia Ultra size 2 (200 particles/L), 4) 50% Cargill Liqualife M-PL / 50% *Artemia* nauplii (100 particles/L + 100 nauplii/L), 5) 50% Zeigler EZ Artemia Ultra size 2 / 50% *Artemia* nauplii (100 particles/L + 100 nauplii/L). Water quality parameters, including ammonia, nitrite, nitrate, hardness, and alkalinity, will be measured on the first and last day of the trials, and temperature, dissolved oxygen, and pH will be monitored daily.

Preliminary results for *E. frenatum* showed that treatments combining liquid diets with *Artemia* produced larvae with total lengths statistically similar to the control (6.46 mm), with average lengths ranging from 5.94 mm to 6.21 mm. In contrast, larvae fed exclusively with liquid diets had shorter total lengths, ranging from 5.13 mm to 5.24 mm. In terms of survival, the control treatment exhibited the highest survival rate (48%), followed by the treatments combining liquid diets with *Artemia* (33% and 34%), while the exclusive liquid diet treatments resulted in significantly lower survival rates (10% and 14%). The remaining experiments are ongoing, and the complete results will be presented at the conference. These results are critical for the freshwater ornamental aquaculture producer as they demonstrate a cost-effective alternative to *Artemia* nauplii, potentially reducing reliance on live feed and lowering production costs. Further research will focus on optimizing the inclusion rate of liquid diets and developing a weaning protocol to determine the optimal timing for transitioning from live feed to liquid diets.

CELL-CULTURED SEAFOOD AND FSIHEMEAL; PRODUCTION OVERVIEW AND THEIR FOOD SAFETY CHALLENGES

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As the demand for sustainable protein sources continues to rise with the growing global population, cellular aquaculture has emerged as a promising complement to traditional seafood production systems, such as aquaculture and wild-capture fisheries. Figure 1 shows the overview of cell-cultured seafood production. Cellular Aquaculture has the potential to address future seafood supply challenges. Currently, a growing number of companies are investing in the development of cell-based seafood and other innovative food products.

Ensuring the safety of these emerging products remains a critical concern. Cell-based seafood must undergo rigorous regulatory approvals before entering the market, particularly in the United States. In this presentation, we will provide a comprehensive overview of the current regulatory framework for cell-cultured seafood production, key food safety considerations, and the path forward for market approval.

Our work includes a simple hazard analysis of cell-cultured seafood production, leveraging established risk analysis techniques such as Hazard Analysis Critical Control Point (HACCP)(See figure 2). We will highlight potential food safety hazards during the production process, as illustrated in Figure 1, which provides examples of specific risks that could compromise product safety. This figure will be included in the presentation to visually demonstrate the importance of monitoring critical control points in the cell-cultured seafood production chain.

By examining the challenges of commercialization, including labeling terminology, consumer perceptions, and the need for cost-effective production methods, this presentation will also address the broader implications of introducing cell-cultured seafood to the market. Drawing on science-based risk assessment tools and regulatory guidelines, we will discuss the necessary steps to ensure safe production and market entry for these innovative food products.

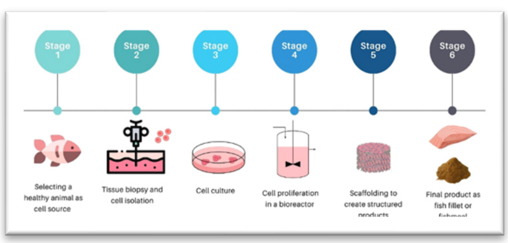

Figure 1: Overview of cell-cultured seafood and fishmeal production. (Credit; Rose Omidvar and Razieh Farzad)

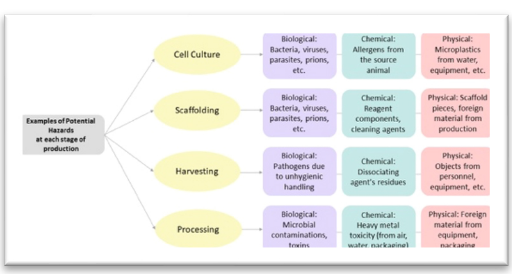

Figure 2. Example of potential hazards associated with cell-cultured seafood and fish meal product. (Credit; Rose Omidvar and Razieh Farzad, UF/IFAS)

IMPACT OF AN INTEGRATED RICE-FISH SYSTEM ON RURAL FARMING HOUSEHOLDS' PROFITABILITY IN NIGERIA: AN ENDOGENOUS SWITCHING REGRESSION MODEL

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Nigeria's current rice and fish monoculture production system has yet to generate the desired profit. The integrated rice-fish farming system is a sustainable agricultural method that has the potential to enhance profitability. This study uses an endogenous switching regression model to assess the impact of the integrated rice-fish system on the profitability of farming households in Nigeria.

Data was gathered from 458 farmers participating in the USAID/FTF project in Nigeria. The findings revealed that the profit of the participating farmers using the integrated rice-fish technology increased by ₦156.00 per hectare, indicating a higher gross margin than non-participating farmers in the integrated rice-fish system. The results of the t-test analysis for the (ATT) and (ATU) of the participating farmers showed a statistically significant difference compared to the non-participating farmers at a 1% significance level. Transitional heterogeneity was observed to be positive for profitability, implying that both participating and non-participating farmers would be more likely to adopt the program in both regimes, given its profitability. The study recommends an input-supporting system for the farmers that could increase farm profits and the location of demonstration plots closer to the wards and wetlands to keep them informed and prepared for the system.

Table 1: Full Information Likelihood Estimates of the Endogenous Switching Regression Model (ESR Model) for Profitability

Variables	Profitability		
	Coeff/Std Er	Coeff/Std Er	Coeff/Std Er
	Selection	Participating	Non-participating
Sex	0.245 (0.139)	-0.132(0.073)	-0.093 (0.065)
Age	-0.003 (0.006)	0.002(0.003)	0.003(0.003)
Marital Status	0.568** (0.241)	-0.029**(0.126)	-0.088 (0.172)
Households size	0.019(0.015)	-0.009 (0.008)	0.026*** (0.007)
Education Level	0.146 (0.143)	-0.075 (0.075)	-0.158** (0.065)
Pry occupation	0.136 (0.228)	-0.069 (0.119)	0.069 (0.115)
Access to extension	0.313** (0.140)	-0.159** (0.074)	0.001 (0.072)
Coop Association	0.080 (0.138)	-0.069 (0.071)	-0.196*** (0.067)
Dist-to-input market	0.005 (0.005)	0.026*** (0.006)	-0.006 (0.005)
Owned Phone	-0.049*** (0.011)	0.105 (0.095)	0.178 (0.091)
Argungu	-0.183 (0.184)	-0.006 (0.189)	0.391 (0.169)
Ikwo	0.128 (0.361)	-0.023 (0.216)	-0.219 (0.183)
Kimba	0.084 (0.407)	-0.270 (0.203)	0.021 (0.176)
Izzi	0.600 (0.387)	0.091 (0.216)	-0.393 (0.197)
Onicha	-0.132 (0.406)	0.076 (0.214)	-0.026 (0.186)
Ngaski	-0.102 (0.403)	-0.209 (0.183)	-0.286 (0.142)
Access to credit	0.415** (0.347)		
Constant	0.033*** (0.790)	1.536(0.942)	1.193(0.792)
Sigma1	0.532*** (0.024)		
Sigma2	0.338*** (0.023)		
rho1	-0.997*** (0.001)		
rho2	-0.196*** (0.361)		
LR Chi2(1)	-410.98303	Wald chi2	47.11
Log Likelihood	87.95	Prob>chi2	0.0001

Table 2: Impact of Integrated Rice-Fish Farming on Profitability in Northeast and Southeast Nigeria.

Activity (outcome)	Regimes	Decision Stage		Treatment Effects	T-value
		Participants	Non-Participants		
Profitability	(ATT)	6105.13	4847.14	1,257.99	5.3187***
	(ATU)	1506.84	5940.13	-4,433.29	37.00***
	Heterogeneity Effects (BH)	BH_1 = 6105.13	BH_0 =5949.13	TH = 156	

UPDATE ON THE CONSERVATION STATUS OF GIANT CLAM SPECIES

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The International Union for Conservation of Nature has recently updated and expanded upon the conservation status of the known species of giant clam (Bivalvia: Tridacninae). Each of the twelve species was given a designation of Data Deficient, Least Concern, Vulnerable, Endangered, or Critically Endangered. Currently, the U.S. National Marine Fisheries Service/ National Oceanic and Atmospheric Administration is also considering the listing of ten of these species as Endangered or Threatened and creating appropriate protective regulations under the U.S. Endangered Species Act.

Giant clams are aquacultured at various locations across the tropical Indo-Pacific region. Many of these captively-reared clams are used in re-stocking efforts where natural populations have experienced declines. And these, along with clams collected from the wild, are commonly exported/traded as food and as live specimens within the global marine aquarium hobby/industry.

However, these changes in conservation status may have a profound effect on their use, trade, and aquaculture. These recent conservation status updates, pending updates, and their possible effects will be presented.

DIETARY EFFECT OF *Cnidoscolus aconitifolius* LEAF POWDER ON GROWTH, HEMATO-BIOCHEMICAL PARAMETERS, AND LIVER HISTOLOGY OF HYBRID CATFISH (*Clarias gariepinus* ♀ *X Heterobranchus longifilis* ♂)

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Phyto-based materials are increasingly being studied as viable alternatives to synthetic additives for enhancing fish performance, stimulating immunity, and promoting environmentally sustainable aquaculture practices. One such plant material is Chaya (*Cnidoscolus aconitifolius*), known for its broad health-promoting effects (Sobrinho et al., 2017). In view of this, the current study was designed to evaluate the impact of Chaya leaf (CH) powder on the growth performance and physiological well-being of hybrid catfish. A total of 225 post-juvenile hybrid catfish were divided into five dietary groups with varying CH inclusion levels, viz., 0% (control), 0.5%, 1%, 2%, and 4%. The fish were fed their respective diets daily at 9:00 h and 17:00 h over 56 days.

At the end of the feeding trial, the results showed that the highest final weight, weight gain (WG; fig. 1), specific growth rate, and thermal growth coefficient were recorded in the group fed CH 0.5%, which is similar to other CH-fed groups but differs significantly from the control ($p < 0.05$). The addition of CH had no effect ($p > 0.05$) on the hematology parameters among the various groups; however, the white blood cells, neutrophils (fig. 2), and lymphocyte counts were found to be higher in CH 4% and CH 0.5 %, respectively, compared to other dietary groups ($p < 0.05$). Similarly, fish-fed CH 4% diets had the highest total protein, albumin, globulin and total immunoglobulin (fig. 2), while lysozyme recorded the highest value in CH 0.5%, which is significantly higher than the control ($p < 0.05$). Furthermore, antioxidant enzyme activities (SOD and catalase) also showed significant statistical differences ($p < 0.05$), indicating a strengthened immune and oxidative stress defense system. The liver histology results revealed that low to moderate inclusion levels of CH powder (up to CH 1%) did not cause any adverse changes. In summary, the results of the current findings showed that the addition of CH powder in the diet of hybrid catfish improved the growth performance, enhanced the immune response, and protected the liver integrity.

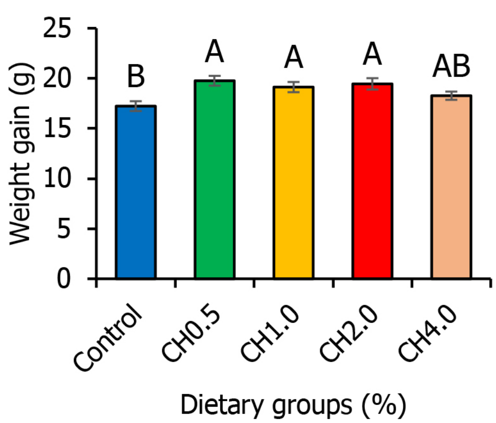

Fig 1: Weight gain (g) ($p = 0.029$)

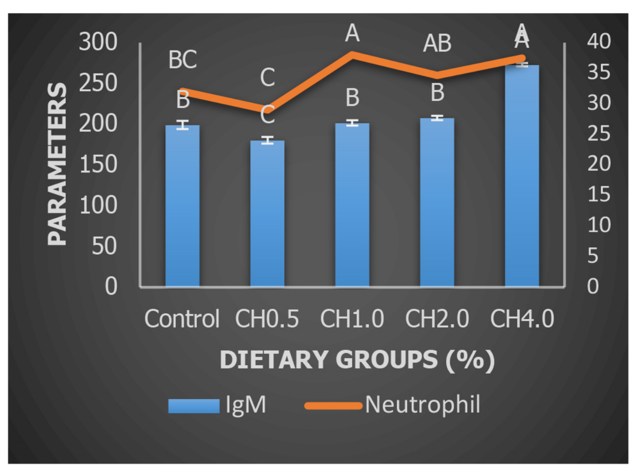

Fig. 2: Total immunoglobulin ($p = < 0.001$) and Neutrophil ($p = 0.002$)

THE EFFECT OF *Ixora coccinea* ALONE OR IN COMBINATION WITH PROPIONIC ACIDS ON ZOOTECHNICAL INDICES, HEMATOLOGICAL PARAMETERS, AND ANTIOXIDANT ENZYME RESPONSES OF NILE TILAPIA (*Oreochromis niloticus*)

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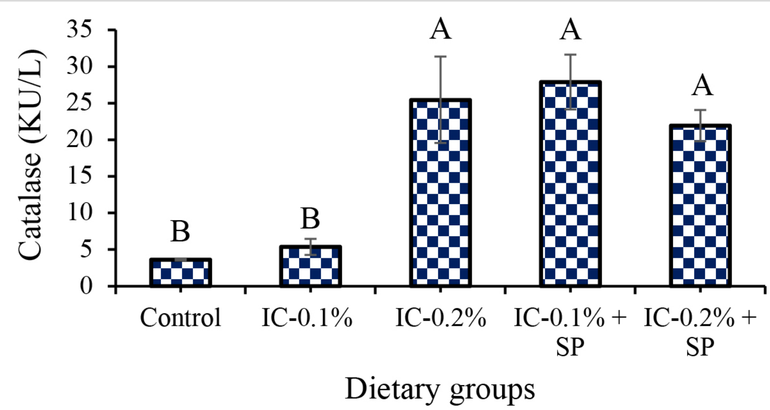
The aquaculture industry is facing considerable challenges due to spatial limitations and rising demand for food fish, resulting in the need to increase production through vertical expansion by increasing stocking densities within existing rearing facilities. However, this approach has resulted in increased competition for resources and crowding stress among fish, adversely affecting their growth and immune responses. Research indicates that dietary supplementation with phytogenic compounds, organic acids, probiotics, vitamins, etc., can mitigate stress effects in fish. Despite extensive studies on the influence of phytogenic substances and organic acids on fish health and performance, there remains a notable gap in knowledge regarding the impact of acidified phytogenic agents on fish. To investigate this, a sixty-day feeding trial was conducted to evaluate the growth and immuno-physiological effects of *Ixora coccinea* (IC) either alone or in combination with 0.2% sodium propionate (SP) in the diets of Nile tilapia.

Five distinct diets were formulated: control, 0.1% IC, 0.2% IC, 0.1% IC + SP (0.2%), and 0.2% IC + SP (0.2%). Two hundred twenty-five fingerlings, averaging 6.69 g ± 0.01, were randomly distributed across fifteen tanks for the trial. The findings revealed that dietary supplementation with IC, either alone or in combination with SP, did not significantly affect growth performance. However, there was a notable increase in neutrophil and lymphocyte counts among fish fed IC- or IC-0.2% + SP and IC-0.1%, respectively, compared with the control group ($p < 0.05$). While no significant variations were observed in red blood cell (RBC), white blood cell (WBC), and hemoglobin (Hb) counts, there was an increase in the activities of superoxide dismutase (SOD) and catalase (CAT) enzymes in the IC- and IC+SP-fed groups, respectively ($p < 0.05$). Furthermore, fish fed the IC-0.1% + SP and IC-0.2% + SP diets exhibited elevated levels of total immunoglobulin (Ig) and total protein compared to the control ($p < 0.05$). In conclusion, the supplementation of IC powder, either alone or in combination with SP, positively influenced immuno-physiological parameters and stimulated CAT enzyme activity without adversely affecting growth performance.



INDUSTRIAL HEMP MEAL: A PROMISING ALTERNATIVE FOR SUSTAINABLE AQUACULTURE

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Aquaculture has greatly contributed to the development of sustainable global animal food production and currently accounts for 57% of food fish consumed by humans. This growth has been largely credited to the changes in consumer food choices and the surge in feed-based aquaculture production. While the unprecedented growth experienced by the sector is noteworthy, the continued dependence on major ingredients commonly used in aquafeed manufacturing, especially fishmeal and fish oil, may hinder the sustainability of this growth. Thus, more effort must be made to increase the alternative ingredient portfolio for aquaculture.

Hemp (*Cannabis sativa* L.) is an ancient annual crop grown for its fiber, seeds, and metabolites, which are used in industrial, food or feed, and medicinal products, respectively. Industrial hemp grain and its co-products are plant-based materials traditionally known for their high-quality and easily digestible protein, health-promoting fats, and appropriate levels of micronutrients, all of which are needed for fish and shellfish growth and physiological well-being. The nutrient component of the grain shows that the whole seed contains 21%–25% protein, 30%–33.9% lipids, 28%–43% fiber, and 4%–10% ash. The protein in hemp seeds is rich in arginine, aspartic acid/asparagine, and glutamic acid/glutamine, in addition to good levels of the sulfur-containing amino acids (methionine and cysteine), which is comparable to other high-quality proteins currently used in aquafeed production. Furthermore, the oil derived from hemp grain is rich in polyunsaturated fatty acids, with linoleic acid (18:2n-6) constituting about 55% of the total fatty acid, while α-linolenic acid (18:3n-3) constitutes 13-17.4%. Moreover, hemp oil has a good level of γ-linolenic acid (18:3n-6) and stearidonic acid (18:4n-3), which are notably absent or only present in negligible amounts in the majority of plant-based oils commonly used in aquafeed manufacturing. The seed also has significant levels of macro- and trace minerals and is a rich source of tocopherols and provitamin A. Aside from the nutritional attributes, hemp seeds contain bioactive compounds with diverse biological properties that are potentially beneficial for fish and shellfish health.

As with other plant-based proteins, hemp seed meal is not without challenges in terms of the presence of anti-nutritional factors, high fiber content, and cannabinoids, especially Δ9-tetrahydrocannabinol (Δ9-THC), known for its psychoactive effect. Nevertheless, the ongoing research effort in our laboratory is geared toward understanding how fish respond to cannabinoids in feeds, their metabolism, and their potential for bioaccumulation in major organs. This study, when completed, will provide valuable information to enrich our understanding of the suitability of hemp grains and their co-products and illuminate the gray area on possible accumulation of Δ9-THC in fish fillets and other notable organs.

IMPACT OF SAMPLING METHOD ON EASTERN OYSTER *Crassostrea virginica* HEMOLYMPH MICROBIAL COMMUNITIES EVALUATED USING CULTURE AND 16S AMPLICON SEQUENCING APPROACHES

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Much of the microbiome research done in oysters, including the *Crassostrea virginica* or the eastern oyster, has utilized hemolymph samples. Hemolymph, the invertebrate analog to blood in that it transports waste and nutrients to and from various tissues, is a useful medium for microbial community analysis as its collection is straightforward, it hosts a complex microbiota, and many bacterial members can reflect or even be predictive of diseased states. However, this requires hemolymph not contaminated with external fluids or other matter. The current gold-standard of hemolymph extraction, the notch method, involves cutting a notch in the oyster shell followed by insertion of a needle into the adductor muscle. Due to the needle's movement through the soft tissue exposed to seawater, and potentially other tissues surrounding the adductor muscle, concerns about potential contamination exist. An alternative hemolymph sampling method, hereby referred to as the hole method, minimizes potential sources of contamination by drilling a hole through decontaminated shell surface above the adductor muscle allowing needle insertion directly into the internal tissue.

To evaluate the potential for sampling method-dependent hemolymph contamination, and to compare the two described methods, hemolymph was sampled from oysters using both the notch and hole methods. Half of the oysters had the notch sample taken first whereas the other half had the hole sample taken first. Plates inoculated with notch sample hemolymph showed bacterial colony growth whereas those inoculated with hemolymph from hole samples did not. Analysis of 16S amplicon data revealed significant differences between the microbial communities of each sample type. Notably, hemolymph samples collected from a hole before notch sampling showed the highest rates of alpha diversity. Overall, these results clearly demonstrate that hemolymph sampling method impacts microbiome composition. Moving forward, researchers should carefully consider hemolymph sampling methods when designing microbiome studies and critically evaluate the potential impact of this method on their results.

CHARACTERIZATION OF HEMOCYTES FROM DIPLOID AND TRIPLOID EASTERN OYSTERS *Crassostrea virginica* WITH A FOCUS ON PROTEIN CONTENT AND ENERGETICS

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Over the past decade a significant area of focus for eastern oyster research has been mortality trends disproportionately affecting triploid oysters. Introduced in the late twentieth century, triploid oysters, carrying three homologous sets of chromosomes, quickly gained popularity along much of the east coast as their rapid growth and meat quality made them appealing for eastern oyster aquaculture. However, across the eastern and gulf coasts there have been a growing number of reported triploid die-offs in late spring and early summer. These mortality events create a significant economic challenge prompting investigation into their cause.

Numerous potential drivers of enhanced triploid mortality including energetic imbalances, altered immune function, and dampened salinity response have been the target of this investigation. However, the vast majority of this research has exclusively focused on the organismal level. As such, there is a critical gap in our understanding of the basic cellular differences between diploid and triploid oysters, particularly those differences that may be connected to reported mortality events. To begin filling this gap, fluorescence-based flow cytometric and confocal microscopy assays were used to characterize the differences in many basic cellular characteristics between diploid and triploid oyster hemocytes. Two significant metrics evaluated as part of this research were total protein and mitochondrial levels. Additionally, flow cytometric variables such as forward and side scatter were used as proxies to compare cell size and complexity along with determining the percent composition of hemocyte populations.

Most interestingly, this approach has demonstrated a shift in triploid oyster hemocyte populations towards a larger percentage of intermediate to large, highly granular, cells. These results suggest that increased overall triploid oyster hemocyte size and granularity may be the result of differences in percent representation of certain cell subpopulations rather than shifts in cell subpopulations along these metrics.

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PRICE DYNAMICS IN THE EUROPEAN MARKET OF FARMED TURBOT (*Psetta Maxima*)

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Concentration in the European turbot industry has been increasing in the last two decades, resulting in a market driven today by three major companies. Price elasticity of demand has been studied using the ex-farm data provided by one of these major actors and the trade flows of turbot between the company's country (Portugal) and the main European market for turbot (Spain). Ex-farm sales data were provided by one of the largest companies (Flatlantic). Export data were downloaded from the External Trade Database of the European Commission. Additionally, exports of wild turbot from the Netherlands to Spain, for testing for potential competition across the two production methods.

Cointegration methods were used for estimating price elasticities, according to the non-stationary properties of the price series. A price elastic demand was rejected in all cases for both farmed and wild products. These results reject a perfect competitive market framework, and suggest an oligopolistic market structure, given the level of industrial concentration and the absence of price competition. Price integration was found across wild and farmed turbot at the production level, where the price series of the Portuguese farm was found to be an exogenous cause of variations in the prices of Dutch wild turbot exported to Spain.

The findings support the consideration of the EU farmed turbot market as an oligopoly, where the main actors avoid price competition in favor of keeping stability in their margins. Further, farmed turbot helps stabilizing the prices of the wild fishery, normally subjected to high volatility.

PREFERENCES AND CONSUMPTION PATTERNS FOR SHRIMP (*Pennaeus* spp.) IN THE SPANISH MARKET

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Consumption patterns of shrimp in Spain have been studied using the classical economic methodologies of hedonic models (Lancaster, 1966) and discrete choice experiments (McFadden, 1973). The hedonic model was used for assessing the attributes of shrimp products that significantly affect the differences in prices paid by consumers. Implicitly these are the attributes affecting the expected utility by consumers and therefore the perceived product value. Product data came from six national supermarket chains, whose prices and salient attributes were collected online on a weekly basis.

According to the results of the model, five attributes (size, production system, brand, species and geographical origin) determine more than 60% of the variation in sales prices. Within them, species and geographical origin are the ones that cause the greatest impact on prices, with the tiger prawn (Pennaeus monodon) and the origin of Madagascar, which have the highest sales prices, as well as the most minority market shares.

Taking this scenario as a reference, excluding the attributes corresponding to the least representative segments, a discrete choice experiment was carried out based on a fractional factorial design considering four factors to determine the probability of choosing different combinations of shrimp attributes.

The comparison of the two models raises interesting findings on the use of alternative extrinsic attributes and their implications on market shares and consumers choices.

ECONOMIC EVOLUTION OF THE MARINE AQUACULTURE INDUSTRY IN THE MAIN EU PRODUCERS AFTER THE COVID-19 PANDEMIC

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In January 2020, the World Health Organization declared a Public Health Emergency of International Concern and a pandemic in March 2020 (COVID-19 pandemic). In response to it, many governments imposed a temporal cessation on large parts of their economies to reduce the propagation of the virus. The COVID-19 pandemic has disrupted worldwide supply chains due to export restrictions and limited freight shipping causing negative effects in many economic sectors with increases in production and transportation costs or decreases in demand and prices of consuming products. Recent surveys suggest that this threat has jeopardized the aquaculture industry throughout the world, having a very negative impact on the profit of the European firms. How the EU aquaculture industry has been affected by the COVID-19 pandemic and which countries have had a better evolution are important questions to analyze.

Within the EU, aquaculture is considered as a key economic activity with a large potential to improve income and employment in coastal and rural areas. Thus, the EU aquaculture provides about one fifth of the EU's domestic seafood supply and has around 15,000 aquaculture enterprises with almost 70,000 employees. In 2018, for example, the sector produced almost 1.2 million tons and was valued at €4.1 billion, being the EU aquaculture production, in volume and value, concentrated in four countries: Spain, France, Italy, and Greece.

A wide representative sample of 879 marine aquaculture firms from the four countries with the largest production in the EU: Greece (127 firms), France (129 firms), Italy (440 firms) and Spain (183 firms) were selected in the BVD's Orbis database for our research. We have employed averaged economic data of these firms for two different periods: the pre-COVID period (2016-2019) and the post-COVID period (2020-2023).

Our results are presented in Tables 1, 2, 3 and 4. According to these results, we can observe that after the COVID-19 pandemic the economic evolution of the EU marine aquaculture has been different in the main production countries, having the best results on average the Greek firms.

TABLE 1. Differences in ROA (%)

Country	Pre-COVID	Post-COVID	Δ	% Var
France	2.07	1.49	-0.58	-28.0
Greece	-2.75	-0.85	1.90	69.1
Italy	-2.42	-2.20	0.22	9.1
Spain	2.31	1.83	-0.48	-20.8

ROA = Return on assets.

TABLE 2. Differences in MOS (%)

Country	Pre-COVID	Post-COVID	Δ	% Var
France	1.81	1.34	-0.47	-26.0
Greece	2.61	4.65	2.04	78.2
Italy	0.62	0.27	-0.35	-56.5
Spain	8.12	9.07	0.95	11.7

MOS = Margin on sales.

TABLE 3. Differences in asset rotation (%)

Country	Pre-COVID	Post-COVID	Δ	% Var
France	117.85	102.23	-15.62	-13.3
Greece	50.87	57.58	6.71	13.2
Italy	135.22	118.49	-16.73	-12.4
Spain	69.75	64.57	-5.18	-7.4

Asset rotation = 100 × Revenues / Total assets.

TABLE 4. Differences in efficiency (%)

Country	Pre-COVID	Post-COVID	Δ	% Var
France	98.19	98.66	0.47	0.5
Greece	97.39	95.35	-2.04	-2.1
Italy	99.38	99.73	0.35	0.4
Spain	91.88	90.93	-0.95	-1.0

Efficiency ratio = 100 × Operating expenses / Operating revenues.

HYDROLOGICAL RESTORATION OF MUANANGOME MANGROVE FOREST IN NAMPULA PROVINCE, MOZAMBIQUE: PROMOTING THE RECOVERY OF NATURAL CONDITIONS FOR MANGROVE REGENERATION

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The Muanangome mangrove forest, located northern Mozambique, was severely impacted by human and natural causes, including salt ponds, sedimentation of natural channels and cyclone Gombe in 2022. These factors obstructed water circulation, compromising water renewal and preventing the natural regeneration of the forest. Furthermore, the unsustainable cutting of vegetation has aggravated ecosystem degradation. This project's main objective was to restore the hydrological conditions of the mangrove, allowing the natural regeneration of the forest and increasing the survival rate of seedlings, while also contributing to increase coastal resilience, and mitigate and adapt to the climate changes. The initiative was carried within the scope of the Blue Future project from Wildlife Conservation Society (WCS, Mozambique) and conducted by the Eduardo Mondlane University, where innovative methods were used to evaluate and quantify ecological condition of mangroves through a Mangrove Conservation Index (MCI), developed by Macamo et al. (2021).

The hydrological restoration was conducted between August and September 2024 and occurred in five main phases: (1) Community engagement: Raising awareness and mobilizing the community, (2) Assessment of environmental parameters: Measurement of salinity, water oxygen, pH and other indicators at 12 control points, (3) Topographic survey: Mapping topography (remote and *in-situ)* to define the depth and ideal path for opening channels, (4) Production of hydrological profiles: Using topographic data to create digital elevation models (DEM), which guided the excavation of channels, (5) Opening of the canals with the participation of the community. The first monitoring phase was carried out in December 2024, evaluating the state of the channels on what regards to silting and the need for maintenance works. 19 canals were opened with a total length of 6.88 km, covering 38 hectares of degraded mangroves. 222 community members actively participated in this activity, 112 men and 110 women. After 4 months and 11 days, variations in environmental parameters were observed: reduction in pH by -1.01±2.27, salinity by -17.94±28.14 and dissolved oxygen by -76.49±36.11. There was a reduction in mangrove competing species (e.g.: *Juncus kraussii*) due to increased salinity in areas previously colonized by swamp vegetation. Community participation was essential to the success of this activity. To ensure the continuity of positive results, regular monitoring of the channels and the planting of *Rhizophora mucronata* along the channel margins are recommended to stabilize the soil. Future monitoring will also include seedling recruitment, carbon sequestration and fauna. This project created an opportunity for partnerships between multiple organizations working on mangroves in different geographic locations of the country, while building capacity of students from Eduardo Mondlane University and local communities in mangrove restoration matters. The initiative represents a successful model for restoring degraded ecosystems and can be replicated in other regions with similar challenges.

A WEB-BASED FRAMEWORK FOR AQUACULTURE SUSTAINABILITY—APPLICATION OF THE FINS MODEL TO TEN BAYS IN EASTERN CANADA

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Introduction
The supply of aquatic products for human food predominantly relies on aquaculture, which is 65% of the global production. Worldwide, the sector has grown at an annual rate (APR) of 6.6% since 2020, but growth in the Western World has been considerably slower, and in some cases inexistent. As an example, Fig. 1 shows that growth in Europe was mainly driven by Norway (salmon) and Turkey (bass and bream). If the UK is excluded due to Brexit, the EU APR for the period shown is 0.4%, compared with 10.4% for non-EU European nations.

North America fares no better, with a 2008-2022 APR of 0.5% for Canada and 2.3% for the USA. The total production for Europe and North America in 2022 is 4.2 million tonnes, 3% of world production, for 12% of the world population.

In addition, the gap between autochthonous supply of aquaculture products in the West and the demand for seafood has widened over the past decades as per capita consumption increases.

In the West, lake and pond aquaculture play a minor role compared to the East, and cultivation is mainly in coastal systems; at the bay scale, aquaculture growth is strongly constrained by sustainability concerns and requires tools that can deal with multi-use challenges. Marine spatial planning (MSP) is a key component in harmonising multiple uses, but there is a need for frameworks that bring together complex models to deal with issues such as sediment organic enrichment and pathogen outbreaks, that occur at very different time and space scales.

Methods
The FINS (Farming In Natural Systems) framework (Fig. 2) combines a set of models that address specific issues related to finfish and shellfish aquaculture.

These include near-field deposition and fate of particulate organics, far-field nutrient enrichment, using ammonia as an indicator, together with oxygen reduction, and for bivalves, chlorophyll (as a proxy for food) depletion.

Detailed models for water circulation are the base for understanding the distribution of key performance indicators (KPI) of aquaculture sustainability. Associated with these circulation models are well-tested physiological models for growth of finfish and shellfish species such as salmon, bass, bream, oysters, and mussels.

The location, type, and dimensions of aquaculture structures are user-defined.

The FINS software can represent different types of cages and other types of structures such as rafts or longlines.

Results and Discussion
The results presented in this paper illustrate the outputs of FINS for different KPI in selected bays in Nova Scotia, Canada.

Fig. 3 shows four layers for Liverpool Bay, Nova Scotia: the bathymetry and residual velocity (from the FVCOM model) are shown for the entire bay, a grid of 30 m diameter salmon cages is shown to the NW of Coffin Island, and the outputs of three models show the effects of salmon cultivation through a 500-day cycle.

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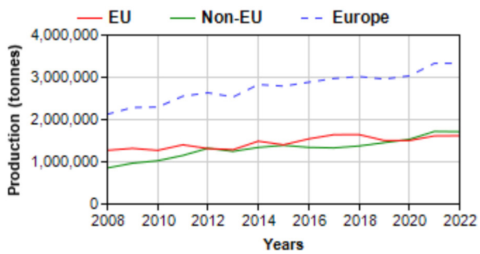

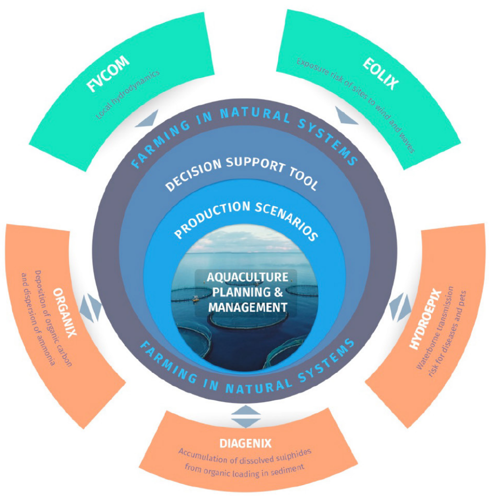

Fig. 2. FINS framework for aquaculture sustainability and growth.

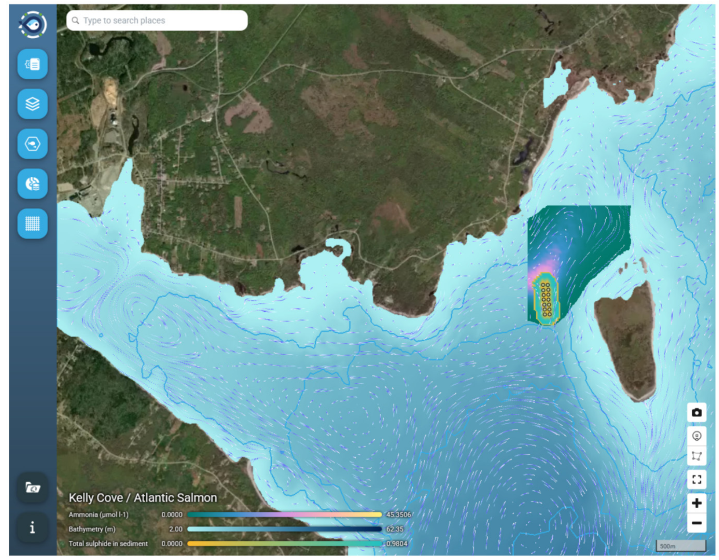

Fig. 3. FINS results for Liverpool Bay, Nova Scotia, showing cage locations for a salmon grid, bathymetry and velocity fields, sulphide in the sediment, and ammonia in the water.

The near-field deposition of particulate organics from uneaten and waste feed is simulated, together with the consequences for sediment diagenesis, in this case with respect to sulphide concentration; far-field dispersal of ammonia is also shown—the residual currents transport the dissolved NH^4_+ plume NE, and the concentrations within this broader, nutrient-enriched area can be used to assess the eutrophication potential of the activity. Pathogen dispersal is also part of the FINS model and allows managers to examine the interaction between existing or potential farms with respect to disease connectivity, promoting animal welfare, natural biodiversity, and ecosystem sustainability.

The relevance of the FINS platform in the context of MSP helps to take spatial planning to the next level. By applying different mathematical models to aquaculture structures placed in suitable areas and examining e.g. the potential effect of pathogen dispersal or eutrophication in adjacent zones, MSP can be leveraged to account for the dynamic nature of coastal ecosystems.

FINS also represents a major technological advance in a platform of this type when compared to conventional desktop solutions. FINS runs in the browser, like any website, and users can access and use it anywhere, from any computer or tablet. In a world that increasingly relies on the Internet of Things (IoT), this is a critical advantage. The option to use the GPU for the graphics primitives in FINS, including design of any type of polygon as an aquaculture structure, animation of residual current flows, and various other features, has led to very significant improvements in speed and functionality.

(Continued on next page)

Acknowledgements
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PHYSIOLOGICAL PERFORMANCE OF THE DIATOM *Chaetoceros* sp. AT EXPONENTIAL AND STATIONARY GROWTH PHASES

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Diatoms, one of the most important groups of microalgae, widely used in aquaculture, play an important role in marine trophic networks and carbon sequestration due to their biomass rich in essential nutrients and their high growth rate. This study provides new insights into the physiological performance of *Chaetoceros* sp., strain CHX1, a coastal diatom isolated from Todos Santos Bay, Baja California, Mexico. Batch cultures of CHX1 were used by triplicate in Erlenmeyer with 800 ml of "f" medium, maintained at 21 °C with continuous irradiance (100 μmol m^{-2} s^{-1}). Growth rate, dry weight, fatty acids profile, pigment content, and photosynthetic parameters were measured at exponential, stationary, and late stationary growth phases. Dry weight was measured gravimetrically, fatty acids with gas chromatography, pigments spectrophotometrically, and photosynthetic activity with a PAM fluorometer.

The growth rate of CHX1 at exponential growth phase was 1.9 divisions day^{-1}, and the generation time was 0.7 days. During the late stationary growth phase, the cells underwent morphological changes, accompanied by alterations in their fatty acid profile and photosynthetic parameters. In the late stationary phase, a drop in pH was observed due to a decrease in the photosynthetic activity of the diatoms (Fig. 1).

The polyunsaturated fatty acids (PUFAs) and pigments (Chl *a,* Chl *c,* carotenoids) content per cell was higher in the stationary growth phase than in the exponential and late stationary phases.

In the exponential phase, higher values of growth rate, α, and Fv/Fm were observed (Table 1). The variations in the photosynthetic parameters of *Chaetoceros* sp. (strain CHX1) are attributed to the growth phase and the type of cells in the cultures. At the late stationary phase, the cells began a resting or dormant process, which led to a decrease in these physiological parameters. The preliminary results show high growth rate, elevated PUFA content, and high irradiance tolerance (E_k) make *Chaetoceros* sp. strain CHX1 a species with significant potential for aquaculture applications.

Table 1. Photosynthetic parameters of CHX1 at different growth phases were: α: initial slope, ETR_{max}: Electron transport rate, E_k: Intensity of saturation, Fv/Fm: photosynthetic efficiency.

Growth phase	α (µmol photon m^{-2} s^{-1})	ETR_{max} (µmol electron m^{-2} s^{-1})	E_k (µmol photon m^{-2} s^{-1})	Fv/Fm
Exponential	0.16 a	308.86	1921.79	0.56 a
Stationary	0.10 b	291.55	2823.58	0.40 b
Late stationary	0.03 c	343.57	2823.50	0.07 c

One-way ANOVA, α=0.05, a>b>c

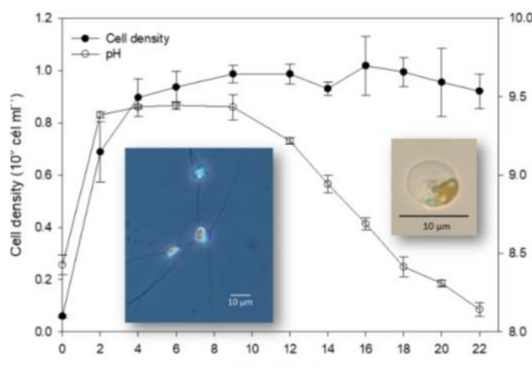

Figure 1. Growth curve and pH dynamics in cultures of strain CHX1. The insets display the morphology of the microalgae.

USE OF PASSIVE BIOACOUSTICS TO CHARACTERIZE THE FEEDING ACTIVITY OF *Penaeus vannamei* WITH DIFFERENT TYPES OF FRESH FOOD

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Bioacoustics, through passive acoustic monitoring (PAM), has been used to investigate shrimp feeding behavior by detecting "click-like" sounds produced by mandible collisions. Previous studies have highlighted variations in these sounds depending on the type of feed (extruded or pelleted). However, little is known about acoustic differences when shrimp consume other food types. To address this gap, this study aimed to acoustically characterize the clicks produced by *Penaeus vannamei* when consuming squid, octopus, mussel, shrimp, and feed (Camanutri 40, CR2 ~ 1.5 mm). Acoustic recordings were conducted in a controlled laboratory environment using shrimp weighing 11.9 g ± 1.4 g. Each food type was offered separately to groups of five shrimp in tanks lined with acoustic foam and filled with 40 liters of water at 10 ppm salinity. For each treatment, 2 g of food cut into 0.5 cm pieces (or 1.5 mm pellets) was provided, and 15-minute recordings were captured using a SoundTrap 300 STD omnidirectional hydrophone. The hydrophone's specifications included a frequency response range of 2 Hz to 60 kHz, a sampling rate of 288 kHz (16 bits), and a sensitivity of -173 dB, allowing sound capture up to 144 kHz. Audio files were analyzed using Raven® 1.5 Pro software, where oscillograms and spectrograms (512 resolution and 50% overlap) were generated. The first 50 clicks per food type were selected for acoustic characterization. Key sound parameters minimum, maximum, and peak frequencies (kHz) and maximum energy (dB) were statistically compared among the food items. The results revealed significant variations in sound parameters depending on the food type. Feed had the highest minimum frequency (5.9 kHz), while squid had the lowest (3.7 kHz). Maximum frequency was highest for shrimp (124.1 kHz) and lowest for feed (68.0 kHz). Mussels exhibited the highest peak frequency (20.2 kHz), whereas squid had the lowest (5.6 kHz). For maximum energy, octopus had the highest value (50.7 dB), and mussels the lowest (32 dB). These differences may reflect the texture and composition of each food type. This study demonstrates that shrimp clicks vary acoustically depending on the food consumed, highlighting PAM as an effective tool for monitoring feeding behavior. Such insights can improve shrimp farming practices, particularly in optimizing feeding strategies during maturation and grow-out phases, ultimately enhancing productivity and sustainability.

Food	Minimum frequency (kHz)	Maximum frequency (kHz)	Peak frequency (kHz)	Maximum energy (dB)
Shirmp	5,76^{ab}	124,10^{a}	10,96^{ab}	43,4^{ab}
Squid	3,79^{c}	93,17^{b}	5,62^{c}	47,8^{a}
Mussel	4,92^{ad}	69,71^{c}	20,25^{a}	32^{c}
Octopus	4,18^{cd}	101,84^{ab}	9,00^{b}	50,7^{a}
Feed	5,90^{b}	68,06^{c}	10,68^{ab}	37,1^{b}

THE EASTERN OYSTER MICROBIOME AND WITHIN-GENERATION ADAPTATIONS RESULTING FROM REPEATED EXPOSURE TO ENVIRONMENTAL STRESSORS

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Acute coastal stressors such as seasonal hypoxia and warming are immediate threats to healthy oyster populations. One mechanism by which oysters could potentially increase their fitness is through the composition of their microbiome, which is known to actively change based on environment and ontogeny. Studying the host-symbiont relationship between oysters and their microbial communities will provide insight into their capacity for rapid, intragenerational adaptation. With this ongoing project, we aim to catalog how oyster microbiomes change in response to repeated and simultaneous exposure to hypoxia and warming. This past summer we completed the first phase of the experiment, exposing eastern oysters (*Crassostrea virginica*) to different oxygen and temperature levels first at three months and then five months of age. Shell height and tissue mass were measured before and after second exposure, and then the oysters were dissected by tissue-type. We plan on performing both 16S ribosomal RNA sequencing and shotgun metagenomics in order to identify bacteria common to those oysters that experienced hypoxia and/or warming. Better understanding how oyster microbiomes respond to real, dynamic stressors will provide aquaculture scientists with new insight into how they can ensure the continued health of their oyster populations.

DIET INFLUENCES SURVIVAL AND GROWTH OF INTENSIVELY REARED LARVAL SAUGEYE *Sander vitreus x S. canadensis*

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We evaluated the performance of five commercial microstarter diets (Otohime, Gemma Micro, Gemma Wean, Optimal Starter, and Omega One Fry) fed to first feeding larval saugeye *Sander vitreus x S. canadensis*. Triplicate tanks of 2,500 larvae (initial weight 2.6 ± 0.6 mg) were stocked at 4 days post-hatch and fed treatment diets for four weeks. Fish were randomly sampled each week to monitor growth and development. Superior growth performance was observed in fish fed Otohime and Gemma Micro and was statistically significant as early as 13 DPH. At the conclusion of the trial, fish fed Otohime and Gemma Micro were approximately 3.6 times heavier than fish fed Gemma Wean and Optimal Starter. Survival was highest in fish fed Otohime (39.7 ± 7.1%), with Gemma Micro performing second best (14.1 ± 3.3%). Survival of fish fed Omega One Fry was too low for analysis (0.70 ± 1.1). Incidence of deformity among the dietary treatments ranged from 0 - 10.7%, primarily manifesting as a malformation of the jaw. Our results highlight the importance of diet for successfully raising saugeye through the larval/ juvenile stage, and provide key information for this critical bottleneck in percid culture.

EVALUATION OF FISH WASTE FERMENTED WITH DAIRY BY-PRODUCT AS AN ALTERNATIVE PROTEIN SOURCE FOR AQUAFEEDS FOR RAINBOW TROUT

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Mixed sex eyed rainbow trout *Oncorhynchus* mykiss embryos were obtained (Spring Lake Trout Farm, UT, USA) and incubated in California trays at the Ohio State University Aquaculture Laboratory (OSUAL). When yolk sac was determined to be almost completely absorbed, larvae (n=62) were randomly stocked to eighteen 15-liter tanks in a recirculating culture system. The experiment was set up in a randomized complete block design with each block being a shelf with six tanks. Six treatment groups were applied in triplicate with five experimental diets which were formulated and produced at OSUAL and a commercial diet. The protein sources of the diets varied and included 50% and 75% raw fish waste, 50% fish waste fermented with a consortium of bacteria, 50% fish waste fermented with a single bacteria strain, and a fishmeal control diet. Fish in each tank were fed three times per day at a restrictive feeding rate. Samples of whole tank biomass were taken monthly to readjust feeding rates and to calculate survival, individual fish mass, feeding coefficient ratio, and specific growth rate. The density of fish was cut to 40 fish per tank at 30 days of feeding (dof) and further to 20 fish per tank at 60 dof until an estimated mean individual fish mass of 10 g was achieved in each diet treatment. When final samples for each diet treatment were taken five fish were flash frozen whole in liquid nitrogen and stored at - 80 °C for proximate and lipid analysis.

The different diet treatments achieved a mean individual mass of 10 g between 92 and 111 dof for all treatment groups, except for the diet with 75% fish waste as the protein source which still had not achieved this endpoint by 122 dof (Fig. 1). Growth was highest in the commercial control, followed by the fishmeal control and then the fish waste diets. The individual mean mass of fish in the 75 % Raw fish waste diet was similar to the other fish waste diets after 30 dof but was lower than these diets by 61 dof. This trend continued to increase in magnitude as the experiment progressed. This appears to be a function of significant decreases observed feed conversion ratio recorded at 92 (1.51 ± 0.05) and 122 (3.87 ± 0.72) dof for this diet which were 1.4-3 folds higher than the other fish waste diets. Results obtained for survival, proximate analysis, and fatty acid analysis of total lipids will also be presented. Differences in survival, growth, and feed conversion ratio of 50% fermented fish waste diets compared to 50% non-fermented were not readily apparent. These results indicate that incorporation of 50% fish waste product, fermented or raw, as the protein source may be acceptable for Rainbow trout growth and survival. Project was funded by United States Department of Agriculture U.S.-Israel Binational Agricultural Research and Development Fund.

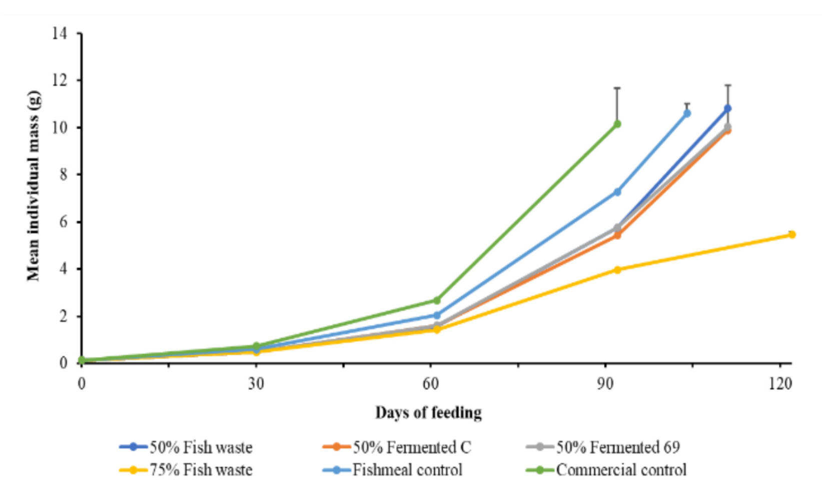

Fig. 1 Mean individual mass recorded throughout the Rainbow trout experiment for each diet treatment. Error bars (standard deviation) are presented for final samples.

A BAYESIAN NETWORK ANALYSIS OF OYSTER HABITAT SUITABILITY

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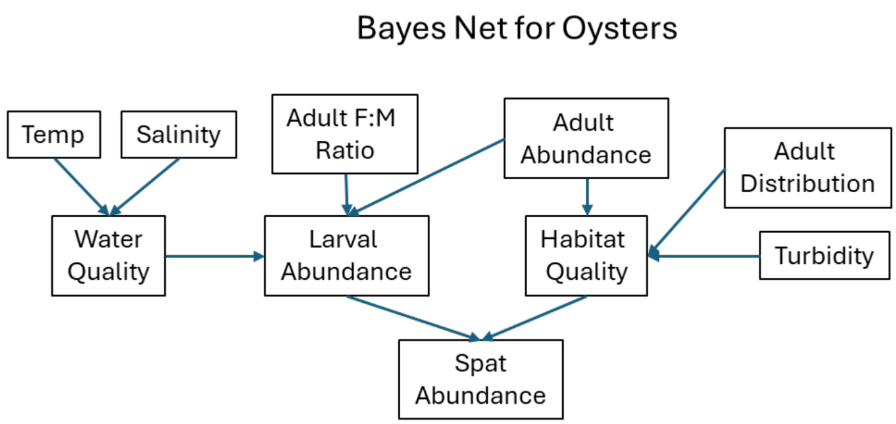
The life cycle of the oyster *Crassostrea virginica* includes four distinct life-stages; gametes, larvae, spat (juveniles) and adults. The success of each life stage is determined partially by the success of preceding stages and partially by environmental water and habitat characteristics. Water and habitat qualities change seasonally during the oyster life cycle, and suitable ranges for oyster survival can differ for each stage. As an example, gametogenesis in the spring may be maximized at temperatures around 21^{o}C, but spawning in early summer may require temperatures of 24^{o}C. A critical step in the life cycle of the oyster is spat settlement, where larvae attach to substrate and metamorphose into the juvenile and adult forms.

A Bayesian network, which is a probabilistic graphical model that incorporates multiple variables, was constructed to examine environmental suitability for spat settlement. Bayesian networks can accommodate categorical and continuous variables and can combine empirical data with expert judgement. If the variables are related to one another causally in the network, scenarios with possible combinations of causal factors can be used to estimate the probability of different outcomes on variables of interest.

To estimate spat abundance, ranges of water quality factors (salinity and temperature), habitat characteristics (cultch and turbidity) and success of preceding life stages are incorporated into a Bayesian network. Prior (unconditional) probabilities are based on available literature, and conditional probabilities consider the uncertainty across the range of consequences for different environmental scenarios.



FECUNDITY, GESTATION LENGTH, AND LARVAL SERIES OF *Thor amboinensis*, THE SEXY DANCING SHRIMP

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Thor amboinensis, the sexy dancing shrimp, is a species of shrimp becoming more popular in the aquarium trade. *T. amboinensis* is prized for its vibrant colors and symbiotic relationships with anemones and corals, making it a popular choice for nano aquaria. The demand for *T. amboinensis* is moderate, with an estimated 20,000-30,000 individuals imported into the US annually. Most of these shrimps are still captured from the wild, raising concerns about sustainability, especially since there is no available population data for the species. Due to their small size and popularity in reef aquaria, *T. amboinensis* is an ideal candidate for aquaculture. However, no published studies to our knowledge have specifically focused on the aquaculture of this species; existing research has primarily examined larval settlement, morphology, and behavior in the wild. This study aims to refine methods for breeding and raising *T. amboinensis* in captivity, addressing a critical gap in the literature and promoting sustainable trade practices. Our primary goals are to determine the gestation period from egg to larval stage, and to quantify the larval yield per brood, essential steps in optimizing production methods for aquaculture.

To understand the fecundity and gestation length of *T. amboinensis*, adult shrimp were placed in 3L Aquatic Habitats™ zebrafish tanks with 1 female and 1-2 males per tank. The shrimp were fed frozen mysis and observed daily for the presence of eggs. The afternoon before hatching, each tank was moved and drained into a larval rearing cone to collect the larvae as they hatched. Fecundity was recorded by counting the number of larvae produced. Larvae were then reared using standard rearing methods and photographed daily with a digital stereoscope to document their development until settlement. The data collected on fecundity and gestation period, along with detailed images of larval stages, will provide valuable insights into the aquaculture potential of *T. amboinensis* as a candidate for ornamental shrimp farming.

2024 GLOBAL REVIEW OF TILAPIA PRODUCTION AND MARKETS

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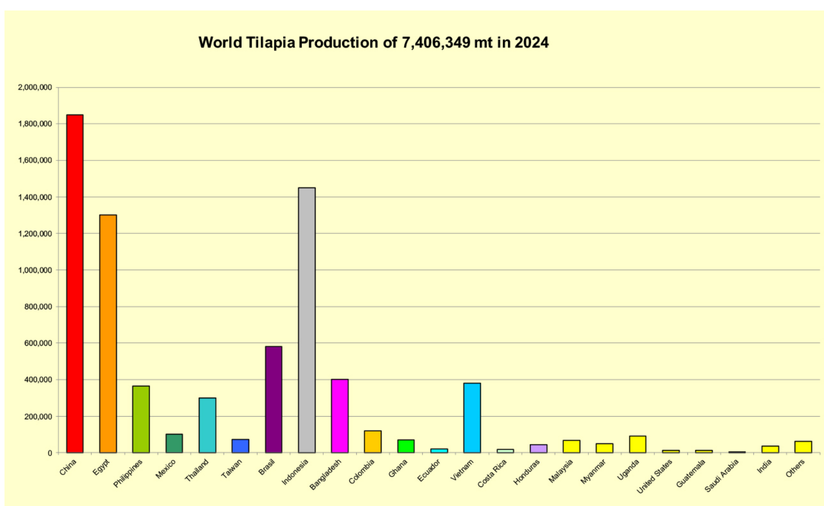
Overall, 2024 was a good year for both tilapia markets and increasing global production. It appears that 2024 saw global production and consumption exceed 7 million metric tons for the first time. Indonesia and Brazil were leaders in the amount of increased production and consumption as both countries have strong domestic markets as well as significant export volumes. While China continues to hold the title for largest producer, consumer and exporter, the production increased slightly, and exports changed little. Domestic sales did increase, especially value-added forms sold into northern and western markets too distant for live fish deliveries.

Egypt increased production and domestic consumption as prices improved for farmers as the economy improved enough for consumers to pay for fresh fish. However, Egypt fell further behind Indonesia as the third biggest producer of tilapia. The Gulf States, continued to be minor producers of tilapia, mostly from brackish water farms, but remain important markets for imported tilapia mostly purchased by employers to feed migrant workforces from Asian and African countries. Saudi Arabia has announced plans to build new tilapia farms, but like last year, these still seem to be in planning stages.

Bangladesh continues to be an important producer with volumes increasing and domestic consumption increasing as well. New farms have been built and existing farms are improving productivity per hectare. Active breeding programs also continue to improve growth and survival rates as well as yield. India is also continuing to increase production from a very low beginning level. Like Bangladesh, India is managing to increase tilapia production without decreasing production of any other aquaculture crop.

Other than Brazil, the rest of the Americas have reported only minimal increases is production and consumption. Ecuador, Colombia, Mexico, Costa Rica, Guatemala, and Jamaica all have domestic sales along with exports to the US.



UPDATE - NEXT GEN USDA - PREPARING HISPANIC AND OTHER UNDERREPRESENTED STUDENTS IN FISHERIES AND AQUACULTURE

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The University of Arizona, New Mexico State University and Santa Fe Community College were awarded grant from USDA-NIFA focused on preparing the Next Generation of professional scientists for various agencies within the USDA. Surveys have reported that most agencies within the USDA (and other Federal Departments) rarely reflect American society. The numbers of Hispanic, Black, Native American and women are significantly lower in the professional ranks than in our general population. This Next-Gen grant is specifically addressing these shortages through a focused program recruiting young people to consider education in fisheries and aquaculture science and then supporting their education with scholarships, internships and introductions to working professionals.

In 2024 the project worked at several levels to recruit and train Hispanic, Black, Native American, Asian American, women and first-generation students. We worked with high schools and community colleges in Arizona and New Mexico to recruit interested students for community college and university level training. Scholarships, work-study, and internships are being used to facilitate their path through 2+2 (community college to university) or 4-year university degree programs. There are also funds for capacity building within the three academic institutions to accommodate additional students and update equipment and supplies. The grant also supports a small number of graduate students focused on areas of identified shortages in professional USDA staff.

USDA agencies looking for increased diversity include the Animal and Plant Health Inspection Service, Agriculture Research Service, Foreign Agricultural Service, Forest Service, Food and Nutrition Service, FDA Center for Food Safety and Applied Nutrition, Division of Seafood Safety, and Natural Resources Conservation Service. While USDA professionals will be our target it is obvious that other Federal agencies also hire professionals in aquaculture and fisheries including the US Fish and Wildlife Service, Bureau of Reclamation, National Marine Fisheries Service, Bureau of Land Management, and Environmental Protection Agency. Of course, Arizona Game and Fish and New Mexico Fish and Game also hire fisheries professionals.

The grant program is supported with industry in-kind contributions with farms and hatcheries offering to host interns for summer work. This kind of real-world experience will be especially important as these young professionals will be able to graduate and contribute their professional duties with USDA from their first day on the job.

GAMIFYING IMPROVEMENT IN AQUACULTURE

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Fed aquaculture is growing, and continuing usage of high fishmeal and oil diets may present financial and environmental bottlenecks in the near future. Most aquaculture certification programs address reducing the use of fishmeal and oil through more stringent FIFO or FCR ratios. However, so far, they have not called for the elimination of marine derived components in compound diets. In 2014, The Fish Free Feed (F3) challenge was created as a collaborative effort between NGOs, researchers, and private partnerships to accelerate and support the scaling of innovative, substitute aquaculture feed ingredients such as bacterial meals, plant-based proteins, algae, insect meals, yeast and fermentation by-products to replace wild-caught forage fish. The Challenge winner produced and sold 86,000 mt of feed and received a prize of US $200,000. The contest changed to F3 – Future of Fish Feed, and in the second challenge, we looked for fish oil replacements, while the third challenge was the carnivore challenge, with $200,000 and $300,000 prizes respectively. Our recently completed challenge was the krill meal replacement challenge. All these challenges are predicated on real challenges faced by the industry. While the prizes may not be significant to the biggest feed companies, the press and public relations generated by these challenges are significant.

We believe this is a viable tool for improving the sustainability of aquaculture production in fed species. As aquaculture continues to provide more and more of the global seafood supply more sustainable aquafeeds will be a necessity.

THE FUTURE OF SHELLFISH FARMING: A GEOSPATIAL ANALYSIS OF PRACTICES AND GROWING TRENDS IN NORTH CAROLINA

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Shellfish farming in North Carolina is a state-managed resource as shellfish leases are granted to farmers on public trust bottom. Over the last 10 years, applications for shellfish leases have surged by 720%. The rapid growth in shellfish farming has transformed industry practices and influenced public perception. Often, these two factors go hand in hand as newer methods, such as floating bags and large-scale bottom cages, have a more pronounced impact on viewshed and navigation than legacy methods, such as planted clutch on the bottom. A preliminary study has identified five areas along the North Carolina coast that exhibit the highest number of shellfish leases per acre and are the subject of ongoing or possible upcoming user conflict. In an effort to better understand and mitigate public conflict, a geospatial analysis of farming methods is underway. An assessment of the distribution of farming methods compared to factors such as bottom composition, yield per acre, and water depth may provide insight into patterns in how farmers choose to produce shellfish. Using these patterns, projection maps can be created to predict how the distribution of growing methods may change as the industry continues to evolve. In 2022, Shellfish aquaculture brought more than $14 million to the local economy and provided commercial fishermen with over 300 new jobs. As managers, it is important to acknowledge these strengths of the industry when we assess how to address user conflict. Since all North Carolina waters are held in public trust, we must make every effort to understand patterns of use to effectively steward the state's resources.

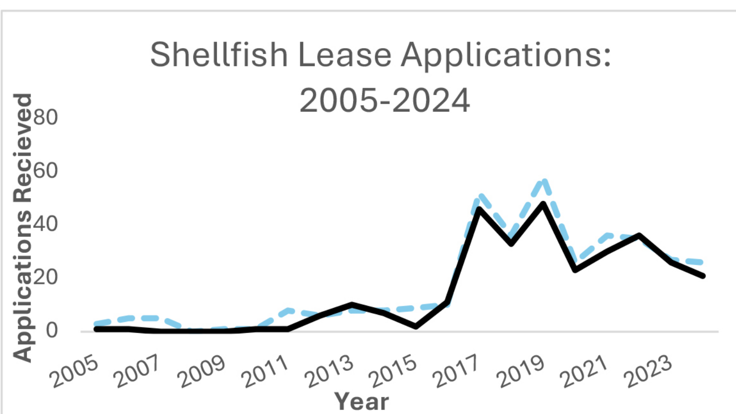

Figure 1: Shellfish lease applications submitted to NCDMF from 2005-2024

Figure 2: Example of a highly efficient shellfish growing method.

BUILDING CLIMATE RESILIENCE AT HOG ISLAND OYSTER COMPANY, CA

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Shellfish growers worldwide are facing increasing climate-related uncertainty - ranging from changes in animal growth and survival to food safety challenges to larger environmental changes in their estuaries. At Hog Island Oyster Company we are working across the fabric of our business (which includes a hatchery, and nursery, two farms and five restaurants) to identify areas of opportunity for building resilience to these climate stressors. Most of these stressors are simply too large for a single entity to tackle, so research collaboration and public engagement are pillars of our approach. In this presentation I will share our story and discuss some of the specific strategies we are pursuing, including breeding for climate resilience, diversifying our "Blue Food" crop portfolio, diversifying our farm locations, and increasing our engagement with technologies that offer an "early warning" system for climate-related changes.

SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF HEALTHY AND WSSV-INFECTED *Litopenaeus vannamei* TISSUES

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Aquaculture is the fastest growing farmed food sector in the world and is a key part of global food security. Crustacean aquaculture, while being one of the most profitable sectors, is threatened by pathogens such as white spot syndrome virus (WSSV), which results in severe stock losses and animal health and welfare problems.

Here, we employed single-nuclei RNA sequencing technology to study WSSV-infected and non-infected Pacific whiteleg shrimp (*Litopenaeus vannamei*) to identify genes involved in the WSSV infection process. To achieve this, we optimized disease challenge protocols in adult shrimps, developed new protocols for nuclei isolation of lymphoid organ, and compiled a cell atlas for the tissue (Figure 1), significantly improving our understanding of both the host transcriptome and the cell heterogeneity present in this tissue. Differential analyses between infected and control animals revealed key cell types and genes involved in the response to WSSV in whiteleg shrimp. The genes identified as important in infection in this study provide crucial insights for the development of effective therapeutic strategies to combat WSSV in shrimp aquaculture.

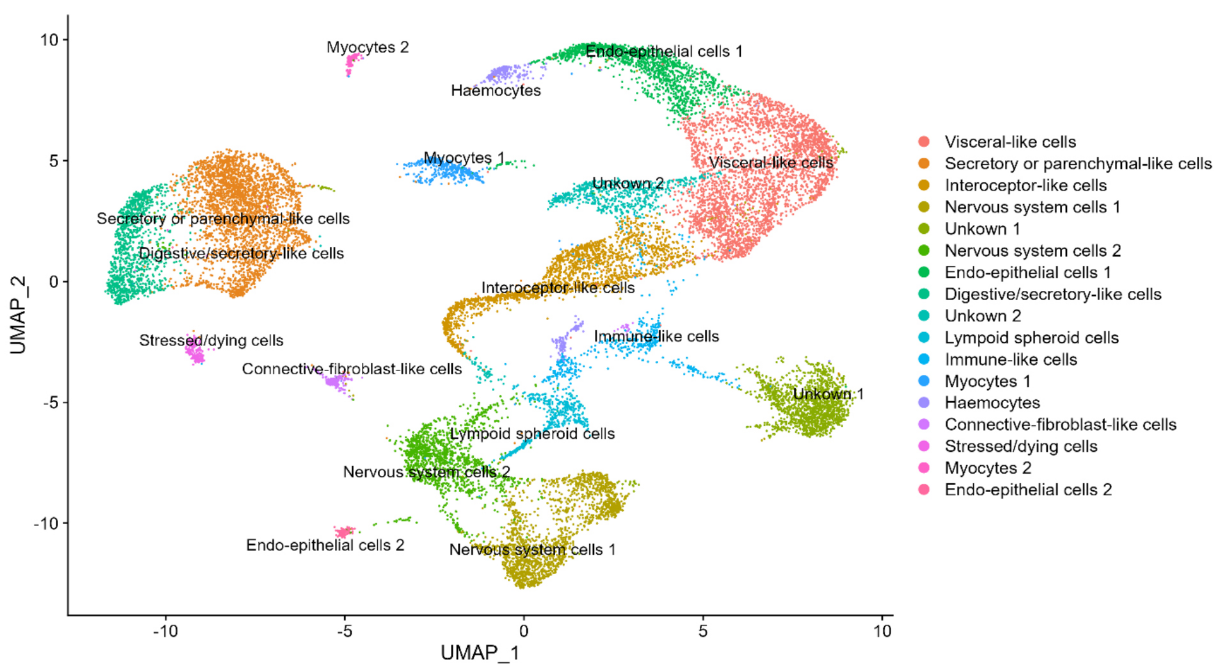

FIGURE 1. UMAP plot of cell clusters from *L. vannamei* lymphoid organ tissue

INTERACTIVE EFFECTS OF SALINITY AND CARBONATE CHEMISTRY ON SURVIVAL AND GROWTH OF EASTERN OYSTER *Crassostrea virginica* LARVAE

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The eastern oyster (*Crassostrea virginica*) is a reef-forming bivalve found naturally along the western Atlantic coast from Canada to Venezuela, including the Gulf of Mexico. The eastern oyster provides habitat structure, filters water, protects environments from degradation and erosion, and is a lucrative seafood product. Despite being essential for the environment and economy, oyster populations have been declining due to natural and anthropogenic events such as overharvesting, disease, and climate change, including potentially excess CO_2 absorption into the ocean.

As oceanic CO_2 levels increase, carbonate saturation state decreases and impedes the synthesis of oyster shell. The estuarine ecosystem is especially vulnerable to changing carbonate chemistry conditions that result from ocean acidification due to its generally low alkalinity and low buffering capacity. Oyster hatcheries, typically located in estuaries, are vulnerable to the negative effects from ocean acidification. A relatively new concept in oyster hatcheries is the utilization of closed recirculating aquaculture systems (RAS) with artificial seawater (ASW) to mimic optimum water chemistry. The use of RAS and ASW can reduce the risk of poor outcomes by providing control over water quality parameters, but little is known about the combined effects of suboptimal aragonite saturation, low pH, alkalinity, CO_2, and salinity on larval growth and survival.

This project examines individual and combined effects of aragonite saturation state and salinity on survival and growth of oyster larvae. Previous research using recirculating larval rearing systems and altered pH has used large-volume systems and examined only a few parameters at a time. This project uses a low-volume, recirculating artificial seawater system to increase the number of treatments and replicates under a manageable workflow to carry out the experimental run. The system supports four salinities, three pCO_2 levels, and one lower river endmember alkalinity comprising twelve 40L combinations in triplicate for a total of 36 4L tanks.

Larvae were spawned from Gulf Coast native broodstock. On day 2, larvae were stocked into the recirculating systems. On even days, a subsample of larvae was counted and measured. The trial continued until larvae were harvested or they reached 18 days of age. Water samples were taken every other day to monitor alkalinity, pH, and salinity. Aragonite saturation state was calculated. Preliminary data analysis using multifactorial ANOVAs suggest that survival is greatly affected by salinity ($p<0.001$) and the interactive effects of salinity and CO_2 ($p<0.01$), whereas harvests are affected by mostly salinity ($p<0.01$).

THE EFFICACY OF STRESS PRIMING AS A TACTIC FOR INCREASING STRESS RESILIENCY OF JUVENILE EASTERN OYSTERS (*Crassotrea virginica*)

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Oyster aquaculture in the Chesapeake Bay is a rapidly growing industry, contributing to both ecosystem restoration and economic growth. In the past decade, Maryland's oyster industry has increased tenfold, providing economic opportunity for coastal communities. These coastal environments are dynamic and while oysters have evolved to cope with environmental variation, long exposures to stressors like low salinity and dissolved oxygen increase mortality and reduce growth and reproduction. Climate change and other anthropogenic impacts (i.e. land-use change, nutrient loading, etc.), exacerbate the intensity and frequency of these exposures which threatens the consistency of aquaculture production and the ecosystem services of farmed oysters.

Stress priming is a strategy by which an organism receives a sub-lethal dose of stress to bolster tolerance to future stress exposures. Based on studies conducted on various taxa, this tactic heightens cellular defenses by inducing frontloading of stress-related genes and causing epigenetic modifications that provide protection during subsequent stress events. While promising, the effectiveness of this environmental stress mitigation tool has not been tested on the eastern oyster. If applicable, this approach could be useful for protecting oyster crops during particularly vulnerable early life stages.

To evaluate the potential for stress priming to be an effective tactic to safeguard juvenile oysters from unexpected low salinity and low dissolved oxygens events, controlled lab experiments were conducted using these stressors. Initial experiments established the intensity and duration necessary to produce a priming effect for low salinity. Preliminary results found that shorter primes (12 hours - 2 days) at 2ppt had less mortality (8.8-11.2%) than a control group (24%) during a long-term stress exposure. Subsequent experiments utilized the most effective prime from the initial round, introduced hypoxia as an additional stressor, and tested both diploid and triploid oysters. Ongoing data analysis includes comparing growth and mortality data as well as processing samples collected for transcriptomic analysis using both existing biomarker tools and RNA sequencing. This study aims to provide insights into the effectiveness of stress priming on juvenile eastern oysters and improve understanding of the molecular underpinnings of the stress response among different stressors and oyster ploidies. The findings may inform future strategies for oyster aquaculture management in changing environmental conditions.

ESTABLISHING A HAWAI‘I-PACIFIC AQUACULTURE CONSORTIUM: REVITALIZATION AND GROWTH OF THE AQUACULTURE DEVELOPMENT PROGRAM

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There is a clear and urgent need to reinvigorate and expand an aquaculture development program for the state of Hawai‘i and Pacific region. Such a program would provide high-level visibility and stature to work across government agencies, non-governmental entities, and community groups on a variety of industry development and support issues.

In 2019, Hawai‘i Sea Grant and partners initiated a NOAA Sea Grant-funded aquaculture project whose aim is to revitalize, solidify, and expand an aquaculture development program via an aquaculture hub consortium that spans Hawai‘i and the US-Affiliated Pacific Islands (USAPI). The consortium is being implemented through a Sea Grant Center of Excellence called the *Pacific Region Aquaculture and Coastal Resource Hub* that consists of over 17 partners from academia, industry, government, and non-profit organizations that conduct integrated research, extension, and education services in support contemporary and Indigenous aquaculture practices in Hawai‘i and the USAPI.

Selected outcomes associated with this hub to date include the hiring of four Sea Grant extension faculty who have been providing education, outreach, and technology transfer services in Hawai‘i and Guam to address the lack of aquaculture extension capacity in the region. Hawai‘i Sea Grant and consortium members have been collaborating on federal- and state-funded projects and activities that support various aquaculture initiatives that include, but are not limited to, examining reproductive bottlenecks in captive finfish broodstock, utilizing genomic selection for improving reproductive output in shrimp, building workforce capacity through internship opportunities, producing and supplying seed stock for grow out in Hawaiian fishponds, conducting husbandry of new and existing commercial species, aquaculture facilities development, and partnering with the aquaculture industry to identify priority needs and actions to address those needs.

CROSS-PACIFIC INDIGENOUS AQUACULTURE COLLABORATIVE NETWORK: CULTIVATING ABUNDANCE THROUGH BIOCULTURAL PRACTICES

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Indigenous aquaculture systems such as clam gardens and fish ponds offer proven approaches–tested over thousands of years–of integrated and adaptive systems capable of expanding local food production without deleterious ecological effects. There is growing evidence that these systems yield net-positive benefits to coastal environments, while also amplifying cultural and economic benefits to local coastal communities. In 2019, Washington Sea Grant, Hawai’i Sea Grant and Alaska Sea Grant came together to catalyze a cross-Pacific regional collaborative with the aim of integrating community engagement, research, outreach and education to advance sustainable Indigenous Aquaculture practices and enhance seafood production in the broader Pacific region. These efforts eventually grew into a thriving community of practice made up of Pacific-region Sea Grant offices that now also includes Guam, together with practitioners from Northwest Tribes and First Nations, Native Hawaiian and Indigenous communities from the Pacific, and organizations and universities. Through virtual and in-person gatherings, the community of practice shares the living traditions from unique places to develop strong cross-cultural learning and support collective efforts. This presentation will provide an overview of Indigenous biocultural systems, and showcase partner activities of the Cross-Pacific Indigenous Aquaculture Collaborative Network over the past 5 years. We will focus on activities by partners in Hawai’i, Washington and Guam that build sustainable coastal food abundance for local Indigenous food sovereignty and well-being.

IMPLEMENTATION OF A GENOMIC EVALUATION PROGRAM FOR ACUTE HEPATOPANCREATIC NECROTIC DISEASE RESISTANCE IN *Penaeus vannamei*

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Acute hepatopancreatic necrosis disease (AHPND), caused by *Vibrio parahaemolyticus* carrying plasmid-borne binary toxin gene, is one of the leading causes of losses in shrimp aquaculture. There are no commercially available therapeutics to control AHPND, but AHPND-tolerant lines have been reported. This suggests that genomic selection could be employed to develop AHPND-resistant lines. We used a genomic selection program to develop AHPND-resistant lines of *Penaeus vannamei* shrimp using a single-step GBLUP approach, using data collected from two experimental challenges involving family lines that differed in their growth performance under grow-out farm conditions.

Experimental bioassays were conducted using Specific Pathogen Free (SPF) *P. vannamei* of 40 family lines (20 fast growth and 20 slow growth lines) and an immersion challenge route using *V. parahaemolyticus* at 4.95×10^7 CFU/ml. Each family line had three replicate tanks and ten animals/ tank (20 L size tank). Animals received a binary mortality phenotype at the end of three days of challenge. Genetic parameters were estimated using a mixed threshold animal model, with a random additive genetic effect modeled for either pedigree or single-step genomic predictions.

The heritabilities (SE) for the first AHPND challenge were estimated to be 0.38(0.08) for the combined slow and fast growth lines. The within-line heritability was 0.16(0.07) and 0.14(0.06) for the slow and fast growth lines. For the second AHPND challenge, heritabilities were 0.31(0.05), 0.47(0.13), and 0.26(0.08) for the combined data, slow growth, and fast growth lines, respectively. The correlations between breeding values predicted with pedigree and the family means were above 0.85. Genomic breeding values (GEBVs) were calculated and allowed for predicting individual performance of SPF shrimp that were not exposed to bacterial challenges. Moreover, GEBVs identified differences between individuals within families, allowing more accurate selection tools. The correlation between growth and mortality performance across families was not significant, indicating the absence of selection trade-offs. However, more data are needed before conclusions.

We are now expanding the genomic evaluation to include genotype data for animals used in the second AHPND challenge. The analysis will include a validation of the GEBVs and enable us to evaluate the estimation of response to selection and define the future steps for a genomic selection program. Additionally, a trade-off analysis between disease resistance and growth will be made to ensure that selecting disease-resistant individuals will not impact growth negatively. Finally, we will correlate GEBVs with histopathology data and quantitative load of *V. parahaemolyticus* in the experimentally challenged animals to better understand the genetic effects of AHPND tolerance on bacterial replication and pathological manifestation in shrimp.

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UNDERSTANDING THE LIFE-HISTORY OF BIGEYE SCAD *Selar crumenophthalmus*

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In the southern region of Florida, the bigeye scad (Selar crumenophthalmus) holds significant popularity as a bait fish. It is commonly utilized by fishermen for kite fishing, targeting large game fish species like marlin. The market value of a dozen live bigeye scad is approximately $180, reflecting its high demand. Despite this popularity, there remains a limited understanding of bigeye scads in their natural habitat, particularly within Florida. Most studies on bigeye scad are conducted in the Pacific Ocean, with minimal research originating from the United States. This project, focusing on the unique aspects of bigeye scad in Florida, aims to enhance comprehension of their life-history, encompassing length-weight relationships (LWR), reproductive patterns, and dietary preferences to help facilitate future commercial aquaculture production.

In collaboration with local bait anglers and markets, a total collection of 350 bigeye scad was analyzed. Monthly collection varied from 10 to 95 individuals. The initial findings reveal a significant increase in LWR and gonadosomatic index (GSI) values from May to August. Conversely, during December and January, both LWR and GSI were notably low. Analysis of stomach contents has unveiled distinct dietary patterns throughout the year, with varying volumes of polychaetes, fishes, and euphausiids in different months. Otolith analysis is currently underway.

Better understanding of the life-history, including age-growth, reproduction, and diet of Atlantic Ocean bigeye scad is crucial in making bigeye scad aquaculture more viable. Aquaculture of bigeye scads holds the potential to mitigate the overharvesting of wild populations, stabilize their numbers, and sustain business operations.

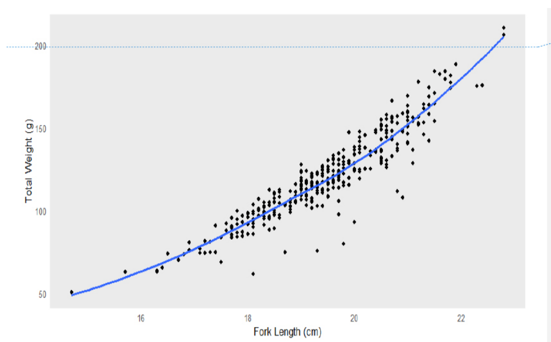

Figure 1) Length-weight relationship of S. crumenophthalmus

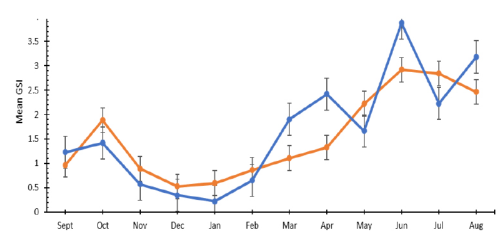

Figure 2) Graph of GSI for male and female S. crumenophthalmus

COLLABORATIVE EDUCATION AND EXTENSION IN SUSTAINABLE AQUACULTURE: THE SAS^2 PROJECT

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The Sustainable Aquaculture Systems Supporting Atlantic Salmon (SAS^2) project is a working model for collaborative aquaculture education and extension, addressing the growing need for skilled professionals and public education in Recirculating Aquaculture Systems (RAS).

SAS^2 has developed a comprehensive framework spanning K-12 to professional development, including youth engagement programs, RAS-specific educational modules, internships, and pathways involving various education levels. Extension efforts work to enhance public awareness and industry connections through educational videos, tours, workshops, and collaborative outreach programs. The impact is evident in increased interest in aquaculture careers (67%), successful job placements post-internship (~40%), and post-program surveys that consistently show heightened awareness of aquaculture's role in sustainable food production.

A cornerstone of SAS^2 is its multifaceted collaboration, exemplified by cross-institutional partnerships in curriculum development and research, integration of Western science with Native knowledge in aquaculture education, and joint initiatives with industry to align education with workforce needs. This approach bridges academic institutions, industry, and diverse communities, cultivating a skilled workforce and informed public essential for the sustainable growth of the RAS industry.

This presentation will share our experiences in implementing the SAS^2 project, offering insights into the diverse educational and extension activities undertaken. We will explore the nature of our collaborations across institutions, communities, and industry partners, highlighting both successes and challenges encountered. By sharing these practical experiences, we aim to contribute to the ongoing dialogue on effective strategies for advancing sustainable aquaculture through collaborative education and extension efforts.

COMMUNITY-SCALE STEELHEAD TROUT OPERATIONS IN NEW HAMPSHIRE PART I: FROM HATCHERY TO HARVEST

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Building upon the work of [1], the University of New Hampshire (UNH) completed the successful grow out and harvest of 4387 kg of cleaned steelhead trout (*Oncorhynchus mykiss*) during the 2023-2024 season. The operation took place at one of the UNH coastal aquaculture sites permitted for stocking up to 7000 juvenile trout in a community-scaled, integrated multitrophic aquaculture (IMTA) system called the AquaFort [2]. The AquaFort has two containment sections, each approximately 6-m x 6-m x 4.57-m, for growing fish. The fish originate as a specific strain of rainbow trout eggs (Riverence), hatched and grown in a freshwater raceway facility in Ossipee, NH. For this season, two size cohorts were investigated. The first 2000 fish cohort was stocked into the saltwater AquaFort on October 24, 2023, at a mean size of 304 grams. The second 2000 fish cohort was stocked in the adjacent containment section on December 1, 2023, at a mean size of 148 grams.

This presentation will focus on the growth and harvesting statistics from late October to early September for the first, higher performing cohort that grew to a mean size of over 2.5 kg, with some individuals approaching 5.5 kg. Datasets will show how the community-scaled system can be categorized as a non-Concentrated Aquatic Animal Production facility by the EPA-National Permit Discharge Elimination System based on small harvest biomass and feeding rates. By maintaining accurate growth and harvest datasets, this seafood system can also be managed to keep stocking densities well below the nominal threshold of 22 kg m^{-3}, appropriate for fish welfare. Most of the fresh products were distributed and sold to local markets in Portsmouth, NH with some hot-smoked processed in Boston, MA. Part II of this presentation will discuss the potential economic benefits and emerging markets of the AquaFort in NH and beyond.

[1] Chambers, M., Coogan, M., Doherty, M, Howell, H. 2024. Integrated multi-trophic aquaculture of steelhead trout, blue mussel and sugar kelp from a floating ocean platform. J. Aqua. 582:740540. https://doi.org/10.1016/j.aquaculture.2024.740540.

[2] Chambers, M.D., Coogan, M., Doherty, M., Berghahn, E., Fredriksson D.W. 2024. AquaFort, a floating, integrated multi-trophic aquaculture system. W. Aqua. Mag. 55(3): 34-36. https://www.was.org/Magazine/2024/03/34/#zoom=z.

OPPORTUNITIES FOR THE CO-LOCATION OF AQUACULTURE AND MARINE ENERGY

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As part of the global transition to clean, renewable energy sources, marine energy should be considered, particularly for at-sea activities. Marine energy includes energy derived from waves, tides, currents, and salinity and temperature gradients. Energy from large rivers may also be included, as it can be captured using similar methods. While marine energy is a developing industry typically used to provide power to the grid, it holds unique potential to power activities in marine or coastal environments. Marine energy is a reliable source of energy, and one that may exist when other renewable resources (like solar or wind) are less present, making it useful for providing full power or for hybrid solutions.

There are several opportunities for the co-location of aquaculture and marine energy (Figure 1). Because marine energy technologies are unique, range in scale, and vary in ideal application, numerous possibilities exist to tailor a fit-for-purpose solution to an aquaculture operation's location, needs, and size. This presentation will detail information from several research projects aimed at understanding the feasibility and opportunities for using marine energy in various aquaculture contexts. The first project assesses the potential for offshore integrated multi-trophic aquaculture and wave energy in Puerto Rico. It identifies viable locations for co-location from a technical perspective through spatial analyses and evaluates social feasibility through community engagement. The second project assesses the potential for tidal and ocean thermal energy to provide power for onshore hatchery and coastal grow-out and net pen aquaculture in Washington and Hawaii. This project includes assessing energy use at existing aquaculture facilities and identifying viable marine energy resources and appropriate technologies at each location.

The third project assesses the application of low-flow tidal energy for nearshore oyster and kelp aquaculture in coastal states. It evaluates marine energy resources near existing farms to identify opportunities.

The research being conducted in these three projects shows promise for furthering the feasibility of co-location between marine energy and various aquaculture operations.

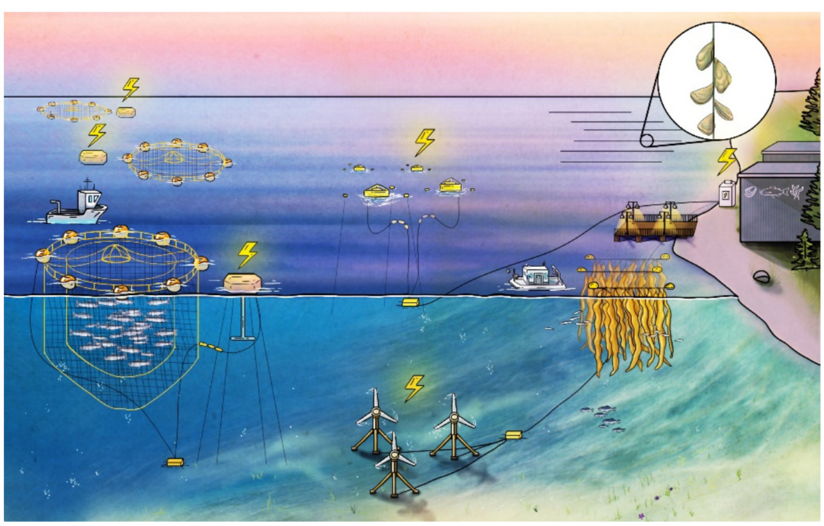

Figure 1. Illustration of potential opportunities for marine energy co-location with various types and scales of aquaculture operations.

BLUE CARBON AND OTHER ECOSYSTEM SERVICES IN MANGROVE FORESTS

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Mangrove forests across the tropics have long been threatened by human development, and their loss represents a reduction in biodiversity and the ecosystem services they provide to local and global communities. Most recently, attention has turned to 'blue carbon' and the potential of mangroves to contribute to climate change mitigation through their ability to sequester carbon dioxide from the atmosphere and store it in their soils at high densities.

This presentation will introduce the various livelihood benefits and ecosystem services provided by mangroves and describe their ability to be an important blue carbon habitat. It will show how blue carbon has become an important driver of mangrove conservation and restoration around the world, helping to turn the tide on the loss of this valuable coastal ecosystem.

EVALUATION OF COMMERCIAL MICRODIETS AS FIRST FEEDS WITH DOMESTICATED STRIPED BASS *Morone saxatilis* IN RAS

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For the past five years, our team has conducted RAS based larviculture research with Domesticated Striped Bass (DSB), *Morone saxatilis*, (distinct from both wild striped bass or hybrid striped bass). The principal goal was to develop tank-rearing techniques and methods to improve overall production during this early life stage through to metamorphosis (about 28 dph). Among areas of investigation include evaluation of commercial, inert microdiets as "first feeds" in 4-8 dph post-larvae.

Artemia nauplii are the most common "first feeds" for many carnivorous marine species, including DSB. These "live feeds" contain excellent amino acid and fatty acid compliments and are readily consumed by the larvae at the onset of feeding. However, the process to hatch and deliver healthy nauplii is expensive and time consuming. Numerous microdiet feed manufacturers market larval diets as "*Artemia* replacement diets". Our work examined more than 15 diets from leading manufacturers and found no diets accepted by DSB larvae during the first feeding stage.

However, when presented, larvae were ATTRACTED to ALL of the diets, but just refused to ingest any of them. Interestingly, we documented that larvae did, infrequently, ingest unhatched decapsulated *Artemia* cysts (Fig 1). From these observations, we attempted to develop diets to mimic the size, shape and compressibility of unhatched cysts and *Artemia* nauplii. These efforts were challenging and only partially successful.

This presentation will review our investigations with commercial diets, discuss manufacturing strategies, compare measured shapes and textures of diets to decapsulated cysts, describe compression analysis techniques to measure and compare cysts and *Artemia* nauplii to feeds. We will also discuss feeding behavior related to feed movement, color, water flow and background contrast.

Figure 1. DSB post larvae (bottom) with ingested decapsulated Artemia cysts

ADVANCING SWIMBLADDER INFLATION WITH DOMESTICATED STRIPED BASS *Morone saxatilis* IN RAS

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For the past five years, our team has conducted RAS based larviculture research with Domesticated Striped Bass (DSB), *Morone saxatilis*, (distinct from both wild striped bass and hybrid striped bass). The principal goal was to develop tank-rearing techniques and methods to improve overall production during this early life stage through to metamorphosis (about 28 dph).

Striped Bass are physoclistous fish, requiring forcible inflation of the swim bladder (SBI) during early larval development, up to about 8 dph. Failure to successfully complete SBI results in skeletal deformities, poor growth and reduced survival (Fig 1). Feeding these larvae usually begins 4 dph-8dph after mouthparts form. A result of feeding, principally *Artemia* nauplii, is the release of uneaten oil that accumulates on the water surface as a thin surface-oil film. This oil film becomes a physical barrier impeding the ability of larvae to access surface air; a process required to complete SBI.

Our first efforts to assist SBI focused on published methods including: surface sprayers, fans, lipophilic pads, and absorptive clay particles, to name a few. All these methods failed. We then found a few early works on various air-driven surface skimmers and made modifications to fit our system. After much trial and error, we have developed a simple method and techniques that have resulted in 80-90% SBI success in post-larvae (Fig 2). Our experience and protocols will be discussed, the most important of which will be highlighted in this presentation.

Figure 1. DSB showing deformities from poor SBI

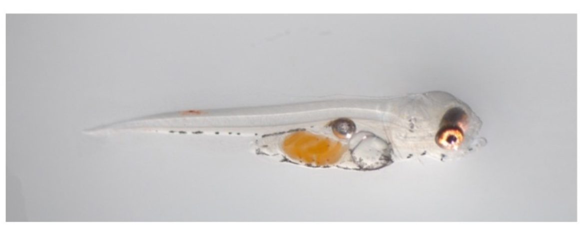

Figure 2. DSB post-larvae with inflated swimbladder

EXPLORING THE SCLAES AND DRIVERS OF BLUE TRANSITIONS OF MARINE WILD-CAPTURE FISHERIES TO MARICULTURE IN CALIFORNIA, U.S.A.

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Commercial capture fisheries worldwide are susceptible to perturbations and sudden declines, also known as *shocks*. While management reforms and diversification within wild fisheries have provided some stability, it is less clear how parallel sources of seafood production, such as aquaculture, fit into these dynamics, past and present. Marine aquaculture (mariculture) is one way to increase seafood supply as demand increases and fisheries landings potentially become more variable in the future.

This study examined historical trends in marine fisheries and mariculture in the state of California, U.S.A, with an aim to empirically understand 'blue transitions', i.e. shifts from wild to farmed production. We explored the potential influence of fisheries shocks and changes to aquaculture policy on temporal trends in mariculture production.

We found both shocks and policy were significant to mariculture growth, but in different and complex ways. Large fisheries shocks in both volume and value appear to statistically correspond to regional emergence and growth of mariculture (within five years), but cumulatively have a negative influence on mariculture growth—perhaps due to loss of maritime infrastructure (e.g., seafood processing). And while increases in policies appeared to significantly increase mariculture production, we found a proliferation of possible restrictive regulations (3:1 restrictive to enabling) likely stymied growth by 3-17x since the 1980s (Figure 1). Although data limitations and misclassification challenged our ability to fully assess wild-farmed dynamics, we also conducted a longer historical analysis of California oyster fishing and farming, which highlighted a full blue transition that informs more recent trends. This study underscores the importance of local and state-level fisheries and policy dynamics in shaping mariculture's role in seafood production.

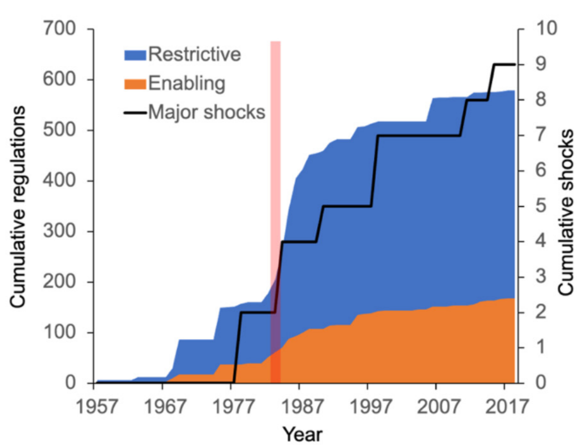

Figure 1. Cumulative aquaculture regulatory policies, enabling (*orange*) and restrictive (*blue*) (N = 747) on the primary axis, with cumulation major shocks of volume and value (Cook's D >0.3) of the four taxonomic groups combined on the secondary axis. The largest major shock (1984) is again depicted by the *transparent red line*

INTEGRATING OYSTER FARMERS INTO OYSTER HABITAT RESTORATION IN THE FLORIDA PANHANDLE

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With oyster reefs in global decline, oyster habitat restoration and management programs need a steady supply of material for reef replenishment; however, finding natural oyster shell can be difficult and expensive. As the oyster aquaculture industry grows, mutually beneficial partnerships with local oyster farmers could provide shell and other byproducts for oyster habitat restoration while also compensating farmers for crop loss. This approach could be especially beneficial in areas such as in Florida where heavy seasonal mortality is common. To determine if this approach is feasible and mutually beneficial, the Florida State University Coastal and Marine Laboratory has partnered with 5 commercial oyster farms in Oyster Bay, Florida. Oyster farmers bag unsellable byproduct in biodegradable mesh bags and deploy material on intertidal restoration sites for a per-bag compensation fee. Restoration sites are surveyed quarterly for success metrics including oyster recruitment, material retention, and restoration reef growth. Drone surveys before and after the deployments will be used to quantify overall physical changes in the restoration sites. Farmer feedback and participation will be solicited to determine whether this approach is beneficial and feasible for industry partners, and to identify roadblocks and solutions for expanding the program into other areas. This project will detail how restoration is progressing and feedback from commercial partners. These collaborations could result in steady supplies of material for oyster habitat restoration and re-shelling programs, without the time-consuming process of collecting and curing shell. These partnerships would integrate the oyster aquaculture industry into resource management and increase beneficial ecological outcomes, while compensating farmers for crop losses.

LIGHT-DUTY AUTONOMOUS UNDERWATER ROBOT FOR OFFSHORE AQUACULTURE

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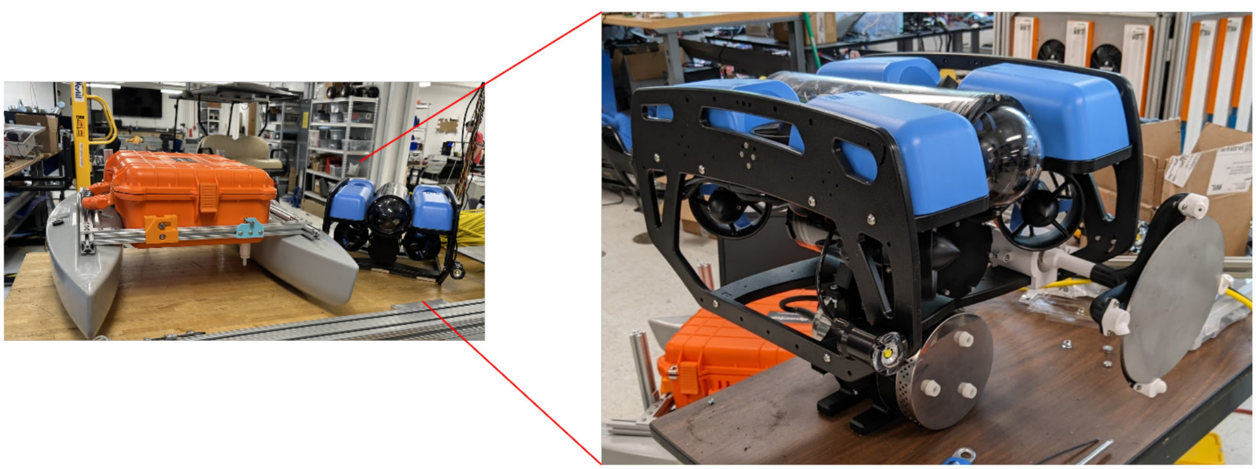
The United States is a minor aquaculture producer, while it is the leading global importer of fish and fishery products. The aim of this project is to design and develop an autonomous, ocean-powered robotic system to support offshore fish farming, which opens new possibilities for advanced technology to aid the proliferation of domestic aquaculture. To operate sustainably far offshore, the system powers itself by harvesting energy from ocean waves. A small-scale autonomous surface vehicle (ASV) serves a dual purpose as both a base system and a wave energy converter (WEC). The ASV deploys an autonomous underwater robot embodied in a small-scale tethered, omnidirectional remotely operated vehicle (ROV), which is termed an AROV. The primary task of the AROV is the light-duty cleaning of fish pens since it operates 24/7.

An ASV and an AROV have been designed and built together with their perception and control techniques to validate the concept of the proposed the proposed robotic system. The AROV, the pose of which is poorly known, has been localized well by identifying its relative position to the fish pen when working on the fish pen and to the ASV when moving from one fish pen to another.



COOPERATIVE LOCALIZATION AND NAVIGATION OF UNMANNED UNDERWATER AND SURFACE VEHICLES FOR OPEN OCEAN ACUACULTURE

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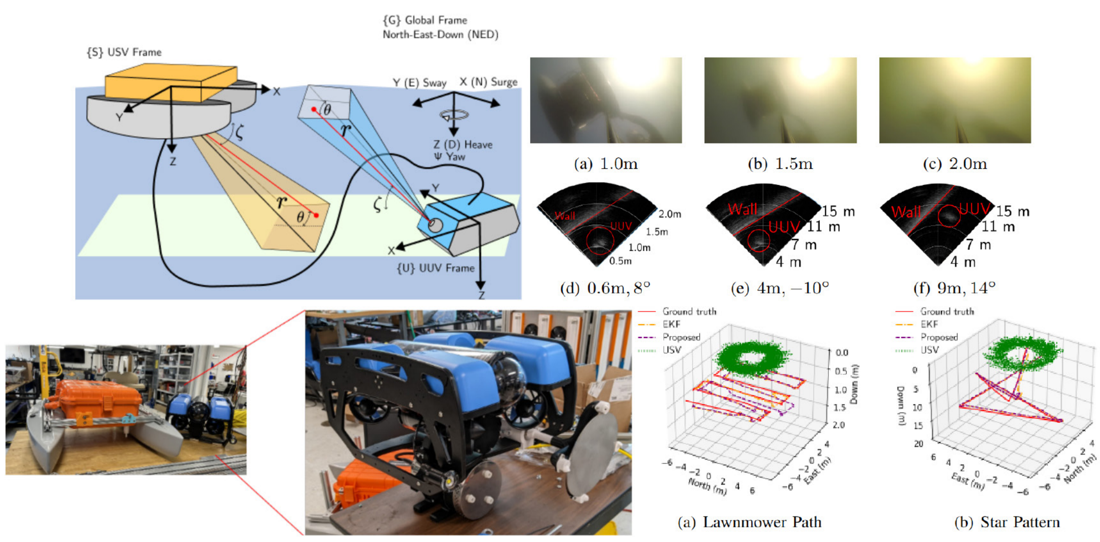
Open ocean aquaculture often needs a set of underwater and surface vehicles for its operation. While it interacts with fish and fish pens, the state of the underwater vehicle is poorly known. The surface vehicle, which is tethered with the underwater vehicle, can thus act as a sensor node to collect information on the surface and better-pose the underwater vehicle.

This paper presents a foundational strategy of the team of unmanned underwater and surface vehicles, which cooperatively localizes and navigates the vehicles. For the localization, the surface vehicle identifies its pose with a Global Positioning System (GPS) and a compass. To localize the underwater vehicle, the surface vehicle is equipped with a sonar sensor whereas the underwater vehicle observes the surface vehicle using a camera. Since the underwater vehicle conducts tasks for aquaculture, the surface vehicle understands the intention of the underwater vehicle through the tethered communication and moves along the underwater vehicle.

Experimental work using a team of unmanned underwater and surface vehicles shows that the proposed strategy allows the cooperative operation of the underwater and surface vehicles.



MULTI SITE GENE TARGETING USING CRISPR TECHNOLOGIES TO IDENTIFY AND DEVELOP HYPOXIA TOLERANT RAINBOW TROUT (*Oncorhynchus Mykiss*)

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Intensified production demands in commercial aquaculture elevate the risk for hypoxic events, primarily due to high-density farming, elevated temperatures, and increased nutrient loading. Hypoxia incidents lead to immediate and enduring negative impacts on fish health, growth rates, and overall facility productivity. Current methods to maintain healthy oxygen levels in hatcheries focus on hypoxia prevention rather than strategies to benefit fish during a hypoxic event.

Using knowledge associated with hypoxia response in other species we have initiated an in vivo CRSIPR gene editing screen to modify numerous target genes involved in hypoxia response in rainbow trout (RBT, *Oncorhynchus mykiss*). The gene editing targets include multi-site-targeting of genes involved with erythrocyte development to promote oxygen delivery. We are also making edits to improve vascular growth of the gills to increase surface area for oxygen uptake, and are editing signaling pathways involved in oxygen regulation and cardiovascular function. The phenotypic screening approach facilitates the characterization of complex genomic elements to better understand the underlying molecular mechanism at work during the physiological response to hypoxic events. Ultimately, this will enhance our insights into genetic traits that promote hypoxia tolerance, allowing us to strategically apply CRISPR technology to develop resilient founder fish for commercial aquaculture operations. The use of current prevention methods for low oxygen combined with the culture of hypoxia-tolerant fish could reduce the risk for negative impact from hypoxic events in commercial aquaculture.

CO-LOCATING REAL-TIME WAVE AND CURRENT DATA TO REDUCE CAPITAL EXPENDITURES AND OPERATING EXPENSES AT OFFSHORE AQUACULTURE SITES

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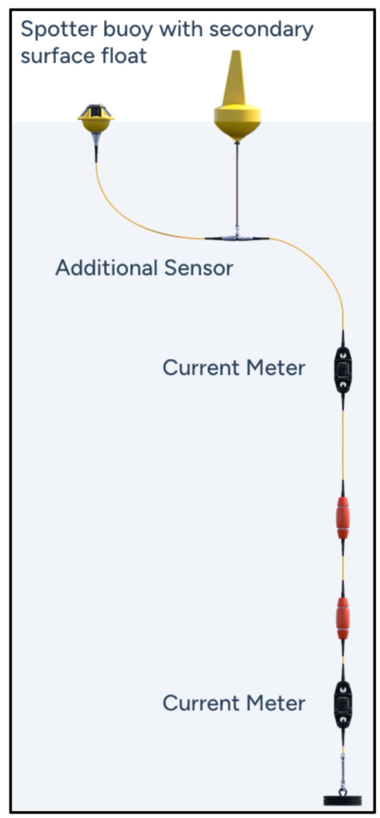
Offshore aquaculture operations face highly variable environmental conditions that can negatively affect efficiency and profitability. Real-time monitoring of waves and currents helps mitigate these effects, providing crucial data that assists in tasks such as farm design and feeding schedule optimization. Traditional environmental monitoring solutions, however, are expensive, cumbersome, and may require separate instrumentation, increasing capital expenditures (CapEx) and operating expenses (OpEx).

Spotter is a modular, rapidly deployable marine sensing platform that delivers real-time surface and subsurface data to power better decisions. Users can attach up to two single-point acoustic Doppler current sensors at depths from 5 to 50 meters, ensuring robust current measurement. Spotter also supports temperature, pressure, dissolved oxygen, and hydrophone sensors, with a CTD and pH probe available by late 2025.

To measure subsurface currents, Spotter integrates the Aanderaa 4830 ZPulse Doppler Current Sensor, known for fast sampling, low power consumption, and high data quality. All observations of current speed and direction made by the sensor are transmitted in real time via satellite and cellular and made remotely accessible via the Spotter Dashboard and API. By co-locating these current measurements with wave measurements made by the Spotter buoy at the surface, operators can better optimize site selection, reduce equipment failure risk, and improve feeding efficiency. Real-time data access enables adaptive management, reducing downtime and increasing resilience to extreme weather.



This co-located monitoring solution lowers CapEx by reducing the volume of sensor deployments and lowering installation costs, and achieves OpEx savings through streamlined maintenance, improved decision-making, and enhanced productivity. Case studies will demonstrate cost benefits and operational improvements, reinforcing the value of real-time ocean data for optimizing offshore aquaculture operations.

EMPOWERING AQUACULTURE: SCALABLE SOLAR-POWERED SOLUTIONS FOR THE WORKING WATERFRONT

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Nauti Sisters Sea Farm, a small-scale, female-owned oyster farm in Maine, leads climate-friendly innovations. In partnership with Shred Electric, we've developed a fully solar-powered electric work barge that operates essential equipment, including a water pump, tumbler, and electric outboard motor. The barge is self-sustaining, powered solely by solar energy. In addition we've also implemented a solar-powered refrigerated unit for transportation of products. Our goal is to create scalable, repeatable solutions for aquaculture electrification, addressing cost and technical challenges while reducing the environmental impact of aquaculture farming.

WHITE SPOT SYNDROME VIRUS (WSSV) GENOMES FROM ECUADOR, PERU, CHINA AND MEXICO ARE NOT INTEGRATED IN THE GENOME OF FIVE PENAEID SHRIMP SPECIES, BUT ENDOGENOUS VIRAL ELEMENTS (EVE) OF WSSV (WSSV-EVE) ARE FOUND IN THE TRANSCRIPTOME OF THE FIRST SPECIFIC PATHOGEN-FREE (SPF) *Penaeus vannamei* DOMESTICATED IN THE UNITED STATES

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Mortalities of cultured shrimp induced by white spot syndrome virus (Viruses; Naldaviricetes; Nimaviridae; Whispovirus; WSSV) have occurred in Ecuador since 1996. Endogenous WSSV-like sequences (WSSV-EVE, Utari et al. 2017) have been reported in *Penaeus vannamei* farmed in Thailand and in expressed sequence tags (EST) libraries prepared from the first specific pathogen-free (SPF) *P. vannamei* domesticated by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP) maintained at the Oceanic Institute in Hawaii.

The WSSV genome sequence form China (AF332093.3, 305119bp), Ecuador (MH090824, 288,997bp), two isolates from Mexico, and 13 complete WSSV genome sequences available in GenBank database were used in this study. Twelve whole genome shotgun (WGS) assemblies and 35 transcriptome shotgun assembly (TSA) databases available for Penaeoidea were used to determine if the WSSV genome, and its nimaviridae family gene *Nimav-1_LVa* (279905bp; Bao et al. 2020; https://pubmed.ncbi.nlm.nih.gov/31947590/), are integrated in the shrimp genome.

WGS searches revealed that none of the WSSV genomes are integrated in the draft genome assembly of *P. vannamei* farmed in China (ASM378908v1; ~1.8-Gb) and in other draft genome assemblies for *P. japonicus*, *P. chinensis* and *P. indicus, P. japonicus*, and *P. monodon*. However, large fragments of *Nimav-1_LVa* are integrated in the genome of *P. vannamei* farmed in China (*Penaeus vannamei* breed Kehai No.1 LVANscaffold_3666 (QCYY01003664, 990704-bp, 428 fragment matches). TSA analyses revealed that many cDNAs and ESTs isolated from SPF *P. vannamei* from USMSFP and other Penaeids are similar to portions of WSSV and *Nimav-1_LVa* sequences, representing putative WSSV-EVE sequences.

To better understand the molecular regulation and evolution of WSSV-EVE sequences, a new, continuous, whole reference genome sequence for *P. vannamei* is needed, particularly considering the variability of current draft genome assemblies for *P. vannamei,* while the expected genome size of SPF *P. vannamei* from an American company is 2.89-Gb (Jeffery & Gregory, 2014).

WHOLE GENOME RESEQUENCING IN TRIPLOID PACIFIC OYSTERS REVEAL AMINO ACID CHANGE SIGNATURES AND SPLICE SITE EFFECT

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Single nucleotide variants, such as SNPs, have been extensively exploited to decode population genetic structure, phylogenetic relationships, and traits of commercial interest in aquatic species. Aquaculture research has applied high-density SNP markers to guide breeding programs based on GWAS approaches. However, single nucleotide variants can alter functional levels, where amino acid changes can improve or repress the performance of proteins and, consequently, their biological functions in organisms with specific molecular signatures. Here, triploid Pacific oysters represent an excellent model for exploring whether triploidy induction alters the signatures of amino acid changes associated with single nucleotide variants and the biological functions where the mutations were identified throughout the genome. In parallel, SNPs have been observed to have potential effects on the spliceosome. Specifically, single mutations can disrupt the splice site, affecting the isoforms of mRNAs and likely the proteome in triploid oysters. This study aimed to explore the differences in the signatures of amino acid changes and the splice site effects of the differential SNP repertoire between diploid and triploid Pacific oysters. Whole genome resequencing was performed in two Pacific oyster families induced to triploidy. Eight diploid and eight triploid individuals were sampled for Illumina sequencing with 30x coverage for each family. A bioinformatics pipeline was designed for SNP calling, filtering by ploidy, and local realignment to improve the identification of nucleotide variations. The amino acid changes and splice site effects were analyzed using the latest genome version for the Pacific oyster, xbMagGiga1.1. Gene ontology analyses were performed on genes differentially annotated with SNP variants and putative splice effects. The results showed no difference in nucleotide mutations and heterozygous/homozygous proportions between 2n/3n oysters. However, nucleotide variations were identified in genes with different functions, where the splice site effects showed strong differences associated with ploidy in oysters. Notably, the assessment between oyster families reveals molecular signatures related to the genetic background, suggesting family-specific nucleotide variations. Future investigations may elucidate how single nucleotide variations drive the phenotype in 3n oysters exposed to environmental stress.

ANTIMICROBIOTA SEA LICE VACCINES

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Global salmon aquaculture is facing a significant threat from pathogens that affect the health and welfare of fish. One of the most common pathogens in the industry is sea lice, and the primary control method is the use of pesticides. Current strategies for improving fish resistance include in-feed additives and genetic selection. However, the immune response to ectoparasites involves interplay with the host's microbiota community during infestation. To develop sustainable strategies in fish aquaculture, it is essential to use the hologenomics approach to uncover the molecular interactions among the host, parasites, and their microbiome. This study aimed to report new sea lice vaccines using the hologenome approach. Here, we challenged Atlantic salmons against sea lice and analyzed them after different immunization strategies using inactivated and live vaccines delivered orally or intraperitoneally. The investigation obtained transcriptional information of skin tissue through RNA Illumina and nanopore sequencing. Additionally, we analyzed the skin's microbiome through full-length 16S RNA nanopore sequencing and functional analysis. The hologenomics approach revealed that the microbiome of Atlantic salmon is a key component in the immune response to pathogens and a novel research field for developing novel fish vaccines.

Acknowledgments: This study was funded by FONDAP grant #1522A0004, FONDECYT #11220307.

MATERNAL EFFECTS AND EGG QUALITY BIOMARKERS IN EASTERN OYSTER *Crassostrea virginica*

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Shellfish aquaculture plays a crucial role in sustainable aquaculture production, with the Eastern oyster, *Crassostrea virginica*, being one of the most important species in the United States. However, the expansion of the oyster aquaculture industry in the Gulf of Mexico (GoM) is restricted by a limited supply of seed and incidences of high mortality in hatcheries. The high demand for quality seed with enhanced growth and survival necessitates improvement in the consistency of larval production to increase commercial hatchery production for the GoM. Ongoing production issues are caused by several factors, with low gamete quality being a significant contributor.

This study aimed to identify biomarkers for egg quality to improve the efficiency of Eastern oyster hatcheries. Five cohorts of diploid oysters were spawned from July through September 2023 using natural spawning methods. Eggs were collected from nine females during each spawn, and a subset was digitally imaged for morphometrics. An additional subset was flash-frozen for fatty acid analysis. The remaining eggs were separated into three groups, each fertilized with a different male. All fertilized eggs from a given female were then pooled, fertilization rates were determined, and eggs were separated into three replicate 800 mL beakers to assess survival. At 24 hours post-fertilization, larvae that had reached the D-hinge stage were counted and measured. Females with the highest and lowest survival rates were selected and categorized as “good” or “bad,” corresponding to >81% and <62% survival, respectively, and egg biomarkers were compared. Egg morphometrics were not significantly different between good and bad eggs. However, fatty acid profiles were different, with good-quality spawns having higher abundances of saturated fatty acids. Docosahexaenoic acid (DHA) levels were correlated with season, with the gametes from the September spawns having higher DHA levels than those from the July spawns.

These findings identify egg quality biomarkers that correlate with 24-hour survival. Future studies will compare gene expression profiles between high- and low-quality eggs to determine molecular mechanisms for game quality. These results will allow hatcheries to prioritize fertilization of high-quality gametes and develop broodstock conditioning protocols that improve gamete quality, enhancing hatchery production and providing a more consistent supply of larvae by minimizing the losses commonly associated with larval rearing.

THE DETECTION OF MERCURY IN INVASIVE CARP TISSUE USING INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETER TRIPLE QUADROPOLE: ARE CARPS A VIABLE SOURCE OF FISHMEAL IN AQUAFEED?

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Invasive carp are in the family *Xenocyprididae* and consist of four species: grass carp, bighead, black carp, and silver carp. They are native to Eastern Asia and were introduced to US waters in the 1960s. Invasive carp have been present in Louisiana since 1976 due to migration and favorable conditions for survival in Louisiana's waters. Invasive carp negatively impact local aquatic life through feeding and impact habitat quality. There are few incentives for local fishermen to target carp, as dockside prices do not compare well to other commercial species. The overall goal of this project is to assess the suitability of Louisiana carp fish meal for use in aquafeeds to create additional market demand. One existing US-based plant in Illinois processes invasive carp into fish meal and fish oil. Ground carp were analyzed for heavy metal contaminants before determining whether Louisiana carp are safe for aquafeed. Fish were sampled from 3 locations- Peoria Ponds in Illinois and then in two commercial fishing zones in Louisiana- Zone 0101 and Zone 0103. For mercury analysis, samples were first digested in acid, microwaved, and analyzed in an Inductively Coupled Plasma Mass Spectrometer with Yttrium as the Internal Standard in two gas modes. An Internal Certified Reference Material, ERM-CE101, trout muscle with a known amount of mercury (0.0219 mg/Kg), was used to validate the sample preparation and method development. The concentration of mercury was analyzed from both the locations Louisiana (0103 and 0101) and Illinois. The concentration of mercury was higher in Louisiana when compared to Illinois and these results are consistent with previous reports of increasing Hg concentrations as you move down the Mississippi River. Louisiana carp could be used as a source of fish meal but care should be taken to keep mercury below the maximum levels allowable for complete animal feed (NRC and AAFCO). Harvesting carp for fish meal production, from both locations, will provide an additional alternative ingredient for use in aquaculture feeds and help to reduce the number of invasive carp.

BACTERIAL MONITORING OF QUEEN CONCH *Aliger gigas* HATCHERY AT THE PUERTO RICO NAGUABO AQUACULTURE CENTER

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The queen conch (*Aliger gigas*) is a marine gastropod predominantly found inhabiting sandy environments and shallow seagrass beds in the Caribbean Sea, the Gulf of Mexico, Florida, and Bermuda. The queen conch holds great fisheries and cultural significance to communities in the Caribbean region, dating back to pre-Columbian times and is considered one of the most relevant species in the area. In 2024 the queen conch was officially listed as a threatened species under the endangered species act. The primary threats are overfishing from commercial, recreational, and illegal fishing activities and habitat degradation. Furthermore, marine environments have undergone significant alterations due to the impacts of climate change and urbanization in coastal regions. Additionally, there has been a marked increase in the frequency of disease outbreaks over the past several decades. The Puerto Rico, Naguabo Aquaculture Center was established in 2019 to serve as a hatchery and nursery for the restoration of the queen conch in Puerto Rico. Since 2021 the USDA-ARS Aquatic Animal Health Research Unit has aided with the development of biosecurity procedures at the hatchery and conducted bacterial monitoring to address sporadic disease events. Since knowledge of queen conch diseases is limited, our objective was to identify and characterize bacteria associated with queen conch aquaculture. To collect bacterial isolates, samples from marine water, egg masses, larvae and juvenile queen conch were collected and inoculated onto a variety of bacteriological media including marine agar, sheep blood agar, TCBS and CHROMagar™ *Vibrio* selective media. Plates were incubated and monitored for 10 d at 28-35°C, and then individual colonies were selected based on morphology and passed to fresh media for isolation and identification. Identification of the bacteria to species was conducted by implementing a multifaceted approach including selective media profiles, Gram staining, Fatty Acid Methyl Esther (FAME) analysis, API 20E strips profiles, and 16S rRNA sequencing. Three predominant bacterial species were identified from the 16S sequencing analysis, including *Vibrio alginolyticus*, *V. parahaemolyticus* and *Pseudomonas mosselii*. These results were supported by FAME and API 20E results that correctly identified the isolates to genus. Given the role of these three bacterial species in finfish and shellfish diseases, research is planned to determine their virulence and potential roles as queen conch pathogens. A greater understanding of queen conch diseases and identifying means of prevention are important for the conservation of this threatened species.

THE DEVELOPMENT OF TRIPLOIDY INDUCTION METHODS FOR HYBRID STRIPED BASS PRODUCTION IN COMMERCIAL AQUACULTURE

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Sunshine Bass are hybrids produced by crossing White Bass females and Striped Bass males. These bass hybrids are an important food fish in the United States. Hybrids show heterosis for survival, growth, disease resistance and tolerance of environmental stressors. Production time for hybrids to reach a marketable weight of 0.6 kg-1.0 kg can range between 10-20 months. During this time, females can become sexually mature and produce eggs. When stressed these females can release eggs which in turn can adversely affect water quality. It is also thought that feed energy is directed toward egg production relative to muscle growth. As with many commercially produced aquaculture species, controlling reproduction can be important to optimize growth and energy allocation efficiency in this production cycle. Triploidization is an effective method to produce sterile fishes. This project aims to evaluate triploidy induction methods that have been used successfully with other commercial aquaculture species. Our goal is determining an optimum methodology for triploid production with special attention being placed on maximizing embryo survival and obtaining high triploidy induction in Sunshine Bass. Hydrostatic pressure shocks were evaluated to produce triploid Sunshine Bass embryos. Four pressures (5000, 6000, 7000, and 8000 PSI) and shock durations of 1.5, 2, 3, 4, and 5 minutes were evaluated. All shocks were administered 4 minutes post-fertilization. Thermal shock treatments were evaluated when administered at 3 or 4 minutes post-fertilization. Cold shock treatments of 2°C or 4°C were given for 10 or 15 minute durations and heat shock treatments of 36°C or 38°C, given for 2 or 3 minute durations. The non-shocked controls were also hatched. The fry produced were evaluated for triploidy using flow cytometry. The treatments yielded very low survival and triploidy percentages. Initially the flow cytometry results indicated heat shock treatments may have higher percentages of triploid induction, relative to cold or pressure shocks. More trials are required to fine tune the triploidization procedure for Sunshine Bass. During the upcoming spawning season, we will evaluate electrical shock as a way of producing triploids, and we will use the Coulter Counter to confirm our triploidy results.

HYDROEPIX: A WATERBORNE SPREAD MODEL FOR INFECTIOUS DISEASES OF FARMED FINFISH

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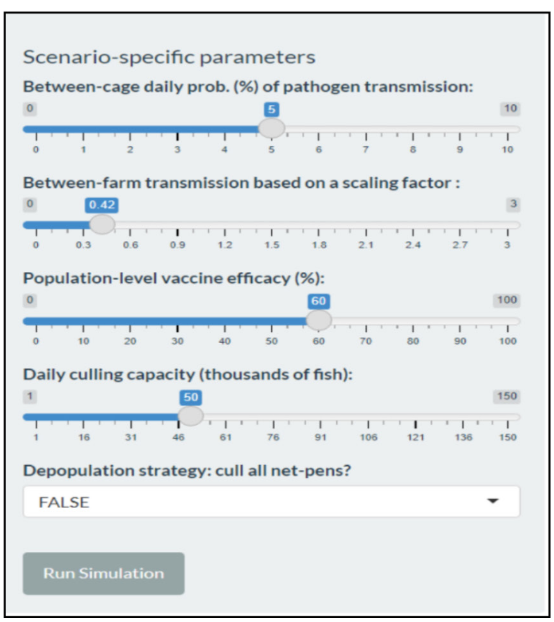
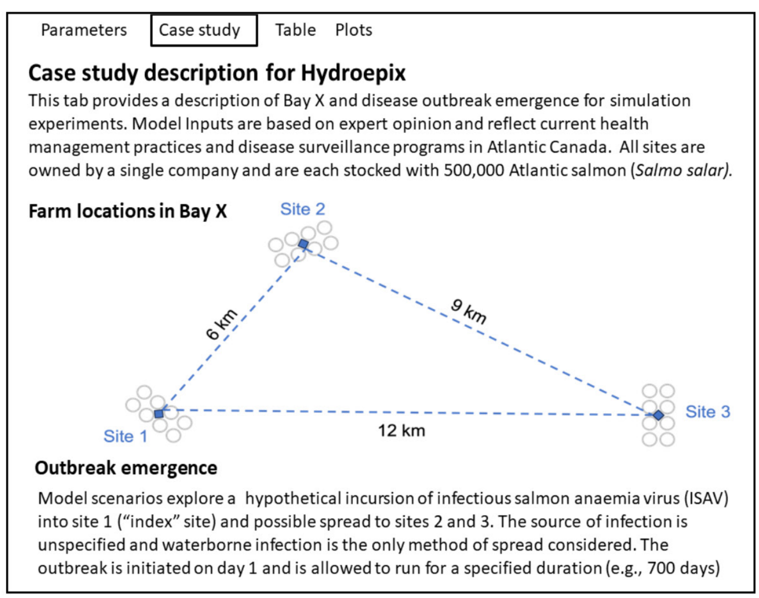
HYDROEPIX is a spatially-explicit simulation framework that models waterborne spread of pathogens within and between net-pens of finfish in the same site, and to nearby farms using the free, open-source software, R. Effects of interventions such as vaccination, early detection and depopulation can be evaluated with the model.

As part of the Farming in Natural Systems (FINS) carrying capacity project, we created a graphical user-interface for HYDROEPIX using the R package "Shiny" to allow users without programming experience to easily adjust parameter values to compare different disease outbreak and mitigation scenarios for a predefined location. The user-interface includes reference documentation and has multiple tabs, including a simulation control tab with sliders to set 5 scenario- parameter values (top left figure). Model inputs are based on Nova Scotia data and documentation, and peer-reviewed references. Inputs are representative of provincial fish health surveillance programs and health management procedures used by salmon aquaculture companies in Atlantic Canada.

We present an example of a simulation run of the Shiny app for infectious salmon anaemia virus infection in Atlantic salmon in a hypothetical bay in Nova Scotia is to demonstrate how the user-interface works and types of outputs that can be compared. The bay includes 3 sites of 500,000 salmon separated by 6, 9, and 12 km in a triangular array (bottom left figure).

The model inputs these distances in a matrix that assumes the risk of waterborne transmission from site 1 to 2 is the same as the risk from site 2 to 1. Ocean circulation effects can be incorporated into the model using an hydroconnectivity matrix as an alternative to a distance matrix. Model outputs are plots of 1) percentage of simulations yielding a transmission event, and 2) magnitude of each outbreak event (e.g. number of infected pens). The model also tracks whether infection is acquired from a net-pen from the same site or from a distant site.





BIOTA NC: HOW A PUBLIC/PRIVATE PARTNERSHIP FOR THE PRODUCTION OF MARINE ORNAMENTAL SPECIES CAN BENEFIT STUDENTS AND AN INDUSTRY

Todd R. Gardner*

The optics of a public/private partnership and the associated opportunities for conflicts of interest often deter academic institutions from engaging in such relationships. Here we report on the specific case of a partnership between Carteret Community College in Morehead City, NC with The Biota Group, a company that specializes in farmed marine life for the aquarium trade.

Five years into the partnership we are seeing numerous benefits, including a college aquaculture lab where all aspects of a hatchery are operating every day of the year, internship and employment opportunities for students and alumni, a steady source of trained employees in an industry where this is often a limiting factor, and at least 12 species added to the Biota's offerings. While conflicts can occur, our experience has been that they are rare and easily resolved as all parties continue to operate in good faith.

CARBOHYDRATE TYPE AND TEMPERATURE EFFECTS ON NUTRIENT DIGESTIBILITY, DIGESTIVE ENZYME ACTIVITY, AND THE INTESTINAL MICROBIOTA OF STRIPED BASS *Morone saxatilis*

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Carbohydrates (CH) are not essential nutrients for fish, but they are a low-cost energy source and are commonly used in the formulation of extruded diets. Additionally, certain CH can be used as an energy source effecting a protein-sparing effect, sparing protein for growth. However, CH can negatively affect fish metabolism, particularly for carnivorous fish. Fish utilization depends on feeding habits, inclusion level and chemical structure of the CH. Striped bass, *Morone saxatilis*, is an anadromous fish of great commercial interest. Baja California, México is the only world region where this species is reared in seawater. However, culture conditions are not optimal, with lower water temperatures resulting in slower growth. Consequently, there is a need to optimized diets to improve growth and reduce costs.

In the present study, four carbohydrate types (i.e., cellulose, dextrin, raw and gelatinized starch) were evaluated, included at 19% in isoproteic and isolipidic diets. The feeding-trial lasted 60 days with juvenile striped bass of 16-18g initial weight distributed in 24 tanks in a recirculating system and cultured at either 16 or 21 °C in full seawater. At the end of the trial, growth performance, digestive enzyme activity, nutrient digestibility, glycogen and lipid content of the liver, and changes in the intestinal microbiota were assessed.

Temperature significantly affected growth performance, feed efficiency, and digestive enzymes, resulting in higher values at 21°C. Dextrin, the simplest carbohydrate, had adverse effects on fish performance while starch resulted in better utilization. Fish feed the dextrin diet showed an increase in amylase activity, highest diet digestibility, but resulted in lower growth and accumulation of liver glycogen. Striped bass appears to better utilize starch compared to simpler CH. Data with respect to gut microbiota will be presented to aid in elucidating our results.

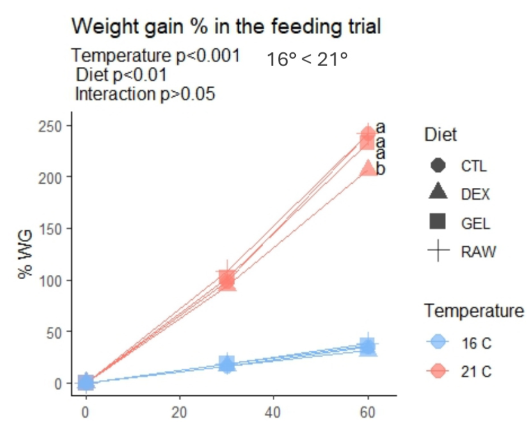

Figure 1. Weight gain of juvenile *Morone saxatilis* fed diets with different carbohydrate types over a 60-day feeding trial, reared at 16 and 21 °C.

Table I. Diet and nutrients apparent digestibility coefficient of *M. saxatilis* fed diets with different CH and reared at 16-21 °C

ADC		Dry matter (%)	Soluble CH (%)	Crude protein (%)
CTL	16°	73.6 ± 3.2 **c**	87.3 ± 3.1 **b**	91.7 ± 1.1 **b**
	21°	72.9 ± 4.4 **c**	88.5 ± 9.0 **b**	91.7 ± 0.6 **b**
DEX	16°	88.8 ± 0.8 **a**	98.3 ± 0.5 **a**	94.3 ± 0.1 **a**
	21°	88.4 ± 0.6 **a**	98.2 ± 0.6 **a**	93.2 ± 0.6 **a**
RAW	16°	83.1 ± 2.2 **b**	83.0 ± 1.1 **b**	94.5 ± 0.3 **a**
	21°	83.8 ± 1.3 **b**	84.1 ± 1.0 **b**	93.7 ± 0.4 **a**
GEL	16°	82.7 ± 2.1 **b**	84.3 ± 0.9 **b**	94.2 ± 0.6 **a**
	21°	84.8 ± 0.7 **b**	86.7 ± 2.4 **b**	94.8 ± 0.5 **a**
ANCOVA				
Temp		P= 0.54	P= 0.42	P= 0.25
Diet		P < 0.0001	P < 0.0001	P < 0.0001
Interaction		P= 0.61	P= 0.92	P= 0.13
IW		P= 0.15	P= 0.29	P= 0.75

CAN RIBBED MUSSEL VALVE GAPING SERVE AS A PROXY FOR BACTERIAL CLEARANCE UNDER VARYING ENVIRONMENTAL CONDITIONS?

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Atlantic ribbed mussels (*Geukensia demissa*) are candidates currently under consideration for microbial pollution remediation in urban estuaries due to their tolerance of degraded environments and their ability to clear bacteria. But the extent and efficacy of this clearance in the face of environmental conditions associated with pollution events, such as rapid salinity decline, are not well characterized. Furthermore, continuous assessment of clearance activity i*n situ* is challenging. Hall effect sensors, which can be used to measure valve gaping, represent an appealing approach to continuously monitor bivalve activities *in situ*. However, the relationship between valve gaping and clearance is not well described in ribbed mussels. This work explores the impact of salinity fluctuations on mussel feeding activities and the potential of valve gaping to serve as a proxy for bacterial clearance.

Ribbed mussels in individual chambers were outfitted with hall effect sensors and magnets on opposing valves. They were then exposed to a gradual, but rapid, salinity fluctuation, with simultaneous delivery of a constant concentration of *Escherichia coli* and 5μm polystyrene microspheres. Valve gaping was monitored continuously via sensors and water samples were collected at the inflow and outflow of each chamber at 15-minute intervals. Particle concentration was then quantified via flow cytometry and percent reduction of particles at each interval was calculated for each individual mussel.

Ribbed mussels were shown to effectively clear *E. coli*. Preliminary results suggest that salinity depression triggers valve closure which corresponds with decreased clearance. These results highlight the role that ribbed mussels can play in remediating pathogen pollution from impacted waterways. Furthermore, they support the potential for hall effect sensors to serve as a proxy for mussel clearance, making continuous monitoring of mussel response to microbial pollution events feasible under field conditions. Ongoing work contrasts response to salinity fluctuations between different mussel populations to evaluate potential local adaptations to different salinity regimes.

SINGLE-CELL TRANSCRIPTOMICS SHEDS LIGHT ON EARLY GERM CELL DIFFERENTIATION IN THE PACIFIC OYSTER (*Crassostrea gigas*)

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Sterile or non-reproductive shellfish are both a market driven need and an ecologically sustainable approach to increasing food production via aquaculture. Current methods for inducing sterility in bivalve shellfish focus on ploidy manipulation. An alternative approach is the induction of sterility by inactivation of genes essential for germ cell (future gamete) formation and development. The power of this biotechnological approach has been realized recently in several finfish species, where suppression of a critical germ-cell specific gene, *dead end* (*dnd*), results in germ cell-free, sterile fish. The shellfish industry is now poised to adopt these technological advances. Unfortunately, the use of this technology in shellfish is hampered by a lack of knowledge of the gene(s) essential for primordial germ cell (PGC) specification in bivalves. To overcome this challenge, we used single-cell RNA-Seq (scRNA-seq), a cutting-edge approach that uses high-throughput sequencing to identify genes expressed in individual cells, to identify genes involved in PGC specification. Using expression of the marker gene *vasa*, we were able to identify cells in cleavage stage and blastula cells likely to represent the germ cell lineage, but had yet to fully differentiate. At the gastrula stage, vasa expression was limited primarily to a single cluster. Genes uniquely expressed in the *vasa*+ gastrula cells include those with functions in transcriptional repression, chromatin architecture and DNA repair. Interestingly, genes with no known homology in other species are also uniquely expressed in this cluster, perhaps indicating novel germ cell specification genes in the bivalve lineage. In addition to the elucidation of genes specific to the earliest germ cells in bivalves, this effort also produced a transcriptional atlas of cell states in early bivalve embryos providing a wealth of information on genes contributing to growth and shell production. This is the earliest developmental stage examined via single-cell RNA sequencing in a lophotrochozoan.

A UNIVERSAL HATCHERY SYSTEM FOR DEVELOPING NEW SEAWEED STRAINS FOR LAND-BASED AQUACULTURE PRODUCTION

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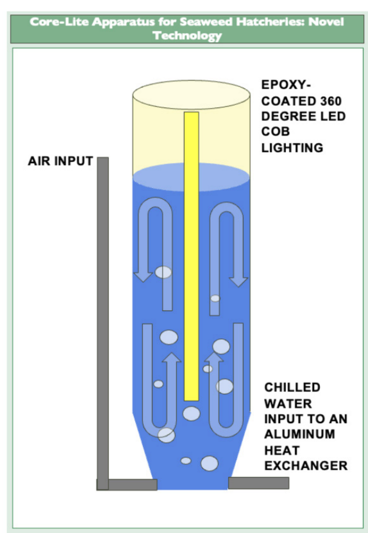
U.S. seaweed farming has the potential to become a significant contributor to seafood production, but the industry is still in its infancy and faces regulatory challenges in developing offshore farms. Land-based aquaculture has the potential to augment seaweed farming and mitigate some of the barriers faced by farmers. In order to expand the land-based seaweed aquaculture industry, there is a need for more low-cost and scalable methods of seaweed production. Our goal is to create novel and accessible hatchery technologies which will reliably produce high density, homogenous macroalgae biomass for use in tumble culture. We have developed an affordable and modular system called the Core-Lit Apparatus for Seaweed Hatcheries (CLASH). Bull kelp (*Nereocystis luetkeana*) and giant kelp (*Macrocystis pyrifera*) have been successfully cultured in the CLASH system. Additional studies seek to determine ideal stocking densities, nutrient supplementation, and light exposure for maximizing productivity. The future of the project aims to introduce new commercially relevant species for aquaculture, create new seaweed strains for land-based farming, utilize CLASH grown kelp for restoration projects, and scale up the use by the industry. This simple, user-friendly, low-cost device has proven to be a promising hatchery tool which could increase the diversity, efficiency, and yields of land-based seaweed farms.



EVALUATION OF WINTER-FEEDING STRATEGIES FOR GROWING NILE TILAPIA (*Oreochromis niloticus)* TO ADULT SIZE

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Aquaculture is the fastest growing animal-producing sector globally. Currently, global supplies of seafood and other products from aquaculture for human consumption is higher than contribution from capture fisheries. Feed cost for intensive aquaculture production accounts for a majority of the operational costs and continues to increase; the opportunity to reduce feed costs through management strategies is a priority to fish farmers. Feeding frequency is known to help reduce cost of feed and prevent wastage from excessive feeding. Limited information is available on feeding frequencies to raise juvenile Nile tilapia to adult size during winter period. Hence, the reason for this study. To examine the influence of two feeding frequencies on tilapia (*Oreochromis niloticus)*, twenty juvenile Nile tilapia with an average weight of 116 ± 1.7 g were stocked in recirculating tanks with three tanks/feeding frequency. Fish were fed *ad libitum* twice or four times daily. Feeding intake was monitored weekly, and fish were weighed biweekly. The current twelve weeks' data revealed numerical but insignificant differences in weight ($P>0.05$). A similar result was observed on the feed conversion ratio and weight gain. The study is ongoing; more information will be presented at the conference.

EFFECT OF TOTAL DISSOLVED GAS PRESSURE ON CUTTHROAT TROUT HATCH AND FRY SURVIVAL

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Elevated total dissolved gas (TDG) levels have been demonstrated to be detrimental in both natural environments and aquaculture settings for many species of fish. The current EPA standard for aquaculture water supplies is that total dissolved gas may not exceed 110% saturation. Gas bubble trauma has been demonstrated for fish species when TDG is above 110% saturation. In hatchery settings utilizing spring or well water, naturally occurring TDG levels of 104% or higher can occur. Detrimental effects of chronic TDG concentration have been variably demonstrated across fish species as well as life stages but limited information is available for cutthroat trout species in early life stages.

To test the effects of elevated TDG on four species of cutthroat trout a 3 x 4 factorial experiment was conducted with TDG concentrations of 100, 104 and 108% saturation with Bonneville, Snake River, Yellowstone, and Greenback cutthroat trout. The culture system consisted of 12 head boxes providing water to 3 tanks each. Four head boxes were allocated to each of the 3 TDG concentrations. The 78 L aluminum tanks received 1 l per min flow through water. Timing of stocking varied for each species due to spawn date. One hundred eggs were stocked in 4 replicate tanks per TDG treatment. Survival and fry size was quantified at hatch, first feeding, and 28- and 56-days post first feeding.

Factorial ANOVA was performed on survival and growth data with TDG and Cutthroat species as the main effects. No interactive effects were observed for TDG concentration and species for survival at any period. Total dissolve gas concentration and cutthroat trout species affected hatch rate with increasing TDG reducing hatch success. TDG did not affect survival from hatch to first feeding, but species effects were observed. Survival at day 28 and 56 were also reduced in the 108% TDG treatment group. Survival rates differed between species with Bonneville cutthroat surviving best and Greenback cutthroat generally surviving worst.

Fish mass at each sampling period were affected by both TDG concentrations and species. Fish mass was lowest for fry reared in 108% compared to 100% TDG with 104% being intermediate. Interactive affects were only observed in the 56-day fish mass with 104 and 108% TDG saturation reducing fish size in Yellowstone and Greenback cutthroat trout and no affect observed in Bonneville and Snake River cutthroat trout. Fish condition score and gill inflammation observations were largely undetectable at day 56. Chronic low levels of elevated total dissolve gas levels appear detrimental to cutthroat fry survival and growth.

TIDAL SALINITY VARIATION MAY PROVIDE A BUFFER FROM LOW-SALINITY EVENTS ON OYSTER HABITAT IN RELATIVELY SHORT FLORIDA ESTUARIES

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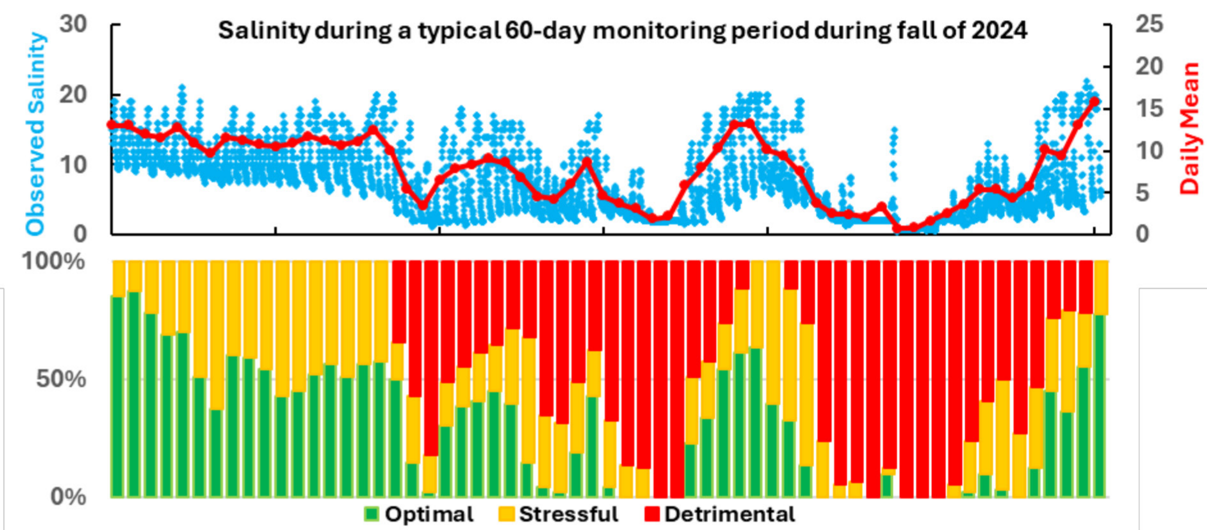
Salinity is a determining environmental factor of the health of oyster reefs in Florida. Many of the estuaries in Florida have small watersheds and tidal amplitudes less than 1 meter. Optimal salinities and habitat for oysters (*Crassostrea virginica*) often occupy a zone less than 10 km, about one third of the total estuarine portion of the rivers. Extreme variation in salinity can occur from localized rainfall events as well as from managed releases of freshwater from upstream structures. In the St. Lucie River (monitored as part of the **C**omprehensive **E**verglades **R**estoration **P**lan = CERP) salinity can vary by 15 on a single tide, and by 20 or more over a few days (see below, top). The percentage of the day where salinity conditions are optimal, stressful, or detrimental to long-term oyster health can decline or improve within just a few days (see below, bottom).

Many studies focus on the metric of daily- or even longer-term mean salinity, but oysters may be able to exploit or benefit from short-term variations during daily tides. While oyster behavior may be limited, the ability to close, alter gape width, and vary filtration rate for feeding or respiration over time frames of an hour or less may allow a refugia from some of the extreme estuarine variability. Oyster health is further complicated by other factors including season, temperature, location in the estuary, reproductive status, and size and age of the oysters. Our study will be used to refine the **H**abitat **S**uitability **I**ndex models used for planning restoration of oyster habitat in CERP estuaries.



EFFECTIVENESS OF LASER AND ACOUSTIC BIRD DETERRENT DEVICES IN AQUACULTURE: IMPLICATIONS FOR SHELLFISH PRODUCERS

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Floating cages have increased shellfish survival, decreased fouling, and increased the quality of shellfish being produced. However, this gear is often attractive to birds which may be spread contaminants such as fecal coliform and campylobacteria. Many methods, including perching deterrents, sound devices, and submerging bags, have been developed and tested to deter birds from aquaculture predation facilities. However, birds often adapt quickly to the presence of these deterrents and their effectiveness is often limited to short-term benefits. Recent advances in acoustic and laser deterrent systems have been found to be effective at orchards and poultry farms and may help reduce avian predation at aquaculture facilities. As such, we used a camera trapping survey to examine the effectiveness, ease of use, and practicality (e.g. cost/benefit) of acoustic and laser deterrent systems on fish farms in Kansas, Michigan, and Missouri. Deterrent systems were programmed to primarily target Great Blue Heron (*Ardea Herodias*) although we considered additional species opportunistically in our analyses. Avian predator abundance was measured before, during, and after the use of each deterrent (laser or acoustic) using camera images analyzed by artificial intelligence (AI). In addition, an integrated pest management (IPM) approach was used to see if the combination of acoustic and laser systems further reduced predation at fish production facilities. The amount of time spent actively feeding significantly decreased ($p<0.05$) in all treatments and at all sites. Additionally, birds were observed actively avoiding or otherwise acting strangely (e.g. sitting, pacing) in areas with the deterrents in place. Discussions will include insight into how our findings may apply to shellfish production and how our results may help producers make informed decisions on reducing avian abundance at shellfish production facilities.

NUTRITIONAL PROFILE AND ANTIBACTERIAL ACTIVITY OF BLACK SOLDIER FLY *Hermetia illucens* LARVAE MEAL AS POTENTIAL PROTEIN SOURCE FOR AQUAFEED

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Aquaculture struggles with unsustainable reliance on fishmeal and soybean, raising costs and compromising feed quality. Excessive antibiotic use further harms water quality and increases disease risks. Plant-based protein alternatives face challenges with amino acid composition and palatability, limiting their effectiveness. *Hermetia illucens* (black soldier fly) larvae present a viable solution, offering high protein and lipid content with antimicrobial properties against aquaculture pathogens. This study evaluates the nutritional composition of *H. illucens* larvae at different instar stages and their antibacterial efficacy against aquaculture pathogens, promoting sustainable and cost-effective solutions for aquafeed development.

H. illucens larvae were collected at different instars (5 stages), cleaned, dried, and ground for nutritional composition analysis. The analysis included crude protein, fat, ash, moisture, carbohydrates, calcium, phosphorus, and fatty acid profiling. For antimicrobial testing, methanolic extract of BSFL was evaluated against four pathogenic bacteria using the agar disk diffusion assay and the minimum inhibitory concentration (MIC).

The nutritional and antibacterial properties of BSFL varied across instar stages. Crude protein (36.96%) and fat (40.90%) peaked in the third instar, while ash and lauric acid (44.76%) were highest in the later stages. Calcium content (3.16%) and carbohydrates were greatest in the fourth instar, while phosphorus and other fatty acids declined with age. Antibacterial activity, assessed via agar diffusion and MIC assays, showed dose-dependent inhibition against *Aeromonas veronii* (7.10 mm), *Edwardsiella tarda* (5.30 mm), *Streptococcus agalactiae* (13.40 mm), and *Vibrio harveyi* (6.50 mm) at 1000 mg/ml. Findings highlight the fifth instar as optimal for aquaculture applications.

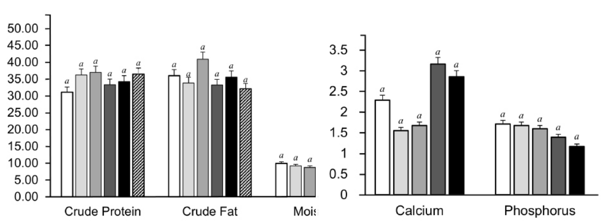

Figure 1. Variation on nutritional composition (%) of *Hermetia illucens* larvae (*a.* Proximate composition and *b.* mineral composition.) in different instar stages. ☐, 1st instar (0-5 days); ☐, 2nd instar (6-9 days); ☐, 3rd instar (10-13 days); ☐, 4th instar (14-16 days); ■, 5th instar (17-19 days) and ☐ mixed.

Table 1. Identified fatty acids components of *H illucens* larvae in different instar stages (calculated by percentage).

Parameters	Results (%w/w)				
	1st instar	2nd instar	3rd instar	4th instar	5th instar
Fatty Acid Methyl Ester					
Capric acid (C10:0)	0.43	0.51	0.47	0.62	0.79
Lauric acid (C12:0)	31.41	28.94	30.5	38.53	44.76
Myristic acid (C14:0)	8.07	7.26	7.36	7.77	7.15
Palmitic acid (C16:0)	22.03	22.49	20.83	19.4	17.46
Stearic acid (C18:0)	4.1	4.12	4.37	3.22	2.28
Oleic acid (C18:1)	21.24	23.66	23.63	18.85	17.25
Linoleic acid (C18:2)	6.63	7.03	6.81	6.02	5.86
Linolenic acid (C18:3)	0.33	0.29	0.33	0.27	0.28

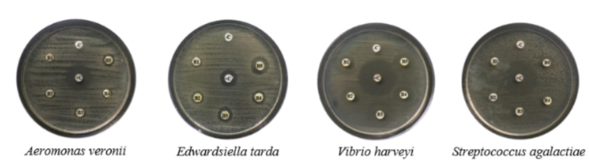

Figure 2. Test result of antibacterial activity of *H. illucens* larvae crude methanolic extract across different instar stages against common pathogenic bacteria in aquaculture (1000mg ml^{-1}). B1- First instar, B2- Second instar, B3-Third instar, B4- Fourth instar, B5 - Fifth instar, +C - Oxytetracycline (positive control), -C - DMSO (negative control)

CONNECTICUT AQUACULTURE WORKFORCE DEVELOPMENT STUDY: SUPPORTING THE NEXT GENERATION OF STUDENTS AND FARMERS

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Introduction

Aquaculture is a valued component of the Connecticut agriculture sector. The aquaculture sector contributes greater than $33 million to the state economy and supports nearly 500 farm jobs statewide. Aquaculture workforce development is strong with three specialized high schools, a network of Agriculture Science & Technology Education (ASTE) centers, and Extension training programs. However, few students stay in CT for post-secondary education or are employed in CT aquaculture jobs. The study objectives were to: 1) This baseline survey has determined the current providers and their location, the curriculum offered and annual recruitment numbers. It has importantly highlighted supply gaps and opportunities and is intended to inform the refinement of future aquaculture education and training, and career pathways.
2) highlight strengths and gaps between industry demand and education supply, and 3) identify current educational linkages and potential future pathways.

In-person interviews and online surveys using Qualtrics^{XM} were used to gather data. At the end of the data collection, a total of 76 individuals participated in the survey. The respondents were distributed among farmers (30), and educators (46). The results from the aquaculture sector show that despite some significant constraints, most of the business owners anticipate growth in output and the numbers of staff they expect to employ over the next 5-10 years. The industry employers' demand for labor and skills can be characterized across four different thematic areas: farm operations, trades related, scientific/regulatory, and marketing/value added. A notable gap is that specialized skills needs are not regularly communicated from the sector to educators. Further, few internships and no apprenticeships exist for future farm-based experiential learning.

A statewide review of the aquaculture education and training supply from tertiary, secondary and extension sectors has determined the current providers and their location, the curriculum offered and annual recruitment numbers. There are gaps between what is taught/trained in aquaculture programs and the skills/experience that employers seek. From the results five key areas for growth were gleaned: marketing and promotion, aquaculture curriculum development including trades skills, infrastructure support and professional development, partnership and pathway development, while promoting diversity, equity, and inclusion.

This study will inform the development of new and improved secondary and post-secondary aquaculture curriculum and increase hands-on learning experiences. This will ultimately better prepare the next generation of farmers and support sectors by establishing transparent, comprehensive & accessible pathways for aquaculture education and training in Connecticut.

THE DUAL ROLE OF AQUACULTURE EXTENSION ALONG HEAVILY POPULATED COASTLINES: EVOLVING TO ADDRESS PUBLIC CONCERN WHILE FACILITATING WORKFORCE DEVELOPMENT

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Introduction
Bivalve shellfish production is a large and growing segment of the U.S. aquaculture industry. In the southern New England region, the number of farms has increased rapidly, particularly along heavily populated coastlines. This growth has been partly facilitated by extension and education staff whose role has been to investigate the development of new species, technology, tools, and training for farmers. However, while these farms still occupy a relatively small area within the coastal zone, further expansion has been met with some concern. In response, extension and education programming has evolved to: (1) expand tools, knowledge and reach for public engagement, (2) expand entry-level workforce training, and (3) improve decision support tools for regulators and public health officials. Extension and education products include:

- *Aquaculture 101 Public Outreach Seminars* - provides global to local perspectives on the need for aquaculture from the standpoints of seafood demand, jobs and economy, and the cultural and ecological role of shellfish
- *Outreach Exhibit at New England Aquarium* - information on shellfish aquaculture in the region including 25 key topical messages, and four hands-on activity kits
- *Outdoor Public Signage* – permanent signage displayed at public coastal access sites provides site-specific information on shellfish harvest and cultivation
- *Video Series* – YouTube Playlist includes a growing series of videos on the cultivation of various aquatic species and methodologies in both commercial and educational settings
- *Fact Sheets* – research-based information on emerging issues in shellfish aquaculture including contaminants (e.g. PFAS), microplastics, and harmful algal blooms
- *Shellfish Aquaculture Workforce Training Courses* – curriculum includes foundational knowledge prospective farmers need to develop a business plan and budget, select a farm site, navigate the regulatory process, cultivate, harvest, process and sell product, and manage production hazards
- *Public Messaging Strategy and Plans* – Messaging strategies were drafted and incorporated into state public health response plans so that audiences would have simple and concise messages that could be communicated to the press and the public about emerging issues (e.g. Vibrio bacteria and associated illness)

Collaborating as a regionwide extension team has enabled staff to develop a more coordinate response to emerging aquaculture sector research, development and training needs, and to public interests and concerns about the social carrying capacity for aquaculture along the coastline.

LARVAL MICROCULTURE OF THE SEA URCHIN, *Lytechinus variegatus*

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Sea urchins are valuable models for understanding genetic control of early deuterostome development, and they can be useful for understanding the interactions between genotype and phenotype. Elucidating such interactions requires genetic tools such as CRISPR-Cas9 and RNAi applied to the genome. Genetically modified (GM) embryos are typically produced in relatively small numbers, ranging from dozens to a few hundred. Standardized larval culture techniques designed to rear small numbers of embryos through the larval and settlement stages will be key for tracking genes through adulthood and creating GM breeding lines of sea urchins. Whereas, embryos and larvae of cold-water species of sea urchins have been used in past years, a warm-water species may be more amendable to GM work across laboratories. *Lytechinus variegatus* is a temperate-tropical species of sea urchin that inhabits the nearshore seagrass communities of the Gulf of Mexico and western Atlantic. The embryos and larvae of *L. variegatus* can be reared at room temperature (approximately 22°C), and larvae begin settlement within three weeks of fertilization, which is much faster than many cold-water species.

Microculture of sea urchins involves growing small numbers of individual larvae in test tubes until the rudiment forms prior to settlement, followed by growing the metamorphosed juveniles in small containers until they can feed on formulated pellets (ca. 3 mm test diameter). Our laboratories are evaluating methods for microculture of *L. variegatus* larvae and juveniles in fully synthetic seawater (Instant Ocean). We investigated housing and feeding strategies for rearing ten larvae through settlement in 20 mL glass test tubes. Sea urchin larvae (full siblings) were housed in 20 mL test tubes containing 15 mL of synthetic seawater at 34 ppt at 22°C with 12:12 light:dark photoperiod. In Experiment 1, tubes were divided equally between one of two devices to simulate oceanic waves: a tube rotator or an orbital shaker (n=10 tubes per device). Larvae were fed 16,000 cells/larva/day of a 1:1 combination of green and red unicellular algae (*Tetraselmis suicica* and *Rhodomonas salina*, respectively). Survival to 8-arm pluteus with rudiment (ca. 16 dpf) was 67.5% for the rotator and 50% for the shaker. In Experiment 2, the tube rotator was used (n=10 tubes, n=10 larvae per tube) and larvae were fed 24,000 cells/larva/day of a 1:1 combination of green and red unicellular algae (*Dunaliella tertiolecta* and *Rhodomonas salina*, respectively). Survival to 8-arm pluteus with rudiment (ca. 16 dpf) was 68%. Settlement success at 30 dpf for surviving larvae was 60%.

Future challenges include improvement of survival and growth demographics of larvae, optimization of feeding strategies, optimization of settlement and metamorphosis, and early growth of juveniles. Successful survival and growth of these early life stages will greatly enhance the utility of the model for both coastal and inland labs. Funding for this work was provided by the NSF EDGE IOS-1923445 and NSF EDGE IOS-2319783.

A NEW MECHANISTIC MODEL OF OYSTER-*Perkinsus* DYNAMICS UNDER CHANGING ENVIRONMENTAL CONDITIONS

Sarah Gignoux-Wolfsohn, Katrina Pagenkopp Lohan, Greg Ruiz, Mariah Kachmar, Lisa McManus

Perkinsus marinus is a protistan parasite that infects the eastern oyster *Crassostrea virginica* and causes dermo disease. Since it was first identified, it has caused large-scale mortalities of oysters along the gulf and east coasts of North America. *P. marinus* prevalence, intensity, and resulting oyster mortality increase with temperature and salinity. Outbreaks occur during summer and fall, when water is warmest and salinity highest. Given the impacts of temperature and salinity on *P. marinus*-*C. virginica* interactions, we expect climate change to further affect this system, with potentially strong impacts in colder locations. Previous models of the *P. marinus*-*C. virginica* system are of two general types: proliferation-based models focused on tracking *P. marinus* abundance or disease transmission models focused on tracking susceptible and infected *C. virginica* individuals. Proliferation-based models show that including temperature and salinity dependencies leads to more realistic model predictions of within-host parasite abundance. As a whole, these models focused on large-scale dynamics rather than on the interactions between an individual host and parasite. Here, we present a simple model tracking the population dynamics of *P. marinus* in the local water volume and within their oyster hosts (Figure 1). We use existing time series data to derive responses for key parameters (filtration, parasite growth rate) to temperature and salinity. We then use the existing model to predict future dynamics under climate change.

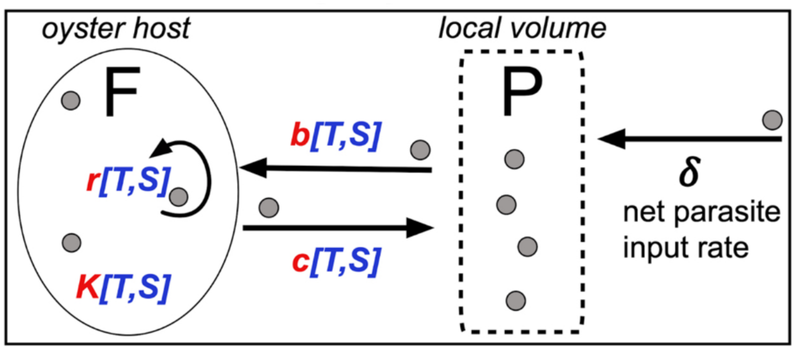

Figure 1. Schematic of the model, showing movement of *P. marinus* between a free-living local volume (P) and the oyster host (F) via rates that are dependent on temperature (T) and salinity (S)

ENHANCING MACROALGAE DIGESTIBILITY WITH PDMA (PARTIALLY DECONSTRUCTED MACROALGAE): DECARBONIZING ANIMAL FEED AND PROMOTING SUSTAINABLE AQUACULTURE

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Conventional animal feeds significantly contribute to greenhouse gas emissions. At the same time, commercial macroalgae farming struggles in many areas due to the paucity of scalable markets. Cultivated macroalgae offer a promising alternative to land-based ingredients, due to their rapid growth and minimal environmental impact (requiring no land area, fresh water or exogenous fertilizer, and absorbing CO_2 and excess nutrients). However, their complex polysaccharides render them largely indigestible by most animals. Herbivorous fish, by contrast, thrive on macroalgal diets, as their microbial consortia in the GI tract are able to deconstruct these polysaccharides into smaller, more digestible sugars and fatty acids. We have co-opted these consortia *in vitro* to produce, PDMA (Partially Deconstructed Macroalgae). Using PDMA for aquaculture and terrestrial ag feeds could provide a commercial driver for expanded macroalgae farming and contribute to mitigating the 7.1 gigaton of CO_2 – equivalent emissions produced by terrestrial livestock annually.

1. **Isolate and identify key microbial consortia:** We collected samples of microbes from various locations in the fish GI tract, and in conjunction with NREL, evolved a microbial consortium with high polysaccharide-degrading activity.
2. **Develop and characterize PDMA:** Using these microbial isolates, we conducted fermentation trials on two macroalgae species (*Agardhiella subulata* and *Halymenia hawaiiana*) to produce PDMA. The resulting PDMA was processed into a shelf-stable, freeze-dried powder for integration into animal feeds at various inclusion levels.

This presentation reviews results of feed trials in a range of species – tilapia, chickens, salmon and shrimp - using PDMA derived from two tropical red macroalgae species (*Agardhiella subulata* and *Halymenia hawaiiana*), with microbiome from chubs (aka drummer, rudderfish; *Kyphosus vaigiensis)*. Growth rates and feed conversion efficiencies, and measures of physiological fitness were not significantly different from animals fed control diets. Feed consumption was greater in PDMA-fed animals, in some instances.

PDMA can provide a scalable, cost-effective, and environmentally friendly alternative to traditional feed ingredients, with the potential to significantly reduce greenhouse gas emissions associated with animal agriculture and provide economic incentives for expansion of macroalgae farming.

ADDRESSING LABOR DEMAND AND PRODUCTION EFFICIENCY IN SHELLFISH AQUACULTURE

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The bivalve shellfish aquaculture industry has rapidly developed over the last two decades, becoming an economically important sector to many rural US coastal communities. Shellfish aquaculture is a sustainable practice that is resource efficient and nutrient dense, thus having a high potential to fulfill global food demands. Expansion of shellfish aquaculture is limited by labor constraints –including labor shortages, high labor costs, and variable working conditions across farms. The process of culturing hard clams (*Mercenaria mercenaria*) and Eastern oysters (*Crassostrea virginica*) has high labor requirements due to biofouling control, gear maintenance and cleaning, and splitting and grading product. Data on labor demands for various shellfish production processes and culture methods are limited.

This research measures labor demands in Virginia and Florida shellfish aquaculture to assess production efficiencies, evaluate potential technological substitutions, and determine optimal workforce development. Biweekly surveys will be used to record labor demands, harvest practices, workforce management, and day-to-day challenges of shellfish farmers. Interviews will be conducted to evaluate commercial shellfish farm employers and employees' views on labor availability, job satisfaction, stressors on productivity, technological substitutions, and industry growth. Data processing include interview analyses identifying emergent themes and generalized linear models assessing factors driving labor demands. A technoeconomic production model will be refined from the results. A productivity benchmarking tool will be developed to improve production efficiency and total product output at individual shellfish farms. By improving our understanding of labor constraints in shellfish aquaculture, expansion can proceed while facilitating economic development and improving sustainability in the sector.

MARICULTURE WORKFORCE DEVELOPMENT THROUGH EXPERIENTIAL LEARNING AT A COLLEGE COMMUNITY CAMPUS IN ALASKA

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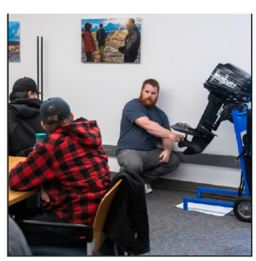
Mariculture is a growing industry in Alaska with an expanding workforce need. Prince William Sound College (PWSC) is located in Valdez, AK, close to budding mariculture efforts. At PWSC I have developed classes and academic programs with a goal of providing field-based, hands-on training of marine scientists and mariculture professionals. Being a community campus allows us to develop unique programming to respond to community needs, while being part of the University of Alaska system connects us to more course offerings and broader educational opportunities for our students.

We approach mariculture workforce development from many angles in an effort to reach as many students as we can and meet them where they are in their education and experience. Our primary mariculture program is a 16 credit occupational endorsement certificate that can be completed in one semester. This includes four core courses, two of which fulfill a UAA general education requirement, and three credits of approved electives. The program focuses on hands-on training and connecting students with industry professionals. We also have community courses in which our maritime tech instructor travels to different communities to teach skills needed in the industry, such as safe boat handling and small engine repair. Students interested in gaining deeper experience with the industry can participate in internships in mariculture as well, with industry professionals, tribal entities carrying out mariculture work, and research institutes participating in mariculture research. In order to reach younger students, we have a field-based mariculture-focused professional development course for K-12 teachers modeling place-based education. The final piece of this education puzzle is a robust student research program, unique for two-year, introductory level campuses, including lab and field projects at our mariculture research and education farm site.

Prince William Sound College focuses on experiential, place-based education to fit local and industry needs. All of these programs have been developed and continue to be improved with industry input. We strive to create accessible, affordable programs that fit industry needs and align with student educational and career goals.



OPTIMIZING ALGAL PRODUCTION AS A SHELLFISH HATCHERY FEED: ALGAL HARVESTING STRATEGIES AND THEIR IMPACT ON FATTY ACID COMPOSITION IN THE MARINE DIATOM *Chaetoceros muelleri*

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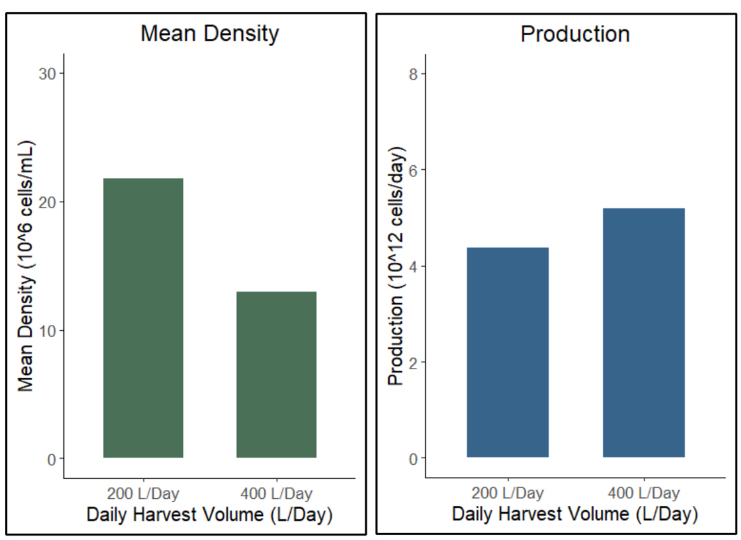
The availability of essential fatty acids (FAs) in algae is critical to the successful development of bivalve larvae in shellfish hatcheries. Among these, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are critical for shellfish development, yet concentrations of these FAs in algal cultures can vary with growth phase and harvesting practices. This preliminary study examines the fatty acid composition of *Chaetoceros muelleri* (CHGRA) grown in Industrial Plankton's automated photobioreactors (PBR 1250Ls) across two steady-state harvesting regimes to evaluate tradeoffs in algal yield and quality.

Cultures of CHGRA were grown under two automated harvesting schedules (200 L/day and 400 L/day), and samples were analyzed for FA composition. Results indicated stable cell densities within each run, though as expected, cultures with a lower harvest rate (200 L/day) showed higher cell counts. However, the 400 L/day regime yielded a 19% increase in algal biomass production, demonstrating a significant boost in daily yield with minimal impact on FA composition.

These findings suggest that higher harvesting rates in automated photobioreactors can optimize algal biomass production without substantial compromise in nutritional quality. The study shows the potential for shellfish hatcheries to further improve larval feed supply efficiency using automated bioreactors.



IMPROVING THE VACCINE EFFICACY OF DOUBLE RECOMBINANT ESC-NDKL1 TO PROTECT CHANNEL CATFISH AGAINST MOTILE AEROMONAS SEPTICEMIA

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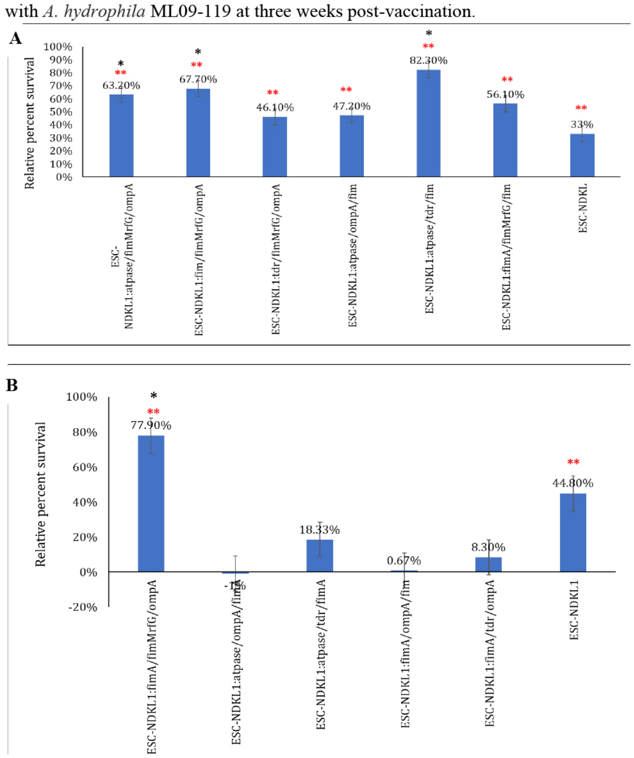
Since 2009, *Aeromonas hydrophila* has emerged as a primary pathogen impacting U.S. catfish aquaculture, causing Motile Aeromonas Septicemia (MAS). The disease has led to severe economic losses, with mortality rates often exceeding 90% in affected ponds and rapid spread across farms. Current therapeutic and preventative strategies against v*Ah* remain limited, highlighting the need for improved solutions.

Our group demonstrated that recombinant v*Ah* surface proteins (OmpA1, Tdr, Fim, FimA, FimMrfG, and an ATPase) significantly protect catfish against MAS. Additionally, the live attenuated *Edwardsiella ictaluri* vaccine strain ESC-NDKL1 is an effective vaccine for ESC and serves as a vector for expressing v*Ah* antigens. Based on this, 18 recombinant ESC-NDKL1 strains expressing one or two v*Ah* surface antigens were developed, with strains expressing two antigens providing enhanced protection compared to those expressing one.

The study aimed to enhance the vaccine efficacy of recombinant ESC-NDKL1 strains expressing two *vAh* antigens. Eleven recombinant ESC-NDKL1 strains expressing three v*Ah* antigens were constructed by in-frame insertion of a v*Ah* antigens into the chromosome of recombinant ESC-NDKL1 strains expressing two v*Ah* antigens and tested on channel catfish fingerlings. Four strains (ESC-NDKL1::fimMrfG::ompA::fimA, ESC-NDKL1::atpase::fimMrfG::ompA, ESC-NDKL1::fim::fimMrfG::ompA, and ESC-NDKL1::atpase::tdr::fim) provided the best protection, with relative percent survival (RPS) ranging from 63.18% to 82.35%. Additionally, the triple recombinant strains induced a stronger antibody response compared to double and single recombinant strains, demonstrating improved vaccine efficacy.

Fig. A and B. Relative percent survival in catfish vaccinated with recombinant ESC-NDKL1 strains expressing three v*Ah* antigens following experimental infection with *A. hydrophila* ML09-119 at three weeks post-vaccination.



ENHANCING ANESTHESIA AND MINIMIZING TOXICITY: EVALUATION OF ESSENTIAL OILS IN NILE TILAPIA (*Oreochromis niloticus*)

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This study evaluates the anesthetic effectiveness and potential reduction of toxicity in Nile tilapia (*Oreochromis niloticus*) through the use of clove, cinnamon, and tea tree essential oils. We evaluated genetic damage, enzymatic activity, and gene expression.

We conducted two experiments. In experiment 1, we individually evaluated the anesthetic efficiency and genotoxicity of these essential oils. In experiment 2, we assessed their combined effects at different concentrations.

Clove oil, particularly at the highest concentration (200 µL/L), induced genotoxic effects and oxidative stress in fish, leading to alterations in gene expression regulation and enzymatic activity. Tea tree and cinnamon oils, at concentrations of 75 and 100 µL/L, required longer times to induce fish to the anesthesia stage and were less efficient in achieving deep anesthesia stage. Notably, combining tea tree and cinnamon essential oils to clove oil showed promise as an alternative to using clove oil alone. This combination demonstrated potential as an effective anesthetic and may help mitigate the genotoxic effects associated with clove essential oil.

Our findings suggest a viable option to minimize potential physiological damage during the anesthesia process in aquaculture, thereby promoting a balance between this necessary procedure and animal welfare.

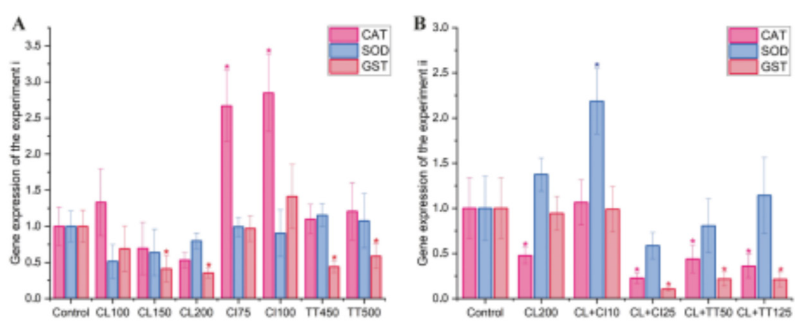

Fig. 3 Relative gene expression of catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST) of *O. niloticus* liver samples anesthetized with different concentrations of essential oils **(A)** in experiment I (clove = 100 µL/L, 150 µL/L, and 200 µL/L; cinnamon = 75 µL/L and 100 µL/L; tea tree = 450 µL/L and 500 µL/L). and **(B)** in experiment II (clove = 100 µL/L, 150 µL/L, and 200 µL/L; cinnamon = 75 µL/L and 100 µL/L; tea tree = 450 µL/L and 500 µL/L). Asterisk (*) indicates the statistical difference to the control group ($p \leq 0.05$). CL = clove oil, CI = cinnamon, and TT = tea tree

BACTERIOPHAGE EFFECTS ON VIBRIO – PROBIONT INTERACTIONS

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Oyster aquaculture farms and restoration projects provide food, economic growth, habitat to other species, and coastal resilience to flooding, storm effects, and erosion. Hatcheries are critical in producing oyster seed required for a rapidly growing aquaculture industry and the restoration of dwindling populations in wild ecosystems. Vibriosis, a major disease caused by various pathogenic *Vibrio* species, affects oysters and other bivalves at the larval stage and has a significant impact on seed production by shellfish hatcheries. A commercial line of a marine probiont, *Phaeobacter inhibens S4* (S4), has been developed to prevent vibriosis in oyster hatcheries. However, there is a large variation in the effectiveness of probiont S4 when used in shellfish hatcheries.

The goal of this project was to evaluate the potential effect of bacteriophages on probiont-pathogen interactions, including boosting or decreasing probiotic action. Lytic vibriophages vB_VcorM-Gr11A (11A) and vB_VcorM-Gr28A (28A) were tested against pathogen *Vibrio coralliilyticus* RE22, probiont *Phaeobacter inhibens* S4, and other bacterial pathogens recently isolated from bivalve larvae, including a highly pathogenic *Vibrio neptunius* strain. These phages showed lytic activity towards both RE22 and S4. Biofilm formation was reduced in RE22 but increased in S4, suggesting phages can modulate processes involved in probiont-pathogen interactions. Furthermore, phages 11A and 28A increased biofilm formation by candidate probiont *Glutamicibacter soli* and decreased biofilm formation by candidate probiont *Pseudoalteromonas galatheae* in a temperature-dependent fashion. Two other phages were isolated from water collected at bivalve hatcheries and showed lytic or lysogenic activity against *V. coralliilyticus* RE22 and *P. inhibens* S4. Functional and genomic characterization of the newly isolated phages is underway. This information will be useful in formulating targeted cocktails of probionts/phages with increased efficacy in the complex microbial environments of shellfish hatcheries.

INVESTIGATING GROWTH POTENTIAL AND FERTILIZATION RATE OF HYBRID EASTERN OYSTERS IN TEXAS

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The eastern oyster, *Crassostrea virginica*, is a valuable benthic organism that plays a crucial role in ecosystem services and supports commercial fisheries and aquaculture industries economically. In the Gulf of Mexico (GoM), research on the genetics of eastern oyster is limited, leaving significant gaps in scientific knowledge about these organisms. The GoM offers a unique environment along the Texas coast, characterized by varying salinity and temperatures across multiple bays. Previous research indicates that eastern oysters in Texas are divided into two populations with distinct environmental tolerances, including a genetic transition zone where naturally occurring hybrid oysters can be found. This research focuses on characterizing growth and reproduction of hybrid oysters, and involves monitoring fertilization rates from both oyster populations and their hybrids, observing their larval development, and development of adult oysters at a local oyster farm in Corpus Christi Bay to monitor and compare growth rates. Currently, environmental stressors and high demands of commercial fishing have led to reductions in Texas oyster populations over time. The results of this study will offer valuable information that can guide the selective breeding of aquaculture, as well as development of conservation and fishery management plans for these vital organisms.

EFFECT OF DIFFERENT LEVELS OF XYLANASE SUPPLEMENTS ON THE GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* REARED IN A GREENWATER SYSTEM

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High amounts of fiber such as non-starch polysaccharides (NSPs), and other antinutritional factors can limit the inclusion of plant-based ingredients in feeds. Complex polysaccharides like NSPs are broken down into simple sugars by carbohydrases, which can be more readily available for absorption. Endo-1,4-β-xylanase is widely utilized in nonruminant nutrition since it degrades Xylan, which is the second most abundant type of polysaccharide in nature into xylose. This enzyme has been reported to enhance nutrient utilization by improving digestibility and breaking down NSP in shrimp fed plant-based protein source diets. Xylanase is not endogenously produced; thus, an exogenous supplementation reduces the digesta viscosity in the gut allowing for a better nutrient diffusion, and an improvement in nutrient utilization. The aim of the research was to determine the effect of different levels of dietary Xylanase on shrimp growth. An 8-week trial was performed in a sixteen-polyethylene tank (805 L) recirculating system with brackish green water. Four levels of xylanase (ECONASE XT25, AB vista., US) were utilized (0, 0.05, 0.1, and 0.2 gr kg^{-1} of feed). Shrimp were stocked at a density of 38 shrimp/m^3, and an initial weight of 0.20±0.0061 g (mean ± standard deviation). Shrimp were fed four times per day using the handfeeding technique. The feed amount was adjusted on a weekly basis. Growth performance parameters (survival rate, final biomass, final average weight, feed conversion ratio) were analyzed, and we found no statistical differences among treatments ($p>0.05$). Additionally, whole-body composition, protein, and phosphorus retention were determined. However, treatments were not statistically different ($p>0.05$). Gut tissue and hepatopancreas were collected, the results of which will be shared in the presentation.

Growth Performance	Basal (0 dose)	Econase XT25 (0.05 dose)	Econase XT25 (0.1 dose)	Econase XT25 (0.2 dose)	PSE	p-value
Survival Rate (%)	100.83	98.35	97.53	99.18	0.859	0.093
Final Biomass (g)	662.5	645.5	641.25	655.38	8.25	0.303
Biomass Gain (g)	656.41	639.42	635.19	649.29	8.25	0.304
Final Average Weight (g)	21.91	21.88	21.92	22.03	0.273	0.981
Feed Conversion Ratio	0.96	0.98	1	0.97	0.013	0.231

EFFECT AND INTERACTIONS BETWEEN XYLANASE, PHYTASE AND A PROTEASE COMPLEX ON THE GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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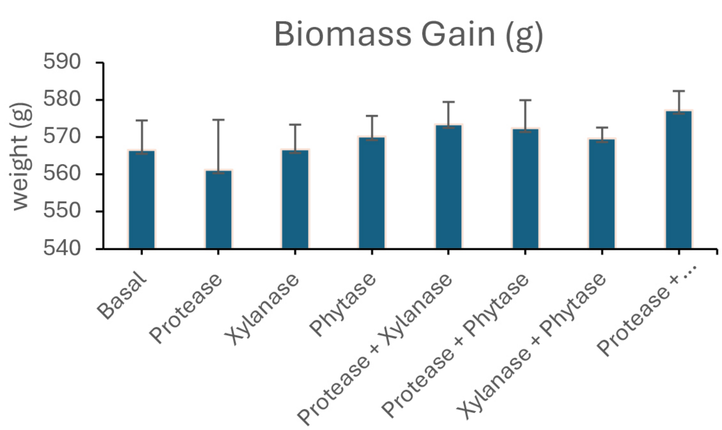
Plant-based ingredients are a cost-effective solution or replacement of animal protein sources in shrimp feeds, however, the presence of antinutritional factors (ANF) like non-starch polysaccharides (NSP) and phytate limit their inclusion. Phytic acid or phytate is commonly found in plant-based ingredients such as wheat, cottonseed meal, soybean meal, maize. When phytate binds to phosphorus (P), it makes it unavailable for monogastric animals, thus, the exogenous inclusion of phytases in the diets aids to increase the digestibility. Additionally, NSP are known to increase gut viscosity which decreases the rate of diffusion of substrates, preventing endogenous enzymes from breaking down feed molecules. The inclusion of exogenous enzymes like xylanase enhances the degradation of NSP into simpler sugars which are more readily available. Therefore, the aim of this study is to evaluate the effect and interactions between xylanase, phytase and a protease complex on the growth performance of Pacific white shrimp. Shrimp (0.23 g ± 0.0083) were stocked at 38 shrimp/m^3 density in a thirty-two-polyethylene tank (805 L) recirculating system using green brackish water. Eight diets, including basal (no enzymatic addition) were formulated to contain 35% and 8% protein and total lipids, respectively. Three diets were made containing an inclusion of each enzyme, two containing the variation of two and one containing all the enzymes. Shrimp were fed using the handfeeding technique for 8 weeks. Growth performance parameters (survival rate, Biomass gain, feed conversion ratio, final average individual weight) were analyzed, although no statistical differences were evidenced (*p>0.05*). Subsequently, ten shrimp per replicate were analyzed for whole-body proximate composition, and protein and phosphorus retention were determined. However, there were no statistical differences among treatments (*p>0.05*). Gut and hepatopancreas tissues were collected, the results of which will be shared in the presentation.



Vibrio campbellii STRAIN M270210 CAN DEVELOP THE CHARACTERISTIC CLINICAL SIGNS AND MORTALITY OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) DISEASE OF SHRIMP

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The increase in shrimp farming practices has resulted in the eutrophication of lagoon systems and the emergence of infectious diseases. Acute Hepatopancreatic Necrosis Disease (AHPND) is a bacterial type of disease that continuously causes ravages in culture ponds. Although it is known that a vast number of bacteria are associated with this disease, strains of *Vibrio parahaemolyticus* have been identified as carriers of the pVA1 plasmid, the causative agent.

In this study, an experimental infection was performed with *Vibrio campbellii* strain M270210, which harbors the same pVA1 plasmid, to observe its capacity to cause the disease and compare its virulence against the *V. parahaemolyticus* strain M0904, reported as highly virulent. Furthermore, we performed a challenge in which both strains were simultaneously inoculated in *Mpc* to test whether or not the mortality of shrimps would be affected compared to the individual treatments. *Penaeus vannamei* shrimp weighing 300 (±0.005) mg obtained from a larval production laboratory (UPL) in Rosario Sinaloa were acclimatized for one week. These were subsequently exposed through a bath challenge with three treatments: *V. parahaemolyticus* (*Vp*) strain M0904, *V. campbellii* (*Vc*) strain M270210, and simultaneous exposure to both strains (*Mpc*). First mortalities were observed at five h, nine h, and six h post-inoculation (hpi), respectively. A 50% survival (Kaplan-Meier estimator) was calculated at 13, 21, and 16 hpi, respectively.

All of the organisms challenged presented the characteristic symptoms of AHPND. By the end of the experiment, significant differences were found between *Mpc* and *Vc* (p=0.049) and between *Vp* and *Vc* (p=0.21) but not between *Vp* and *Mpc* (p=0.71). These results suggest some antagonistic effect between both bacterial strains because the combined inoculation of strains (*Mpc* treatment) showed a delay in mortality compared to the inoculation of only *V. parahaemolyticus* (Vp treatment).

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THE CONSERVATION FUND FRESHWATER INSTITUTE: RECIRCULATING AQUACULTURE RESEARCH PROGRAM UPDATES

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The Conservation Fund Freshwater Institute (TCFFI; Shepherdstown, West Virginia, USA) has a longstanding history of research and innovation in the area of recirculating aquaculture systems (RAS), primarily thanks to funding support from the United States Department of Agriculture's Agriculture Research Service (USDA-ARS). Since the 1980s, TCFFI has supported the growing RAS aquaculture industry through addressing stakeholder-driven research priorities, providing open access to our scientific publications, and engaging in consultation services, conference presentations, private, state, tribal, and federal outreach, and educational courses. Our upcoming 5-year USDA-ARS research plan (2025-2029) includes projects focused on early-rearing culture environment optimization for triploid Atlantic salmon *Salmo salar*, reducing early maturation in Atlantic salmon, assessing heritability of superior Atlantic salmon performance in RAS, optimizing RAS parameters for coho salmon *Oncorhynchus kisutch*, converting RAS waste to sellable products, and assessing and developing precision agriculture technologies applied to RAS. These research plans will be discussed in detail, as well as a summary of findings from our most recent USDA-ARS research cycle (2019-2024).

INVESTIGATING RAS WATER DISINFECTION WITH LOW-DOSE PERACETIC ACID

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Peracetic acid (PAA; CH_3CO_3H) has shown promise as an efficacious, environmentally-friendly disinfectant in aquaculture; however, PAA is not currently approved in the United States for treating systems when food fish are present and is only permitted for use in aquaculture as a surface disinfectant. There is significant interest in the aquaculture industry for using PAA as a water disinfectant to reduce or eliminate potential opportunistic pathogens (e.g., *Flavobacterium* spp). The efficacy of PAA in killing a range of bacteria, fungi, yeasts, and viruses has been demonstrated, and its rapid degradation into non-toxic byproducts of acetic acid, hydrogen peroxide, and water largely eliminates pollution and discharge issues. Previous research on PAA application in aquaculture settings has primarily focused on disease prevention through PAA's bactericidal activity. Additionally, several toxicity studies indicate that low-dose PAA treatments are tolerated by commercially raised rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) at various life stages. Intensive fish production technologies, such as recirculating aquaculture systems (RAS) commonly used to raise these species, rely on microbial-based treatment processes, including nitrifying biofilters. Bacteria established on biofilter media are integral to converting toxic fish metabolites (i.e., ammonia) into less harmful nitrate. Because PAA is a broad-spectrum antimicrobial product, nitrification could be impaired if biofilters are exposed to sufficient concentrations of PAA, due to the destruction of nitrifying bacteria. Disruption of nitrification, even if temporary, can result in significant adverse outcomes for fish, as ammonia and nitrite can be highly toxic when above certain concentrations. As such, baseline research is essential to identify biofiltration-safe protocols for administering PAA in RAS. Several studies investigating low-dose PAA application in RAS have been carried out at The Conservation Fund Freshwater Institute (Shepherdstown, WV, USA) in recent years, and this presentation will describe these investigations in depth and will summarize our research findings.

CHARACTERIZING TOLERANCE THRESHOLDS OF GENETICALLY DIVIDED EASTERN OYSTER *Crassostrea virginica* POPULATIONS IN TEXAS

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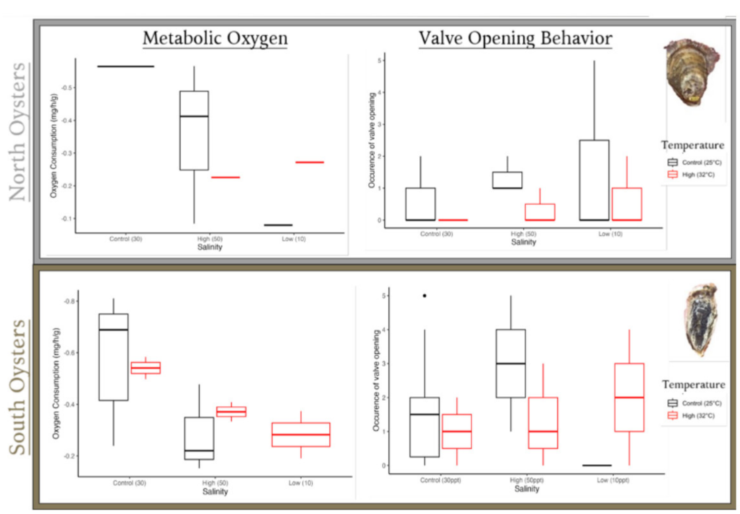
The Eastern Oyster (*Crassostrea virginica*) is Texas's most valuable coastal resource, providing critical habitat for fish and invertebrate species, shoreline stabilization, and improving water quality. The Texas oyster mariculture industry was created in 2019 to guide oyster growing operations and protect their valuable environment from ecological decline. Texas is the last Gulf of Mexico bordering state to allow an oyster aquaculture industry; however, by providing this economically important industry, Texas oyster growers can support their surrounding environments and provide income, jobs, and another source of seafood to coastal restaurants and communities. Over the next decade, Texas' top commercial fishery will be threatened by population growth, coastal development, pollution, and flooding. These local threats will interact with global environmental changes (i.e., warming temperatures and acidification). With these valuable ecosystems in rapid decline and the birth of industry to restore and rehabilitate these ecosystems at the forefront of Texas's economic opportunities, it is vital to understand the stress tolerances and adaptive capacities of the Eastern Oyster.

This Texas oyster population is divided into two genetically different sub-species found in the Northern and Southern regions of the state, with Corpus Christi Bay acting as the transition zone between the divergent populations. However, few scientific conclusions have been made regarding why there is such vast genetic variation between Texas's two populations of oysters. Therefore, using an intermittent flow respirometry technique, this project will characterize the individual tolerance thresholds of the genetically different sub-populations to a range of salinities under high temperature. The results of this work aim to help predict future risk and resilience of the South Texas oyster population dynamics, aquaculture production, and restoration of ecosystem services. The risk assessment created will contribute to the resiliency of Texas' coastal oyster reef habitats to climate change by filling the knowledge gap surrounding stress tolerances to dramatic salinity fluctuations and evaluating how the genetically different oyster populations will respond to interacting local and global stressors.



COLLABORATIVE FARMER-SCIENTIST EFFORTS IN EVALUATING THE IMPACTS OF MARICULTURE IN ALASKA

Melissa Good*, Sean Crosby, Seawan Gehlbach, Lexa Meyer, Caitlin McKinstry, Nick Mangini, Erik O'Brien, Lindsay Olsen, Alf Pryor, Thea Thomas

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The Mariculture Research and Restoration Consortium (Mariculture ReCon) is a collaborative initiative focused on understanding the environmental impacts—both positive and negative—of mariculture farms in Alaska's coastal waters. Central to this project is the partnership between local seaweed and shellfish farmers and scientists, with farmers actively participating in much of the data collection. Working together, they deploy environmental sensors, conduct plankton net tows, collect eDNA samples, perform benthic surveys, and more, ensuring that the research is both scientifically rigorous and reflective of real-world farming conditions.

Farmers across Prince William Sound, Kachemak Bay, and Kodiak Island, alongside scientists, are helping to assess how mariculture influences water quality, habitat, and marine species. This cooperative research approach allows for a comprehensive understanding of both the potential benefits of mariculture, such as ecosystem support and water quality improvement and any negative effects, including changes to species interactions and nutrient flows.

Through this collaboration, a community of practice is emerging, where farmers and scientists not only share data but also knowledge, skills, and innovative techniques. Regular meetings and exchanges create a space for mutual learning and problem-solving, fostering an environment where both groups continually improve mariculture practices. This community of practice strengthens the mariculture industry while contributing valuable insights into the environmental footprint of mariculture.

REBOOTING THE NORTHEAST AQUACULTURE EXTENSION NETWORK: A SKILLS TRAINING AND MENTORSHIP PROGRAM FOR EXTENSION PROFESSIONALS

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The Northeast Aquaculture Extension Network (NAEN) is a group of extension professionals in the Northeast region of the U.S. This group has previously received funding for extension workgroup projects from the Northeast Regional Aquaculture Center (NRAC) to deliver a variety of regionally important aquaculture extension products and services. Although the 2010 NAEN program was highly successful, generating $377,500 in value for the aquaculture industry (Fairchild et al., 2017), the subsequent lack of NRAC extension projects and the generational turnover of NE aquaculture extension agents has led to lost momentum, comradery, and institutional knowledge. In the past 3+ years, the Covid-19 pandemic has compounded some of these issues. Agents that have been hired during the pandemic have not received the same level of mentoring and training that has occurred in the past due to the nature of the virtual environment. It has been shown that extension professionals have felt high levels of stress during the pandemic and have had trouble with work-life balance (Israel, 2020).

Concurrently with the gap since the last NAEN project, there has been an influx of funding toward aquaculture development nationally, and heightened interest in expanding the aquaculture industry in a sustainable manner. Funding has largely come through the NOAA Sea Grant network, as well as other state and federal sources. Regionally, this funding has enabled an increase in the number of aquaculture extension professionals in the Northeast, though with fewer robust peer-to-peer connections. The compounding factors of the loss of NAEN projects, the pandemic, and increasing aquaculture funding have left a gap in professional development and network building among the extension community. Structured training and mentoring programs play major roles in advancing career development (Cummings et al., 2015) and success in extension roles (Kutilek and Earnest, 2001).

This project has implemented a training and mentorship program for aquaculture extension professionals in the region that reflects and capitalizes on the changed landscape since the previous NAEN project. In doing so, we are revitalizing the NAEN to continue and expand the delivery of regionally important aquaculture value for stakeholders.

OPTIMIZING CARBON STORAGE IN AQUACULTURE SYSTEMS: A STUDY ON SPECIES SELECTION AND FARMING PRACTICES

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Aquaculture ponds, often neglected in national carbon budgets, present significant potential for carbon storage. This study investigates the carbon absorption rates of pond sediments in relation to fish species selection, pond age, and fish farming practices. A standardized soil sampling protocol was employed, collecting sediment samples of 200-300g from a depth of 20 cm using a hand core sampler, based on the methodology from Gilbert et al., 2021. To ensure consistency, all samples were collected by the same individual and analyzed at the Oklahoma State University Soil Testing Lab. Soils from channel catfish, koi, and bluegill ponds were sampled, with four catfish ponds, two koi ponds, and two bluegill ponds included in the study. A questionnaire gathered data on physical, chemical, and fish farming management factors. The results showed that carbon absorption rates varied significantly between species, with bluegill ponds having the highest organic matter and total carbon content, followed by catfish and koi ponds. Carbon absorption was also analyzed in relation to pond age and management practices such as stocking rate, feed, and total production, with bluegill ponds again demonstrating the highest absorption rates. This study underscores the potential for improving carbon storage in aquaculture ponds, emphasizing the importance of fish species, pond age, and management practices in carbon sequestration strategies. These findings offer valuable insights into the role of aquaculture systems in mitigating climate change impacts.

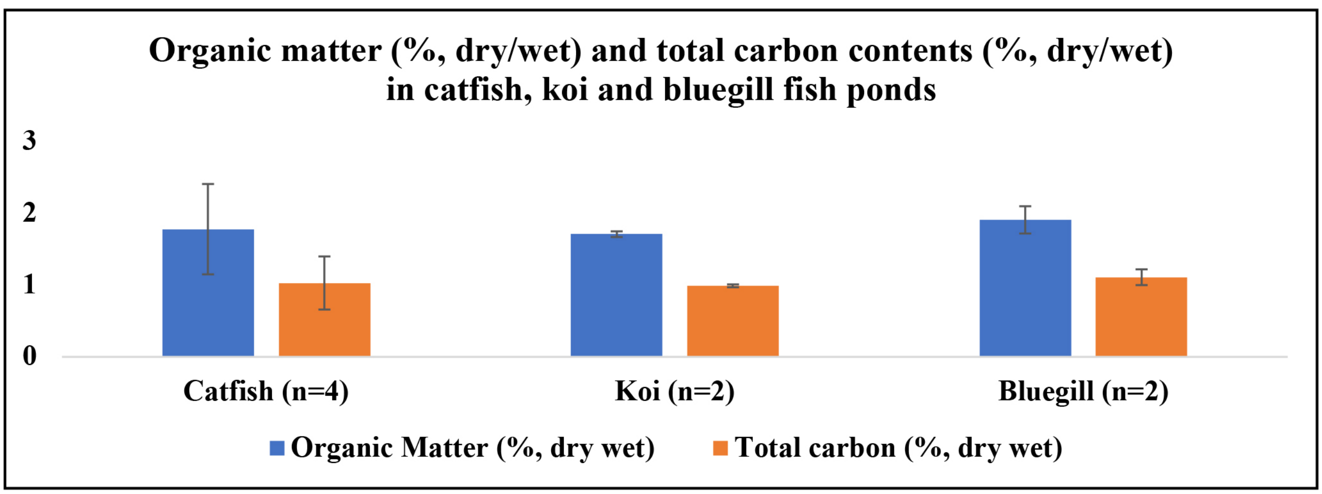

Figure 1: Organic matter (%, dry wet) and total carbon contents (%, dry wet) in channel catfish, koi and bluegill fish ponds (source: current research)

NUTRIENT DYNAMICS AND GROWTH PERFORMANCE IN AN NUTRIENT FILM AQUAPONICS SYSTEM: A STUDY ON FISH AND VEGETABLES

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Aquaponics integrates fish farming with soilless plant cultivation (hydroponics) in order to establish a symbiotic system where fish waste provides nutrients for plants, with bacteria facilitating the conversion of ammonia to nitrates. In contrast, hydroponics involves plant growth using nutrient-enriched water. This study aims to compare the growth rates and production of lettuce and fish within an aquaponics system. Koi fish were raised under controlled conditions, receiving a daily feed of 3% of their body weight, while lettuce seedlings were transplanted into a system utilizing reverse osmosis-treated water. Daily monitoring of total dissolved solids (TDS) and electrical conductivity (EC) ensured nutrient optimization. Water samples were tested for ammonia, nitrite, nitrate, pH, and dissolved oxygen using API strips. Preliminary results from the koi fish showed an average weight of 14.94g with a standard deviation of 4.25g across 21 samples. Lettuce growth displayed an average weights ranging from 1.95g to 34.91g, and standard deviations from 0.26 to 30.23g. These findings suggest that the aquaponic system facilitates effective nutrient transfer from the fish tank, promoting lettuce growth. Future research will compare these results to soil-grown lettuce and pond-grown fish to evaluate which method produces higher growth and yields, providing insights into sustainable agricultural practices for both leafy greens and fish production.

Fig. 1: Lettuce Growth in Nutrient Film Hydroponics Bed: Tube #1-Row 1-4

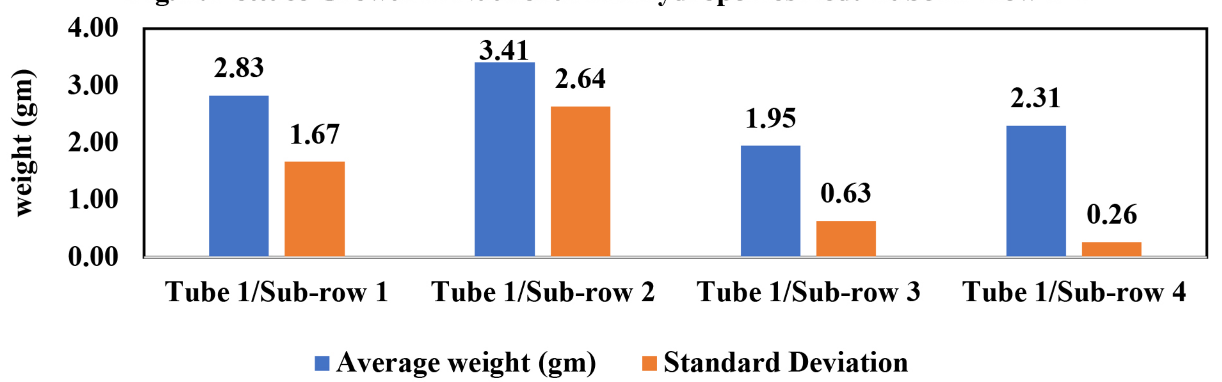
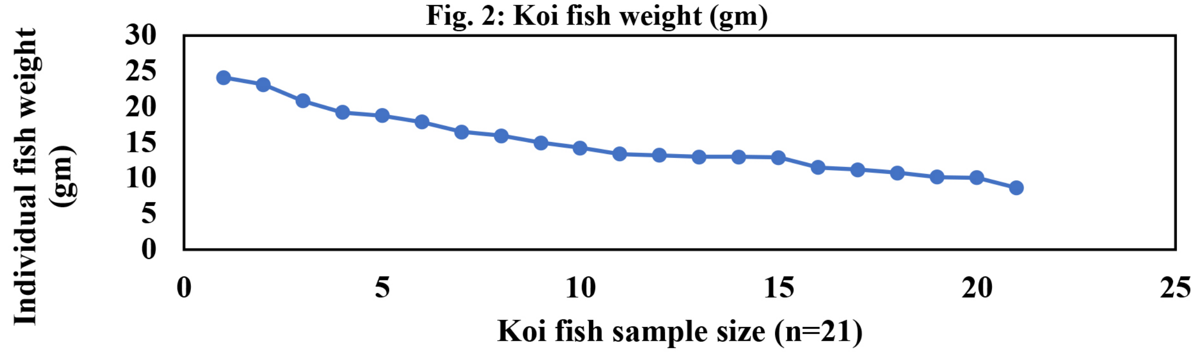


Fig 1-2: Lettuce and koi fish growth in nutrient film aquaponcis system

Fig. 2: Koi fish weight (gm)



EFFECT OF CEREAL GRAIN CHOICE ON THE EXTRUDED FEED CHARACTERISTICS AND GROWTH PERFORMANCE OF RAINBOW TROUT *Oncorhynchus mykiss*

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Dietary grain sources can impact nutritional status, economic efficiency, and environmental impacts of aquatic animals. This study evaluated the feed quality and growth performance of rainbow trout fed extruded diets formulated with various sorghum grains and wheat. Four practical-type diets were formulated to be isonitrogenous and isolipidic at 45% protein and 18% lipid using wheat (GW), non-waxy red sorghum (NRS), waxy red sorghum (WRS), or waxy white sorghum (WWS) as the primary carbohydrate source. Diets were extruded using a Wenger X-20 single screw extruder and fed to rainbow trout (mean initial weight ± SD, 48.3 ± 0.70 g; 15 fish per tank) over a 14-week growth trial in a recirculating system at 15C with five replicate 400L tanks per diet. Feed manufacturing energy consumption varied due to grain choice, with GW requiring the highest total energy (532kJ/kg) and NRS the lowest (442 kJ/kg). Water stability and pellet durability (PDI) were significantly altered by grain choice; water stability was lowest in WRS (86.6%) and highest in GW (87.8%). PDI was highest in WRS (93.4%) and lowest in GW (86.0%). GW and NRS showed superior floating ability (100%), with WRS having the lowest (53.5%). Feed conversion ratios (FCR) were significantly affected by grain choice; WRS-fed fish had the highest feed intake (155.8%bw/d) and FCR (0.96). GW and NRS-fed fish exhibited lower feed intake (~140%bw/d) and improved FCR values (0.86 and 0.88, respectively). Visceral index and filet ratio also showed significant differences suggesting the need for further investigation to define the physiolocial implications of dietary grain type on nutrient utilization by rainbow trout. Overall, the results suggest that sorghum grains, both waxy and non-waxy, offer promising alternatives to wheat in aquafeeds, supporting rainbow trout growth with improved levels of pellet durability and acceptable water stability.

TABLE 1: Response of rainbow trout (mean initial weight of 48.3 ± 0.70 g) fed diets with different cereal grains within a 14-week period. Values represent the means of 5 replicates. Data were analyzed by ANOVA.

Diet	Feed Intake %bw/d	FCR (g feed/g gain)	Avg Fish Weight (g)	Weight Gain (% Increase)	Survival (%)	Hepatosomatic Index (%)	Viscera Index (%)	Filet Ratio (%)
GW	139.7[b]	0.86[b]	421	772	100	1.2	10.0[ab]	59.3[a]
NRS	141.8[b]	0.88[b]	417.	758	99	1.3	10.8[ab]	57.4[ab]
WRS	155.8[a]	0.96[a]	431	799	100	1.3	11.2[a]	57.4[ab]
WWS	151.8[a]	0.94[a]	418	764	100	1.2	9.6[b]	58.9[ab]
P-Value	<0.0001	0.0009	0.7206	0.5206	0.4182	0.3871	0.0102	0.0336

define the physiolocial implications of dietary grain type on nutrient utilization by rainbow trout.

IMPACT OF PARTICLE SIZE ON PHYSICAL QUALITY AND IN-VIVO PERFORMANCE OF GRAIN SORGHUM-BASED FEED FOR TILAPIA *Oreochromis niloticus*

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This research investigated the impact of grind size in wheat and sorghum-based floating feeds for Nile tilapia. The study looked at expansion metrics, pellet durability, water stability, and animal performance. Diets were ground through either a 0.61, 1.02, or a 1.27 mm hammermill screen before being extruded through a Wenger X20 single screw extruder. Processing conditions were kept similar for all treatments. As particle size decreased, mechanical energy inputs increased (267 to 359 kJ/kg), and pellet expansion increased resulting in lower bulk densities (453 to 367 g/L). Specific expansion was significantly affected by grind size in sorghum-based diets, with expansion ratios increasing as grind size decreased (1.45 to 2.15). Specific length (mm/g) followed similar trends in both grains; as grind size decreased, values increased for both wheat and sorghum-based feeds (327-361 mm/g and 335 to 371 mm/g respectively). Water stability results indicated that all diets were similarly stable in water (~80% by dried pellet weight). Sorghum-based diets led to better growth performance, with all sorghum-based diets resulting in larger tilapia than wheat-based diets. Tilapia fed the medium grind sorghum diet had the most efficient feed conversion ration (FCR) of 1.03, while fish fed the wheat-based fine grind diet had the largest (1.13). These results indicate that grain sorghum can be successfully incorporated into diets for Nile tilapia with positive impacts on both physical feed qualities and growth rates of the fish. While grind size may impact processing parameters and feed qualities, there were limited impacts on digestibility and growth rates, with indications that grinding energy consumption can be minimized while still obtaining better feed conversion. Overall, sorghum demonstrated strong potential as a viable, sustainable alternative to wheat in tilapia feeds.

Table 1: Response of juvenile Nile tilapia (mean initial weight 3.83 ± 0.03 g) fed diets with different extrusion sizes of sorghum and wheat within a 12-week period. Values represent the means of four replicates.

		Final Biomass (g)	Final weight (g)	Weight Gain (g)	Weight Gain (%)	Total feed per fish (g)	FCR[1]	Survival (%)
Sorghum	0.61	1617.3[ab]	81.94[ab]	78.10[ab]	2031	84.51	1.09	99
Sorghum	1.02	1778.6[a]	88.93[a]	85.08[a]	2218	86.41	1.03	100
Sorghum	1.27	1640.5[ab]	83.09[ab]	79.20[ab]	2036	87.00	1.10	99
Wheat	0.61	1469.3[b]	74.42[b]	70.61[b]	1850	79.36	1.13	99
Wheat	1.02	1590.9[ab]	80.63[ab]	76.77[ab]	1985	85.31	1.11	99
Wheat	1.27	1509.8[ab]	75.49[ab]	71.75[ab]	1918	80.89	1.13	100
PSE[2]		29.96	1.52	1.51	37.96	0.92	0.01	0.39
p-value[3]		0.0278	0.0454	0.0452	0.0815	0.0716	0.2409	0.8424

EVALUATION OF SORGHUM-BASED DIETS AND EXTRUSION ENERGY INPUTS ON THE PHYSICAL QUALITY AND GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* FEED

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This study investigated the impact of process energy inputs (high, medium, low) on the physical qualities of extruded feeds and animal performance when fed wheat and sorghum-based diets. Specific thermal energy (STE) and mechanical energy (SME) were calculated to assess their effects on starch gelatinization, bulk density and water stability. Six experimental diets, sorghum and wheat-based with three different levels of energy inputs each, were extruded using a Wenger X-20 single screw extruder and fed to shrimp (mean initial weight ± SD, 0.82 ± 0.05 g) over a 42-day growth trial in a semi-closed recirculating system with 36 tanks. STE was manipulated by varying the amount of water and steam addition during preconditioning. For both wheat and sorghum-based diets, reduced PC steam input led to reduced STE (236.8 to 113.4 kJ/kg for wheat; 279.8 to 120.4 kJ/kg for sorghum) which increased SME (228 to 260; 237 to 288 kJ/kg respectively) and lower overall energy inputs in both diets; as energy inputs decreased, sorghum-based feeds exhibited decreased starch gelatinization (89.55 to 82.97%) and water stability (77 to 59%), whereas those values were similar for all wheat-based diets (98-94% gelatinized; 80-77% water stable). Wheat-based pellets had the highest sectional expansion index (1.58 to 1.43), while sorghum-based pellets ranged from 1.32 to 1.26. Bulk density, sinking percentage, and pellet durability was similar across all treatments. Apparent digestibility coefficients (ADC) for protein and energy were higher in wheat-based diets; however, there were no significant differences in shrimp performance or feed conversion ratios. These insights highlight the critical role of energy management in extrusion processing, especially for sorghum, where increased thermal energy is required to maintain feed quality. This research demonstrates the viability of sorghum as an alternative carbohydrate source in shrimp feeds.

Table 1: Percent of pellets remaining by mass after soaking for one hour, and degree of gelatinization of dried pellets

Diet	Water Stability (%)	Degree of Gelatinization (%)
W95	80.0 ± 0.00^{a}	98.67
W80	79.0 ± 1.41^{a}	97.89
W60	77.0 ± 4.24^{a}	94.34
S95	77.0 ± 1.41^{a}	89.55
S80	72.0 ± 2.83^{a}	86.43
S60	59.0 ± 1.41^{b}	82.97
Two-Way Anova		
Model	0.0007	-
Grain	0.0004	-
Thermal Energy	0.0017	-
Grain*Thermal Energy	0.0092	-

EFFECT OF DIET AND GUT MICROBIOME ON SEA URCHIN (*Lytechinus variegatus*) PERFORMANCE AND TOXICOLOGICAL OUTCOMES

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Sea urchins are frequently collected, grown and maintained in aquaculture for developmental and toxicological studies where they serve as model organisms. They are also raised for their gonads as a food source, called uni. Applied studies have used lab-acclimated sea urchins as subjects to demonstrate how sea urchin populations respond to various stimuli with minimal mention of the gut microbiome. Changes in gut microbiome diversity have been observed in a variety of animal models during maintenance in lab settings. Such lab-induced microbiome changes can cascade to influence their animal host, potentially impacting research outcomes and their consistency as the gut microbiome affects the host organism's development, susceptibility to pathogens, and ability to metabolize and acquire nutrients. The effects of probiotic supplementation and/or fecal microbiota transplantation (FMT) in sea urchins have not been explored to our knowledge. Due to the relationship between microbial diversity and gut function, dysbiosis may dramatically impact urchin health and, consequently, the quality and consistency of urchins raised for food and applied research. Since urchin microbiomes are sensitive to abiotic and dietary conditions, standardization of husbandry is essential for researchers seeking reproducible experimental results. To test the effect of diet and gut microbiome on performance and toxicological outcomes wild sea urchins (*Lytechinus variegatus*) were collected at Port St. Joe, Florida. They were placed in a 30 tank recirculating system with there being 10 tanks per treatment with 4 urchins individually maintained per tank. Sea urchins were fed a standard reference diet, a standard reference diet with FMT and the EPA approved diet of lettuce for 10-weeks. At the conclusion of the experiment indicators of sea urchin performance for weight gain, survival, and changes in test diameter, gut, gonad, and lantern weights were measured. Gut microbiome was measured and compared for urchins fed the 3 different diets as well as the microbiome of the wild sea urchins. Toxicological outcomes were measured between the wild and laboratory fed sea urchins. This was done to examine the effect of toxicological outcomes on reproductive potential. Data from these outcomes will be presented.

EXPANSION AND RESEARCH DEVELOPMENTS AT THE UMCES OYSTER DEMONSTRATION FARM AT HORN POINT LABORATORY

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Shellfish aquaculture is widely regarded as an environmentally friendly source of animal protein, providing numerous ecosystem services such as water filtration and habitat creation. As global interest in sustainable food sources grows, there is increasing enthusiasm for expanding oyster aquaculture to meet rising demands while promoting ecological benefits.

But despite oysters being cultivated for millennia, modern aquaculture practices—especially off-bottom production systems—are far from optimized. New gear types and innovative production methods require rigorous research to maximize oyster yields and improve understanding of their ecological interactions. While oysters offer clear co-benefits, potential negative environmental impacts must also be investigated. Research farms play a crucial role in advancing aquaculture science, offering full-scale experimental settings robust studies.

To help meet the needs of the oyster industry, the UMCES Oyster Demonstration Farm at Horn Point Laboratory, located on the Choptank River in the Chesapeake Bay, will be expanding from its original 3-acre site to a 16-acre farm this year. This expanded farm enables in-depth research into bottom culture, reef creation, and intertidal infrastructure with oysters. The facility will assess various aquaculture production systems, including on-bottom production and harvesting, off-bottom methods such as float cages, suspended culture, and experimental gear aimed at optimizing oyster growth and evaluating the environmental impacts of these systems. On-bottom production in newly designated zones can now include evaluation of artificial substrate types, including artificial shells, clam shells, and stone to assess oyster recruitment and harvestability. These areas will also be used as test beds for robotic and drone assistance in farm management. By studying diverse planting, management, and harvesting techniques, this research farm will advance the efficiency and sustainability of oyster farming practices.

DESIGN ITERATIONS OF A USV DESIGNED FOR OYSTER AQUACULTURE

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Development in marine aquaculture is necessary to avoid overfishing and other ecological damage among a rise in growing demand for seafood. Our team at MIT Sea Grant worked with a local shellfish aquaculture company, Ward Aquafarms, to increase the productivity of oyster farming. To prevent biofouling and ensure water flow in oyster baskets, a worker in a kayak periodically flips thousands of baskets. This is a physically demanding and slow to complete process due to baskets that weigh up to 30 kg (60 lbs). The Oystermaran is a catamaran style surface vessel designed to autonomously maneuver through rows of oyster baskets and flip each basket.

In 2021, version 1 of the Oystermaran was developed in response to this problem. Oystermaran v1 was able to successfully flip baskets. However, field testing revealed key limitations with the design such as the critical ability to autonomously navigate through oyster fields. It would get stuck between the crowded rows and slide on top of the baskets. The flipping mechanism was also unreliable due to the baskets slipping off the flippers and lacked any ability to shake the oyster baskets to redistribute the oysters.

In 2023, version 2 of the Oystermaran was developed to improve upon the maneuverability and flipping reliability issues of version 1. To address the snagging issues, all of the components were inset into the hulls. Oystermaran v2 also added two lateral thrusters to enable side to side movement to navigate through other rows blocking its forward motion. Additionally, new nose geometry was developed to prevent rising over the baskets. The flipping mechanism was also altered to increase flipping reliability by preventing slipping and an ability to shake the baskets.

In 2025, version 3 of the Oystermaran is being designed to build upon the successes of version 2 and create a more final design. Another new vessel, the hull is being made thinner to reduce interference with other rows through a redesign of the upper frame. Further iterations of the forward thruster channels will improve hydrodynamic efficiency. Furthermore, Oystermaran v3 will improve basket flipping reliability by moving components to prevent interference with other baskets in the same row.

REPLACING CORN OIL WITH BLACK SOLDIER FLY OIL IN WALLEYE (*Sander vitreus*) FEED: EFFECTS ON GROWTH, HEALTH, AND TOLERANCE TO TEMPERATURE SHOCK

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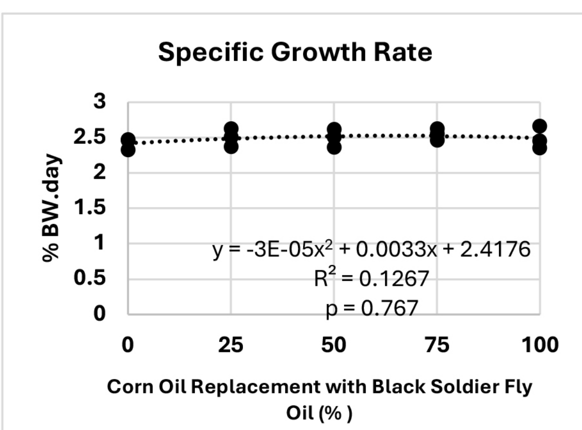
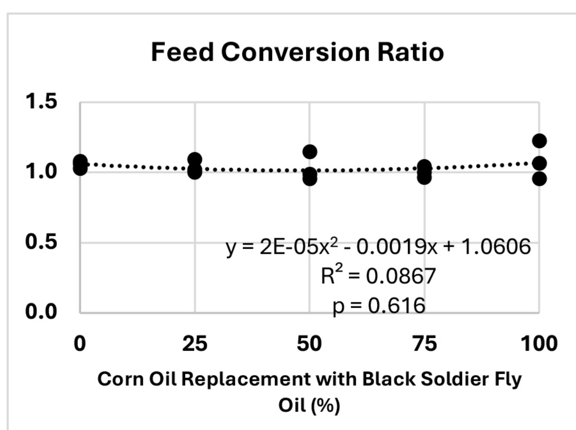
Black soldier fly is one of the insect species receiving increasing research on its use as alternative protein and lipid sources in aquatic feed. As a lipid source, black soldier fly oil can provide up to 50% lauric acid, which has been shown to function as a potent antimicrobial and is beneficial to fish growth and health. However, overfeeding this oil may also cause negative impacts due to the limited contents of other essential fatty acids required by targeted fish species. Thus, it is critical to understand the acceptance of this alternative lipid source when used in feed formulated for different species of fish. Our goal of this study was to determine the potential application of black soldier fly oil in feed for juvenile walleye (*Sander vitreus),* an economically and environmentally important fish species in the Midwest region. Specifically, we evaluated the growth, health, nutritional composition, and tolerance to temperature shock of walleye fed diets containing various levels of corn oil replaced by black soldier fly oil. The basic dietary lipids contained 8% corn oil and 6% menhaden oil. Corn oil was replaced by black soldier fly oil at levels of 0%, 25%, 50%, 75%, and 100%. The protein and carbohydrate levels were the same across all the test diets. The feeding trial was conducted on walleye with an average initial body weight of 7.4 ± 0.06 g (n=18). Fish were raised in a flow-through water system (21°C) with three replications for each dietary treatment. Based on the 8-week preliminary observation, there was no significant difference in the weight gain (%), specific growth rate (% body weight. day), and feed conversion ratio (($p>0.05$). No significant difference in mortality was observed due to the different dietary treatments ($p>0.05$). These results indicate that black soldier fly oil did not impair walleye feeding and can completely replace corn oil under the current testing conditions without negatively impacting growth or feeding efficiency. A final conclusion will be made based on the overall growth performance, fish health, nutritional composition, and temperature tolerance when this feeding trial is concluded at the end of the 10-week feeding period. The outcome of this study will provide insight into understanding the potential application of black soldier fly oil in walleye feed.





USAS STUDENT SUBUNIT SHOWCASE

Julianne M. Grenn[1]* and Abdulmalik Oladipupo

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USAS Student Subunits will share engagement, activities, outreach, and accomplishments over the past year. Session culminates with discussion to share ideas, ask questions, and troubleshoot common issues.

SEA LETTUCE *(Ulva spp.)* AND OYSTER CO-CULTURE: A POTENTIAL CLIMATE CHANGE MITIGATION STRATEGY FOR OYSTER FARMERS

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This project explored the feasibility of Eastern oyster (*Crassostrea virginica)* and sea lettuce (*Ulva* spp.) co-culture with a field experiment deployed at a shellfish farm in Virginia. We examined how relative oyster density (empty, low, high – with high density at 700 oysters and normal density at 400 initially, then divided in half for each subsequent split (decreasing density of oysters in bags for biological reasons)) fully crossed with relative *Ulva* density (empty (0 g), low (150 g), high (300 g)) in Zapco™ floating baskets affects 1. dissolved oxygen (DO), chlorophyll-a, and pH, 2. oyster performance, and 3. *Ulva* performance, all within oyster grow-out containers.

Twice per month from May - October 2024, diurnal water samples were collected from the inside of floating oyster grow-out bags using a peristaltic pump, and water parameters were assessed in real-time with a sonde. Oyster samples were collected periodically from each bag (n=15 per bag) and processed in a laboratory to determine if performance varied among treatment groups. Once per month, *Ulva* was removed from individual bags, weighed, and redeployed at the high or low Ulva stocking density threshold $\pm$ 10 g.

Water: In a representative water sampling event on June 10, 2024, *Ulva* stocking density significantly affected DO and pH ($p \leq 0.01$ for all), while neither *Ulva* nor oyster stocking density affected chlorophyll-a levels within bags ($p > 0.05$). The presence of *Ulva* was associated with higher DO and pH levels. **Oysters:** Oyster height (measured from umbo to bill), dry tissue weight (DTW), and dry shell weight (DSW) were all significantly affected by oyster stocking density ($p < 0.01$), where the oysters were smaller in the higher density treatment. Oyster height was also significantly impacted by *Ulva* density, where the presence of *Ulva* was associated with smaller oysters. ***Ulva*:** In terms of overall production, *Ulva* yield from July – September was highest in the 'normal' *Ulva* stocking density treatments with either no oysters (130-185% increase from initial 150 g of *Ulva*) or 'normal' oyster stocking density (75-130% increase from initial 150 g of *Ulva*). High oysters/high *Ulva* produced the least amount of *Ulva (*2-20% increase from initial 300 g of Ulva*)*.

Results suggest that *Ulva* can improve DO and pH conditions within oyster grow-out containers, but an oyster growth penalty is associated with this co-culture practice (potentially because *Ulva* may reduce water flow in the containers). Even with this growth penalty, *Ulva* could still be a useful tool for farmers experiencing the effects of climate change via ocean acidification and decreased DO related to rising water temperatures.

USAS STUDENT SUBUNITS

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Student sub-units are vital to the USAS organization. They further USAS outreach, bringing the benefits, activities, and news of USAS to students at their institutions. Student sub-units also provide increased opportunities for student involvement in USAS and WAS activities and help students to organize and participate in aquaculture-related activities on a local scale.

NATIONAL AND INTERNATIONAL RESOURCES FOR EDNA APPLICATIONS TO AQUACULTURE

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Over the past two decades, methods for analyzing DNA from the environment (eDNA) have improved and can now be useful for several practical purposes, such as early detection of harmful species and ecosystem assessment. Despite this progress, eDNA is an evolving field with few established protocols, and thus still daunting to implement. Here I review two large-scale eDNA efforts and highlight how they, and eDNA in general, can be used in aquaculture. First, I will discuss the Maine-eDNA Index Sites survey that sampled eDNA monthly at 12 sites in coastal Maine over three years. Samples were metabarcoded with methods targeting bacteria, microbial eukaryotes, invertebrates, and vertebrates allowing us to explore how coastal ecosystems and aquaculture-relevant species changed seasonally, latitudinally, and across years. Then I will discuss the GOTeDNA (Guidance on optimal eDNA Sampling periods) project, which is an international effort to collate and summarize species detection data across eDNA time seriess. GOTeDNA currently provides a user-friendly Shiny app that enables practitioners to quickly identify the optimal time of year to sample many species and the eDNA methods that are best for their purposes. Overall, both Maine-eDNA and GOTeDNA provide useful insights and resources for those who seek to use emerging eDNA methods in aquaculture.

OYSTER FARMING – IS IT FOR YOU?

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The off-bottom cage culture commercial oyster farming industry has seen significant growth in the last few years and with that there are tools and publications that have been developed to increase the sustainability and resilience of these companies. This presentation will provide a brief overview of these products such as online training courses, the Oyster Farming Resilience Index, Storm Preparation, the Oyster Community of Practice and the Commercial Oyster Aquaculture Sector Training Program. Additionally, there have been recent changes to USDA/RMA programs that are available to the industry that will be referenced.

While these materials are intended to be used by commercial oyster farmers in the Gulf of Mexico they are also to applicable to other species and in other areas of the country. Oyster farming using containerized gear systems is relatively new industry in parts of the Gulf and this presentation is intended to raise awareness of the information available to those interested in or already participating in the industry to help them with decision-making and increase the opportunities for success.

ALABAMA - MISSISSIPPI OYSTER COMMUNITY OF PRACTICE

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The purpose of the Alabama-Mississippi Oyster CoP is to:

1) establish a network of interested oyster farmers, wild oyster harvesters, restaurants, management agencies, and other members of the oyster industry to determine common challenges within the oyster community
2) implement solutions to the challenges facing the oyster industry through sustained engagement
3) enhance collaboration within and across the oyster farming and wild harvest sectors by identifying challenges and solution
4) solve problems collaboratively through sustained engagement with the oyster industry over the long-term
5) create a list of priority issues, tradeoffs, and decisions to help guide meetings and create innovative solutions

TREMATODE MANAGEMENT IN CATFISH AQUACULTURE

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The trematode *Bolbophorus damnificus* is a persistent threat to US catfish aquaculture. The complex, indirect life cycle involves the American White Pelican (AWP, *Pelecanus erythrorhynchos*), the planorbid snails (*Planorbella trivolvis* and *Biomphalaria havanensis*) and catfish (*Ictalurus* spp.). Trematode eggs released with the feces of AWP are dispersed in the aquatic environment, where they hatch into free-swimming miracidia. These miracidia infect snails, which release cercariae that are infective to catfish. Encapsulation of the metacercariae results in a parasite induced anemia in the catfish host, which leads to morbidity, and in severe cases, death. Reduced feeding activity, as well as increased susceptibility to bacterial infections in trematode infested catfish stocks causes significant production declines. Due to federal protections of the AWP, the most practical point of control is the snail intermediate hosts. Current practices rely largely on pond margin treatments of copper sulfate (CSP; $CuSO_4$•5 H_2O) equating to ~0.5-1.0 ppm Cu^{2+} (2-5 ppm CSP). However, copper's phytotoxicity can be harmful to beneficial algal blooms, resulting in catastrophic oxygen depletions. Copper toxicity to catfish also increases with temperature. Consequently, during the growing season when temperatures are highest and snail hosts are most abundant, the safety margins for copper are lowest. Studies designed to optimize CSP treatments revealed wild-caught snails have poor survival compared to laboratory reared cohorts, which may indicate poor snail vigor from handling, environmental stressors or parasitism. Copper sensitivity of laboratory-reared *P. trivolvis* was compared to wild-caught individuals actively shedding parasites or not. Snails parasitized with a common trematode, *Alloglossidium kenti*, served as proxy for the effects of trematode parasitism on copper sensitivity. Results indicate laboratory-reared snails were most resistant to repeated doses of CSP, followed by non-shedding pond snails. Pond snails actively shedding cercariae at the time of collection were most sensitive to CSP, highlighting this as a targeted approach, as parasitized snails are the primary target of control. In this context, it is hypothesized trematode eggs are dormant during cold periods, with miracidia hatching in a somewhat synchronized fashion when water temperatures increase in the spring, aligning with snail emergence from winter estivation. The effects of CSP on trematode egg hatching was also evaluated, with *Drapanocephalus spathans* serving as surrogate for *B. damnificus*. Trematode eggs exposed to a range of CSP (0.2 – 12 ppm CSP; ~0.05-3 ppm Cu^{2+}) indicate doses >4.0 ppm CSP (>1.0 ppm Cu^{2+}) may preclude trematode egg hatching, although these levels are likely impractical. This work also revealed limited hatching of trematode eggs at temperatures $\leq$23 C, supporting assertions of winter dormancy. Combined, these works advance our understanding of trematode and snail life histories and offer foundational data to develop best management practices against trematode infestations in commercial catfish aquaculture.

THE IMPACT OF ACUTE SALINITY STRESS ON EARLY-JUVENILE EASTERN OYSTER RESPIRATION Q_{10}

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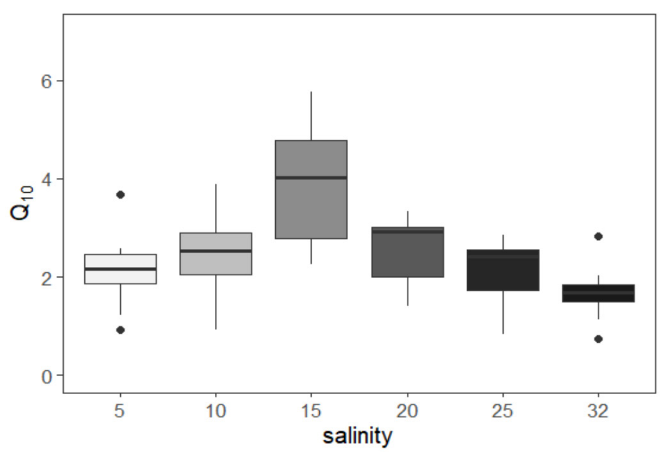
The eastern oyster (*Crassostrea virginica*) represents a growing industry in the US and in other locales. As a "blue food", oysters are a sustainable source of nutrition with limited associated environmental burden. A major limitation to oyster production, however, are the dynamic environments in which they are cultivated. With a large natural geographic distribution, the eastern oyster can tolerate a wide range of temperature and salinity variation, but these fundamental changes have large consequence for production. Further understanding the metabolic costs associated with environmental variation and the mechanisms employed to cope with them is a first step in the identification of biomarkers relevant to maintain and enhance production. As global temperatures continue to increase, coastal zones are experiencing increased storm activity and receiving un-paralleled volumes of rainfall in short periods of time. The resultant coastal flooding can cause acute but substantial departures from ambient salinities along estuarine habitats where oysters are cultivated. Here the impacts of acute salinity changes on the respiratory and metabolic activity of early-juvenile eastern oysters (<10 mm), a critical bottleneck in the life-history of this species that are vulnerable to environmental variation, are quantified. Individual juveniles were incubated in calibrated respiration vials at 15 and 25°C across a salinity gradient (5-32) to determine impacts on respiration Q_{10} (see Figure) and changes in mass-specific metabolism. As salinity decreased to 15, respiration Q_{10} was elevated relative to higher salinity treatments, representing an increase in mass-specific metabolism associated with maintaining ion homeostasis. At salinities below 15, respiration Q_{10} was similar to that of higher salinity treatments but was associated with decreased valve opening and activity. Future studies identifying how the resultant energy is allocated along salinity gradients will provide insight into resilience to environmental stress and the strategies that organisms use to mitigate those limitations.



ADVANCING HUMANE SLAUGHTER METHODS FOR WHITE STURGEON (*Acipenser transmontanus*): EFFICACY OF NON-PENETRATIVE CAPTIVE BOLT STUNNING

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Traditional sturgeon slaughter practices, involving a two-step process of percussive stunning followed by exsanguination, often fail to deliver humane outcomes efficiently due to the robust and cartilaginous anatomy of these large fish, which typically necessitates multiple strikes to achieve insensibility. This inefficiency not only complicates the slaughter process but also raises significant welfare concerns, highlighting the urgent need for improved methods. In response, this study evaluates the efficacy of a non-penetrative captive bolt (NPCB) gun as an alternative method aimed at enhancing animal welfare during the slaughter of farmed white sturgeon (*Acipenser transmontanus*). Utilizing a Jarvis HPS-1 NPCB, the research applied concussive strikes at various pressures (120, 135, and 145 PSI for juveniles, and 175, 200, and 225 PSI for adults) prior to exsanguination and immersion in an ice bath. Subsequent histological examinations of cartilage sections from the brain area were conducted to assess the extent of physical damage and to correlate strike pressures with intracranial hemorrhage. The results indicated a significant relationship between increased pressure and the occurrence of meningeal-to-cerebral hemorrhage in juveniles, with no significant differences observed in adults. Recovery monitoring revealed that 100% of juveniles and 90% of adults did not recover post-stunning with exsanguination, validating the NPCB's potential to maintain insensibility effectively. However, the absence of immediate brain death in some cases suggests that while NPCB stunning significantly improves upon traditional methods by reducing the need for multiple manual strikes, further research is essential to refine this technique. Optimizing pressure settings is crucial to consistently achieve immediate brain death with a single strike, aligning the slaughter process with the highest standards of animal welfare and addressing the unique challenges posed by the anatomical characteristics of sturgeon.

GENOMIC INSIGHTS INTO BAY SCALLOP (*Argopecten irradians*) POPULATION STRUCTURE AND ENVIRONMENTAL ADAPTATIONS

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The bay scallop (*Argopecten irradians*) is a species of substantial economic and ecological importance, found along the U.S. Atlantic coasts, from New England to the Gulf of Mexico (Fig. 1). Despite its significance, populations of *A. irradians* have suffered significant declines due to factors such as overfishing, habitat degradation, climate change, and disease outbreaks. In 2019, New York's bay scallop population was devastated by a mass mortality event linked to an outbreak of the apicomplexan parasite Bay Scallop Marosporida (BSM). This event, driven by rising water temperatures, highlighted the urgent need for genomic data to better understand and protect *A. irradians* populations. Genomic data can be crucial for developing strategies such as marker-assisted and genomic selection, which could improve scallop resilience to environmental stressors and disease. Historically, *A. irradians* has been divided into three extant subspecies: *A. irradians irradians*, found along the Atlantic coast from Massachusetts to North Carolina; *A. irradians concentricus*, distributed along the Gulf of Mexico; and *A. irradians amplicostatus*, primarily located in the western Gulf. Although these subspecies exhibit subtle phenotypic differences, they are difficult to distinguish based solely on physical traits. Our genomic analysis aimed to fill the gap in knowledge regarding these subspecies, as most previous genomic data were limited to scallops introduced to China from the USA and Canada in the 1980s and 1990s. This study harnesses advanced genomic techniques, including genotyping-by-sequencing (GBS) and whole-genome sequencing (WGS), to investigate the genetic and genomic diversity of the species, with the goal of informing future conservation strategies and improving aquaculture practices. The study involved sequencing and analyzing 960 scallop samples from six U.S. states. Our findings revealed significant genetic differences across populations, with distinct clustering based on geographic regions. By identifying genetically distinct populations and understanding their evolutionary history, this research establishes a basis for targeted management practices to help protect bay scallop populations from disease threats and global environmental changes.

Figure 1. A variety of color and pattern variations in *Argopecten irradians from New York*, commonly known as the bay scallop. Photo: S. Tettelbach

LAYING THE FOUNDATIONS TO TRANSFORM *Mercenaria mercenaria* AQUACULTURE IN THE U.S.: THE HARD CLAM SELECTIVE BREEDING COLLABORATIVE

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The hard clam, *Mercenaria mercenaria*, is extensively aquacultured from Massachusetts to Florida. The maintenance and growth of this aquaculture industry rely on hatchery production of seed clams, with billions produced annually to fulfill aquaculture and restoration needs. In this context, the production of quality seed capable of surviving under harsh biological and environmental conditions is a major priority for the aquaculture community. This collaborative project builds on partnerships between Sea Grant programs, scientists and extension teams in five Atlantic states to develop hard clam selective breeding programs using state of the art genomic tools. In this framework, the team produced a chromosome-level assembly of the clam genome and used that resource as a reference for the characterization of the species genetic diversity across its range. Through a series of stringent selection criteria, single nucleotide polymorphisms (SNPs) identified in *M. mercenaria* were filtered and a subset of 66,543 SNPs was used to produce SNP arrays for high-throughput genotyping of clams (Figure 1). The array incorporates markers for detecting the clam pathogen *Mucochytrium quahogii* (formerly Quahog Parasite Unknown, a.k.a. QPX), enhancing its utility in disease management. Performance evaluation highlighted the importance of sample preservation on genotyping results, while concordance testing demonstrated the array's reliability, with an average allele call agreement of 99.64% across multiple tissue types. More importantly, the produced arrays showed high efficiency and robustness in differentiating clams from different geographic origins and in contrasting the genotypes of clams resistant or susceptible to QPX disease and heat stress. Overall, the produced array represents a powerful and robust genotyping tool offerring unprecedented insights into the species' genomic architecture and population dynamics. Genomic selection using this tool is ongoing to identify clam stocks resistant to QPX disease and heat stress. Through coordination and extension, we are working to transform hard clam aquaculture by promoting the use of these modern approaches to enhance industry sustainability and resilience.

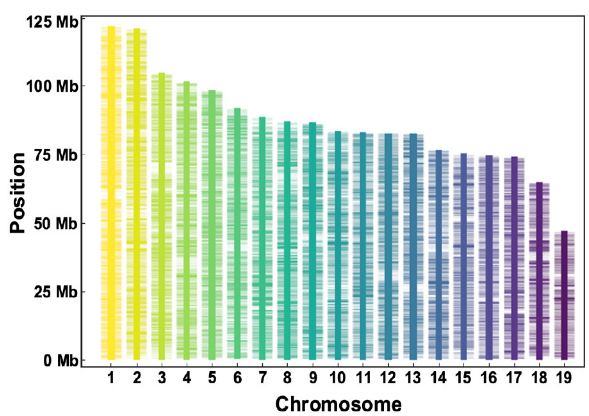

Figure 1. Chromosomal distribution of SNPs on the hard clam 66K SNP array.

THE ROLE OF CANNIBALISM AND DISEASE ON THE FUNCTIONAL RESPONSE OF JUVENILE BLUE CRABS

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Hematodinium perezi, a parasitic dinoflagellate, infects the hemolymph of blue crabs (*Callinectes sapidus*) and is found in high prevalence (> 80%) in juvenile crabs throughout the coastal bays of Virginia. In the blue crab–*Hematodinium* system, metabolic exhaustion from *H. Perezi* contributes to high mortality. This study investigated the role of cannibalism in the transmission of *H. perezi* by quantifying the functional response. We hypothesized that infected juveniles would experience a higher proportional mortality than their healthy conspecifics. This hypothesis supported a type II functional response, allowing for the functional elimination of *Hematodinium* within the population as infected juveniles are removed through the effects of cannibalism. Between July and October of 2024, we ran mesocosm experiments to evaluate the proportional mortality of infected and uninfected juveniles (10-30 mmCW). Individual tanks harbored artificial seagrass mats (1075 shoots/m^2) and sand to four densities of prey (n = 1, 2, 4, & 8) and 1 cannibal >60 mmCW. Infected juvenile blue crabs followed a type III functional response. These results suggest that infected crabs experienced a reduced encounter rate with the predator due to their lack of movement from metabolic exhaustion at heavy infections. Although there seems to be a relative low-density refuge for infected juveniles, cannibals still predated on both, and did not share a preference for infected or healthy juveniles. This has important implications for the fishery at large as *Hematodinium* can persist at low densities. These findings highlight the resilience of *Hematodinium perezi* within blue crab populations in endemic regions, demonstrating that cannibalism, while influential, is insufficient to fully eradicate the parasite due to the presence of low-density refuges for infected juveniles.

SEX DETERMINATION AND SEX DIFFERENTATION OF *Penaeus vannamei* SHRIMP

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Sexual dimorphism exists in *P. vannamei* shrimp (ZZ/ZW determination system) with females having a higher growth rate than males[1-2]. A mono-sex culture program (ALL-FEMALE SHRIMP) may increase the economic return of shrimp farming by mapping the sex-determination region in this species, which may assist in improving economic relevant traits like reproduction and growth, and study recombination rates and transposable elements (TE). A TE (*RTE-3_LVa*) has been identified as a potential female sex marker in *P. vannamei*[3].

Garcia et al. (2024) reported a genetic map that yielded 15,256 single nucleotide polymorphisms (SNPs) assigned to 44 linkage groups (LG). LG18 was found to be the largest for both sexes, whereas LG44 was the shortest for males and LG31 for females[4]. They identified a sex-determining region in LG31 with 21 statistically significant SNPs[4], the most important SNP was previously identified as a sex-linked marker[5] and was able to identify 99% of the males and 88% of the females[4]. The oplophorus-luciferin 2-monooxygenase, serine/arginine repetitive matrix protein and spermine oxidase genes were identified as candidates with possible participation in important processes of sexual differentiation in shrimp[4]. Yu et al (2017) identified a sex-determining loci in LG18 of the reference genome[5]. Two microsatellites in sex LG of shrimp (LG4, *ShrimpMap*[7]) are similar to the sex-linked marker in LG18[4].

To confirm the ZZ/ZW determination system, efforts are underway to sequence the genome of a wild female *P. vannamei* from Ecuador using long-read sequencing to assemble 45 chromosomes, even if it will be more challenging to assemble than ZZ males. Considering that there are only five draft genome assemblies available for *P. vannamei*[6], a new chromosome-based genome is needed.

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DISPERSAL LIMITATION OF CARBON DECOMPOSITION-ASSOCIATED TAXA SHAPES REGIONAL DIFFERENTIATION OF MANGROVE BACTERIAL COMMUNITIES

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The plant species component of mangrove communities is highly similar among geographic locations even across the global scale. However, the rhizosphere bacterial communities are highly geographically structured. To uncover the mechanism shaping bacterial community assembly and connectivity at the regional scale, we compared 136 samples of rhizosphere and bulk sediment from 17 mangrove sites along the coast of southeast China.

We found that the community similarity between sites was majorly correlated with geographic distance, as indicated by PCoA, variance partitioning analysis (VPA), beta diversity decomposition, and the phylogenetic-bin-based null model analysis (iCAMP). Consistently, dispersal limitation was found to be the dominant mechanism shaping bacterial community assembly. The proportion of dispersal limitation is positively correlated with geographic distance, which is mostly contributed by the phylogenetic bins belonging to Proteobacteria. There are also some bacterial taxa that are constraint by both geographic distance and soil carbon content in dispersal ability. We conducted a cellular automata simulation, showing that specialists are enriched in high-carbon areas through higher birth rates and dispersal preferences, while generalists distribute more evenly in the entire spatial space.

These results elucidate the mechanism underlying the high dispersal limitation across geographic distances of the phylogenetic taxa associated with carbon decomposition, which further shapes the differentiation of bacterial communities among different sites at the regional scale.

SHELLFISH BREEDING AT RUTGERS HASKIN SHELLFISH RESEARCH LABORATORY

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Rutgers University has a long history in shellfish breeding. The breeding program was initiated by Dr. Harold Haskin in 1960 to breed eastern oysters for resistance to MSX (multinucleated sphere X caused by protozoan *Haplosporidium nelsoni*) disease. The development of MSX resistance was rapid, and significant improvement was achieved after five generations of selection, with selected oysters surviving 8.8 times better than the susceptible control. Dermo disease (caused by protozoan *Perkinsus marinus*) appeared in Delaware Bay in 1990 and since then, selection for dermo resistance became a priority. In 1998, fast growth and shell shape were added as target traits for eastern oyster breeding. Over the years, new genetic materials were incorporated multiple times to maintain genetic diversity in selected lines. Two disease-resistant eastern oyster strains were developed and released to the industry: Haskin NEH® derived from Long Island Sound and Haskin DBX derived from Delaware Bay. The two strains have demonstrated fast growth and strong resistance to MSX and Dermo. In 1993, the breeding program developed tetraploid technologies for the production of triploid oysters, which have been widely used by oyster farmers in the US and globally. Recently, genomic selection was applied to improve dermo resistance in the eastern oyster, which demonstrated higher efficiency than traditional selective breeding. The breeding program has also expanded to include bay scallops and hard clams. For bay scallops, selection targeted fast growth with the goal of producing a crop that can be harvested within the year. For hard clams, genomic selection is being tested for improving field survival under QPX (Quahog Parasite Unknown) disease or heat stress.

The shellfish breeding program is supported by three Rutgers facilities. The Haskin Shellfish Research Laboratory at Port Norris, NJ is the main lab where most of the genetic analyses take place. The Cape Shore Laboratory in North Cape May is the main breeding station for the eastern oyster that consists of a research hatchery and an experimental farm. The Cape Shore Farm is intertidal with moderate salinity (18 – 22) where oysters are exposed to MSX, Dermo and thermal stress (-5 to 40 C), an ideal environment for breeding disease resistance and environmental resilience. The New Jersey Aquaculture Innovation Center (NJAIC) also in North Cape May provides support for breeding high-salinity species such as bay scallops and hard clams. The NJAIC is a commercial scale hatchery that produces seeds for shellfish farmers in New Jersey and the region.

GENETIC IMPROVEMENT OF OYSTERS AND THE PROSPECTS OF GENOMIC SELECTION

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Oysters are major aquaculture species worldwide. Oyster farming in the US has been growing rapidly during the past two decades partly due to genetic improvements of cultured stocks. The oldest recorded breeding of oysters began in 1960 when Dr. Harold Haskin started breeding eastern oysters for resistance to MSX (multinucleated sphere X disease, caused by protozoan *Haplosporidium nelsoni*). The development of MSX resistance from selective breeding was rapid, and significant improvement was achieved after five generations of selection. Selection for dermo (caused by protozoan *Perkinsus marinus*) resistance was effective but relatively slow. Tetraploid oysters were developed in 1993 and enabled commercial production of triploids. Triploid oysters produced from tetraploids offered significant benefits including faster growth, improved meat quality and sterility. The use of triploids has transformed oyster farming in several major producing countries with triploid production accounting for 30 – 70% of farmed oysters. The release of disease-resistant and triploid oysters has contributed significantly to oyster farming in the US. The genome of the Pacific oyster was sequenced in 2012, and rich genome resources have been developed during the past two decades. Genomic data have provided unprecedented insights into the biology and evolution of oysters. With the discovery of genes for production traits, gene-editing provides a new approach for genetic improvement. In oysters, gene-editing has succeeded in the laboratory although no gene-edited animals with altered phenotypes have been produced. Single-nucleotide polymorphism (SNP) arrays have been developed for oysters to enable genomic selection. Preliminary results indicate that genomic selection is effective in improving traits with low heritability such as dermo resistance. As genotyping cost continues to decline and prediction models are optimized, genomic selection may become a common approach to genetic improvement of oysters and contribute to oyster farming.

EFFECT OF VARYING DIETARY ENZYME INCLUSION LEVELS ON THE GROWTH PERFORMANCE OF FLORIDA POMPANO *Trachinotus Carolinus*

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Florida pompano is prominent for its high feed conversion efficiency, rapid growth, and high market demand. Xylanase, in comparison with protease, is not produced endogenously in Florida Pompano. The aim of this trial was to evaluate the effect of varying enzymes inclusion levels on the growth performance of the Florida Pompano. The basal diet was formulated to contain 40 % protein, then supplemented with commercial sources of protease or xylanase at multiple levels of 0.2, 0.4 and 0.6 g kg-3 to form a total of seven tested diets including the basal. Twenty fish were stocked with the initial weight of 8.30 ± 0.27 g in a 36 polypropylene tanks (0.8 m3) recirculating system. Five replicates were assigned for each diet, except the basal with 6. Pompano were fed according to the body weight (4.5 %- 7 %) four times a day. Feed input was adjusted according to visual observations and bi-weekly FCR. Overall, there was no statistically significant difference between diets and level of inclusion. However. there were significant differences between protease and xylanases. In conclusion, despite no statistical significance, protease and xylanase increased biological response of Florida pompano compared to the basal numerically. Therefore, inclusion levels will be dependent on the enzyme of choice.

Table. 1 Growth response of Florida Pompano fed various levels of commercial protease and xylanase.

Diet	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Survival rate (%)	FCR
Basal	55.38	577.87	577	90.83	1.84
Protease-0.5x	59.88	607.55	606^{B}	92.00	1.75
Protease-1x	59.82	625.68	625^{B}	94.00	1.71
Protease-2x	55.93	571.37	539^{B}	93.00	1.81
PSE	2.21	2.20	27	4.61	0.09
p-value	0.230	0.222	0.198	0.933	0.458
Xylanase- 0.5x	61.29	629.29	638^{A}	92.00	1.74
Xylanase-1x	60.66	606.08	619^{A}	94.50	1.69
Xylanase-2x	62.66	648.13	658^{A}	93.33	1.67
PSE	2.12	2.07	23	2.36	0.07
p-value	0.145	0.138	0.128	0.506	0.933
Two-way ANOVA					
Level	0.711	0.69	0.611	0.488	0.301
Type	0.071	0.06	0.049	0.172	0.059
Level × Type	0.119	0.11	0.104	0.488	0.159

Means not sharing any letter are significantly different by the Tukey's HSD-test at the 5 % level of significance

IMPROVING MICROALGAL RESEARCH AND PRODUCTION WITH LOW-COST TECHNOLOGIES

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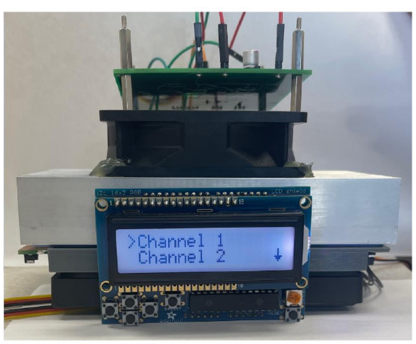
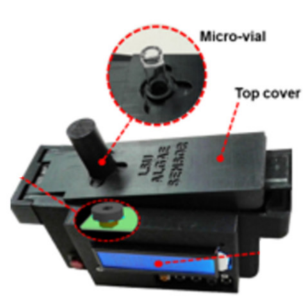
The interest on microalgal cultures interest has been undergoing changes not only on their multiple applications, that go from water treatment, biofuels and bioproducts, nutraceuticals, pharmaceuticals, high value food supplements, animal feed, indicator organisms of environment health, cosmetics, agricultural amendments and many others. The fast-doubling rates of microalgae makes them prime candidates for biomass production for these applications. Nonetheless, the promise of microalgal biomass has not been fully realized, as most of the products targeted for these cultures are commodity products.

Screening for higher value products requires equipment that is not always available to producers and researchers, such as computerized bioreactors, spectrophotometers, flow cytometers and other equipment that is out of reach for many entities. Even in pond production, where the scale makes the operations less expensive for unit mass produced, monitoring equipment that can be transported to the field can be expensive.

In this work, we explored the use of technologies such as 3-D printers, consumer level microprocessors and low-cost fabrication to produce devices that will allow research, production and monitoring of microalgal cultures. With the 3D printing technology going down in cost continuously, and with freely available software to run the printers, developing and producing these devices is within the reach of any interest entity. Coupling 3D printing with low cost, consumer level electronic devices, from microprocessors, to fabrication parts, open the doors for worldwide distribution.

In this work, we will discuss the role of low-cost open hardware for algal culture and research, with some examples developed by our team, from handheld florescent monitoring systems, growth monitoring plates, self-powered lights, and other devices, to reduce the costs associated to microalgal research, monitoring and production. Through interdisciplinary partnerships, the development of these devices' benefits form the feedback of different stakeholders. Some of these devices are being developed as open-source, and can be disseminated as fabrication files, accompanied by assembly and operation instructions. This strategy allows a wider distribution of the devices, and reduce the cost of disseminating the resulting products, by eliminating physical shipment of the devices.





SPECIES SORTING BY SEAWEED ULVA AND MARINE PERIPHYTON GOVERNS MICROBIAL ASSEMBLY ALONG MARICULTURE EFFLUENT TREATMENT

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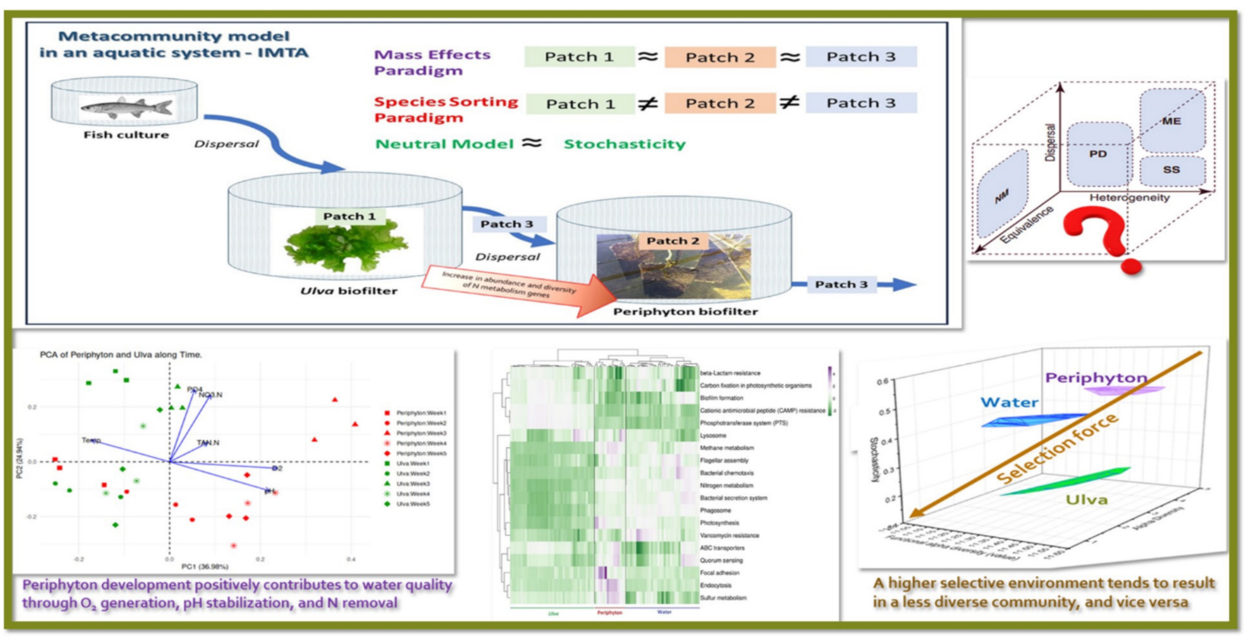
Plant-based biofilters with seaweeds or periphyton efficiently remove the excess nitrogen in mariculture effluent while forming a protein-rich biomass. Yet, little is known about the microbial assemblies in such biofilters. Metacommunity theory, the study of communities spatially connected via dispersal, is among the central pillars of microbial ecology. It evaluates which of the four paradigms of patch dynamic, species sorting, mass effects, or neutral model best explains community dynamics.

While theoretical research endorsed the neutral model in aquatic environments, empirical studies primarily support mass effects and species sorting paradigms. Here, we study metacommunity theory in a two-step biofilter with *Ulva fasciata* for ammonia removal and a sequenced marine periphyton biofilter for polishing and nitrate removal. During five weeks, microbial assemblies in the three patches of *Ulva*, periphyton, and water were analyzed following 16S rRNA gene amplicon sequencing.

Our results of community structure, diversity, and functionality support the argument that species sorting, operating through environmental heterogeneity, is the central force that drove microbial community dynamics in all three habitats. Determinism was a leading force across all patches, highest in *Ulva*, medium in the ambient water, and lowest in periphyton. This trend coincides with community diversity, suggesting that a less diverse environment tends to impose a more selective force and vice versa. Function-wise, genes related to nitrogen and sulfur metabolisms were higher in periphyton than in the water and *Ulva* assemblies. Our results proved against the common thinking that mass effects would overrule in such a small-scale aquatic system.



MICROBIAL CONTRIBUTION TO FOOD DIGESTION IN THE GUT OF ALGIVOROUS SEA URCHIN: A NOVEL RESOURCE OF ALGAL POLYSACCHARIDES DEPOLYMERIZING BACTERIA

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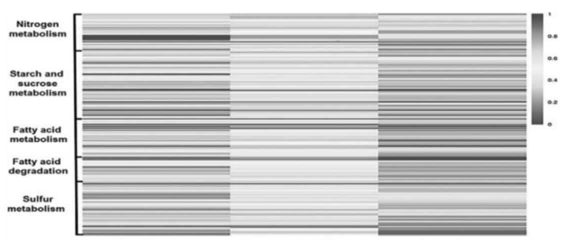
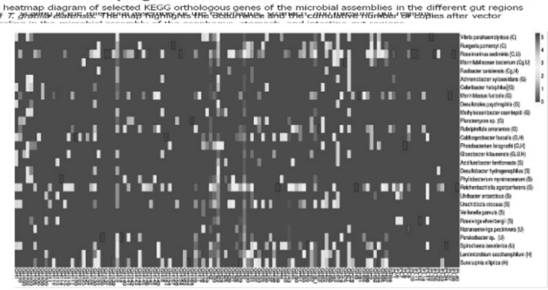
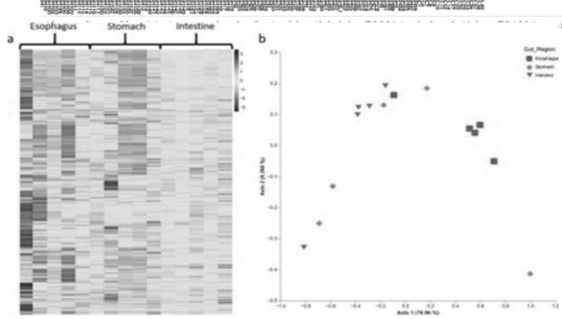
An ecological insight into the spatial arrangement in the gut bacterial community of the algivorous sea urchin *Tripneustes gratilla elatensis* will improve our knowledge of host-microbe relations concerning the involved taxa, their metabolic repertoire, and the niches of activity. We hypothesized that alterations in the bacterial compositional structure under different diets and along the gut are associated with the potential contribution to food digestion. Toward this goal, we investigated the bacterial communities in the sea urchin's esophagus, stomach, and intestine when fed a mono-specific diet of either *Ulva fasciata* or *Gracillaria conferta,* or an algal-free aquafeed. The study combined 16S rRNA amplicon sequencing, followed by bioinformatics analyses of community structure, interactions, and the prediction of their functional genes.

Sea urchins fed with *U. fasciata* grew faster, and their gut microbiome network was rich in bacterial associations (edges) and networking clusters. Bacteroidetes was the keystone phylum in the gut, with few microbes being central hub nodes that maintained community connectivity. Communities with distinct features in the esophagus and intestine evidenced spatial distribution. Bacteria that can contribute to *Ulva* digestion are common in the stomach and intestine and consist of genes for carbohydrate decomposition, fermentation, synthesis of short-chain fatty acids, and various ways of N and S metabolism. The various bacterial genes for the degradation of algal polysaccharides may be valuable for biomass biorefinery processes.



A heatmap diagram of selected KEGG orthologous genes of the microbial assemblies in the different gut regions





A comparative analysis of the KOs in the gut microbial assemblies of T. gratilla elatensis. (a) A heatmap diagram of the KEGG orthologous genes in the three main gut regions of the esophagus, stomach, and intestine. Each gene orthologue is colored according to its number of KOs in each of the five biological replicates (for each gut region); color varies from dark blue (0) to dark red (20,098). (b) A principal-coordinate analysis demonstrates the dissimilarity between the microbial assemblies in the different gut regions regarding their potential functions based on the measured, weighted UniFrac indices (n = 15).

EVALUATION OF ALTERNATIVE PROTEIN SOURCES IN THE DIETS OF FINFISH USING GROWTH, PHYSIO-BIOCHEMICAL AND MOLECULAR APPROACHES

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Aquaculture is one of the fastest-growing food industries globally. According to FAO 2024, the world aquaculture production in 2022 achieved a new record of 130.9 million tons (94.4 million tons of aquatic animals), a 6.6% growth from 2020. This increase was mainly from finfish aquaculture production. However, aquaculture production has increasing challenges, including disease mitigation and feed costs. As this industry grows, finding sustainable alternatives to traditional fishmeal (FM) in fish diets has become a key focus. This work summarizes the findings of our three published growth studies that evaluated the potential of insect and clam meals as alternatives to FM in finfish diets using growth performance, physio-biochemical, and molecular approaches.

Insect meal studies: For the first study, a 12-week growth trial was conducted to evaluate the effects of FM substitution by defatted mealworm meal (50% and 100%DMM) and whole mealworm meal (50%WMM) in the diets of Atlantic salmon parr. The study found: (i) statistically similar growth performance between treatments; (ii) dietary DMM significantly increased plasma immunoglobulin M and up-regulated the expression of immune genes compared to the control diet (FM-based diet); and (iii) mealworm inclusion significantly changed gut microbial beta-diversity and the most common genus in all treatments was *Pseudomonas*. In a second recently conducted growth study (factorial design), Atlantic salmon parrs were fed with a control diet (FM diet) and six test diets [three levels of defatted black soldier fly larvae (DBSFL) meal and two soy protein levels] for 12 weeks. The study suggested that DBSFL meal, up to 15% of the diet, could replace FM without compromising Atlantic salmon growth and health; and alleviate inflammation caused by anti-nutritional factors derived from soy proteins.

Clam meal study: A 12-week feeding trial was conducted to assess the effects of FM replacement by clam meal (10%, 20%, and 30%CM of the diet) in the diets of Florida pompano juveniles. The study found: (i) significantly higher final weight in fish fed 10% and 20%CM diets than those fed the control diet; (ii) no significant difference in plasma health parameters; (iii) similar gut microbial diversity between the treatments; and (iv) the most dominant genera across all treatments was *Vibrio*, which could produce hydrolytic enzymes such as amylase, lipase, cellulase, and chitinase, that assist fish to breakdown dietary components.

Overall, these studies suggest that both insect and clam meals offer promising, sustainable alternatives to traditional fishmeal in aquaculture diets. Not only do they support finfish growth and health but also positively influence immune responses and gut microbiome, highlighting their potential to help meet the growing demand for sustainable aquafeeds.

THE EFFECTS OF OCEAN ACIDIFICATION ON PACIFIC OYSTER LARVAE FROM STRESSED AND NON-STRESSED BROODSTOCK

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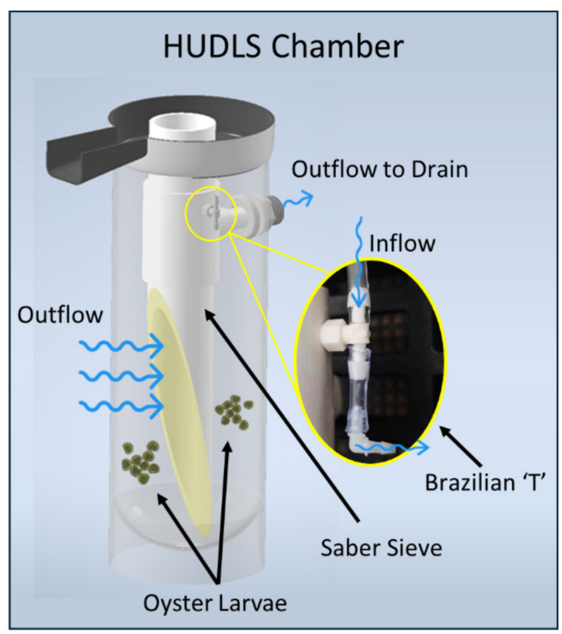
Along the US West Coast, climate change is intensifying and prolonging the upwelling of low-pH seawater, which is particularly harmful to calcifying organisms like shellfish. Commercial oyster hatcheries, such as the Whiskey Creek Shellfish Hatchery, Netarts Bay, Oregon, have faced reduced oyster seed production during periods of coastal upwelling of seawater with low aragonite saturation states (Ω_{arag}).

This presentation describes a multi-generational experiment investigating OA effects on Pacific oyster (*Crassostrea gigas)* larvae. These larvae were produced from stressed or non-stressed parental broodstock and reared in the Hatfield Ultra Larval Density System (HUDLS) that provides consistent flow-through culture conditions (Figure 1). Stressed parental broodstock had been subjected to hatchery OA stress as larvae and heat stress as adults at an intertidal field site. Non-stressed parental broodstock had been reared under normal hatchery conditions as larvae and at a subtidal field site as adults. The fastest-growing parental oysters were genotyped from each group, and unrelated individuals crossed to produce two groups of larvae that were reared in either acidified (7.5-7.6 pHt) or normal (8.0-8.1 pHt) seawater in the HUDLS.

We found an adverse effect of OA on shell growth at 1-day and 10-day post-fertilization (dpf), regardless of parental stress exposure; furthermore, 10-dpf larvae from stressed parents reared in acidified conditions were significantly larger than those from non-stressed parents. A higher proportion of larvae from non-stressed versus stressed parents were retained (a measure of viability) at all stages and treatments, with significant differences observed under OA conditions. The larvae from stressed parents exhibited significantly lower metamorphic success, resulting in lower spat yields from stressed parents under all seawater conditions at 35 days post-fertilization (dpf).

These results indicate that parental stress during larval (OA) and adult (heat) stages adversely affected the production of offspring.

Figure 1. HUDLS chamber including a saber sieve with the Brazilian T anti-overflow device. Larvae are reared in the chamber space around the saber sieve



LEVERAGING THE R-STRATEGY AND UNMANNED AERIAL VEHICALS TO OVERCOME PREDATION IN SHELLFISH RESTORATION

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The importance of bivalves to estuarine ecosystems is well known and documented in the scientific literature. Repatriation of clams has been established as a viable means of aiding in ecosystem restoration. Unfortunately, this work is time and labor intensive requiring large amounts of funding to be impactful. The need to find novel approaches to clam restoration has arisen to optimize restoration dollars while accelerating ecosystem restoration. Blue Ocean Quest in collaboration with the SJRWMD and the University of Florida have experimented with a novel approach leveraging native clam reproductive strategies to deliver millions of juvenile clams to restoration sites via heavy lift drone. This approach negates aquaculture techniques of growing adult clams and repatriating with cover nets. Trials of aerial dispersal indicates the mean survival of clams was 33.6% (±8.0%). This can be accomplished for approximately one third of the cost of clam repatriation via the traditional methods.

The traditional method for clam restoration has been to raise clams in the nursery to a size of 15-25 mm then out-plant them at a density of up to 50 clams per square feet and covered with protective cover netting for a period of 1 to 2 years. The theory is that the clams will spawn, and the larvae released into the water column to then be carried by the tides and eventually settle on the bottom to continue the life cycle.

The heavy-lift drone approach is accomplished through a patented device attached to the drone that deploys clam seed. The clams are 2 to 3 mm in size and are deployed in a homogenous manor at a density of approximately 20/sq ft or 1,000,000 per acre. Once the clams are deployed, they bury into the bottom and complete the life cycle. This mimics what occurs in nature with the early stages of life protected in a nursery. A scientific study of this method has shown a 33% survival rate after 8 months. Predation is expected and welcomed as it naturally feeds the food web and helps restore the fisheries.

Using the survival rate that has been observed, an economic comparison of the traditional method vs the drone seeding method can be made. With an expected outcome of 1,000,000 clams of 15 to 25 mm in size being introduced into the IRL, the total cost using the traditional method is $88,500 vs the Drone seeding method cost of $29,300. This results in a saving of $59,200 or 67% using the drone method as opposed to traditional.

ADVANCING DOMESTICATED STRIPED BASS *Morone saxatilis* LARVICULTURE USING RAS TECHNOLOGY

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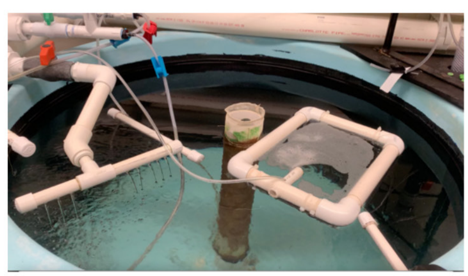
For the past five years, our team has conducted RAS based larviculture research with Domesticated Striped Bass, *Morone saxatilis*, (distinct from both wild striped bass or hybrid striped bass). The principal foci include system optimization, inert feeds, nutrition, and digestive physiology to improve production during this early life stage. We encountered numerous surprising and unexpected challenges to our basic larviculture protocols. This resulted in additional studies to improve these methodologies and technologies. This presentation will highlight these efforts from a systems approach as outlined below.

Feeding larvae usually begins 4 dph-8dph after mouthparts form. Feeding this early produces excess surface oil, impeding critical swim bladder inflation. We have enhanced the efficiency of our surface oil skimmer, examining airstone placement, lipophilic pads, and surface retainers.
In addition, water depth and water flow rate affect the ability of larvae and fry to eat and impact energy expenditure. We will discuss initial efforts to understand these variables as related to post larval and fry production. Control of these factors is enhanced in recirculating systems.

Artemia nauplii are the most common first feed for carnivorous marine species like domestic striped bass. The process to hatch and deliver healthy nauplii is expensive and time consuming. Our lab has shifted from using a common decapsulation protocol to one using a magnetic cyst shell removal process. This change has reduced labor, while also providing a superior product clean of hatching debris. In parallel, biofiltration performance has been improved.

Larvae require feeding around the clock. Examination of gut contents showed food retention time of about 3 hours, throughout most of the larviculture period. Wi-fi controlled peristaltic pumps were used to maintain uniform provision of Artemia 24/7. Some challenges remain with these units. Systems produced successfully and improvements are underway for season two.



Left: Replicated RAS with 12 tanks, feeders and unit operations. Right: Skimmer to enhance surface properties for larval swim bladder inflation. (Photos: Hall, Frinsko)

OPTIMIZATION OF WATER QUALITY PARAMETERS FOR SURVIVAL AND GROWTH OF EASTERN OYSTER *Crassostrea virginica* LARVAE IN CULTURE

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The Eastern Oyster provides essential ecosystem and economic services. However, oyster populations have declined significantly due to various natural and anthropogenic stresses. Aquaculture can potentially meet the demand for commercial and restoration purposes, but hatcheries have yet to consistently meet demand due, in part, to water quality/chemistry problems. Salinity, temperature, pH, dissolved oxygen, and carbonate chemistry were monitored at three hatcheries along the Gulf coast during April-October from 2022-2024. Water quality was compared among the hatcheries, and associations with larval growth, percent hatch, and survival were assessed.

Data from Auburn University Shellfish Laboratory (AUSL), a flow-through hatchery, the University of Southern Mississippi (USM), a recirculating hatchery, and Bay Shellfish Company (BSC), a flow-through hatchery, showed that, on average, BSC produced larger larvae than USM and AUSL across different ploidies. USM's percent survival in 2022 and 2023 was 34.9% and 28.4%, respectively; AUSL's was 26.8% and 43.6%, respectively; and BSC's was 36.1% in 2023. Individual water quality parameters influenced larval growth (MANOVA). Temperature and salinity interactions significantly impacted growth across all sites, while temperature and pH interactions were significant at all sites except AUSL. The interaction between salinity and pH was notable only at BSC, and interactions between aragonite saturation and both salinity and alkalinity were significant across all sites, suggesting variations in environmental factors have a substantial impact on larval development. The significance of interactions suggested that larval growth was influenced by the combination of environmental factors rather than individual ones. Segmented regression models initially suggested a potential positive effect on growth below the breakpoints at a temperature of 23.3°C, a pH of 7.71, a salinity of 23.19 ppt, and a calcium of 5.002 mmol/Kg; and a potential positive affect above breakpoints at an alkalinity of 1929.85 μmol/kg, and an aragonite saturation state of 0.232Ω. Further analysis is needed to clarify these findings and their implications for improving larval production.

To better assess how water quality, temperature, salinity, and pH affected larval survival, the data from all locations in 2022-2023 were combined. Linear mixed models (LMM) analysis revealed that only pH significantly influenced survival (negatively). In a LMM including interaction terms, there were no significant linear interactions among any of the factors. A generalized additive model (GAM) that looked for non-linear relationships among water quality parameters showed that interactions between pH and salinity, and interactions between temperature, pH, and salinity were significant. These results indicated that survival depended on the individual factors and their combined effects. The results from both models offered unique insights that can improve hatchery practices. Further refinement of these models is needed to determine optimal water quality parameters that enhance survival in hatchery settings.

AUTONOMOUS VEHICLE SYSTEMS IN OYSTER AQUACULTURE: FINDINGS FROM NSF/ USDA NATIONAL ROBOTICS INITIATIVE AND FUTURE NEEDS

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Increasingly, autonomous and automated vehicle systems (e.g. surface, underwater, aerial) in aquaculture are applied in North Carolina and other locations. This talk will provide an update on our project focusing on using multiple vehicles (primarily aerial and surface) to assess and manage oyster aquaculture. Modeling and assessing water quality to better determine optimal timing for harvest; and optimizing the process are critical. This update will include field data and modeling efforts to assess location, time, flow, temperature, nitrate, pH and other water quality parameters and link these to measured bacterial loads (captured manually) under different conditions.

Updates on software including "digital twin" modeling in different situations; open source code including enhanced learning and use of multi-vehicle automated systems will be included. Preliminary work with collaborators in nearshore oyster leases to assess effectiveness, speed, accuracy and improve understanding of limits to autonomy and human-robot interfaces are ongoing.

The coastal environment is dynamic and subject to high energy events, but is also an extremely productive zone. These systems should enhance sustainability, improve monitoring and productivity, and may be able to provide improved information on coastal water quality, biological and ecological conditions, thus allowing improved decision making by farmers, managers and others.

This presentation will provide an update on current work, relevant findings, and direction of future work, in the hopes that coastal and offshore zones will enhance wise use of automated systems in aquaculture.

Figure 1: This fleet of dual paddlewheel autonomous surface vehicles may also be in communication with aerial vehicles and should enhance human decision making and management.

CHARACTERIZATION OF ALKALINE PHOSPHATASE (E.C.3.1.3.1) IN FRESHWATER FISH SPECIES (*Coptodon zillii*, *Heterobranchus bidorsalis*, *Clarias gariepinus* AND *Chrysichthys nigrodigitatus*)

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Alkaline phosphatases (APs) are homodimeric enzymes that catalyze the hydrolysis and transphosphorylation of phosphate monoesters**.** Alkaline phosphatase activity was determined in different tissue homogenates (flesh and bones) of species four fish species- *Coptodon zillii, Heterobranchus bidorsalis, Chrysichthys nigrodigitatus* and *Clarias gariepinus.* The bone was found to have the highest activity of alkaline phosphatase. The K_m and V_{max} values for the fish bone alkaline phosphatase (FBAP) were estimated to be 0.0106mM and 2.1730 µmol/min/mol for *C. zillii*, 0.0074mM and 3.1868 µmol/min/mol for *H. bidorsalis*, 0.0226mM and 8.9928 µmol/min/mol for *C. gariepinus* and 0.0104mM and 6.0753 µmol/min/mol for *C. nigrodigitatus* respectively. The pH optimum of the enzyme for the categories of fishes was estimated to be 9.0. Divalent metals such as Mg^{2+} and Ca^{2+} enhanced enzyme activity but have an inhibitory effect at concentration above 1.5 mM. The highest activity of the enzyme in the bone of the four species of fishes in this study suggests that most of the organic phosphates in the species are hydrolysed principally in this tissue. This enzyme also plays the role of metabolic regulations.

EFFECT OF ROOM TEMPERATURE AND FEED & FEEDING FREQUENCY ON BERRIED CRAB PRODUCTION, SURVIVAL AND GROWTH OF MUD CRAB (*Scylla olivacea)* LARVAE

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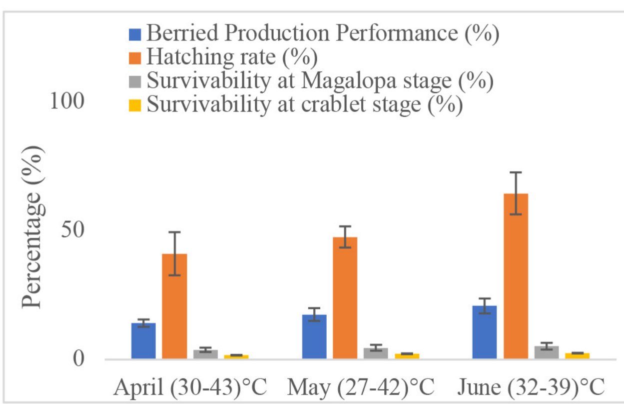
Presently, the stocking of mud crab (*Scylla olivacea*) aquaculture in Bangladesh is based primarily on wild-caught juveniles and causing the decrease of wild stock. To overcome this, the experiment was conducted to test the effect of different room temperatures of the hatchery in the month of April (30-40^{0}C), May (27-42^{0}C) and June (32-39^{0}C) on berried crab production, hatching and larval survivability. Another, experiment was carried out in the larva rearing tanks (LRT) to test the effect of feed and feeding frequency on the growth and survivability rate at different zoea stages. The experiment had a completely randomized design, with three treatments in triplicate. The treatment consists of T1 (artemia; four times a day), T2 (artemia + processed feed; six times a day) and T3 (artemia; six times a day). For Zoea-I and Zoea-II stages, umbrella type artemia (1.0 ind/ml) and for the late zoea stages (Z3 to Z5) artemia nauplii (5 ind/ml) was provided as feed. However, processed feed was supplied only for T2 at a rate of 1.0 g/ton of culture volume of water. One-way ANOVA indicated that room temperature variation had significant ($p < 0.05$) effect on berried crab production and hatching. The highest berried crab production and hatching rate were observed in June (32-39^{0}C) which were 20.83 ± 2.80% and 64.55 ± 8.14% respectively. Larval survivability in each stage also differed significantly by the room temperature variation and the highest survival rate was observed in June (32-39)^{0}C. Consequently, the highest survivability at the megalopa and crablet stages was also recorded from the room temperature of 32-39^{0}C which was 5.20 ± 1.30% and 2.52 ± 0.13%. On the other hand, feeding treatments varied significantly ($p < 0.05$) in the zoea size and survivability at different zoea stages. Treatment T2 produced the highest survivability rate at different larval stages which was 90.67%, 79.67%, 60.33%, 41.67%, and 33.00% for Zoea-I, Zoea-II, Zoea-III, Zoea-IV, and Zoea-V stages respectively. T2 feeding treatment produced the highest survivability in crablet stage which was 2.90%. The highest larval size in each stage was also recorded by the T2 feeding treatments. So, the feeding combination of T2 might be applied in hatchery operation.



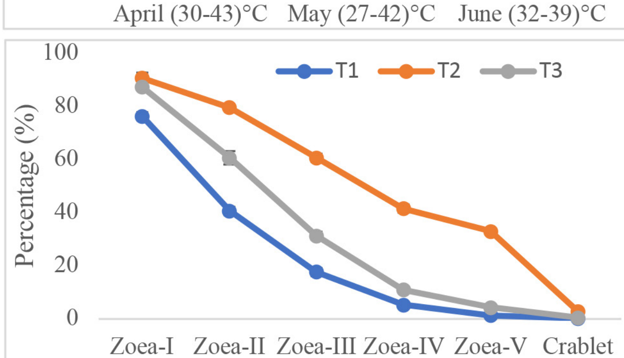


Fig. 2 Survivability at different larval stages

GENOTYPING AND ANTIMICROBIAL SUSCEPTIBILITY OF COLUMNARIS-CAUSING BACTERIA ISOLATES FROM TEXAS AQUACULTURE AND SPORT FISH

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Columnaris disease affects a variety of freshwater fish, including all commercially important species worldwide. In the southeastern USA, it is the most reported disease in catfish farming, according to case summary reports by the Aquatic Research and Diagnostic Laboratory at Mississippi State University. This disease is caused by a group of Gram-negative, yellow-pigmented bacteria known as columnaris-causing bacteria (CCB). CCB includes four distinct species with host associations, namely *F. columnare*, *F. covae*, *F. davisii*, and *F. oreochromis*. Historically, *F. columnare*, *F. covae*, and *F. davisii* have been isolated from columnaris disease cases in the southeastern U.S.

Severe columnaris disease outbreaks recently impacted commercial catfish farms and sport fishing ponds in south Texas. During these outbreaks, hybrid catfish (*Ictalurus furcatus* x *I. punctatus*), hybrid tilapia (*Oreochromis mossambicus x O. niloticus*), red drum (*Sciaenops ocellatus*), and other sport fish species such as largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and crappie (*Pomoxis* spp.) were affected. However, there is limited data on the prevalence of specific CCB species in Texas aquaculture and recreational fish. The study aimed to identify the predominant species of CCB associated with columnaris disease outbreaks and evaluate the isolates' antimicrobial susceptibility profiles to guide treatment strategies.

Putative CCB isolates (n=40) were recovered following the standard microbiological procedures from the different fish species submitted to the Fish Health and Disease Laboratory at Texas A&M University during columnaris outbreaks throughout 2023-2024. After DNA extraction, a multiplex polymerase chain reaction (PCR) was conducted using four reference CCB strains provided by the USDA-ARS Aquatic Animal Health Research Unit (Auburn, AL) as control to identify the most prevalent species among the samples. 60% of the collected isolates (n=40) were identified as *F. covae* (n = 24), followed by *F. davisii* (n = 16). Notably, no isolates were categorized as *F. columnare* or *F. oreochromis*. Antibiotic susceptibility tests were performed on representative isolates (n = 13) following established protocols using FDA-approved antibiotics for food fish. *F. covae* isolates showed an average zone of inhibition of 36.09 ± 2.67 mm against oxytetracycline (30 µg), while *F. davisii* exhibited an average zone of inhibition of 32.83 ± 0.73 mm. The average inhibition zones for florfenicol (30 µg) were 45.03 ± 0.18 mm and 42.72 ± 0.09 mm for *F. covae* and *F. davisii*, respectively. In contrast, *F. covae* isolates displayed an average zone of inhibition of only 5.75 ± 0.58 mm around Romet-30 discs, and all *F. davisii* isolates showed no zone of inhibition. This research emphasizes the need to develop alternative therapeutic targeting the most prevalent CCB species to prevent future columnaris disease outbreaks in Texas effectively.

DO CERTIFICATION PROGRAMS MAKE MEANINGFUL REDUCTIONS TO THE ENVIRONMENTAL IMPACTS OF AQUACULTURE?

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Certification programs have made notable strides in promoting more sustainable practices. Some of the key environmental areas where certification programs have led to positive changes include improvements in feed sourcing and feed management, reduced dependence on antibiotic use and better disease control, better waste treatment and effluent control, and habitat protection. Certification programs have led many producers to adopt improved farming practices, although adoption of practices often leads to procedural compliance without assurance of environmental impact reduction. Overall, the effectiveness of certification programs in reducing environmental impacts has been mixed. Certification has been more successful in reducing direct, farm-level impacts than in addressing the larger, systemic issues affecting entire ecosystems.

Certified production represents only a small portion of total global aquaculture, limiting the overall impact on sustainability. Certification levels for the most farmed species group globally – the carps – are estimated to be <5%, and in China, the world's largest aquaculture producer, only about 5-10% of domestic aquaculture production is certified. Certification programs focus on large-scale operations or high-value and widely traded species, such as salmon or shrimp. These programs are more prevalent with export-oriented production compared to production of species farmed for domestic consumption or by small-scale farmers.

Certification typically focuses on farm-level practices, but significant environmental impacts may occur elsewhere in the value chain. For example, although there have been efforts to reduce fishmeal and fish oil use in feeds, alternative ingredients like soy also have substantial environmental impacts. Thus, farm-level certification standards have had limited success in promoting truly sustainable feed solutions.

The unit of certification in nearly all programs is the individual farm and certification programs do not comprehensively address the cumulative impacts of multiple farms operating in the same region, such as the effects of nutrient loading (eutrophication), the spread of pathogens causing disease outbreaks, habitat degradation and fragmentation, and biodiversity impacts. Certification programs do not consider the limits of shared natural resources and ecosystem services.

Certification can create incentives for farms to intensify their operations, as certified products often provide access to particular markets or higher market prices. This can lead to more farms seeking certification in already high-density regions, exacerbating environmental pressures and offsetting gains made through certification.

To fully deliver on their promise, certification programs need to evolve by adopting more holistic, inclusive, and adaptive approaches that go beyond individual farm certification to ensure meaningful reductions in environmental impacts. A broader approach requires coordination and collective action by producers, regional-level spatial planning, and other integrated management and policy approaches.

DO WE NEED INNOVATION TO GROW AQUACULTURE?

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John Hargreaves has 44 years of experience in research, teaching, training, and development. For the last 18 years, he has been a freelance consultant on commercial aquaculture and development projects. He has worked with producers employing intensive ponds, biofloc systems, partitioned and recirculating ponds, net pens, raceways, recirculating aquaculture systems, and hatchery and nursery systems. He has broad international experience in Latin America, Asia, Africa, and the Middle East, working with many commercially important finfish, crustaceans and molluscs in freshwater, brackishwater, and marine systems. He was Vice-President of WAS in 1999, named a Fellow of WAS in 2017, and editor of World Aquaculture magazine for 11 years (2012-2023).

METABOLIC EFFECTS IN RAINBOW TROUT *Oncorhynchus mykiss* TISSUE DUE TO LOW DIET PH

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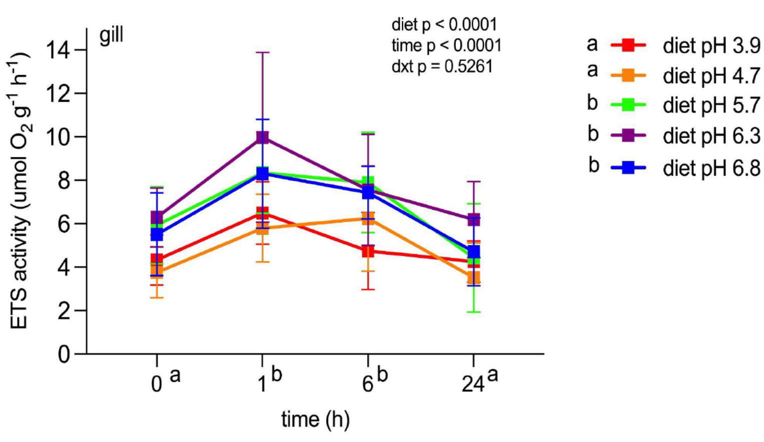
Trout are a major aquaculture target species in the United States. Diet directly influences production efficiency and diet developments provide a practical and manageable means of enhancing farm performance. In this study, we focused on the effects of diet pH on energy expenditure in trout.

Trout require an acidic stomach environment for digestion. To achieve a low pH in the stomach, the fish must spend energy pumping H^+ into the stomach lumen and, consequently, HCO_3^- into the blood. If the fish were able to obtain these H^+ from a low pH diet, it is possible that they would not need to spend the energy sourcing them from the body. For this project, the metabolism of stomach, gill, and muscle tissue was measured to determine the effect that diet pH has on energy expenditure before and after feeding in rainbow trout (*Oncorhynchus mykiss*).

Ten experimental diets with a pH ranging between pH = 3.9 and 6.8 were fed to rainbow trout over twelve weeks. There were three tanks per diet with thirty fish per tank. During the trial, gill, stomach, and muscle samples were collected before and after feeding and frozen at -80°C for later analysis. Each sample was then homogenized, and the supernatant was collected. The electron transport system (ETS) microplate assay was used to measure metabolic activity with a spectrophotometer.

It was found that in rainbow trout gill, fish fed a diet pH of 3.9 and 4.7 had a lower ETS activity than diets with a higher pH (Figure 1). These results were similar to oxygen consumption measured at the whole animal level and support our hypothesis that energy used for digestion would decrease with a decrease in diet pH. This may be explained by a reduced acid-base disturbance with feeding and decreased need for ion transportation at the gill. ETS activity was also seen to increase 1 to 6 hours after feeding and then decrease at 24 hours. This verifies the ability of the ETS assay to measure "specific dynamic action" (a predictable metabolic increase after feeding).



FACILITY MANAGEMENT APPROACHES FOR LARGE-SCALE AQUATIC HUSBANDRY

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Efficient management of aquatic animal husbandry is essential to the sustainability of live animal populations in aquatic genetic stock centers. However, achieving this requires innovative strategies to ensure uniform and consistent care across research animals. Since 2014, the Aquatic Germplasm and Genetic Resources Center (AGGRC, www.aggrc.com) has expanded from several aquaria to 14 distinct husbandry spaces (Figure 1), now including 24 recirculating systems supporting over 850 animals from 18 aquatic species including fishes, amphibians, and invertebrates. This increasing scale of operations, coupled with species-specific requirements, has highlighted the limitations of traditional task management and record-keeping methods.

To address these, we have focused on centralizing husbandry operations and developing an advanced management platform tailored to the unique demands of aquatic animals. We partnered with a team of industrial engineering students at Louisiana State University to develop a low-code, open-source mobile application for husbandry management using Microsoft PowerApps. The system includes several key features including streamlined task assignment, comprehensive documentation of daily care activities, accessible instructional resources for student workers, longitudinal water quality monitoring, automated emergency notifications, and animal-specific history information for research. Importantly, the app also ensures compliance with the detailed record-keeping requirements of the Institutional Animal Care and Use Committee (IACUC) and other oversight bodies to foster animal welfare.

This system enhances operational efficiencies, promotes centralized awareness among staff and researchers, and supports improved animal care practices in aquatic genetic stock centers. By modernizing husbandry management, we can further the sustainability of the AGGRC and its mission to support aquatic species repository development.

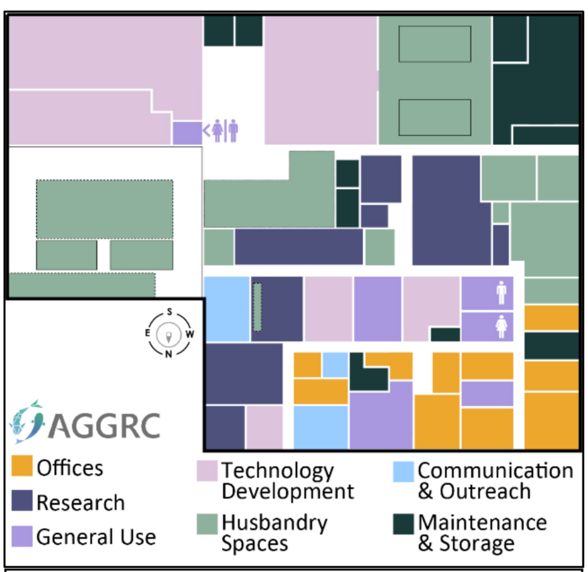

Figure 1. AGGRC wayfinding map with rooms categorized by function. The AGGRC occupies ~3 acres adjacent to LSU's main campus in Baton Rouge, Louisiana.

DIVERSIFYING SHELLFISH AQUACULTURE: ESTABLISHING NURSERY REARING METHODS FOR RAZOR CLAMS *Ensis leei*

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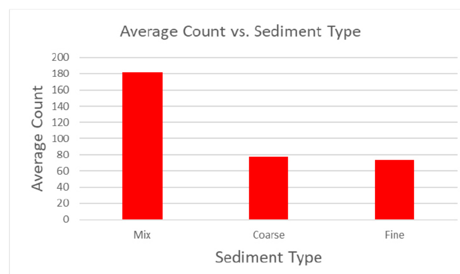
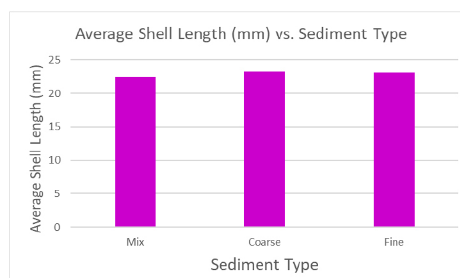
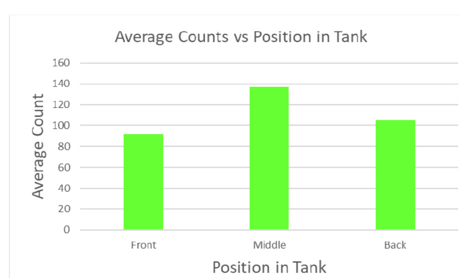
Along the east coast of the United States there are a plethora of shellfish farms that produce around $170M in shellfish products such as oysters, scallops, and various species of clams. Most of these earnings are from oysters and hard clams. This can be detrimental since having a very narrow selection of shellfish may result in a collapse in the event of a disease outbreak, climate change, and cause fluctuations in the market. It's in the shellfish industry's best interest to diversify the market in order to address these concerns. A viable candidate would be the razor clam. Razor clams, *Ensis leei* can be found all throughout the northeast of the United States, and it is a valuable crop for shellfish farmers all over that region, that can reach up to $6/lb. There have been attempts in the past to lay down the foundation of a razor clam industry by proposing to condition, spawn, settle, and develop methods to successfully establish nursery rearing for a large sample of post-set razor clam spat. The purpose of this research is to target and resolve known bottlenecks in the early development stage. Earlier research has pointed out that sediment type may be a factor in decreasing mortality rates in post set razor clams.

Three raceways were partitioned into three separate sections, and each section housed a type of sediment. The tested sediments were fine, coarse, and mixed sand. Mixed sand was comprised of fine sand and natural mud harvested from Lowes Cove. Once the sediment in the tank was filled and seawater was added, the tanks were left untouched for two weeks in order to promote a natural microbiome.

During this time, broodstock was spawned and larvae were introduced into the nursery. After 14 days settlement occurred and the larvae were moved from the upwellers to the experimental sediment tanks. Then spat was then distributed into each partition. We allowed two weeks to pass before collecting the first core sample, which was used to identify the health, and density of the recently settled clams.







HOW NORTH CAROLINA HAS APPLIED NEPA CUMULATIVE IMPACT LANGUAGE TO PROPOSED SHELLFISH LEASES

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Shellfish lease applicants in North Carolina are required to select their own proposed lease site, which is then approved or denied by the NC Division of Marine Fisheries (DMF). Applicant defined areas are required to meet the standards set by NC Marine Fisheries Commission (MFC) rules, NC General Statutes, regulatory closures, and federal permitting requirements. Once the shellfish lease application meets these standards, it proceeds to a public comment period, where only comments specific to the proposed lease, that cite public trust use concerns (excluding viewshed), are considered. As a result of aquaculture industry growth in specific localized areas, the NC General Assembly required the DMF to study and implement MFC rules to address user conflict issues related to shellfish leases. The MFC then adopted rule amendments study aimed at reducing user conflicts, including the addition of cumulative impact language to limit the number of acres leased in any area.

Cumulative impact language is derived from the National Environmental Policy Act (NEPA) to be included in Environmental Impact Assessments of actions by Federal Agencies. The framework for assessing indirect, direct, and cumulative impacts required by the NEPA is outlined in Council of Environmental Quality (CEQ) regulations (40 CFR §§1500-1508).

The inclusion of this language in the MFC shellfish lease rules has allowed for evaluation of a shellfish lease application to expand to include disproportionate effects due to the existing shellfish leases nearby. Through the triggering and completion of a Cumulative Impact Analysis (CIA), the DMF can characterize comparison values for the protected public trust usage in an area and comparison values for the impact of the proposed and existing leases on the public trust rights. The DMF has developed and implemented this policy into its review of shellfish lease applications in an effort to further resolve user conflicts and find consensus between shellfish lease applicants and the local public.

Figure 1: Localized Growth in Shellfish Leases

DEVELOPMENT OF IMPROVED FISH STRAINS USING GENOME EDITING TECHNOLOGY IN JAPAN

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Genome editing technology allows for precise cutting of specific sequences in the genome, enabling a rapid and accurate introduction of useful traits. In aquaculture, this technology has been used as a powerful tool for the development of improved lines within a few generations. Notably, deletion-type genome editing introduces changes that could occur naturally in populations; for this reason, its impact on food safety is considered as equivalent to that of fish found in nature or those subjected to traditional breeding methods.

Regional Fish Institute Ltd. is a startup company established in 2019 that has been focusing on genetic improvement of aquaculture species, by combining genome editing and breeding technologies from both Kyoto and Kinki Universities, respectively. Using Crispr-Cas9 technology we developed an improved breed of red sea bream (*Pagrus major*) that show increased filet by knocking out the myostatin gene (*mstn*), a member of the TGF-β family with a key role in inhibition of muscle growth. In September 2021, our company completed the notification procedures with Japan's Ministry of Health, Labour and Welfare (MHLW) and Ministry of Agriculture, Forestry and Fisheries (MAFF) for this strain. Subsequently, two other strains, one of tiger puffer (*Takifugu rubripes*) and another of olive flounder (*Paralichtys olivaceus*), both carrying mutation in the leptin receptor gene (*lepr*) and displaying fast growth due to increased appetite compared to other strains, were developed and subjected to the same notification procedures with MHLW and MAFF. Currently, those genome edited strains are being farmed experimentally in land-based fish farms in Japan and their respective products are available for purchase on online sites and other Japanese platforms. With focus on both native and exotic aquaculture species, we are expanding the genome editing technology to other aquaculture species, broadening the target genes and traits, developing new genome editing tools, and integrating genome editing with other classical and cutting-edge biotechnologies such as conventional selective breeding, sex control, chromosome manipulation, surrogate broodstock and genome selection.

EFFECT OF PROBIOTICS ON LARVAL SURVIVAL, GROWTH, AND SETTLEMENT SUCCESS OF THREE SHELLFISH SPECIES

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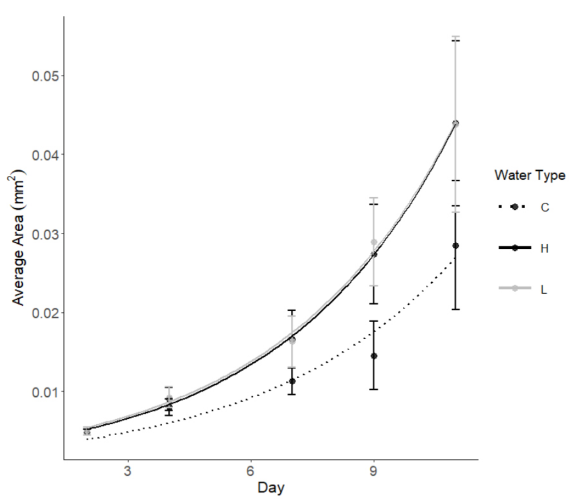
Shellfish aquaculture relies on a consistent supply of larvae for restoration, research, or mariculture. Research investigating the addition of probiotics, consisting of viable microbial spores or live microorganisms, has been shown to enhance growth, survival, and disease immunity in a variety of shellfish species. Commercially available probiotics are widely used in the shrimp industry, but these products have not been evaluated for bivalve culture. Previous studies have reported *Bacillus sp*. can enhance the production of *Crassostrea* (oyster) and *Argopecten* (scallop) species. This study identified whether a readily available probiotic comprised of *Bacillus sp*. would improve growth, survival, and competency for multiple commercial shellfish species in the Gulf of Mexico: the eastern oyster (*Crassostrea virginica),* bay scallop *(Argopecten irradians),* and hard clam *(Mercenaria mercenaria).*

Larval cultures closely mimicked commercial densities and techniques used in shellfish aquaculture. Treatments consisted of a control (no probiotic), high probiotic ($1\ x10^{10}\ CFUs 1\ x10^{10}\ CFUs$), and low probiotic ($5\ x10^{9}\ CFUs 5\ x10^{9}\ CFUs$) each consisting of three replicates. Seawater treated with probiotics was 'aged' for 24hr prior to larval addition. Survival counts were recorded every two days, and images were captured for growth measurements. Competent larvae were harvested, counted, and settled in replicated downweller systems. Seed were counted after a grow-out period of two weeks.

C. virginica cultures had the most benefit from probiotic additions, with an increased larval survival and growth rate in both probiotic treatment levels (P <0.05) in the fall culture and an increased survival rate (P <0.05) in the spring. *M. merceneria* and *A. irradians* cultures lacked significant increases in growth and survival during the larval phase. However, the *M. merceneria* fall culture reported a significant increase in competency and seed production in probiotic treatments compared to controls. These results suggest species-specific enhancements are shown for some bivalves when using commercial probiotics marketed for other species.



COMPARISON OF COMMERCIALLY AVAILABLE MICRODIETS WITH AN OPEN FORMULA REFERENCE DIET WHEN WEANING SERIOLA FROM LIVE FEEDS

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Seriola, like many other marine finfish, are first fed cultured live feeds and then weaned onto formulated microparticulate feeds (microdiets) during the later larval stages. Selecting appropriate microdiets for these early-stage animals is of great importance because early nutrition can affect both short-term production efficiencies (i.e. survival and larval duration) as well as the long-term growth trajectory and robustness of the fish. However, little is known about the specific nutritional requirements of *Seriola* larvae making it difficult for producers to select appropriate microdiets from those that are commercially-available for marine finfish. Moreover, feed companies do not disclose the complete formulation or nutrient composition of these diets making growth studies difficult to interpret. Open formula diets, on the other hand, are those wherein the formulation and composition are in the public domain and provide valuable research tools in animal nutrition. In addition, open formulas, or aspects of open formulas, can be directly adopted by industry if desired.

A growth trial with *Seriola dorsalis* larvae was conducted at Hubbs-SeaWorld Research Institute, wherein the growth, survival and biochemical composition of the fish were compared between those fed an open formula microdiet produced at the University of Maine and four microdiets produced by commercial feed companies. The open formula microdiet, made via microextrusion followed by marumerization (MEM), has been proposed as a reference diet (i.e. open formula reference diet; OFRD) that could be used for wide range of marine finfish species. Three of the diets produced by feed companies were commercially available (Algonorse-Trofi [EWOS], Advance [Alltech Coppens] and Otohime [Marubeni Nisshin Feed Co.]) and the fourth was a closed formula experimental diet produced by Zeigler Bros. Larvae were fed these microdiets starting on 17 days post hatch (dph) and were co-fed with *Artemia* until 34 dph after which the fish only received the microdiets; the trial ended when fish reached 52 dph. Each dietary treatment was reproduced across four replicate tanks. A repeated measures ANOVA (REML personality) indicated that larval dry weights (mg larva^{-1}), measured throughout the growth trial, were the highest for fish fed Algonorse-Trofi (EWOS) and were only matched by those fed Otohime. The open formula microdiet resulted in similar larval dry weights when compared to all microdiets evaluated in this study except Algonorse-Trofi. Larval endpoint survival was not significantly different among treatments and had a grand mean of 45%. The nutrient composition of the microdiets were measured and compared with larval whole body compositions in order to identify specific nutrients that may have contributed to the observed growth trends. Ultimately, this study serves to help producers select from currently available products and provides insight into key nutritional parameters that should be considered when formulating *Seriola*-specific microdiets. Furthermore, our results support the use of this open formula microdiet as an adequate reference diet for *Seriola* larvae.

THE USE OF COMPLEX PARTICLES FOR ORAL VACCINATION OF SABLEFISH *Anoplopoma fimbria* AGAINST ATYPICAL *Aeromonas salmonicida*

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The application of finfish vaccines in aquaculture has reduced the use of antibiotics and led to greater disease prevention in farmed fish. Traditional vaccination methods, like injection and immersion (bath) vaccines, have drawbacks that make their use less than ideal in large-scale aquaculture operations. Injectable vaccines, while highly effective, require fish to be individually handled, which is time-consuming and stressful for the animals. Furthermore, these vaccines are most effective when administered to larger fish (typically over 20 grams), limiting their utility in younger or smaller fish. Immersion vaccines, on the other hand, are less labor-intensive but have lower efficacy due to the challenges of ensuring adequate vaccine uptake. In contrast, oral vaccines have the potential to overcome several of these limitations. Oral vaccination methods allow for the administration of vaccines through feed, eliminating the need for injection or fish handling altogether. However, the development of effective oral vaccines has been slow due to challenges with 1) vaccine encapsulation, 2) ensuring fish ingest the vaccines, and 3) achieving sufficient protection of orally vaccinated fish. This project aimed to address these challenges by developing a novel oral vaccine platform that could be used in both freshwater and marine finfish aquaculture systems. Specifically, whole-cell *Aeromonas salmonicida* vaccines were encapsulated within liposome-based complex particles. The efficacy of this oral vaccine was tested through immune response and pathogen challenge trials with sablefish and trout.

Results from these trials showed that the oral vaccine was as effective as bath vaccines in providing protection against *A. salmonicida*. Furthermore, the oral vaccines also proved to be effective boosters when combined with other vaccination strategies, offering additional flexibility in managing disease prevention protocols. Despite these promising results, further research is needed to 1) evaluate this platform for use with additional pathogens and 2) optimize the oral vaccination methodologies and formulations for improved economy. However, once these challenges are overcome, this oral vaccine platform presents a potential tool for finfish aquaculture by offering a versatile and non-invasive vaccination strategy. It could significantly reduce labor costs, improve animal welfare, and further reduce reliance on antibiotics, which are key goals in sustainable aquaculture practices. In conclusion, the oral vaccination platform developed in this project holds promise for improving disease management in aquaculture, offering an alternative to immersion-based methods, and possibly transforming the way vaccines are administered in commercial fish farming.

PROGRESS AND CHALLENGES FOR RESTORATIVE AQUACULTURE IN HAWAIʻI AND THE U.S. AFFILIATED PACIFIC ISLANDS

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Restorative or regenerative aquaculture is characterized by the use of aquatic species and aquaculture practices that provide environmental benefits including bioremediation, water quality improvement, shoreline protection and provision of aquatic habitats. Moreover, as natural resources also serve as cultural resources for many Pacific Islanders, restorative aquaculture activities can provide social and cultural benefits as well. Hawaiʻi's unique circumstances and history continue to shape the development of restorative aquaculture. There are over 1,500 years of history related to the traditional Hawaiian fishponds (*loko iʻa*) and other cultural practices such as the integrated agriculture-aquaculture in the *ahupuaʻa* system and marine fish feeding. The U.S. Affiliated Pacific Islands (USAPI) also have a long history of successfully culturing native species, such as coral, sponges, pearl oysters, and bivalves that can be used for restorative purposes. Stakeholders in the region are also now taking steps to revive fisheries stock enhancement efforts.

Recent advances have been made in developing native species such as bivalves (e.g. *Dendostrea sandvichensis*, *Pinctada margaritifera*), seaweed (e.g. *Gracilaria* spp., *Codium edule*) and fish such as Flathead Grey Mullet (*Mugil cephalus*), Pacific Flagtail (*Kuhlia* spp.) and Rabbitfish (Siganids). Seaweed (*limu* in Hawaiian) occupies a special place with a long history of Hawaiians and immigrant populations using dozens of species for subsistence and medicinal purposes. A review of successful efforts will be presented and the challenges preventing further progress described. The USAPI represents a valuable test bed for aquaculture development; lessons learned can be transfered to other locations, if regulatory obstacles can be lessened.

While the advisability of using native species is widely acknowledged, the species tested so far are in different stages on the development spectrum. There is a general need to further refine production methods to optimize efficiency, and assess the environmental value for each species. Aquaculture in Hawaiʻi is still primarily limited to land-based systems or the *loko i*ʻa because of severe regulatory inhibitions making the use of open water extremely difficult, which in turn limits research and development of locally appropriate systems and practices. While the role of the *loko iʻa* in supporting fisheries and providing habitat is increasingly recognized, little economic value accrues to the organizations which manage the fishponds. Carbon or "green" credits may offer a partial solution.

The foundation for restorative aquaculture in the Pacific Islands region is actively being laid; however, further progress relies on the ability to more efficiently produce more native species, obtaining data to assess their functionality for environmental purposes, elimination of irrational policy and regulatory barriers, establishment of financial incentives and more consideration of the business proposition for the private sector to participate in this field.

INCREASING THE EFFICIENCY OF OYSTER TISSUE STORAGE FOR DNA EXTRACTION WITH AN ETHANOL-FREE METHOD

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With the increasing demand for high-throughput genotyping in breeding programs, the aquaculture industry is under pressure to find more efficient methodologies. Tissue from finfish is often preserved by placing a small fin clip onto chromatography paper and allowing it to dry. Storing tissue on chromatography paper reduces storage space and material costs compared to the typical method of storing samples in ethanol. Additionally, transportation of samples is simplified by avoiding the use of flammable liquids, and laboratory processing time is reduced because tissue can be quickly taken from chromatography paper using a tissue punch. While this method has proven successful for storing fin clips from finfish, it is unknown whether bivalve tissue stored in a similar manner will retain DNA integrity.

We compared genomic DNA isolated from oyster mantle tissue preserved in ethanol to that isolated from adductor muscle and mantle tissue preserved on chromatography paper. DNA was extracted and purified from all samples using the Kurabo QuickGene DNA tissue kit S (DT-S). The mantle samples on chromatography paper were extracted twice, once using one punch (3mm diameter) of tissue and once using two punches. The mantle samples preserved in ethanol yielded more DNA than the other treatments, but all mantle samples yielded sufficient DNA for common genotyping techniques (SNP arrays, amplicon sequencing, single target PCR) and whole genome sequencing (Table 1). Samples will be genotyped with a 60k SNP array and genotyping success for each treatment will be presented.

Table 1. Mean ± SD concentration, yield, and purity of extracted DNA from oyster tissue preserved in ethanol (EtOH) or on chromatography paper.

Storage	Tissue type	Amount of tissue used	Concentration (ng / uL)	260/280	260/230	Yield (ug)
EtOH	mantle	approx. 5mg	431 ± 386	1.8 ± 0.04	1.9 ± 0.24	21.6 ± 19.3
Paper	adductor	1 punch	18.4 ± 18.4	1.8 ± 0.09	1.3 ± 0.31	0.92 ± 0.92
Paper	mantle	1 punch	88.8 ± 32.1	1.8 ± 0.02	1.7 ± 0.19	4.44 ± 1.60
Paper	mantle	2 punch	220 ± 137	1.8 ± 0.03	1.7 ± 0.23	11.0 ± 6.86

RHIZOSPHERE SULFUR OXIDIZING COMMUNITIES DOMINATE PLANT-MICROBE INTERACTIONS IN MANGROVE ECOSYSTEMS

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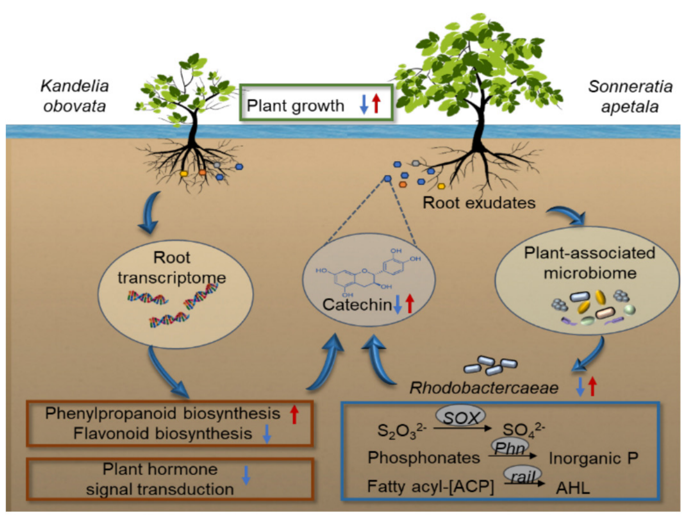
Mangrove ecosystems play critical roles in global climate change and carbon sequestration. Understanding the mangrove-microbe interaction and their mechanisms is a central question in ecology. To address this question, we investigated key microorganisms and their potential mechanisms for mangrove-microbe interactions by integrating metabolomics of root exudates, transcriptomics of root tissues, and metagenome sequencing analysis of sediment microbial communities in a pot experiment with a native mangrove species (*Kandelia obovata*) and an introduced mangrove species (*Sonneratia apetala*).

We found that mangrove root exudate profiles were correlated with rhizosphere microbiomes, showing significant ($p < 0.05$) influences on the taxonomic or functional profiles between those two mangrove species. Specifically, the concentration of flavonoid catechin exhibited the most correlations with the abundance of microbial groups and functional genes, and flavonoid biosynthesis pathways were mediated by the transcription factor MYB118 of mangrove roots. Also, sulfur-oxidizing Rhodobacteraceae was strongly correlated with catechin, which could be utilized as a carbon source, and recovered Rhodobacteraceae metagenome-assembled genomes (MAGs) showed genetic potentials for urea degradation, nitrite reduction, phosphate mineralization and regulation, and acyl-homoserine-lactone synthesis, which could promote mangrove growth and increase the plant-derived carbon by detoxifying sulfide, regulating phosphorus turnover, and mediating quorum sensing. In addition, the higher microbial necromass carbon in the carbon-rich mangrove sediment was related with sulfur oxidation, and chemoautotrophic sulfur-oxidizing Burkholderiales could be key microbial groups for transforming plant-derived carbon to microbially-derived carbon. These findings reveal a key role of sulfur oxidizing microbial communities in mediating plant-microbe interactions, providing novel insights into plant-microbe interaction mechanisms in mangrove ecosystems.



FLORFENICOL AND ERYTHROMYCIN MEDICATED FEEDS ARE SIMILARLY EFFICACIOUS IN REDUCING MORTALITY FROM *Lactococcus petauri* INFECTIONS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Piscine lactococcosis caused by *Lactococcus petauri* is one of the most serious emerging threats to wild and farmed fish in the Americas. This bacterial disease commonly presents as a peracute-acute hemorrhagic septicemia, resulting in high mortality rates and substantial losses in animal life and financial revenue. There are no commercial vaccines for piscine lactococcosis in the United States, and treatment options are limited and understudied. Florfenicol (Aquaflor®) is a broad-spectrum antibiotic approved for use in finfish aquaculture and erythromycin (Aquamycin 100®) an Investigational New Animal Drug (INAD) with activity against gram-positive bacteria. The effectiveness of these antimicrobials in controlling lactococcosis was investigated through cohabitation challenges in a susceptible host species, rainbow trout (*Oncorhynchus mykiss*).

Shedder fish were intracoelomically injected with *L. petauri* (~1.5 x 10^4 CFU) and introduced to naïve tank populations maintained at either 13 or 18°C. Medicated feed treatments were initiated after the first observed mortality. Experimental tanks received either 15 mg/kg Aquaflor® for 10 days or 100 mg/kg Aquamycin 100® for 21 days. Negative and positive control tanks received antimicrobial-free feed. Both antimicrobials were effective in halting disease progression. At 18°C, cohabitant survival was significantly higher ($p < 0.05$) in the florfenicol (100%) and erythromycin (93%) treatment tanks compared to the positive controls (60%). There were no mortalities in shedder or cohabitant fish at 13°C. Bacterial persistence in surviving fish was assessed by culture and quantitative PCR (qPCR). By qPCR, *L. petauri* DNA was detected in 21% of positive control fish, 7% of erythromycin-treated fish, and 0% of florfenicol-treated fish at 13°C, compared to 29%, 14% and 14% in the respective groups at 18°C. However, culturable *L. petauri* was only re-isolated from 14% of the untreated positive control fish at either temperature. These results indicate that early intervention with florfenicol or erythromycin can limit mortality and spread of *L. petauri*, and that lower water temperatures may reduce disease onset, improving options for managing lactococcosis in aquaculture.

FISHING FOR DISEASE DETERMINANTS IN PATHOGENIC *Lactococcus* SPECIES

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Lactococcosis is a disease of concern for aquatic animal health, affecting commercially and ecologically valuable species across freshwater and marine systems worldwide. Control of the disease is hindered by limited options for treatment and prevention, conflated by a lack of information on the three etiologic agents – *Lactococcus petauri, L. garvieae,* and *L. formosensis*. Historical misidentification has obscured the individual contributions of these closely related species to global disease, but preliminary studies indicate they have important differences in host-specific pathogenesis and immunogenicity relevant to disease management. To better understand the genetic background underlying these characteristics, we used comparative genomics to identify putative species-specific and shared virulence factors between the lactococcosis-causing bacteria (LCB). Twenty-four isolates of *L. petauri* (n=14), *L. garvieae* (n=6) and *L. formosensis* (n= 4) from aquatic and terrestrial clinical cases in the US and Canada were submitted for pangenome analysis in EDGAR 3.2. Dispensable genomes were screened for potential determinants of host virulence between the species and strains. The core genome was evaluated for shared virulence factors that could be targeted for cross-protective vaccine design. Conservation was confirmed for genes of interest in an expanded sample set by nucleotide and amino acid alignments against a custom database populated by all publicly available LCB genomes. Subcellular location and antigenicity of proteins with 100% conservation and high homology were predicted using UniProt and VaxiJen, respectively. We identified several species-specific genes of interest, and at least six well conserved virulence factors that are promising targets for further exploration.

ECONOMIC CONTRIBUTION OF THE U.S. SALMONID INDUSTRY

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The U.S. farmed salmonid sector, primarily consisting of Atlantic salmon and rainbow trout, plays a pivotal role in the national economy. This sector is crucial in terms of employment, economic output, and tax revenue. An economic contribution analysis was conducted using data from a 2016 national survey of U.S. salmonid producers updated to 2022 dollars. Input-output modeling was facilitated by the IMPLAN (MIG Inc.,) online software and database. In 2022, the sector directly employed 1,524 individuals with a labor income of $79.8 million, contributing significantly to rural economies. The total economic impact was substantial, with a direct output of $198.5 million, and a total impact of $378.8 million when considering indirect and induced effects (Table 1). The salmonid industry interacts extensively with various other sectors such as animal feed manufacturing, construction, financial institutions, grain farming, and healthcare, demonstrating broad economic linkages.

The U.S. industry faces significant competition from imported products, affecting both market prices and growth opportunities. Regulatory costs and restrictions, particularly those concerning environmental impact, significantly affect operational costs and expansion capabilities. The U.S. salmonid industry could have achieved significant growth had expansion plans not been prevented by regulatory decisions. Expansion of salmonid production by 25%, 50%, or 75% could have boosted the economic output by $100 million to nearly $300 million, potentially supporting 2,000 additional jobs. Consequently, the industry's total economic size could have reached between $500 million and nearly $700 million, with total employment ranging from just under 3,000 to nearly 4,000 jobs across the nation, depending on the extent of growth achieved. The economic contributions of the U.S. salmonid industry were estimated at the farm level and did not account for contributions from market channel businesses. Sales into recreational markets generate substantial additional impacts from expenditures by anglers that were not accounted for in this analysis.

Table 1. Economic contribution of the U.S. salmonid industry, 2022.

Type of contribution	Employment (no. of jobs)	Labor income	Value added	Economic output
Direct	1,524	$79.8 million	$132.4 million	$198.5 million
Indirect	377	$30.2 million	$46.0 million	$108.9 million
Induced	352	$23.0 million	$40.5 million	$71.3 million
Total	2,254	$133.0 million	$218.9 million	$378.8 million
Multiplier	1.48	1.67	1.65	1.91

REDUCING PH IMPROVES COPPER EFFICACY FOR HARMFUL ALGAL BLOOM AND OFF-FLAVOR MANAGEMENT

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Harmful algal blooms (HABs) severely disrupt aquatic ecosystems by degrading water quality and producing unpalatable off-flavors in aquaculture products. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) has been used for decades as an affordable and FDA-approved algaecide to manage HABs. However, growing concerns about negative environmental impacts and the diminishing efficacy of treatments over time have prompted the exploration of alternative strategies. This project evaluated the synergetic effects of low-dose $CuSO_4 \cdot 5H_2O$ treatments following pH manipulation with carbon dioxide (CO_2). The addition of CO_2 lowers pH, which is hypothesized to increase Cu toxicity to cyanobacteria. To test this, large ~1600 L mesocosms were placed in a eutrophic pond and treated with a low dose (0.33 mg/L $CuSO_4 \cdot 5H_2O$) or a micro dose (0.08 mg/L $CuSO_4 \cdot 5H_2O$), which are 20% and 5% of the standard dosing recommendations, respectively. These were tested alongside untreated controls. Half of each of the treatments also received a CO_2 addition that dropped the pH in the enclosures from 9.15 to 7.58 for the first 3 days of the experiment. The addition of CO_2 for the micro dose of $CuSO_4 \cdot 5H_2O$ resulted in 14% greater removal of cyanobacteria in the first 24 h and 20% more removal of off-flavors in the first 3 d, which was significantly more effective than treatments without CO_2. After one week, treatments with $CuSO_4 \cdot 5H_2O$ and CO_2 additions continued to significantly reduce harmful cyanobacteria (>94%) and off-flavors (>95%); however, beneficial green algae were significantly promoted (+512%) in these treatments, which was not observed in treatments without CO_2 or the controls. This approach ultimately mitigated cyanobacteria and off-flavors while preventing the unintended consequences of $CuSO_4 \cdot 5H_2O$ treatment, all while using just 5% of the copper dose that is typically recommended. These findings offer crucial insights for water resource managers on how to best optimize $CuSO_4 \cdot 5H_2O$ use for effective and sustainable HAB mitigation.

STRIPERHUB: STRIPED BASS (*Morone saxatilis*) AQUACULTURE

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StriperHub is one of the National Sea Grant Aquaculture Hubs coordinated by North Carolina Sea Grant. The StriperHub integrates diverse Sea Grant programs (IL/IN, OH, MD, NC, NY, NH), industry partners, government researchers (NOAA and USDA), policymakers, cooperative extension agents, and academic scientists to consolidate and streamline striped bass (*Morone saxatilis*) aquaculture efforts in the US through demonstration of commercial culture, economics, and marketing. By value, >80% of seafood products consumed in the US are imported, accounting for over $18 billion in the annual US trade deficit. The Sea Grant StriperHub centered in North Carolina will help to address this deficit by developing striped bass as a candidate aquaculture species and expanding hybrid striped bass (*M. saxatilis* x *M. chrysops*) aquaculture to strengthen the domestic seafood industry and boost the economies of coastal and rural communities. This collaboration will define striped bass markets and economics of production, develop education and training programs, clarify regulatory permitting and licensing procedures, and promote comprehensive outreach and visibility among likely producers and consumers of this new seafood product, which is now available in markets along the Eastern US Coast. For continued development of the Sea Grant StriperHub, we anticipate acquiring additional partners (academic, government, and private sector) as the success of striped bass aquaculture continues to expand from the East Coast to nationwide. Specific StriperHub program goals include:

1. Continue development of the Sea Grant Aquaculture Hub: A nexus to commercialize striped bass as a major aquaculture industry (The Sea Grant StriperHub);
2. Improve seed stock production, distribution, growout, and better define parameters for production economics of domestic striped bass aquaculture;
3. Continue marketing strategies, market economics, permitting clarity, and business models for domestic striped bass aquaculture; and
4. Expand communication, outreach, extension, and training to support domestic striped bass aquaculture development

Specifically, the StriperHub will continue its efforts in broodstock and seedstock production and distribution to cooperating farmers and collaborators to ensure consistent commercial striped bass production. Additionally, members are performing an evaluation of reduced frequency feeding strategies to limit feed input costs at commercial density; are synchronizing batch spawning to improve seedstock production; and expanding the number of commercial domestic striped bass producers.

JUST KEEP SWIMMING:ESTABLISHING A SUSTAINABLE FISH AQUACULTURE OPERATION AT GEORGIA AQUARIUM

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This study will present the current efforts being made in ornamental aquaculture by the Aquatic Sustainability Team at the Georgia Aquarium. It will explore the growing importance of culturing fish in the public aquarium space and the roles that public aquariums and their collaborations with academic institutions play within both the aquarium and aquaculture industries. We examine the role of the Association of Zoos and Aquariums (AZA) and the goals established by their Aquatic Collection Sustainability Committee (ACSC) for the present and future of aquaculture in accredited facilities. The presentation also highlights current aquaculture practices in public aquariums, specifically at the Georgia Aquarium, including live food cultures, broodstock management, larval rearing techniques and juvenile grow out. Current efforts being done by participating facilities will be highlighted such as the Aquarium Larval Production, Larval Culture Project and the Egg Catalog to name a few. Lastly the study concludes with proposing future directions for the industry and the role the aquariums will continue to play. This research aims to contribute to the advancement of sustainable aquaculture practices, specifically in public aquariums, to support conservation efforts and to serve as a place of continuing education for both aquarists and guests interacting with these species once they are on exhibit.

INNOVATION IN AQUACULTURE SYSTEMS: THE ROLE OF THE CIRCULAR BIOECONOMY IN SUSTAINABILITY IN ARID ZONES

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The implementation of circular bioeconomy principles in aquaculture is crucial for sustainable development, particularly in resource-limited regions such as La Guajira, Colombia. Based on recent research on intensive tilapia farming and zero-effluent circular bioeconomy approaches, as discussed by Zimmerman et al. (2023) with technologies like Biofloc, bio-RAS, and multitrophic systems, this study examines the feasibility of Recirculating Aquaculture Systems (RAS) as an innovative and sustainable solution to enhance aquaculture productivity while reducing environmental impacts.

The integration of RAS with circular bioeconomy strategies allows for water use optimization, implementation of functional feeds that boost productivity, and efficient waste management in aquaculture systems within this arid region. Through local case studies (Riohacha and Fonseca) and an analysis of bioeconomic models, the benefits of RAS were evaluated in addressing challenges such as resource scarcity, economic efficiency, and social inclusion.

The results highlight the potential of RAS to reduce environmental footprints, improve food security, and promote resilient economic growth in La Guajira. This article proposes the large-scale adoption of bioeconomy principles in aquaculture, tailored to the environmental and socioeconomic conditions of La Guajira, offering insights that can be applied to other regions in Latin America.

EXPANDING THE USE OF FARMER-RUN BIPHASIC VIBRIO TEST KITS TO MONITOR SEAFOOD SAFETY

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Oyster aquaculture is a growing industry that contributes to the production of sustainable seafood. Oysters may accumulate microbial human pathogens during filter feeding and, as they are often eaten raw, there are seafood safety concerns associated with their consumption. The current FDA-approved testing methods for these species in oysters are time consuming and/or require extensive resources to conduct, which limits the ability to investigate *Vibrio* at the farm level. To improve understanding of *Vibrio* at a finer scale, simple and inexpensive biphasic assays have been developed that produce results in approximately 24 hours (Fig. 1). These assays can be performed by oyster farmers without specialized equipment, improving knowledge of *Vibrio* abundances at specific sites and providing opportunities to test practices that reduce these potential pathogens, leading to increased safety for the consumer.

This project, funded by a two-year grant, seeks to 1) expand the use of Vp and Vv biphasic assays to oyster farms in the northern Gulf of Mexico (GoM), 2) assess the assays for specificity, ruggedness, and comparability to FDA-approved methods, and 3) develop a proficiency test for participating growers. This presentation will provide a one-year update on these objectives. To date, a laboratory has been established at one commercial GoM farm. Vp assays are rugged, performing statistically the same with different lots of reagents. Testing on mantle fluid demonstrates that Vp levels are stable with refrigeration for up to 3 days (Fig. 2), indicating it can be used for proficiency testing. Use of mantle fluid provides a real-world robust challenge that incorporates diverse natural strains and competing bacteria while avoiding "dangerous goods" shipping status required for laboratory inoculated pathogens.

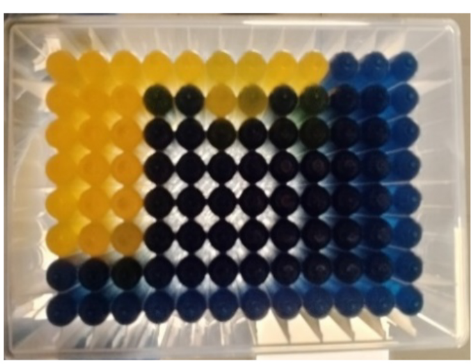

Figure 1. Results from a *Vibrio parahaemolyticus* biphasic assay. Positive samples changed the Vp media from blue to yellow.

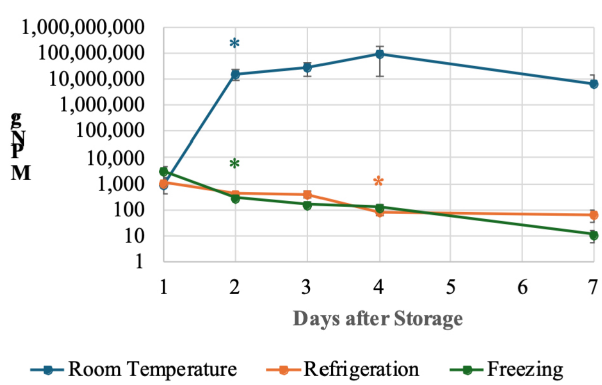

Figure 2. Change in Vp over time in mantle fluid held in different storage conditions. Asterisks indicate the first day where a significant change was observed as compared to the initial sample.

HUSBANDRY OF THE CALIFORNIA GIANT SEA CUCUMBER *Parasitichus californicus*: UPDATE FROM ALASKA

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The California Giant Sea Cucumber *Parasitichus californicus* was once a very important commercial dive fishery throughout Alaska but harvest levels have declined in recent years due to a litany of reasons including sea otter predation, conservative management practices, environmental changes manifested by slow growth rates and sporadic recruitment.

The Alutiiq Pride Marine Institute has been refining culture techniques for P. californicus as a possible tool for wild stock enhancement, species preservation and potential aquatic farming.

A major obstacle for hatchery production at APMI has been the procurement of healthy brood stock due to logistical constraints. Commercial harvesters from Southeast Alaska must dive to collect the adults often several hours from port, match airline schedules to get the broodstock to Anchorage and then transported to Seward completing a 1,200-mile journey. The shipping stress causes an up to an 80% evisceration rate and 20% mortality. The recovery and acclimation time impact their natural reproduction schedule.

Despite these challenges APMI has managed to spawn and culture juveniles for almost twenty years anticipating the eventual need for hatchery produced juveniles. Temperature, feeding strategies and culture practices are routinely modified in attempts to increase survivals.

An impediment to releasing sea cucumber for enhancement is the inability identify hatchery produced seed to monitor their growth, survival, and behavior. APMI has been experimenting with calcein dye as a marking tool. Soaking the sea cucumbers for 1 hour in a calcein dye has resulted in mark retention for up to two years in the facility.

In 2024 APMI started a collaborative project with the Canada Department of Fisheries and Oceans Bioinformatics/Molecular Genetics lab by providing juveniles for stock assessment studies who are using the methylation of rDNA as an aging tool.

In 2024 broodstock were retained at APMI into September, several months beyond the supposed reproductive cycle. Despite their loss of weight, the adults were “stress” spawned by elevating the temperature 16°C water (ambient is 12°C). Surprisingly, they quickly spawned with competent larvae indicating that timing and appearance may not be an indicator of their reproductive potential.

PRODUCTION OF XENOGENIC CATFISH BY TRANSPLANTING CULTURED BLUE CATFISH, *Ictalurus furcatus* STEM CELLS INTO CHANNEL CATFISH, *Ictalurus punctatus* TRIPLOID FRY

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Xenogenesis has been identified as a potential technique for hybrid catfish (channel catfish, *Ictalurus punctatus* ♀ × blue catfish, *I. furcatus* ♂) embryo production. This process can be accomplished by transplanting germline stem cells from a diploid donor fish into a sterile recipient. Currently, freshly extracted stem cells are used, which presents challenges as germline stem cell production depends on the donor's size, age, and seasonal cycles. Thus, culturing stem cells would solve this issue by providing a year-round supply which can significantly enhance the efficiency of the xenogenesis process. The present study aimed to compare the effectiveness of using fresh versus short-term cultured stem cells for germ cell transplantation. During the present study, triploid channel catfish fry were injected at 5 days post-hatch (DPH) with either freshly isolated or cultured blue catfish stem cells labeled with PKH26 dye. Stem cells cultured as both attached and unattached to the culture container were evaluated separately. Growth performance and survival of recipient fish were assessed at 45 and 90 DPH. Additionally, colonization of donor cells in the recipients was quantified using PKH26 dye fluorescence to calculate the percentage of cell and cluster areas. PCR and fluorescence image data were used to determine the percentage of xenogens.

At 45 and 90 DPH, no significant differences in body weight or total length of fry were observed between treatments ($P > 0.05$). However, fluorescence image analysis revealed that recipient fish injected with cultured-attached stem cells exhibited the highest percentage of cluster area ($P < 0.05$) compared to those injected with freshly extracted or cultured-unattached stem cells at both sampling periods at 45 and 90 DPH. Additionally, a significant increase in percentage of cluster area was observed from 45 to 90 DPH in both cultured-attached and freshly transplanted treatments. The percent cell area at 45 DPH was significantly higher in cultured-attached treatment ($P < 0.05$) than other treatments, while no significant difference was noted at 90 DPH between freshly transplanted and cultured-attached treatments ($P > 0.05$). PCR analyses showed that a higher proportion of xenogens (>80%) were found in recipient fish injected with cultured-attached or freshly extracted stem cells, compared to those injected with cultured-unattached cells (< 60%). Overall, the findings from the current study demonstrate that cultured-attached stem cells exhibit comparable performance to freshly extracted stem cells across all measured variables. Therefore, cultured-attached stem cells present a promising option for future germ cell transplantation to support xenogenesis, as they can be readily available under controlled culture conditions, enhancing the efficiency of germ cell transplantation for commercial-scale hybrid catfish production.

PERCEPTIONS OF MUD BLISTER WORMS IN WEST COAST ESTUARIES

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Changing ocean conditions are increasing the prevalence of Mud Blister Worms (MBWs) (Polydora spp.) in aquacultured Pacific oysters across the Pacific Northwest, including Washington, Oregon, and Northern California. The presence of these worms in oyster shells can jeopardize their marketability, making it crucial to understand the perceptions of consumers, industry professionals (such as restaurant purchasers and wholesalers), and oyster growers regarding MBWs. While previous research has shown that MBWs do not render oysters unsafe for consumption, this understanding is not widely recognized by the general public, posing marketing challenges for oyster growers amid rising MBW populations. This study analyzed surveys and interviews with individuals, professionals, and growers, offering valuable insights into the perceptions held by these three groups toward MBWs in Pacific oysters from the PNW.

SPATIAL MANAGEMENT FOR AQUACULTURE PLANNING: DEFINING NEEDS FOR ASSISTING COUNTRIES

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Coastal marine ecosystems are areas vulnerable to pollution, habitat destruction and resource depletion. Multiple human activities, including aquaculture, can further jeopardize important and often fragile ecosystem services as well as have a negative impact on biodiversity. In recent years, coastal aquaculture has become a promising economic sector in terms of its potential contribution to food security, economic development, employment and livelihood opportunities. However, the expansion of the sector needs to tackle issues concerning environmental sustainability, spatial conflicts and regulatory concerns. To address these multiple challenges and to foster the sustainable development of the sector, there is an ever increasing need to adopt integrated and forward-looking spatial planning strategies that are key for the development and management of human activities.

In recent years, the Food and Agriculture Organization (FAO) and several Regional Fisheries Management Organizations have spearheaded activities aimed at integrating aquaculture into spatial planning frameworks and how best to achieve this. While there is a wide consent in the role of the sector in contributing to global food security challenges, countries recognize the importance of adopting an ecosystem approach to the development of aquaculture so to minimize negative interactions concerning aquaculture activities, the environment and the social context. In October 2024, FAO and the Italian Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) organized an expert workshop to stimulate discussion and share lessons learnt on how spatial planning has been successfully implemented and difficulties faced in the process. The workshop resulted the development of strategic objectives and concrete actions that countries should take to advance spatial planning in offshore and coastal aquaculture for 5 key priority areas: 1) Streamlining governance and institutional frameworks; 2) knowledge support for spatial planning in aquaculture; 3) criteria and guiding elements for allocation of space, co-use of marine space, land and sea interactions and adaptation to climate change; 4) capacity building, awareness and capacity extension; and 5) marine conservation and restoration. This is now being developed into a roadmap for implementing marine spatial planning for aquaculture development as a complement to the new FAO Guidelines for Sustainable Aquaculture. This presentation will summarize this road map and FAO's resulting work to improve spatial planning for aquaculture development.

LESSONS FROM THE CREATION OF AN URBAN AQUAPONICS LABORATORY IN MEDELLÍN, COLOMBIA

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Aquaponics in urban environments is emerging as an alternative to provide food that meets protein and vegetable needs with low carbon footprint, close to the consumer and at a reasonable cost. The Universidad Pontificia Bolivariana (UPB) in Medellín, Colombia, has built a laboratory as part of the “Technologies in Urban Agriculture” research program. The laboratory is a 160 m2 greenhouse (Fig. 1) located in the middle of the city that houses several projects, with the Urban Aquaponics Laboratory (UAL) occupying the largest space. The purpose of this space is to serve both research and teaching.

UAL is divided into a laboratory for experimenting with new food sources for tilapia and a demonstration aquaponics facility with two tanks of 2000 l each and different hydroponic systems (substrate, NFT and floating rafts). The limited space available has been a challenge that has developed our creativity to make the best use of the space.

Some of the differentiating elements of the UAL are the solar panel system with energy storage for up to 4 hours, which supplies energy to what we call “essential” equipment (pumps and blowers), so that less energy is consumed and there is a backup in case of a power outage. We also developed a facility to test new foods, such as Andean oilseeds and insects, to reduce costs and dependence on imported inputs. Control systems were developed to regulate the temperature of feed tanks and turn other equipment on and off.

We have also made mistakes in design and implementation, but we have learned great lessons from them that we would like to pass on to all those who venture into the development of new projects, whether in research or business. The design and implementation of this space has required the application of knowledge in biology, hydraulics, IoT, electricity, electronics, materials and a lot of common sense. One of the characteristics of the laboratory was the use of easily available or recycled materials, so that the system can be easily replicated by the users of the different trainings that will be carried out. The sum of all this knowledge and experience will serve to support STEM education at different educational levels.

The implementation of these kind of facilities for education and research in urban aquaponics is an opportunity for knowledge of this technology to reach more people and contribute to food sovereignty and security in a more sustainable way.

Figure 1. Urban Agriculture Lab in UPB Medellín, Colombia.

AQUACULTURE IN AFRICAN – IS IT GROWING FAST ENOUGH?

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The 2022 paper coordinated by the University of Bonn and titled *Prospects for Aquaculture Development in Africa – A Review of Past Performance to Assess Future Potential* (Hinrichsen *et al.*) focused on contextualising the aquaculture output of African countries to allow for an depth of understanding beyond an absolute reported tonnage. The current work builds on this and looks at aquaculture growth imperatives in Africa to sustain the current levels of per capita fish consumption.

Africa's population is fast approaching 1.5 billion (2024), making it the second most populous continent after Asia. The average population growth rate has remained above 2.45 percent from the year 2000, and it is expected that Africa will house 2.5 billion people by 2050. This rapidly increasing population results in a rapidly increasing need for food; especially aquatic foods, and specifically foods produced from within Africa. Additionally, the youthful population of Africa needs skills and jobs, both of which can be addressed by an expanded aquatic food sector.

The 2024 yearbook of the FAO indicates that African capture fisheries yield stands at 10 358 thousand tons (FAO, 2021), with aquaculture at 2 322 thousand tons. This means that the contribution of aquaculture to fisheries supply in Africa is a mere 18,3%; in sharp contrast to the rest of the world where aquaculture supply has surpassed that of fisheries. Moreover, if ignoring the contribution of imports and exports, African per capita fish availability stands at less than 8,5 kg per annum; also in sharp contrast to the global average which is approximately 20 kg per capita per annum. As it is well known that capture fisheries, globally and in Africa, has largely reached capacity, Africa has to look to aquaculture to maintain and increase its per capita aquatic food supply.

In this paper we look critically as to whether aquaculture in Africa is growing fast enough and at the rate at which aquaculture needs to increase to (i) maintain and arrest the current declining levels of per capita fish consumption and (ii) to grow African per capita fish consumption to address the current lag behind global consumptions rates.

ESTABLISHMENT OF ELECTRICAL EUTHANASIA PARAMETERS PRIORITIZES ANIMAL WELFARE IN *Danio rerio*

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Zebrafish *(Danio rerio)* play a major role in scientific aquaculture as the second most used model organism in biomedical research. However, animal welfare surrounding the end of life demonstrates mixed efficacy and aversion behaviors across all life history stages. Both adult and embryonic zebrafish display high resistance and aversion to widely used chemical agents for the euthanasia of laboratory fishes. Electrical euthanasia has been shown to produce a 100% mortality rate and inhibit consciousness within 1 second of current application. With the proper voltage gradient and length of exposure, electricity provides an alternative and improved method of euthanasia over chemical and thermal applications. Since parameters for electro-euthanasia of zebrafish have not been established, the objectives of this study are to determine effective settings and operational procedures for pre and post-hatch embryos and adults.

Two novel electrical systems, the EFS-WASP1 and WASP3 (Electro Fishing Services Ltd. Ireland) were utilized for the euthanasia of pre and post-hatch embryos (12hrs-5 days post fertilization [dpf]) and adult zebrafish (21-40mm), respectively. Embryos (n=5 replicates per treatment, 10 embryos per replicate) were transferred into a square plate electrode exposure chamber (width 3.3cm) and exposed to continuous and pulsed direct current with a rectangular DC waveform at 50Hz with a 50% duty cycle in a water conductivity of 175 µs/cm. The optimal exposure period was initially assessed utilizing the manufacturer-recommended voltage gradient (30V/cm for pre and post-hatch embryos) during pilot testing. Experimental trials sought to validate the minimum voltage gradient and time to achieve 100% mortality. Embryos were randomly exposed to a voltage gradient of 30V/cm for 30-120sec and assessed for survival immediately following and at 5min, 30min, and 12 hours post-exposure.

Adult zebrafish (n=5 replicates per treatment, 3-5 fish per replicate) were initially exposed to manufacturer-recommended settings, 2.8V/cm in a circular electrode with voltage gradients reduced by 0.4V/cm every five consecutive trials achieving 100% mortality. All fish were assessed for indicators of recovery (operculation, coordinated movement) at times 0, 5, and 30min post-exposure. Handling controls were placed in the euthanasia tank but not exposed to current and monitored, and non-handling was left undisturbed for all tested stages.

Post-hatch embryos at 4dpf had 100% mortality utilizing a 90sec exposure to 30V/cm, while 5dpf post-hatch embryos required 120sec exposures of 25V/cm. Adults between 30-40mm experienced 100% mortality following a 90sec exposure of 2.0V/cm, while adults between 21-25mm and 25-35mm had 100% mortality following an exposure of 2.0V/cm for 150 sec. Results show electricity is an immediate acting and viable alternative to traditional euthanasia methods, which require long exposure periods, up to 20 min. Electro-euthanasia provides a more humane ending for zebrafish at all tested life stages by reducing the necessary exposure time during the euthanasia process.

THE LIMITS OF CERTIFICATIONS: WHY COMPLEX OCEAN CHALLENGES NEED HOLISTIC ECOSYSTEM CENTRIC SOLUTIONS

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Our oceans are facing unprecedented challenges. Global threats such as climate change and biodiversity loss are threatening marine ecosystems worldwide, including those essential for aquaculture and wild capture fisheries. Place-based threats, such as industrial activities within marine protected areas, further compound these impacts. Tackling these issues requires comprehensive solutions implemented through better management practices and robust governance.

To date, certifications and standards have functioned as a risk management tool primarily used by retailers to claim sustainability in their supply chains. Aquaculture certifications, which are primarily applied in the United States and European markets, can provide guidance on good practice, and recognize responsible actors where management policies are insufficient to provide that assurance. However, their farm-specific application struggles to drive the comprehensive changes required to safeguard our oceans. This panel will explore the necessity of implementing stronger policies, and how emphasizing improved regulations and governance can safeguard vital ecosystems. Pew's efforts to promote healthy oceans revolve around preserving crucial marine habitats and reforming global fisheries management, including those used for aquaculture feed. We will present key policies such as ecosystem-based fisheries management and creating essential and well-managed protected areas needed to sustain healthy and intact ecosystems, along with the current challenges we face in achieving global implementation.

Holistic ecosystem centric governance efforts are vital for the health of our oceans and the future of fisheries and aquaculture, and they are key elements of both the Sustainable Development Goals and the Global Biodiversity Framework. Currently, certifications fall short in recognizing these critical issues within their criteria, permitting aquaculture farms to function within marine protected areas and omitting ecosystem-based criteria in feed standards. It is essential to not only push certifications to include stronger ecosystem protections but to ultimately adopt comprehensive, ecosystem-centric international governance for fisheries and aquaculture. Without robust governance, there is a significant risk of continued ecosystem and biodiversity degradation, which jeopardizes aquaculture supply chains. Retailers and markets need to go beyond depending solely on certifications in their seafood policies by actively engaging with their suppliers and advocating for improved, ecosystem-based management strategies.

ASSESSING POTENTIAL CAUSES OF BIVALVE LARVAL CRASHES IN HATCHERIES ALONG THE COASTAL UNITED STATES

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The overall goal of the Bivalve Hatchery Health Consortium (BHHC) is to aid the bivalve aquaculture industry in the United States coast by assessing potential causes to hatchery run crashes along with providing suggestions for methods to manage these crashes. In recent years, there has been a major increase in larval crashes in these hatcheries, however, what is causing these crashes is not well known.

The BHHC collected incoming water (before water treatment), larval tank water, and larval samples from production runs experiencing unusual losses (unhealthy), as well as from normal (healthy) runs. Larvae from the healthy and unhealthy runs were washed in filtered sterile artificial seawater (FSSW) and incubated overnight in FSSW to allow pathogens in the larvae to shed (shed water). Incoming, tank, and shed water were size fractionated (Figure 1) to eliminate potential pathogens based on size. Healthy larvae (5-10 days post fertilization) were exposed to FSSW (negative control), the non-fractionated water (positive control), and the 0.22 micron-filtered water (eliminating bacteria and parasites, retaining viruses and toxins).

The relative percent survival (RPS) of larvae exposed to fractionated water compared to survival of larvae exposed to FSSW was calculated after 20 hours of exposure. Preliminary results showing mortality of healthy larvae exposed to 0.22 micron filtered water from some of the hatcheries indicate that crashes may be due to incoming water quality issues, toxins, or viruses (Table. 2). Additionally, as a year-round alternative to the larval assay, we are developing a less labor intensive and faster water screening assay utilizing hemocytes from adult bivalves. Results from these assays and other diagnostic tests are shared with the hatcheries along with a document of suggested issues and changes to be applied to their next hatchery run. The BHHC has been set to continue another year of sampling runs during the 2025 production season.

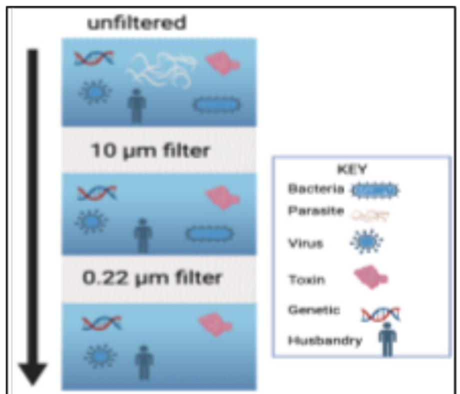

Figure 1
Potential crash causes are present at each of the filter levels we are going to be filtering to. Pathogens are not to scale.

Table 2
This table is showing the RPS to a control treatment of 28ppt filtered sterile seawater(FSSW). Hatchery codes are used to keep anonymity of the hatchery and R# signifies the run number. Standard deviation is in parentheses. Possible crash causes are also shown.

Hatchery	Incoming Water Filtered	Culture Water Filtered	Shed Water Filtered	Possible Crash Cause
Hatchery 1-R1	0 (0)	-1 (9)		genetic/bacterial
Hatchery 2-R1			-41 (9)	virus/toxin
Hatchery 2-R2	-85 (2)	-41 (3)	-27 (5)	virus/toxin
Hatchery 3-R1	2 (3)	7 (2)	2 (4)	genetic/bacterial
Hatchery 4-R1	-44 (6)	-36 (7)	12 (1)	virus/toxin
Hatchery 4-R2	-29 (6)	-32 (3)		virus/toxin
Hatchery 5-R2	-6 (3)	1 (3)	-4 (5)	genetic/bacterial
Hatchery 6-R1	-18 (6)	-9 (3)	-3 (4)	virus/toxin
Hatchery 7-R1		-33 (4)	-8 (4)	virus/toxin
Hatchery 8-R2	0 (3)	2 (5)	-3 (2)	genetic/bacterial

IMPLICATIONS OF AGE-BASED MORTALITY FOR ATLANTIC SURFCLAM *Spisula solidissima* POPULATION DYNAMICS

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The Atlantic surfclam, a biomass dominant of the benthic ecosystem of the Middle Atlantic Bight (MAB), supports a lucrative fishery on the continental shelf of the U.S. east coast. Federal stock surveys, ongoing since the 1980s, have provided extensive demographic information for Atlantic surfclams, which show regional variability in population characteristics along the MAB. Population mortality has been identified as a process contributing to the observed population variability. To investigate mortality effects a non-linear age-dependent mortality equation was constructed and fit to the observed Atlantic surfclam age frequency distributions for the entire MAB population and to populations in three sub-regions that represent the southern, middle, and northern regions of the MAB. Age-dependent mortality rates were then obtained for populations that represent the entire MAB and the three sub-regions. The resulting age-dependent mortality rates, and demographic parameters (growth rate and asymptotic length) calculated for each sub-region using the von Bertalanffy growth function, were input to a population dynamics model to assess trends in Atlantic surfclam density along the MAB.

The age frequency pattern for the three sub-regions showed a rapid decrease of survivors at young ages, a small constant decrease at mid ages, and another rapid decrease at old ages. This pattern was represented by two mortality peaks. A small mortality peak with a maximum at about an age of 5 years was obtained for each sub-region. A larger mortality peak was obtained in each sub-region, with a maximum that moved from 20 to 25 years old for all but the middle sub-region, which reached a maximum at 30 years of age. Demographic parameters calculated for the sub-regions show an increasing south-to-north gradient in asymptotic length and growth rate of 7.5% and 31.4%, respectively.

Simulations showed that the mean density of the Atlantic surfclam population reached lower equilibrium densities for age-dependent mortality relative to constant mortality. Similar results were obtained for the mean densities per size classes. Age-dependent mortality rates produced a gradual decrease of survivors throughout all size classes; whereas constant mortality rates resulted in an initial decrease of densities that remained constant until the last size class where densities dropped close to zero. Comparison of simulated and observed Atlantic surfclam densities was done using root mean square error and bias was estimated with mean percentage error. Both statistical indices showed values close to zero for simulations that used age-dependent mortality rates. These results suggest that Atlantic surfclam populations experience age-dependent mortality and inclusion of this morality is needed to accurately represent population densities and trends on the MAB continental shelf.

SEASONAL DYNAMICS OF A NOVEL OYSTER GROWING REGION

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Global change has dramatically impacted aquaculture in the US. For the Pacific oyster (*Crassostrea [Magallana] gigas*), disease prevalence and summer heatwaves result in regular mortalities on the US West Coast. High latitude Pacific oyster farms in Alaska may be more resilient to climate change due to cold water temperatures at the lower limit of the oyster's physiology. However, the environmental dynamics and productivity of this highly seasonal and glacially influenced nearshore region, and the impacts on the Pacific oyster physiology, are poorly understood. This research provides an assessment of the seasonal dynamics of oceanography, phytoplankton communities, and oyster tissue quality over three years of sampling at a Southeast Alaska oyster farm. The results of this study provide a better understanding of nearshore dynamics in the estuarine environment of Southeast Alaska's inside waters to aid in future site suitability analyses and improve our understanding of Pacific oyster exposure to stressful and beneficial conditions. This region is also prone to spatially and temporally discrete harmful algal blooms (HABs), and the data collected in this study improve our understanding of the conditions that contribute to HAB events.

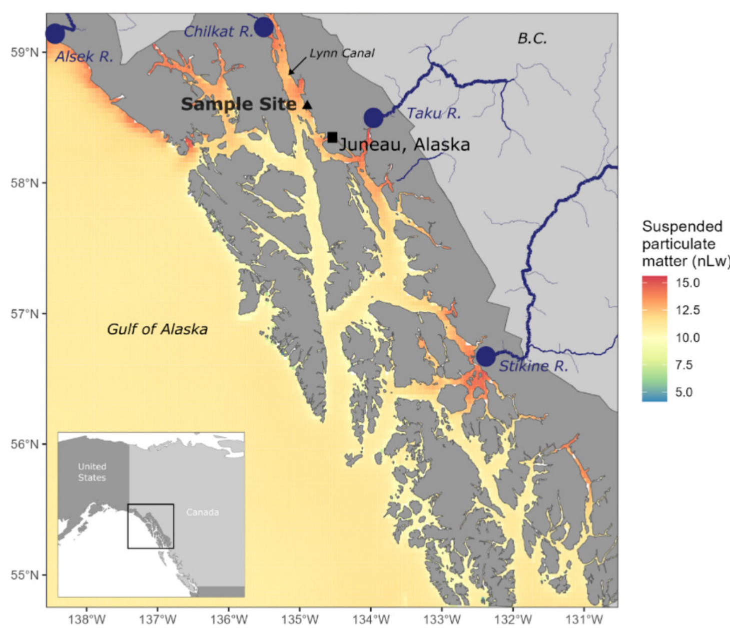

Fig 1. Map showing sampling location and suspended particulate matter concentrations in Southeast Alaska as determined by normalized water-leaving radiance (nLw 671nm). Large transboundary glacial rivers are shown in blue.

OPPORTUNITIES AND CHALLENGES FOR ALASKA KELP AQUACULTURE

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Kelp aquaculture is a nascent and growing industry in Alaska where cold, nutrient-rich, and uncontaminated waters result in high quality biomass. There is considerable support from regional and federal initiatives to expand the blue economy sector, and in Alaska much of that support is focused on kelp and shellfish aquaculture. This presentation will review the opportunities, challenges, and ongoing research in three select topics that are key to sustainable industry expansion: (1) aquaculture site suitability, (2) ecosystem interactions with aquaculture, and (3) kelp stabilizing and processing. Regarding site suitability, there are ample opportunities for aquaculture expansion in the extensive coastal zone of Alaska, however low population densities and vast distances between inhabited communities result in little infrastructure and logistical challenges for both cultivating kelp and getting it to target markets. Investigations are underway that will inform optimized farm placement from both marine spatial planning and organismal physiology perspectives. Ecosystem interactions can constitute a service or a detriment depending on the nature of the interaction, which may also vary spatially and temporally. We are conducting research to understand how species of interest are interacting with kelp aquaculture, and developing in situ strategies and permitting tools to maximize benefits while minimizing interactions seen as harmful, such as Pacific herring spawning on cultivated kelp. Finally, stabilizing cultivated kelp in a cost effective manner has proved challenging in Alaska's cold and wet climate. Research is underway to develop processing methods suitable to the climate and economies of Alaska, and local products are being developed to incorporate kelp into local manufacturing that may require less transport and stabilization. The expansion of the kelp aquaculture industry is full of exciting possibilities and formidable challenges. Knowledge exchanges and collaborations with other US and foreign regions that have mature or growing kelp aquaculture industries will likely aid in sector growth in Alaska.

ASSESSMENT OF CHROMOSOMAL INVERSIONS IN EASTERN OYSTER POPULATIONS IN THE GULF OF MEXICO

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The eastern oyster (*Crassostrea virginica*) is a vital component of coastal ecosystems and the aquaculture industry in the United States, and the use of genomic technologies have shown promise for understanding the population dynamics of wild populations, informing restoration and fisheries management decisions, and accelerating genetic improvement in oyster breeding programs. Recent population-level genome resequencing efforts have revealed the presence of several large chromosomal inversions in the eastern oyster genome, and the genetic and phenotypic impacts of these polymorphic inversions have yet to be assessed. In this presentation, we discuss current progress towards discovery and characterization of chromosomal inversions in the *C. virginica* genome, as well as the potential implications for population management and selective breeding.

A RESEARCH OYSTER FARM IN CORPUS CHRISTI BAY ASSOCIATED WITH TEXAS A&M UNIVERSITY - CORPUS CHRISTI AND TEXAS A&M AGRILIFE RESEARCH

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Texas A&M University - Corpus Christi (TAMUCC) and Texas A&M AgriLife Research (TAMAR) established a 1/3-acre research oyster farm in Corpus Christi Bay, TX in 2021 to support the nascent Texas oyster aquaculture industry and to assist in the development of a selective breeding program for Texas oysters. Located in 1.5–2.5 m water depth with sandy substrate and salinities of 30-40 PSU, the farm consists of two parallel anchored lines of floating cages. The farm site is adjacent to the TAMUCC University Beach and is easily accessible from the campus of TAMUCC.

The farm site supports several ongoing research projects and is also utilized as an evaluation site for the TAMAR/TAMUCC oyster breeding program, which is developing regional breeding populations for the northern and southern coast of Texas. The farm currently also supports education through field trips, hands-on student/internship training, and is incorporated into the curriculum of a hands-on graduate/undergraduate course (*Mariculture Techniques*) at TAMUCC.

IMPROVING RESILIENCE OF HATCHERY-REARED BLUE MUSSELS *Mytilus edulis* TO OCEAN ACIDIFICATION WITH DIET AND SEAWATER BUFFERING

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As wild blue mussel seed declines in the Gulf of Maine, mussel growers are relying increasingly on hatchery production to stock their farms. While mussel populations are currently under pressure from predation by the invasive European green crab, ocean acidification (OA) is expected to be another stressor – especially among earliest life stage mussels – as climate change progresses. Traditionally, shellfish hatcheries have implemented seawater buffering to mitigate low pH in their incoming seawater. Here, live microalgae diet optimization is proposed as an alternative strategy for rendering blue mussel larvae inherently more resilient to OA in a hatchery scenario. Following an initial microalgae growth and nutritional analysis phase, 4 diets – each consisting of a flagellate and a diatom species – were created to emphasize different nutritional parameters (industry standard, high calorie, high DHA, high EPA/protein). A first experimental trial tested larval/early juvenile response (survival and growth) to a 2-level seawater pH treatment (7.80/present day, 7.30/year 2100) and a 4-level diet treatment, and a second experimental trial tested response to the pH treatment, a reduced 2-level diet treatment, and a 2-level seawater buffering treatment (soda ash buffering, no buffering). Each trial was followed by a field deployment of experimental mussels at each of 2 mussel farms in different locations along the Maine coast (Blue Hill Bay Mussels and Bangs Island Mussels) to reanalyze mussel responses after they achieved market size. The experimental trials tested the efficacy of diet optimization for improving blue mussel resilience to OA, as well as its interaction with seawater buffering.

SEASCAPE GENOMICS OF EASTERN OYSTER *Crassostrea virginica* IN AND AROUND BEAUFORT INLET, NORTH CAROLINA

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Complex interrelationships between biological traits and environmental conditions influence the genomic diversity and population structure of marine species. Offspring of broadcast spawning organisms are subject to oceanographic, benthic, and chemical conditions that influence survival and connectivity, with the potential to produce genomic divergence between populations at closely located sites. This study aims to use restriction site-associated DNA sequencing (RADseq) to characterize fine-scale genomic differentiation between eastern oyster populations in and around Beaufort Inlet, North Carolina. RADseq libraries have been prepared from 29 oysters from each of seven beds in Bogue Sound, Back Sound, North River, and surrounding bodies of water, for coverage of 203 total individuals from a maximum of ~20 km apart. These beds were selected from a geographic area spanning the swift flowing and well trafficked Beaufort Inlet entrance, and additionally represent a range of environmental conditions, including salinities and proximities to intense anthropogenic development. The resulting sequence data will be analyzed to determine genomic differentiation between sites, putative markers of selection, and association between markers of selection and local environmental conditions. This research will help elucidate connectivity patterns of oysters in the area of study as well as provide insights on potential fine-scale genomic adaptation in benthic marine species.

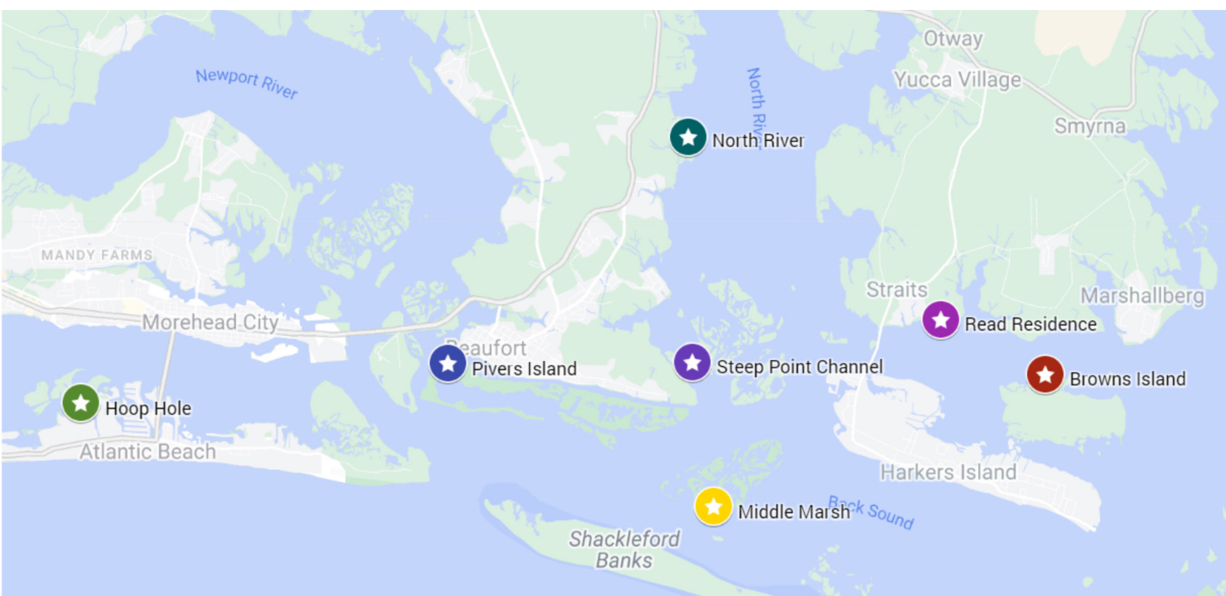

Figure 1: Map of sample locations.

BLUE MUSSELS AND SUGAR KELP: POSSIBLE CO-CULTIVATION STRATEGIES FOR A CHANGING OCEAN

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Without mitigation strategies, ocean acidification lessens U.S. shellfisheries profitability by weakening shells and byssal threads and disrupting larval development. Cultivated seaweed can raise seawater pH in the immediate vicinity of a sheltered coastal seafarm, but this remediation capacity is unclear when considering a warmer and more acidic ocean. We performed a two-factorial tank experiment with contemporary conditions (~12±2 °C, 400±100 μatm pCO_2) and projected conditions (+4°C, +600 μatm pCO_2) for the Gulf of Maine. Within these treatments, blue mussels were grown with (~150g) and without (0 g) sugar kelp, *Saccharina latissima* for 50 days (n=6 tanks). Custom 35L flow-through tanks (1.5L/min) each housed 10 juvenile mussels (~2-4 cm shell length) and ~100 spat (0.5 cm). Reported are resultant seawater carbonate chemistry conditions (e.g., total alkalinity and dissolved inorganic carbon), mussel fitness (growth, shell morphometrics, meat mass) and kelp growth (wet weight, linear extension). Shell thickness for juvenile mussels increased 1.62 to 5.84% when co-cultivated with kelp in both ambient and future conditions and meat mass increased 6.47% in future conditions. Only meat mass of spat increased 14.72% with kelp, and only in future conditions. Gut contents are currently being analyzed with recently developed qPCR primers (COI gene) to quantify gene copy numbers of *S. latissima* therein to identify mechanisms of kelp influence on mussel productivity, be they a result of phytoremediation of CO_2-driven acidification or kelp detritus feed subsidies. Kelp-mussel co-cultivation may improve mussel fitness now, and in the future, with variable impacts across mussel size classes.

A RESTORATIVE APPROACH TO WHITELEG SHRIMP *Penaeus vannamei* AQUACULTURE

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Global aquaculture production is on the rise and whiteleg shrimp (*P. vannamei*) comprises a dominant share of the global seafood trade. Indeed, shrimp is the primary seafood import in the United States, principally sourced from low latitude developing nations where traditional, extensive approaches to shrimp aquaculture have resulted in the destruction of hundreds of thousands of hectares of mangrove habitats as mangroves are cleared for shrimp ponds. In Indonesia alone, *P vannamei* production increased by over 800% in the period 2000 to 2016, while commodity production (driven by shrimp aquaculture) was responsible for 75% of mangrove loss (799,940 ha). Shrimp aquaculture has destroyed mangroves across the globe and led to the loss of their critical ecosystem services, while also stifling production as traditional shrimp ponds are prone to pathogen induced production disruptions. Inadequate regulatory and enforcement mechanisms, along with limited market incentives to modernize production practices have stagnated innovation, but Climate Smart Shrimp employs a unique methodology to restore mangrove habitat (hydrologic and seed stock restoration) and grow *P. vannamei* yield (responsible intensification), delivering benefits to livelihoods and ecosystems. This modernized approach provides a scalable model for blended production and restoration.

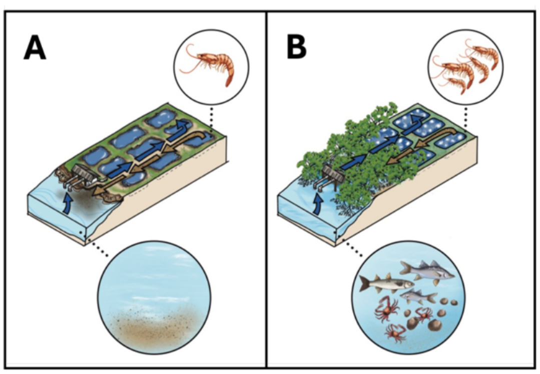

Figure 1. Traditional extensive shrimp ponds (A, left) have destroyed mangroves, but the CSS approach (B, right) can restore mangroves while responsibly intensifying production.

FROM A FULL GUT TO A FULL GONAD; USING GNRH TO EXAMINE THE LINK BETWEEN REPRODUCTIVE AND NUTRITIONAL PHYSIOLOGY IN ABALONE

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Historic over-fishing reduced white abalone(*Haliotis sorenseni*) populations, resulting in their listing as an Endangered Species in 2001. Captive breeding efforts, led by UC Davis Bodega Marine Laboratory, have increased production substantially over the past decade, but still fall short of the target 100,000 animals a year necessary for recovery. This shortfall is largely due to difficulties inducing gametogenesis and spawning. The diet of the *H. sorenseni* can impact the number of gametes they produce, and lipids have been shown to play an important role in larval development and survival in many marine invertebrates. This led us to explore the effects of a high lipid diet on abalone hormone levels by adding (seeding) a high lipid alga, *Nannochloropsis* sp., to their environment and collecting central nervous tissue (CNS) to measure the reproductive hormone, Gonadotropin-Releasing Hormone (GnRH). GnRH regulates reproductive behavior and gametogenesis. Using an ELISA assay to detect GnRH, we observed that females increased their GnRH production while males decreased their GnRH production, in response to the high-lipid diet. In addition, we saw that the high-lipid diet had beneficial impact on body length under heat stress. Examining the environmental factors that stimulate the release of GnRH can prove to be useful for increasing production, as well as aid in future studies focusing on white abalone reproduction.

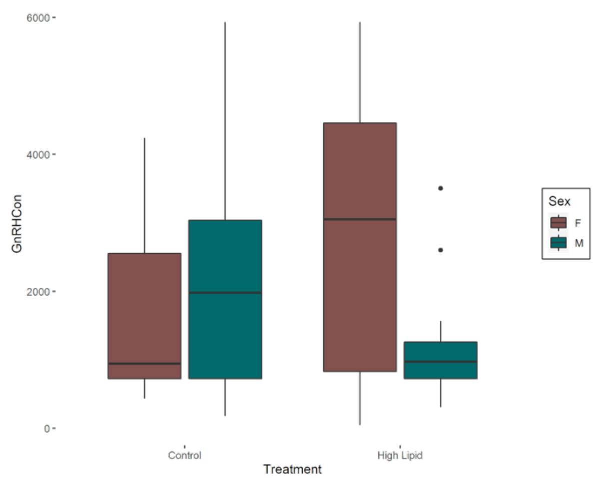

Fig 1. Sex differences in GnRH levels on a given diet: high lipid vs control. While there was no difference seen in the GnRH production between males (2219 pg/g) and females (1618 pg/g) in the control diet (P = 0.162), there was a difference seen

AN OVERVIEW OF FOOD SAFETY POLICIES IN BANGLADESH

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Despite significant advancements in Bangladesh's economy, the country is confronted with the daunting task of ensuring the supply of safe food to its residents, given its rapidly increasing population of over 160 million people. This is because food adulteration and contamination pose a serious risk to the public's health in Bangladesh. Considering the alarming issues, this paper aims to provide a comprehensive review of Bangladesh's food safety policies and regulations (including fisheries, livestock, and poultry) with gaps and deficiencies as well as proposing a pathway to address them. The study also explored the involvement of various government agencies in assuring compliance and safety in the food industry, especially in the fisheries sector.

This review found that although several food safety policies exist in Bangladesh, they are not exercised properly. Inadequate enforcement and lack of cooperation among the authorities, excess food adulteration and contamination practices, difficulties of mobile court systems, and consumers' dissatisfactions are the major issues reported in this review. Throughout the review, limitations and gaps in the food safety policy landscape are identified, emphasizing the need for resource allocation, enforcement, and greater public awareness. Establishing a robust institutional framework that encompasses the formulation of food safety policy, education of both public and commercial sectors, elimination of barriers to policy enforcement, and implementation of a consistent monitoring system will effectively address the issue of food safety policy.

MARKET SALES AND DEMAND DYNAMICS OF OYSTERS IN THE USA: EVIDENCE FROM RETAIL LEVEL SCANNER DATA

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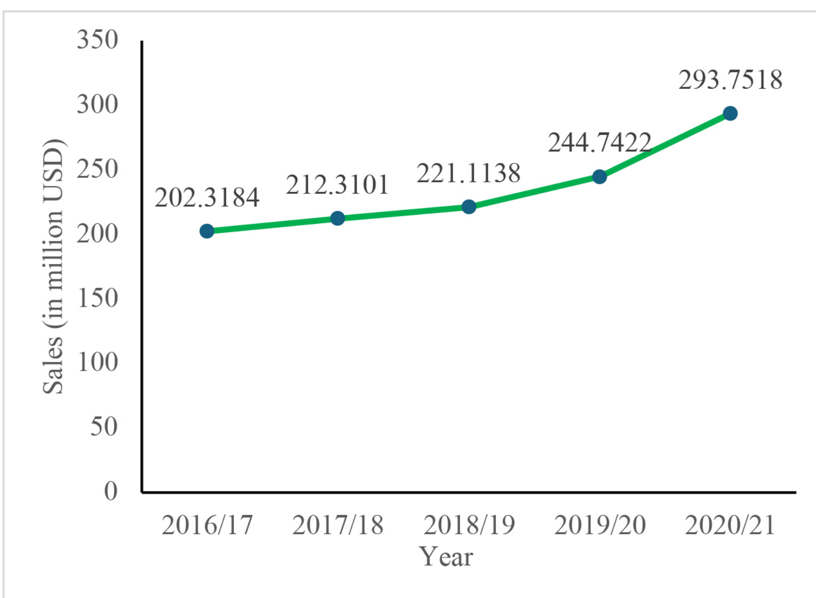
The U.S. oyster market has experienced considerable fluctuations in sales and demand, influenced by factors like seasonal availability, price variability, and customer preferences. However, there is limited understanding of the specific market dynamics at the retail level, particularly in terms of how these factors influence oyster demand over time and across regions. Thus, this study examines the market sales and demand dynamics of oysters in the U.S., utilizing comprehensive retail-level scanner data from 2016 to 2021. This research estimated that the total sales value of oysters has been rising annually, peaking at roughly $293.75 million in 2020-21. We have identified seasonal changes in oyster sales, with peak sales occurring in November and December of a particular year. The analysis revealed that the sales of non-value-added oysters and whole oysters surpassed those of other categories and forms. Moreover, frozen and refrigerated products constituted the highest category for total oyster sales, succeeded by shelf-stable items and entrées. The sales exhibited variation across distinct geographical market locations, package sizes, and oyster types (e.g., oyster, Pacific oyster, Atlantic oyster, etc.). This study additionally measured the demand elasticities for oysters in the U.S. market. The own, cross, and expenditure elasticities of oysters varied according to different parameters. Finally, his research identified key determinants of oyster demand, including pricing trends, temporal effects, promotional effects, and regional consumption patterns. The findings elucidated consumer responsiveness to price fluctuations and the influence of the retail market, furnishing essential information for producers, retailers, and policymakers seeking to comprehend and improve the oyster market.



STUDIES ON THE PARASITIC FAUNA OF OPEN WATER FISHES IN THE NORTHEAST BANGLADESH

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The present study was aimed to know the present status of parasitic fauna and its prevalence, intensity and abundance in the northeast part of Bangladesh and to evaluate the histopathological alterations of different parts of the fish organ due to parasitic infestation. The present status of parasitic fauna was studied on 17 different fish species collected from Singari Beel, Erail Beel situated at Golapganj Upazila, Kawadighi haor situated at Sadar Upazila and Surma River from October 2020 to March 2021 (six months).

During the investigation 189 individuals of different fish species were examined. Among them 91 fish were found infested with 860 parasites from 7 groups namely Digenea, Nematode, Acanthocephala, Crustacea, Protozoa, Monogenea and Cestode. A total of sixteen different parasitic genera were identified from the hosts examined as *Myxobolus* sp., *Tetrahymena* sp., *Dactylogyrus* sp., *Djombangia* sp., *Argulus bengalensis*, *Euclinostomum multiceacum*, *E. heterostomum*, *Camallanus intestinalus*, *Procamallanus viviparous*, *Gnathostoma spinigerum*, *Camallanus pearsei*, *Pallisentis ophiocephali*, *Pallisentis nandai*, *Pallisentis goboes*, *Lytocystus indicus*, *Senga ophiocephalina*. The highest prevalence (100%) was observed in *Channa striatus* and the lowest (30.76%) was observed *in Mystus cavasius*. The highest abundance and mean intensity were observed at 27 and 43.2 percent respectively in *Mastacembelus armatus*. The lowest abundance (0.77%) was observed in *Nandus nandus* and lowest mean intensity (1.8%) in *Clarias batrachus*. Parasite infected fishes were euthanized and histological samples from different organs showed multiple pathological alterations in gills, kidney, liver and gastrointestinal tract. Moreover, for the first time in Bangladesh, *S. ophiocephalina* were identified by Polymerase Chain Reaction (PCR) and generated PCR product size around 700 bps. The obtained 28s RNA F and 28s RNA R sequence of *S. ophiocephalina* matched with 1121– 1463 bps (Identity- 100%) of the 28s RNA gene of *S. magnum*, *S. lucknowensis* and *S. visakhapatnamensis* strain 28S ribosomal RNA gene, partial sequence (GB Accession number KR780913.1, KR780891.1 and KR780890.1).

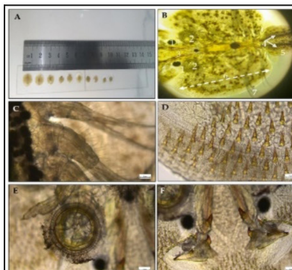

Figure-1: A. *Argulus* sp. measuring with scale. B. 1. Eye; 2. Sucking cup; 3. Antenna; 4. Abdomen; 5. Testis; 6. Thorax; 7. Thoracic appendages. C. Thoracic appendages of argulus (40X magnification). E. Sucking cup of Argulus sp. (40X). F. Extended view of antenna (40X).

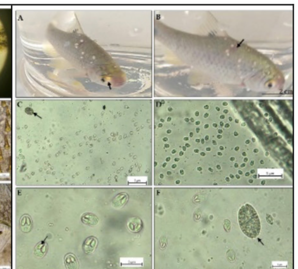

Figure 2: (A) Severe infection on skin characterized with white pox like oval spots in adult live olive barb (*P. sarana*) (B) Hemorrhagic skin (C) oval shaped and ciliated Myxobolus sp. (D) Wet mount of skin biopsy (F) *Tetrahymena* sp.

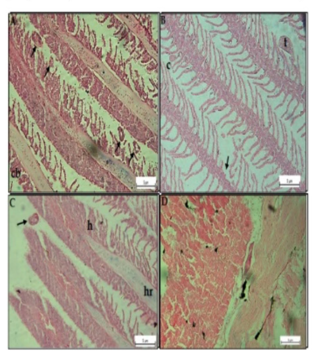

Figure 3: A. Multiple parasites gill lamellae; B. Affected gill from (*P. sarana*) showing broken secondary gill lamellae; C. gill histology presented gill filaments including hyperplasia (h) and hypertrophy; D. Skin section containing loss of epidermis, collagen fibre breakdown.

INFLUENCE OF OYSTER DENSITY AND BIOFOULING ON CHL-A CONCENTRATION AND PHYTOPLANKTON COMMUNITY IN OYSTER AQUACULTURE BAGS

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Oyster aquaculture has grown rapidly in recent years due to the declining wild populations. As the industry expands with the increased growth of the oyster aquaculture industry in the US, farmers consistently seek ways to optimize yields. Oyster farmers commonly make decisions about stocking density and control (or not) of biofouling with a focus on maximizing yield while reducing labor. These decisions can produce micro-environments that harbor conditions that differ substantially from the surrounding waters, influencing the growth and health of oysters. One key factor that can be impacted by these micro-environments is the availability of phytoplankton.

We investigated the effects of oyster stocking density and biofouling control on chlorophyll-*a* (chl-*a*) concentration and phytoplankton community composition in oyster grow-out bags at a commercial oyster farm located on the lower York River in Virginia. We hypothesized that increased oyster density and increased biofouling (resulting from a lack of air-drying measures taken to control biofouling) would decrease chl-*a* concentration and phytoplankton abundance, while creating a phytoplankton composition distinct from the surrounding water. The experimental design included three relative levels of oyster density (High, Normal, and Empty as a control) and two levels of biofouling (Fouled/Clean). Chl-*a* levels, a proxy for phytoplankton biomass, were measured across all six treatment combinations, each with three replicates. Additionally, a detailed phytoplankton abundance and community analysis was conducted for High Oyster Density/Fouled, High Oyster Density/Clean, and Empty of Oysters/Clean treatments. Water samples for chl-*a* and microscopy analysis were collected 13 times between September 2022 and June 2023.

Our results indicate that the High Oyster Density/Fouled treatment showed reduced phytoplankton abundance and chl-*a* concentration and altered phytoplankton species composition (but not in the High Oyster Density/Clean treatment). This reduction is attributed to increased phytoplankton consumption by more oysters combined with restricted water circulation due to biofouling. Biofouling control emerges as a critical strategy to ensure a consistent food supply for oysters and promote optimal growth.

APPLICATIONS OF SMALL AERIAL DRONES FOR INTERTIDAL SHELLFISH FARM MANAGEMENT AND PRODUCTION

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Advances in remote sensing technologies for precision agriculture, including small unoccupied aerial vehicles (UAV) or drones, have increased in recent years and become more cost effective and accessible for public consumers to operate. We investigated the use of a small, "off-the-shelf" aerial drone to collect high-resolution (1 cm/px) imagery of intertidal shellfish farms in Washington state to demonstrate applications for inventory development, vegetation mapping and monitoring for nuisance species. Drone flights were completed during summer low-tides (June – August) in 2023 and 2024 on shellfish farms in Willapa Bay and Puget Sound, WA. Imagery was collected with a DJI Mavic 3 multispectral drone with RGB, visible light camera (20 MP) and multispectral camera with green, red, red-edge, and near infrared sensors (5 MP). Images were post-processed and georeferenced into orthomosaics using the photogrammetry software Agisoft Metashape v.2.1.2. Raster orthomosaics were imported into ArcGIS Pro to identify benthic features of interest: bottom grown Pacific oysters (*Crassostrea gigas*), aquaculture gear (anti-predator clam nets, oyster longlines and flip bags), macroalgae (*Ulva* spp.), eelgrass (*Zostera marina*), and ghost shrimp burrows (*Neotrypaea californiensis*). Benthic features visible in the raster imagery were either heads up digitized (aquaculture gear) or classified (on-bottom Pacific oysters, eelgrass, macroalgae, shrimp burrows) using object-based image analysis (OBIA). For the process of OBIA, raster images were first segmented using groups of neighboring pixels (objects) that share similar attributes. Following image segmentation, training data was created for each benthic feature to be classified. Using the Support Vector Machine (SVM) algorithm, images were classified with inputs from the training data. Any mis-classified areas were re-classified to improve the final classified image. Classified benthic features were converted from raster to vector polygon layers and summary statistics were calculated to determine total areal cover (acres) of each feature. Shellfish biomass estimates were calculated by multiplying average planting or stocking densities for each culture type by total areal cover. Orthomosaics and classified layers can be further utilized by farms in a GIS or web map environment. Repeat drone image collections and classifications can facilitate change detection of benthic features over time at farm-scale, including the distribution of sensitive habitats (eelgrass) or nuisance species (*Ulva* spp., ghost shrimp). This study demonstrates the use of classified orthomosaics from high-resolution (1 cm/px) drone imagery as a potential tool for intertidal shellfish farm management including biomass estimates for inventory development.

USING METAGENOMIC SEQUENCING TO SURVEY MICROBIAL DIVERSITY OVER A ONE YEAR PERIOD IN A RAS-AQUAPONICS SYSTEM RAISING WALLEYE *Sander vitreus*

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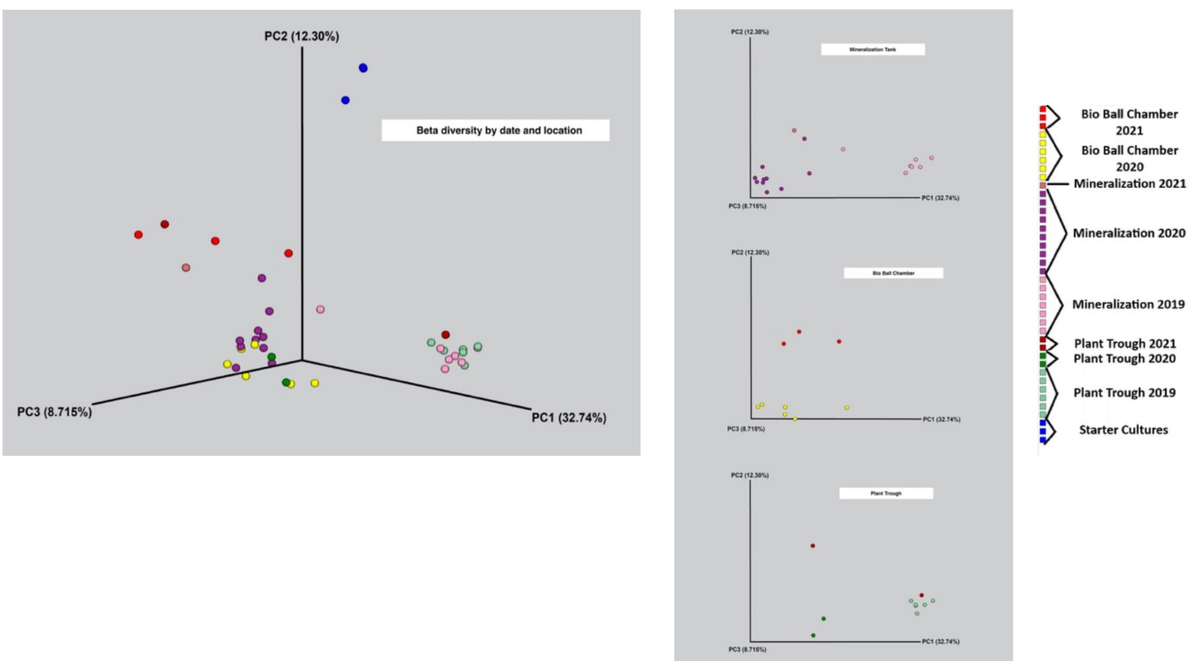
Microbial communities in various components of a RAS system raising Walleye (*Sander vitreus*) paired with an aquaponics system were surveyed using metagenomic sequencing over a one-year period. Components sampled included the mineralization tank, biofilter and plant trough. The system was seeded with a commercially available mixture of nitrifying bacteria and the nitrifying community assemblage was also followed over-time.

Results showed ammonia-oxidizing bacteria (AOB) played a major role in ammonia oxidation compared to ammonia-oxidizing archaea (AOA) while nitrite-oxidation was dominated by species of the *Nitrospira* rather than *Nitrobacter* nitrite-oxidizing bacteria (NOB).

Results showed that were significant differences in microbial abundance and diversity in and between the three components of the system. In addition, over time the community make-up also varied. The significance of these change will be of importance to culturists.



FUNDING FOR AQUACULTURE INNOVATIONS: PAST, PRESENT & FUTURE OPPORTUNITIES

Dylan Howell

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With the U.S. aiming to reduce its $20 billion seafood trade deficit and meet the rising demand for sustainable protein, aquaculture innovation has never been more essential—or more investable. Yet, funding for innovation in this space has historically been difficult due to high capital costs, long development cycles, and investor hesitation toward biological and ocean-related risks. However, with rising seafood demand, climate change pressures, and the need for sustainable protein sources, the industry is at a turning point where innovation is critical for scaling sustainable solutions. With growing federal and state support, increasing private sector interest, and breakthroughs in technology, the funding landscape is shifting rapidly.

In this talk, we will provide a high-impact overview of how aquaculture funding has evolved, where the biggest opportunities lie today, and how scientists and entrepreneurs can secure the capital needed to bring their innovations to market. With increasing public investment from U.S. agencies, alongside a surge in impact-focused venture capital, now is the time to scale solutions in aquaculture. HATCH Blue is the world's leading supporter and investor in early-stage aquaculture entrepreneurs. We play a critical role in bridging the gap between cutting-edge research and commercial success.

Over the past 7 years in supporting 500+ entrepreneurs, investing in 70+ startups and scaling their businesses, during this talk we will provide insider knowledge on how to secure funding, leverage public and private capital, and utilize our global network to accelerate innovative science, ideas and ventures.

THE SUPPLEMENTATION OF AUTOCHTHONOUS PROBIOTIC, *Lactococcus lactis* MA5, IMPROVES ACUTE HYPOXIA STRESS RESISTANCE FOR HYBRID CATFISH (*Ictalurus punctatus* x *I. furcatus*)

Jing Huang*, Heather R. Jordan, Caitlin E. Older, Matt J. Griffin, Peter J. Allen, David J. Wise, Ana Beatriz S. Farias, Penelope M. Goodman, J. Grant Reifers, and Fernando Y. Yamamoto

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Hypoxia is an ongoing challenge during aquaculture production. With the recent intensification of catfish production systems to reduce production cost, hypoxia has become a greater challenge, affecting growth performance and disease resistance. Feed supplemented with probiotics have shown stress regulating responses, growth promotion, and immune modulation in animals. In our previous study, an autochthonous lactic acid producing probiotic, *Lactococcus lactis* MA5, demonstrated beneficial effects as a dietary supplement for hybrid catfish, increasing survival after *Aeromonas hydrophila* infection. In this study, lyophilized MA5 was evaluated as a dietary supplement to ameliorate hypoxia stress in a 56-day feeding trial. In total, 450 hybrid catfish juveniles (initial weight 16.4 ± 1.6 g) were equally distributed into 18 tanks as a completely randomized design with three experimental groups (Control, 10^4 and 10^6 CFU/g of MA5, N=6) in a flow-through system. The lyophilized probiotic was top coated with 1% saturated oil on a commercial feed. After the feeding trial, a significant increase in body weight was observed for fish fed 10^6 CFU/g compared to the control group. Upregulation of the *GPx1*, *iNOS*, and downregulation of *hsp70* mRNA expression in the intestine was observed for fish fed MA5-containing diets. Digesta samples were collected from the posterior intestine for microbiota analyses after the feeding trial, and a high relative abundance of *Lactococcus* spp., *Cetobacterium* spp., and *Plesiomonas* spp. were found in all the treatment groups. Ten remaining fish per tank were subjected to an acute hypoxia challenge, to investigate the potential stress regulation of MA5. Under acute hypoxia stress, fish fed with probiotics diets displayed an increase in serum glucose and cortisol levels with higher levels of hemoglobin, total protein, and red blood cells when compared to the fish fed the control diet. In addition, the remaining fish were subjected to a bacterial challenge via exposure to *Edwardsiella ictaluri* S97-773. Significantly higher survival was observed after being fed 10^6 CFU/g of probiotics diet (10^6 CFU/g: 84%, Control: 66%) for 56 days. In conclusion, MA5 dietary supplementation can regulate the stress response when fish are exposed to an acute hypoxia stressor, with possibly benefits observed as a result of oxidation regulation. Additionally, MA5 supplementation may confer higher survival upon *E. ictaluri* challenge.

ASSESSING THE COMPLETE REPLACEMENT OF FISHMEAL WITH ANIMAL BY-PRODUCTS OR SOYBEAN MEAL IN CHANNEL CATFISH FINGERLING DIETS: IMPACTS ON PRODUCTION PERFORMANCE AND DISEASE RESISTANCE

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Catfish is one of the most important freshwater fish produced in the United States; however, the production cost of catfish is continuously increasing. To maximize production efficiency and reduce costs, affordable and sustainable alternative protein ingredients are needed. In this study, three commercial animal by-products and soybean meal were tested as protein ingredients to replace fishmeal in catfish fingerling diets. In total, 750 channel catfish juveniles (*Ictalurus punctatus*) were equally distributed in 25 aquaria, and each tank was assigned one of the five treatment groups [soybean meal (SBM), fishmeal (FM), animal protein concentrates A and B (APC-A/B), and poultry by-product meal (PBM)] (n=5, 30 fish/tank, initial weight = 15.3g). The tanks operated as a recirculating aquaculture system and treatments were distributed in a completely randomized design. Growth performance and water quality were recorded throughout the feeding trial. Fish fed SBM diets demonstrated a significantly lower growth performance and feed efficiency compared to the other groups, and increased intraperitoneal fat and viscerosomatic index compared to the APC-B treatment. Intestinal microbiota presented a higher relative abundance of *Pediococcus* and *Oscillospirales* in fish fed the PBM and APC-A diets, respectively. Interestingly, fish receiving APC-A showed the lowest lysozyme activity while fish fed PBM diets had the highest activity. A catfish bacterial pathogen, *Edwardsiella ictaluri*, was cultured to challenge the remaining fish and assess whether dietary treatments compromised their disease resistance. The PBM had a significantly higher survival after the infection when compared to SBM (PBM: 48.2%, SBM 31.8%). In conclusion, PBM shows promise as an alternative ingredient in catfish diets as it is less expensive when compared to FM, and it does not have deleterious effects but rather promotes antimicrobial activity and survivability after *E. ictaluri* infection. In comparison, fish fed SBM diets had an increase in visceral fat, reduced growth performance, feed efficiency, and increased disease susceptibility, which are undesirable responses for sustainable aquaculture production.

INVESTIGATING THE EFFECTS OF AUTOTHCHONOUUS PROBIOTIC, *Lactococcus lactis* MA5, ON EARLY LIFE STAGE MICROBIOTA, EMBRYO QUALITY, AND SIZE DISPARITY FOR HYBRID CATFISH *Ictalurus punctatus* × *I. furcatus*

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The practice of intensive farming in the catfish industry has been increasing along with the odds of disease outbreaks. In addition, hybrid catfish size disparity is problematic for the processing plants, which may lead to financial penalties to farmers. A balanced intestinal microbiota can be beneficial for disease resistance and may assist on standardized growth performance. In this study, an autochthonous probiotic, *Lactococcus lactis* MA5, was tested in early life stage hybrid catfish. The egg and intestinal microbiota, embryo quality, and body indices were investigated after rearing the fish for 92-days in a pond-like environment.

Parent fish (female: channel catfish; male: blue catfish) were obtained from the Warmwater Aquaculture Research Unit (USDA-ARS), and used to obtain the gametes. Eggs were collected and divided into a control and two probiotic groups. The first probiotic group (TRT) was introduced to MA5 during fertilization; eggs and sperm were mixed with 1.03×10^7 CFU of MA5/mL, and incubated in individual McDonald type hatching jars in a flow-through system for 48 hours (N=3). Embryos were collected for microbiota characterization and tested for egg quality-related gene expression levels. After hatching, 3,000 fry were moved to an indoor aquarium system for an intermediate nursery with 500 fish/tank (70-L). Fish were fed with a commercial feed to satiation three times a day. The second probiotic group (IM) was exposed to MA5 at this point; 6 tanks of 15-day post-hatched naïve fish were immersed with 2.1×10^7 CFU/mL of MA5 in 10-L aquarium tanks for 20 mins. Finally, catfish fry (~0.5 grams) were moved to outdoor tanks operating as a recirculating system using pond water (n=3) and reared for 92 days. The body weight, total length, and intestinal microbiota were assessed. The result shows the TRT group has less body weight and total length variation compared to the other groups. No genes were found to be significantly differentially expressed, however there was a trend of lower *hsp70* expression in the TRT group compared to the control. Relative abundances of approximately 23-39% were observed for *Lactococcus* spp. after 48 hours of fertilization in TRT embryos. Weight gain was not affected by MA5. In summary, the autochthonous probiotic MA5 may be a valuable for decreasing size disparity without affecting embryo quality, when introduced during egg fertilization.

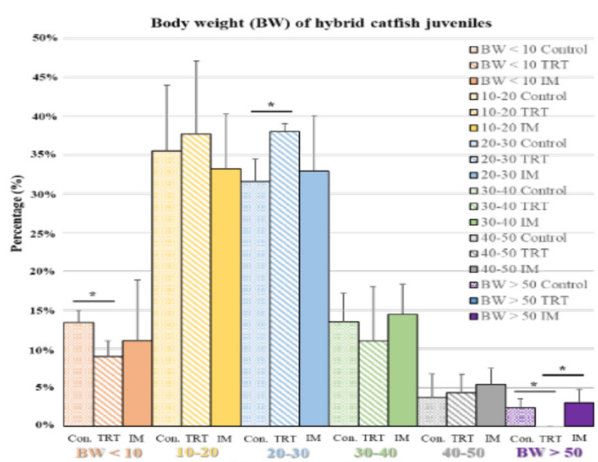

Figure 1. Body weight of the hybrid catfish after receiving probiotic MA5 during fertilization (TRT) and fry immersion (IM) compared to naïve fish (Con.). Data analyzed using pair T-test with triplication, *p<0.05.

BIVALVE HATCHERY HEALTH CONSORTIUM

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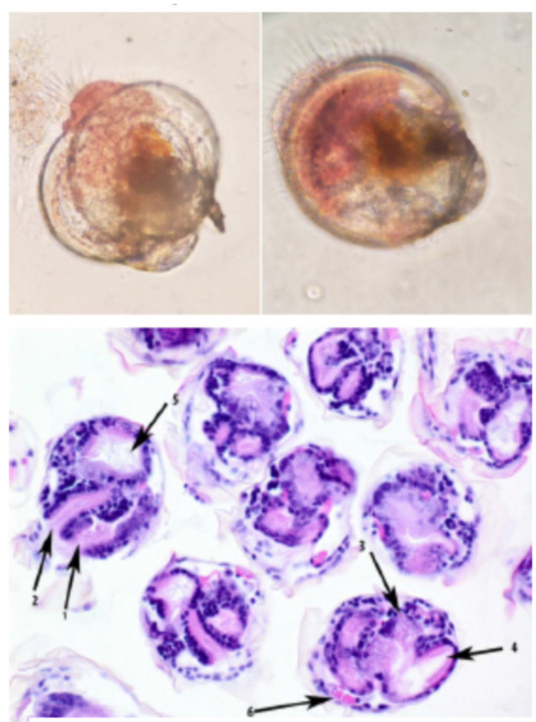
Unexplained larval crashes are a problem for shellfish aquaculture. The goal of the "Bivalve Hatchery Health Consortium (BHHC): Managing Larval Mortalities in Hatcheries" is to support the growth of the bivalve shellfish industry in the USA and beyond by: (1) identifying the causes of bivalve hatchery larval mortalities and crashes through an integrated, collaborative, and proactive approach to sample collection and analysis; and (2) developing strategies and protocols to manage and minimize larval crashes in hatcheries. Members of the BHHC include pathologists, ecologists, physiologists, analytical chemists, hatchery owners, managers, and workers, and extension specialists.

This interactive workshop will focus on discussing findings from the 2024 hatchery sampling season. The BHHC coordinating team developed a sampling kit designed to collect algae, water, and larvae from spawning to post set, including both "good" (successful performance) and "bad" (lower performance or crashes) larval runs. Samples from more than 30 production runs were received from a mix of commercial and public/research hatcheries. Hatcheries provided data relevant to larval performance indicating low performance or crashes in about 30% of the production runs sampled. Results from aggregated data (to ensure hatchery confidentiality) will be presented from microbial, histological, and toxicological assays. In this workshop, we seek recommendations for improvements to the sampling protocols for the 2025 hatchery season, and look to recruit new members, including the Gulf and West Coasts as broad participation will help in addressing this critical issue of larval crashes.



Eastern oyster larvae from larval crashes Photos by Gomez-Chiarri (top) and R. Smolowitz (bottom, histological section showing evidence of mycosis)

BEYOND THE BASICS FOR BUSINESSES AND PRACTITIONERS

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Rhode Island Sea Grant is launching “Professional Aquaculture Training: Beyond the Basics for Businesses and Practitioners,” January 2025. Aquaculture is a growing and vital part of the Rhode Island and regional maritime seascape. This course is a hybrid (live, in-person and virtual) 12-week course that aims to provide an advanced understanding of aquaculture in six main topic areas. 1) Economics of Shellfish Farming 2) Staying Healthy in Aquaculture 3) Women and Minorities in Aquaculture 4) Keeping the Peace in Aquaculture 5) Effective Communication In Aquaculture and 6) 21st Century Farming Principles, Practices and Considerations.

This course will provide participants with information so they can continue to competently and confidently improve farm businesses and operations by minimizing conflicts, and take advantage of state and federal resources. It is clear from recent, contentious lease applications that the state’s aquaculture industry will have to do more and make changes if it is to help ensure a more favorable future. This includes ensuring the use of best management practices, more effective communication strategies when engaging with the public, and participating meaningfully in research and outreach, all of which are part of this effort. There are no official prerequisites, but it is highly recommended having taken an aquaculture course available to the public. Weekly guest presenters from the experts will either be available by a pre-recorded video or during a hybrid class.

EVIDENCE SUGGESTS INSIGNIFICANT RISK OF CONTAMINATION FROM BIRDS

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The presence of birds on shellfish farms has drawn increasing attention due to perceived potential fecal contamination. We evaluate the potential risk of bacterial contamination at two surface culture oyster farms in Willapa Bay, WA, through seasonal observation of migratory and resident birds perching on the floating gear, and enumeration of fecal coliform in water within the floating culture arrays. Visual and remote camera observations (Figure 1) estimated bird presence during seasonal migrations in spring through late fall 2024. When birds were present on the surface gear, water samples were collected from at least three locations within the culture arrays. Additional reference samples were collected 100 m away from the arrays, upstream of tidal flow. Collections followed standard protocols for shellfish growing waters and were transferred to an accredited lab within 24 hours. Fecal coliform analysis was performed by Am Test Laboratories in Kirkland, WA, using standard method 9222D. Results to date demonstrate that both aquaculture sites meet shellfish water quality standards, as specified by the National Shellfish Sanitation Plan (NSSP) Guide for the Control of Molluscan Shellfish. Excluding reference samples, the median most probable number (MPN) during bird presence was 3 MPN (4 samples >43) at one farm site, and 2 MPN (never >43) at the second site. Therefore, both sites did not exceed the fecal coliform standard (median >14 MPN per 100ml, and ≤10% samples >43 MPN per 100ml) when sampling targeted periods of substantial bird presence on surface culture oyster farms. These results suggest that fecal coliform contamination from birds on surface gear is not a significant risk at these sites.

Figure 1. Floating oyster aquaculture array with perching gulls on 8/30/2024 in Willapa Bay, WA, USA. Image captured by an on-water Reolink camera situated on a pole, with remote upload.

WE ARE GRASPED BY WHAT WE CANNOT GRASP: THE HIDDEN PHYSIOLOGICAL EFFECTS OF LONG-TERM EXPOSURE TO NITRITES IN FINFISH

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Intensive aquaculture of finfish species is often associated with a variable concentration of nitrogenous compounds in water. These compounds are derived from ammonia excretion by fish after protein catabolism and posterior bacterial denitrification of ammonia into nitrite and nitrate in water. Since the accumulation of these compounds can be toxic for fish, aquaculture practices include the maintenance of low levels of ammonium and nitrite (the most toxic forms). However, due to the high cost of water renewal, long term biofiltering, sudden fluctuations in water quality, etc., fish are often exposed to low concentrations of nitrite for long periods of time. Until now, it has been assumed that exposures to low concentrations of nitrite that don't elicit an obvious stress response in fish are acceptable for fish culture. However, our research showed that long-term and low concentration nitrite treatments had a dramatic effect on sensory organs like the nose, mucosal microbiomes, brain function, oxidative stress and embryo neural development in several finfish species. Conversely, no other known nitrate/nitrite acute physiological effects were detected at the sublethal concentration tested (methemoglobinemia, loss of appetite, behavior, etc.). Thus, although no immediate effects are shown under chronic nitrite treatments, it is clear that fish are faced with morphological and physiological changes that will threaten their health in the long-term. Our research also sugestted alternative approaches to palliate the nitrite effects in aquaculture farms.

Understanding the broad physiological effects of water-borne nitrite as a toxin at specific concentrations for each fish species has a broad biological significance with impacts on neurobiology, animal behavior and aquaculture.

IMPROVING ACCESS TO AQUACULTURE CAREERS FOR UNDER-REPRESENTED GROUP

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Great deal of effort has been put forward in trying to increase the diversity of the aquaculture workforce, gains have been relatively slow. The primary attempts up to now have involved making additional positions available and/or directing hiring practices to try to influence this change. These methods have met with some success, but generally not the degree of success that was desired. This talk will focus on the fact that several areas have been ignored when trying to increase the recruiting outreach for under-represented groups. Among these areas that have been ignored are included cultural parameters including food, housing and religion) , mobility parameters, and the general concept of how to make new personnel feel welcome in an environment that is new to them. Examples will be given to exemplify how the lack of attention to these areas directly blocks the success of recruiting efforts designed to bring in groups that have not traditionally been involved in aquaculture. The presentation will also how directed verbiage in job postings and an emphasis on career upward mobility can also help to move the diversity needle forward.

APPLICATION OF SPERM STUDIES FOR ENHANCING ENDANGERED SPECIES CULTURE, CONSERVATION, AND REPRODUCTIVE SUCCESS

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Understanding sperm quality and its impact on reproductive success is essential for the aquaculture, conservation, and management of protected fish species. At the Fish Conservation and Culture Laboratory at the University of California, Davis, where all species were maintained under controlled environment, we studied the sperm in three California smelt species: the endangered delta smelt (Hypomesus transpacificus), the threatened longfin smelt (Spirinchus thaleichthys), and the introduced wakasagi (H. nipponensis), and applied the knowledge to inform the aquaculture practices and conservation management. Using advanced imaging and analysis tools, including the OpenCASA plugin and Scanning and Transmission Electron Microscopy (SEM and TEM), we examined sperm motility and ultrastructure (Figure 1). Key findings revealed that sperm quality, characterized by motility traits such as velocity and motility, was significantly affected by preservation time, salinity, recovery phases, and body size. Notably, sperm motility in delta smelt peaked within five seconds post-activation, highlighting the importance of precise collection timing. Delta smelt, longfin smelt, and wakasagi exhibited unique responses to sperm activation salinity, underscoring species-specific adaptive strategies. We also looked into the morphology of their sperm, revealing structural differences that may influence motility and fertilization outcomes. In summary, a comprehensive assessment of sperm quality across these smelt species under aquaculture conditions provides valuable knowledge for enhancing hatchery efficiency and supporting broader conservation efforts. By integrating these findings, aquaculture operations can better align with conservation objectives, ensuring the sustainable propagation of protected fish populations.

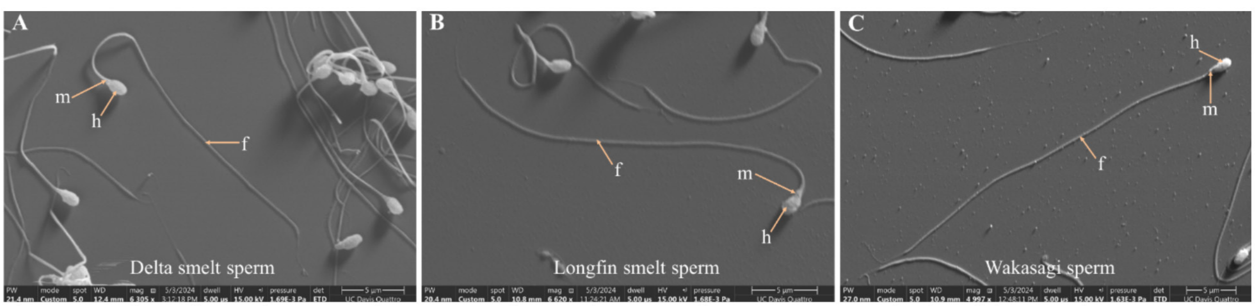

Figure 1. Scanning Electron Microscopy (SEM) images of spermatozoa of (A) delta smelt (Hypomesus transpacificus), (B) longfin smelt (Spirinchus thaleichthys), and (C) wakasagi (H. nipponensis). Figures show the overview of spermatozoa, displaying the round head and short midpiece, which is anchored between the head and the flagellum. Here, h – sperm head, m – sperm midpiece, and f – sperm flagellum. Scale bars and additional information are displayed at the bottom of each image.

UNVEILING NEW FRONTIERS FOR ASSESSING HEALTH IN FISH WITH PHYSIOLOGICAL, GUT MICROBIAL BIOMARKERS AND ALLOSTATIC LOAD INDICIES

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Aquatic stressors range from environmental, e.g., warming temperatures and hypoxia, to aquaculture-related practices, e.g., transport, handling, and crowding, and can act in synergistic, additive, or multiplicative ways. The energetic cost of exposure to fluctuations or heightened neuroendocrine response from stressful events is termed allostatic load (*AL*). Principles of allostasis and allostatic load (*AL*), the energy needed for resistance and recovery from stressors, have been widely applied to assess chronic stressors' impacts on physiological adaptation, mostly in mammals and humans. As *AL* cannot be measured directly, measurements of the effects of stressors, including adrenaline and cortisol, as well as metabolic and morphological biomarkers, have been used to construct allostatic load indices (*ALI*s). No *ALI*s exist for fish, yet health and condition assessment is critical for fisheries and aquaculture. Also, ALIs do not include biomarkers for gut microbial health. Yet the gut microbiome's composition is crucial to animal longevity and health in combating stressor effects by enhancing immune function. Case studies including, red drum (*Sciaenops ocellatus*) and Mozambique tilapia (*Oreochromis mossambicus*) investigate the impact of gut microbial health in a laboratory setting on growth, physiological condition, based on the addition of different probiotic strains, 1) as a water-soluble probiotic and 2) as a custom industry-formulated, probiotic-enhanced starter feed. ALIs, derived from biomarkers of morphology, growth, metabolic activities, and alpha and beta diversities from 16S rDNA sequencing, suggest that probiotics improved gut microbial health, increasing fish survival, growth, morphological and physiological condition indices, as well as feed conversion ratios. ALIs for other fish species are encouraged as a way to assess the impact of stressors on the health and for the future of all fish.

THE INTEGRATED CULTURE OF FISH, SEA CUCUMBERS, AND TWO-STAGE MACROALGAE IN A LAND-BASED CASCADE IMTA SYSTEM: PERFORMANCE AND WASTE REMOVAL EFFICIENCIES

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Nitrogenous compounds such as ammonia and particulate organic nitrogen are the main waste components of marine fish aquaculture effluents. These compounds are also regarded as nitrogen sources for seaweeds and solid waste feeders. In order to efficiently re-use waste containing nutrients and particulate organic matters produced in marine fish aquaculture, we developed a land-based IMTA system to co-culture white seabass (*Atractoscion nobilis*), warty sea cucumber (*Apostichopus parvimensis*), and macroalgae *D. mollis* and *U. lactuca*. The performance of co-cultured species and waste removal efficiencies of system were evaluated.

In this IMTA system, sand-filtered seawater flowed by gravity through a four tier system of 700L circular tanks. The order of tiering was: 1) *A. nobilis*, 2) *A. parvimensis*, 3) *U. lactuca*, and 4) *D. mollis* in Phase 1 of the Trial. In Phase 2, the order *D. mollis* and *U. lactuca* was reversed. This experiment was conducted from March through to June with three replicate tier groups. The initial stocking densities of *A. nobilis*, *A. parvimensis, D. mollis* and *U. lactuca* were 30 kgWW/m^3, 16 ind./tank, 4 kgWW/m^2 and 1 kgWW/m^2 with densities reset monthly for *A. nobilis* and weekly for seaweed, respectively. The temperature ranged from 13°C to 21°C. *Devaleraea mollis* tanks were shaded at 60% to decrease ectocarpus growth. The seawater exchange rate was 63vol./day.

A. nobilis grew 0.63 and 0.73%/day, with an *FCR* of 1.55 and 1.43 in Phases 1 and 2, respectively. The growth rate of *A. parvimensis* was 0.077±0.090%/day fed with the waste from *A. nobilis* tanks, and the apparent digestive ratio and removal efficiency on solid waste was 63.65% and 42.07%. The average total ammonia nitrogen (TAN) of 0.078 mg/L (peaking at 0.20 mg/L) in influents of seaweed tanks resulted in an average productivity of 20.22 gDW/m^2/d for *U. lactuca* and 14.66 gDW/m^2/d for *D. mollis* in Phase 1. In Phase 2, the productivity of *U. lactuca* increased to 25.90 gDW/m^2/d, while *D. mollis* gradually decreased to negative productivities due to increasing temperatures. The average TAN removal efficiency was 35.88% and 32.51%, and 54.51% and 21.17% by *U. lactuca* and *D. mollis* in Phase 1 and Phase 2, respectively. The pH in the seaweed tank effluents was significantly greater than the influents. Based on the nitrogen balance, the largest proportion of nitrogen was the yield of *A. nobilis* (34.94%), followed by the excretion yield of *A. nobilis* (17.39%), while seaweed production and leftover waste contributed 9.73% and 9.29% to the total input of nitrogen, respectively. 25.15% of nitrogen was not directly measured (i.e. "black box" nitrogen) including N_2 production, trapped in plumbing, assimilation by other autotrophs, and lost with the effluent discharge. Based on these results, 2.36 additional tanks for both *U. lactuca* and *D. mollis* (5 tanks total), to assimilate all TAN excreted by *A. nobilis*, and additional 1.4 times the biomass of *A. parvimensis* are needed to digest leftover waste. This study showed that *A. nobilis* can supply nutrient-enriched effluents to co-cultivated *A. parvimensis* and seaweeds to increase system efficiencies and diversify seafood production. Future trials need to be conducted to refine the operation of this IMTA.

SPAWNING, AND OPTIMIZING TEMPERATURE AND SETTLEMENT INDUCERS IN LARVAL REARING OF THE WARTY SEA CUCUMBER, *Apostichopus parvimensis*

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The high commercial value and the increasing demand for sea cucumbers has resulted in over-exploitation, and is likely negatively impacting vulnerable benthic ecosystems. Overfishing is catalyzing the need to develop aquaculture technologies for sea cucumbers to meet the market demand and to create new economic opportunities. Warty sea cucumber (WSC) *Apostichopus parvimensis* is one commercial species along the Pacific coast of the U.S. Wild populations of WSC have reached levels of concern due to overfishing, and it is now listed on the International Union for the Conservation of Nature Red List of Threatened Species. Thus, there is an urgent need to develop WSC aquaculture to take pressure off wild resources and offer the capability of restoring the depleted stocks through out-planting of cultured animals, while simultaneously supplying data to help improve fishery management.

In this study, we present the results on artificial breeding of WSC, describing the spawning induction, larval development, optimizing temperature and settlement inducers in larval rearing, aimed at developing rearing protocols for this species. Four methods to induce spawning in WSCs were tested - neuronal peptide (NGLWYamide) injection, thermal shock, algal/diet stimulation and mechanical shock. Peptide injection proved to be the most effective method for obtaining healthy WSC gametes. To test the upper thermal tolerance of WSC larvae, survival and development were measured under four temperature treatments of 22, 24, 26 and 28 °C to the pentactula stage. The upper limit for WSC larvae was 24 °C (Fig 1). At 26 °C and beyond, the larvae stopped developing and shrank. Settlement of larvae at the Doliolaria stage was induced by exposing them separately to several kinds of food inducers (Fig 2). Larval WSC exhibited similar settlement rates using attached *Navicula* sp., attached wild diatoms, *Spirulina* powder painting, and AlgaMac 3050, AlgaMac Protein Plus. The results of this study will help establish hatchery production protocols for WSC and aid in the further development of the aquaculture of this species.

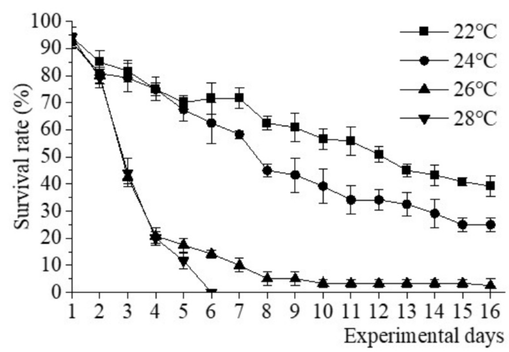

Figure 1. Survival rate of *A. parvimensis* larvae cultured at temperatures of 22°C, 24°C, 26 °C and

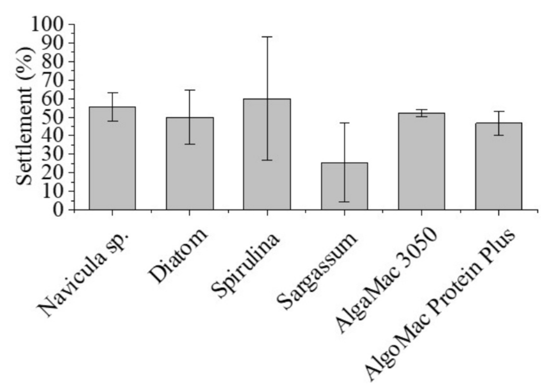

Figure 2. Settlement of *A. parvimensis* larvae in different cues.

CURRENT STATUS OF THE PROPAGATION OF BASIL IN AQUAPONIC SYSTEMS – A LITERATURE REVIEW

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Basil (*Ocimum sp.*) is a commercially important herb that has been coupled with several aquatic organisms in aquaponic systems. The goal of this literature review was to aggregate the current knowledge on the status of basil production in aquaponic systems, by identifying the different aquatic organisms cultured with basil, hydroponic subsystems, cultured basil species/ varieties, and address research gaps. Of the one hundred reviewed publications, about 90% of the reviewed publications used sweet basil (*Ocimum basilicum* L.) and only three publications used a different basil species; holy basil (*Ocimum tenuiflorum*), lime basil, (*Ocimum Americanum*), or lemon basil, (*Ocimum africanum*). Of the publications that used sweet basil, Genovese was the most commonly used variety (21 articles), followed by Italian large leaf (6 articles) and purple ruffles (4 articles) varieties. Between the reviewed publications, 38.7% used deep water culture (DWC), 31.1% used media bed (MB), and 17.9% used nutrient film technique (NFT) to grow basil. Tilapia was the most commonly cultured species with basil in aquaponic systems (44%), followed by catfish (14%) and carp (9%; Figure 1). The amount of feed for tilapia *Oreochromis* sp. cultured per square meter of grow space per day ranged between 20.3 and 81.6g. Values ranged between 29.2-68.9g of fish feed/m^2 grow space/day while culturing *Pangasius* sp., 20-25 g of fish feed/m^2 of grow area/day while culturing catfish (*Ictalurus* sp.), and 4.4-16.9 g of fish feed/m^2 of grow area/day while culturing carp (*Cyprinus* sp.). The variability in the fish feed-to-plant ratio suggests a need for establishing a recommendation for a species-specific optimal feed input that supplies sufficient nutrients for basil. The current review suggests further investigations comparing different basil varieties, cultivating different basil cultivars with different fish or crustacean species, using alternative hydroponic types, and evaluating feed inputs for establishing a recommendation for species-specific optimal conditions in aquaponic systems.

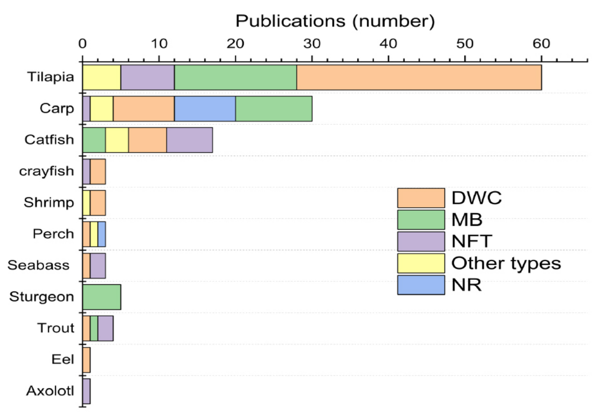

Figure 1: The aquatic organisms cultured with basil via different hydroponic components (Total peer-reviewed publications number: 93) regardless of the category (human consumption, ornamental, or conservational purposes). Some articles used multiple fish and/ or hydroponic component types.

EVALUATION OF CALCIUM BENTONITE CLAY ON NUTRIENT DIGESTIBILITY OF TILAPIA (*Oreochromis niloticus*)

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Clays have been used for centuries as 'ancient medicine' for their therapeutic benefits. One specific clay, calcium montmorillonite, has historically been used not only as an anti-caking agent in animal feeds but also has been verified to possess the ability to bind toxins and alleviate infectious diarrhea. The present study evaluated the effects of a calcium bentonite clay (4TX) supplement on tilapia's nutrient digestibility. An indirect method was utilized for the determination of digestibility coefficients with yttrium oxide (Y_2O_3) included at 0.1% as the non-digestible marker. Three diets containing either 0% 4TX (T_1, Basal), 0.5% 4TX (T_2), or 1.0% 4TX (T_3) were tested with each diet fed to Nile tilapia (120 ± 4.7 g/ fish, mean ± SE) in triplicate aquaria connected as a closed recirculating system. Water quality parameters (dissolved oxygen, ammonia, pH, alkalinity, and nitrite) were measured daily to ensure optimum environmental conditions. Fecal samples were collected 4 h post-feeding every day for 21 days, pooled per tank, dried at 60°C overnight, and stored at -18°C before analysis. Results showed non-significant (P 0.05) Differences in apparent dry matter digestibility (ADMD), apparent crude protein digestibility (ACPD), or apparent crude lipid digestibility (ACLD) are due to the 4TX inclusion level in the diet. The apparent digestibility of indispensable amino acids also was found statistically non-significant in all three diets. Orthogonal polynomial contrast and regression analysis of ADMD, ACPD, ACLD, and amino acid availability showed non-significant differences among all three experimental diets. Overall, the addition of 4TX to the diet to potentially reduce the adverse effects of mycotoxins did not appreciably affect the digestibility of various nutrients by tilapia.

ROOSTING BIRDS POSE CHALLENGES FOR SOME MASSACHUSETTS OYSTER GROWERS, CAN AN EYE SAFE LASER DETERRENT MITIGATE RISK?

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The use of floating gear to culture oysters, *Crassostrea virginica*, has grown over the last several decades as both the industry and technology has advanced. While floating gear is not permitted in all areas or for all farms, some oyster farms and even some municipal shellfish programs use floating gear extensively due to advantages in survival and growth. Floating gear does have its draw backs, including the attractiveness to local bird populations that utilize the gear as a roosting space, including species of terns, gulls, and cormorants. This issue came to a head in 2024 when *Campylobacter jejuni* illnesses were reported from commercially consumed oysters and birds were implicated through trace back to a growing area utilizing floating gear.

A large array of bird deterrents have been attempted by Massachusetts oyster growers and their effectiveness was discussed at a roundtable type meeting of growers. No universally effective options are apparent but an approach without significant gear modification was desired. One method, the laser scarecrow developed by the University of Rhode Island, has shown effectiveness in deployments at cornfields and orchards. Trial deployments are underway on shellfish farms in fall of 2024 and will be discussed in the context of the challenges of the marine environment, and farms in a public space.

REAL-TIME WATER QUALITY MONITORING IN SHELLFISH GROWING AREAS, MAKING IT ACCESSIBLE

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Cape Cod and South-Eastern Massachusetts is home to a thriving shellfish aquaculture industry. These aquaculture farmers, as well as regulators and wild harvesters, rely on water quality conditions to operate, and accurate data can help to properly operate their business. Changes in water temperature, chlorophyll concentration or dissolved oxygen are important as farmers make decisions to manage their farms and harvest the product. Having access to real time information specific to the growing area, as well as long term data is valuable for both farmers and regulators to track seasonal trends as well as changes over time. Cape Cod Cooperative Extension has been maintaining long term monitoring at sites across the region for over 20 years at some locations, but has lacked an ability to provide data in real time at most of these sites.

To make the data available in near real-time, water quality instruments need to be connected to telemetry devices. This equipment can often be cost prohibitive and technically challenging at sites that are intertidal in nature. Working with some lower cost telemetry equipment (In-Situ Inc.) and a software engineer (Interactive Oceanographics) for web hosting, Cape Cod Cooperative Extension has been able to provide a more cost-effective option to bringing telemetry to all six locations. These devices work in conjunction with water quality sondes and currently upload to a custom public website designed. The website updates every few hours and allows any interested party to view real time data, graph recent data or download raw data. Archived and quality-controlled data is always freely available upon request.

BIOFLOC TECHNIQUE WITHIN RECIRCULATING AQUACULTURE SYSTEMS FACILITATES ACHIEVEMENT OF PROSPEROUS NITRIFICATION CYCLE AND SUBSEQUENTLY FOSTERS DIVERSITY IN FRESHWATER MICROBIOME AFTER ADJUSTMENT PERIOD

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In recirculating aquaculture systems (RAS), a significant challenge involves managing the biological cycling of nitrogenous waste. The biofloc technique (BFT) represents a recent advancement technology, enabling high-density culture with limited or zero water exchange. However, there is still a lack of information concerning the microbial diversity derived from BFT, particularly in relation to nitrogen cycling. We hypothesized that in a RAS, the nitrification cycle would become fully functional after a distinct adjustment period following BFT treatment, and that the microbiome structure during this period would differ significantly from that during subsequent stabilization periods. To investigate this, we observed the dynamics of freshwater microbiome and water properties subsequent to BFT and ammonium chloride treatment during the pre-treatment maturation phase for Japanese eel cultivation using 16S rRNA gene amplicon sequencing and nitrifier-specific qPCR. Our research demonstrated that water properties and freshwater microbiome underwent dynamic alterations over time. Through random forest analysis, we identified that nitrate, final product of the nitrification cycle contributes most significantly to microbiome diversity. Building on this, we elucidated the relationships between nitrogen compounds, nitrifier abundance, and microbiome diversity in the nitrification cycle using structural equation modeling. Furthermore, we observed a sharp increase in nitrifier abundance starting from three weeks after BFT treatment, and we determined that several microbiome structure indices significantly differ between the adjustment period and stabilization period. Our study provides a detailed and multifaceted understanding of the changes induced by BFT in RAS, highlighting its potential application in promoting water quality through nitrification and subsequent microbiome alterations.

CSARP: FIVE YEARS OF COLLABORATIVE EFFORTS TO REDUCE ANTIBIOTIC USE IN CHILEAN SALMON AQUACULTURE

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The Chilean salmon farming industry utilizes more antibiotics than any other salmon-producing country, including Norway, Canada, Scotland, and the Faroe Islands. This high antibiotic use is primarily driven by the need to combat the endemic bacteria Piscirickettsia salmonis. This bacterium causes significant mortality in farmed salmon, particularly during the marine phase of production, and currently lacks effective vaccines. As the leading cause of antibiotic treatment in Chile, P. salmonis has prompted the industry and government to actively seek solutions to reduce antibiotic reliance and mitigate associated risks.

While Chile boasts one of the highest rates of environmental certification in the salmon farming industry, antibiotic use remains a significant challenge. Addressing this issue effectively requires a concerted and collaborative effort across the sector

In 2019, the Chilean salmon farming industry and the Monterey Bay Aquarium Seafood Watch launched the Chilean Salmon Antibiotic Reduction Program (CSARP), with the goal of reducing antibiotic use in that country's industry by 50% by 2025.

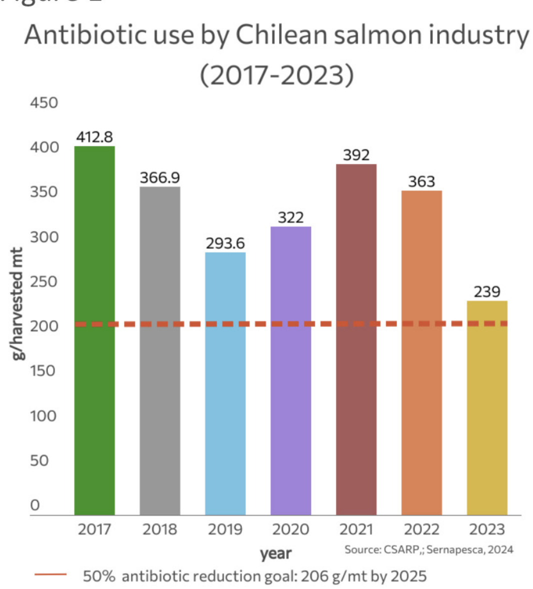
CSARP collect data from 17 Chilean salmon farming companies, representing over 90% of national production. The dataset includes over 6 million tons of harvested salmon from 2017 to 2023.

Antibiotic use in Chilean salmon farming fluctuated between 2020 and 2023. Notably, since the inception of the CSARP partnership, overall antibiotic use has decreased by 42%. (fig 1)

The significant progress in reducing antibiotic use demonstrated by some companies highlights the effectiveness of the CSARP model in promoting sustainable aquaculture. To recognize these achievements and encourage broader industry participation, the Aquarium and its partners launched CSARP+, an initiative designed to acknowledge and incentivize companies actively implementing strategies to minimize antibiotic consumption.

Reducing the use of antibiotics is a complex challenge that requires industry action and collaboration with government, academia, and nongovernmental organizations.

Figure 1



PRODUCTION AND SANITARY STRATEGIES AS KEY REDUCTION DRIVERS IN MORTALITY AND ANTIBIOTIC USE IN CHILEAN SALMON FARMING

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Chile holds the position as the second largest producer of the renowned Atlantic salmon (*Salmo salar*) globally, with a production output reaching 758,000 metric tons in 2022. Like most intensive animal food production systems, salmon farming utilizes antibiotics to manage disease outbreaks. While a downward trend in antibiotic use has been observed in recent years, 389.8 grams of antimicrobials per harvested ton were administered in Chilean salmon production last year. Notably, *Piscirickettsia salmonis*, a bacterium endemic to Chile, is the causative agent of a disease responsible of mortality and for over 90% of antimicrobial treatments in Chilean salmon aquaculture.

This study evaluates the association between production strategies, farming variables, and mortality due to SRS (*Piscirickettsia salmonis*) and antibiotic use in Atlantic salmon harvested in regions X and XI of Chile between January 2021 and June 2023. Health, production, and environmental variables were analysed at the cage level. The results indicate that production and health strategies best explain SRS mortality and antibiotic use.

Factors associated with lower SRS mortality and antibiotic use include shorter growing cycles, higher growth rates, specific genetics, use of functional diets, and a longer period before the first SRS outbreak. The analysis also revealed an association between the use of chitin inhibitors as an antiparasitic strategy and lower SRS mortality and antibiotic use.

Generalized linear mixed models (GLM) identified factors influencing SRS incidence, including time to first outbreak, mortality from sea lions and low oxygen, spring harvests, stocking in 2021 and 2022, use specific diet and breeding programs (fig 1).

Antibiotic use was associated with factors such as time to first outbreak, total mortality, temperatures above 15°C, stocking in specific years, use of oral antiparasitic treatments, and use of specific diet.

Finally, model predictions for antibiotic use were evaluated in relation to SRS mortality and three control strategies: genetics, functional diets, and vaccination. The results suggest that genetics (QTL-SRS) and specific diet are associated with lower antibiotic use.

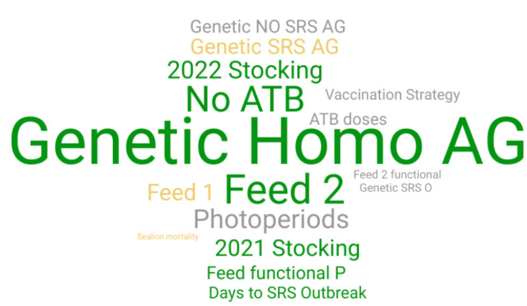

Fig 1, Word Cloud of Generalized linear mixed models (GLM) identified factors influencing SRS incidence.

DIAGNOSTIC OF TOXIGENIC *Vibrio* Spp. IN WHITE SHRIMP *Litopenaeus vannamei* POST-LARVAE COLLECTED FROM TRANSPORT TANKS PRIOR TO STOCKING IN SHRIMP FARMS LOCATED IR SONORA, MEXICO

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Shrimp farming is an extremely important sector in food production worldwide, and it is considered that the biggest problem it faces are disease outbreaks. Various infections are attributed to the genus *Vibrio*, which cause significant mortalities (up to 100%) from the early days of shrimp culture (SENASICA, 2018). Within this bacterial genus, exists highly toxigenic strains capable of producing a binary toxin PirAB, which primarily affects the shrimp hepatopancreas. (Cuéllar-Anjel, 2013). The objective of the present study is to determine the presence of toxigenic *Vibrio parahaemolyticus* in white shrimp post-larvae collected from transport tanks.

Post-larvae samples were collected from 26 containers, 12 from Sonora (Hatcheries LA, LC and LE), and 14 from Sinaloa (Hatcheries LB, LD and LF). *Vibrio* spp. strains were isolated and purified from a homogenate of postlarvae in 2% NaCl solution using specific media. The molecular diagnostic of purified strains was carried out with real-time PCR (IQ REAL AH; and molecular identification of strains by partial amplification and sequencing of the 16S rRNA.

A total of 21 colonies of presumptive *V. parahaemolyticus* were obtained, however, by PCR only five strains were confirmed (toxin gen carriers). In addition, the molecular identification revealed a high identity of the species *V. parahaemolyticus* (1 strain) and *V. campbellii* (3 strains).

The shrimp post-larvae analyzed, despite not showing apparent signs of infection, were carriers of the toxigenic agent *Vibrio* spp. Additionally, the molecular identification shows high affinity with *V. parahaemolyticus* and *V. campbellii*. It is recommended to reinforce the surveillance and health analysis of shrimp broodstock and larvae production in hatcheries.

Table 1. Identity of bacterial strains using the BLAST Algorithm (NCBI)

Hatchery	Strain ID	Sequence length (bp)	Bacterial identity	Identity (%)
LA	C3	1412	*Vibrio campbelli*	99
	C6	1421	*Vibrio parahaemolyticus*	100
	C15	1428	*Vibrio campbelli*	99
L B	C2	1428	*Vibrio campbelli*	99

1. Cuéllar-Anjel J. (2013) Síndrome de mortalidad temprana (EMS) Enfermedad de la necrosis aguda del hepatopáncreas (AHPND). The center for food security & public health.
2. SENASICA (2018). ACUERDO mediante el cual se dan a conocer en los Estados Unidos Mexicanos las enfermedades y plagas exóticas y endémicas de notificación obligatoria de los animales terrestres y acuático.

PRELIMINARY DATA ASSESSMENT OF SEMIAUTOMATIC AQUACULTURE LARVAL REARING SYSTEM FOR RED DRUM INTENSIVE JUVENILE PRODUCTION

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The marine fish production by aquaculture exceeded wild catch. However, marine fish aquaculture remains undeveloped in the United States despite high consumer demand for marine fish. On the other hand, it is predicted that wild fish populations will be overexploited, so the production necessary to satisfy the demand for fish derived from the increase in the world population will have to be provided by aquaculture. However, the limiting factor for marine aquaculture is the availability of juveniles to stock grow out facilities. To supply juveniles, we develop innovative semiautomatic intensive larval rearing technique to reduce space, labor and the final production cost per juvenile.

Red Drum (*Sciaenops ocellatus*) fertilized eggs were collected from natural spawn obtained from Whitney Lab broodstock. We follow the embryo develop and vitelline reserve before the larval rearing start. The egg incubation was done in a two 1.8 m^3 tanks, maintaining a density of 100 larvae L^{-1} in an flow through system. The rotifer were cultured using three 250 L tanks, maintaining densities of 800-1500 rotifers mL^{-1}, to produce up to 100 million rotifers daily, sufficient to sustain optimal larval feeding densities of 15-20 rotifers mL^{-1}. A single refrigerated unit maintains microalgae (Rotifer Diet™) at 4°C, continuously pumping this high-density feed to the rotifer tanks. After that, the rotifers were transferred directly from culture tanks to larval rearing tanks using peristaltic pumps. An average of 30 million of rotifers were moved to enrichment tanks (three units, each 75.7 L) to enhance their nutrient content. The *Artemia* was produced in three 132 L tanks, generating up to 35 million nauplii daily.

This system has demonstrated preliminary successful production runs, including 75,000, 95,000 and 130,000 pre-metamorphosed juveniles harvested after 23 days of hatch in each 1800, liters larval rearing culture tank. The temperature varied from 27 to 21 Celsius, oxygen average was 6 mg/L and pH of 7.9. The automation between tanks optimizes production efficiency. This new technology development increases larval survival and directly could benefits on-farm profitability as well as helps in sportfish and conservation efforts to reduce pressure on the natural stocks. This compact semiautomatic system provides a viable and sustainable pathway for future larval rearing aquaculture.

COMPARATIVE GROWTH ASSESSMENT OF AFRICAN CATFISH (*Clarias gariepinus)* FED SWEET POTATO (*Ipomea batata*) LEAF MEAL AND RICE BRAN AS ALTERNATIVE FEED MEAL

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The study compared growth performance of *Clarias gariepinus* fingerlings fed sweet potato leaf meal and rice bran as alternative feed meal. Five diets, that contain 0%, 25%, 50%, 75% and 100% inclusion levels were formulated and designated as SPM_1 SPM_2 SPM_3 SPM_4 SPM_5 and RBM_1 RBM_2 RBM_3 RBM_4 RBM_5. Highest initial weight in $SPLM_3$ (50%) differ significantly ($p>0.05$) from $SPLM_1$ (0%) while RBM4 (75%) can be compared to RBM_1 (0%). Final weight and body weight gain follow similar trend with $SPLM_2$ (50%) recorded highest, which differ significantly ($p>0.05$) with other diets. Decrease of SPLM inclusion level increase fish weight. Highest SGR in $SPLM_2$ (25%) can be compared favorably with $SPLM_1$ (0%) and other diets while RBM_2 (25%) differ significantly ($p>0.05$) with RBM_1 (0%). Diet $SPLM_5$ (100%) can be compared with other diets in SGR with RBM3 (50%) that differ significantly ($p>0.05$) with only RBM1 (0%). Highest FE in $SPLM_1$ (0%) can be compared favorably with $SPLM_3$ (50%) and $SPLM_4$ (75%) in feed acceptability. RBM_5 (100%), which is highest differ significantly ($p>0.05$) with RBM_1 (0%). Apart from RBM4 (75%) other diets were accepted by the fish. It is therefore recommended that 25% inclusion level of SPM should be used in the diet of *C. gariepinus* for better performance than even 75% inclusion of rice bran, study on lower inclusion level of SPM need to be carried out, this type of study need to carried out on other cultured species of fish such as *Heterobranchus* (catfish) and even Tilapia, study on the use of other processing methods of sweet potato leaf and rice bran for feed formulation should be carried out and this type of study need to be carried out in other culture system.

FOOD AND FEEDING HABITS OF THE CICHLIDAE IN TAGWAI RESERVOIR, NIGER STATE, NIGERIA

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Four hundred and seventeen (417) samples comprising of one hundred and eighty-six *Sarotherodon galilaeus* and two hundred and thirty-one (231) *Coptodon zillii* were collected on monthly basis for four (4) months (March, 2021 to June, 2021) from the catches of local fishermen operating on Tagwai Reservoir, in Niger State, Nigeria. The fresh samples were transported in ice - chest box to the Biology Department laboratory of Ibrahim Babangida University (IBB), Lapai, Niger State, where analyses were carried out. Eight (8) different types of items that constituted plant and animal materials were found as food in the stomachs of each of *S. galilaeus* and *C. zillii*. This included detritus, sand, algae, plant material, nematode, plankton, seed and unidentified material. Plant material was highest (30.30%) followed by detritus (17.32%) then algae (16.01%) and lowest was nematode and unidentified material each with 2.16%. Feeding intensity of both species of fish was high due to low percentage of empty stomachs recorded during the period of study. 157 stomachs of *S. galilaeus* out of 186 examined had food while 181 stomachs of *C. zillii* out of 231 examined had food. *S. galilaeus* and *C. zillii* are omnivore and herbivore respectively based on their feeding habits. Diet overlap or similarity showed moderate level of association in diet and less competition for food between the two species. There is need to examine other aspects of biology, such as growth, fecundity, age of these fishes in the reservoir. This study can be used as baseline information for carrying out similar study in other water bodies.

WATER AND SEDIMENTS FROM RIVER ELEMI, SOUTHWEST NIGERIA

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Abstract

ɔduction

y metals and microplastics are pollutants of the tic environments because of their toxicity, stency, non-biodegradable and tendency to nulate in organisms. This menace in the tic ecosystem is of increasing concern lwide due to their impacts on the entire ystem and human health. This study aimed at ssing bioaccumulation concentration of heavy ls and microplastics in selected fish species, r and sediments from River Elemi.

ıds

ıles Collection

Samples

*:hromis niloticu*s and *Clarias gariepinus* samples were ıt from the fishermen at the landing center of the and they were placed in ice- box, transported to the ıtory for further analysis. The total length (L) of the ıas measured to the nearest 0.1 cm from the tip of nout to the extended tip of the caudal fin. Body t (BW) of individual fish was measured to the st 0.1g with Electronic sensitive weighing balance removing adhered water from the body.

ır samples

samples were collected at 0.5 m below the water e, filtered in pre-cleaned liter bottle acidified by g 5ml of concentrated nitric acid (HNO3) and kept alysis.

nent samples

ıent samples were collected using Ekman Grad and in polythene bags and later transported to ıtory air dried at room temperature

y metals Analysis: Were analysed using Atomic ption Spectrophotometer (AAS) Bulk Scientific model 'GP by the calibration plot method. Three processes involved; standard preparation, equipment ation and sample analysis (APHA, 2017).

ɔplastic Analysis: was carried out using Fourier form Infrared (FTIR) spectrocopy at the Central ːe Laboratory, OAU, Ile – Ife, Osun State using ːr-Transform Infrared (FTIR) Spectroscopy (Shim et.)17) to confirm polymers present.

ılts

esults showed that mean concentrations of all the metals analyzed *in C. gariepinus* were higher than *O. niloticus* and followed this order: 15±0.006ppm)>Zn(1.93±0.04ppm)>Cu(0.31±0.003p Mn(0.14±0.003ppm)>Pb(0.019±0.001pp)>Cd(0.01± ppm)>Co(0.006±0.001ppm). The higher ːntrations recorded in *C. gariepinus* may be due to ːding habits been a benthic feeder as heavy metals to accumulate more at the benthic layer of water s. The mean concentrations of heavy metals in follow this order: Iron>Zinc>Cu>Mn>Pb>Cd>Co FTIR analysis of microplastic results showed nent bands around 536.73 cm^{-1}, 581.4 cm^{-1} in fish ːs stomach contents analysed, 529cm^{-1} in water 544cm^{-1} in the sediment, which further confirm the nce of toxic heavy metals i.e. coloured ions tion metals used as additives like Cu, Co, Fe and bserved wave length spectra peaks of 1,636.30cm^{-1} ːter, 1,636.71cm^{-1} in sediment and 1,625.15cm^{-1} in ndicate the presence of polyethlene terephthalate ı, silicone and polyamide polymers. River Elemi and fish species need proper monitoring to prevent r contamination with heavy metals and plastics.

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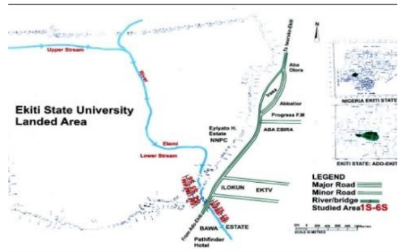

Figure 1: Map showing River Elemi (The Study Area)

Plate 1: A Typical Plastic Polluted River

Table 1: Mean concentration values of heavy metals in water, sediments and fish species from River Elemi

Heavy Metals	Fish Species		Water	Sediment
	Oreochromis niloticus	*Clarias gariepinus*		
As (ppm)	0.016±0.004	0.02±0.001	0.06±0.004	0.19±0.002
Cd (ppm)	0.005±0.001	0.01±0.000	0.03±0.002	0.08±0.004
Cu (ppm)	0.27±0.004	0.31±0.003	0.21±0.001	0.74±0.003
Co (ppm)	0.003±0.001	0.006±0.001	0.016±0.004	0.05±0.003
Fe (ppm)	2.73±0.004	3.15±0.006	0.94±0.006	28.74±0.005
Mn (ppm)	0.12±0.004	0.14±0.003	0.10±0.004	0.51±0.005
Ni (ppm)	0.008±0.001	0.017±0.001	0.03±0.002	0.15±0.006
Pb (ppm)	0.024±0.003	0.019±0.001	0.07±0.002	0.18±0.002
Zn (ppm)	2.19±0.05	1.93±0.04	0.26±0.03	8.51±0.08

Table 2: FTIR Spectra Standard of Wave Length Numbers for Identification of Types of Microplastics Polymers

Microplastics			Spectra wave number (cm^{-1})						
Poly propylene(pp)	1000	1200	1400						
Polyamide (PA)	1700	1600	1300						
Polystyrene(PS)	1800	1600	1450	1200	1000				
Polyethylene there phthalate (PET)	1750	1600	1650	1620	1550	1500	1480	1450	1400
Polyethylene (PE)	1580	1500	1400						
Silicone	1410	1350	1300	1250	1110	1050			
Polycarbonate (PC)	1750	1550	1500	1450	1300	1150	1100	950	900
Polyurethane (PU)	1700	1550	1500	1450	1300	1150	1100		
Polyvinylchloride (PVC)	1800	1600	1450	1300	1050				

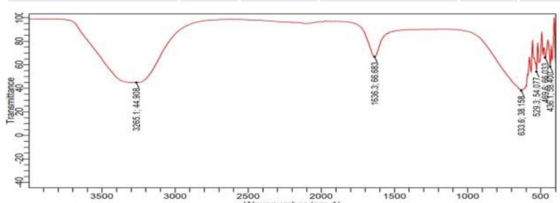

Figure 2: Wavelength frequency and Intensity of FTIR result of the Water from Elemi River

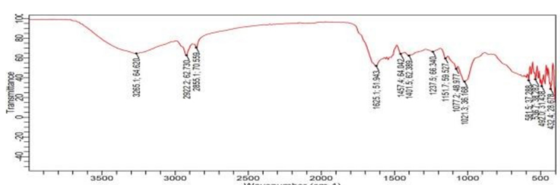

Figure 3. Wavelength frequency and Intensity of FTIR result of the Fish Stomach from Elemi River

Conclusion

This study ascertains the presence of heavy metals and microplastic contamination in fish (Oreochromis niloticus and Claris garipinus), sediment and water samples collected and analyzed from River Elemi. This is an indication of the microplastic pollution status of the river. It also shows that freshwaters are sinks for plastics, heavy metal and serve as channels into the marine environment. In view of this, proper management of plastic and other anthropogenic wastes to prevent drainage into aquatic environment is recommended.

Acknowledgement

The authors acknowledge staff of Central Laboratory, Faculty of Science, Ektiti State University, Ado-Ekiti, where the analyses were carried out.

CHOLESTEROL CONTENT OF AQUATIC MOLLUSKS: PERIWINKLE AND OCTOPUS IN OKESIRI COASTAL WATER BODY, ONDO STATE, NIGERIA

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This study examined the cholesterol content and nutritional composition of periwinkle (*Tympanotonus fuscatus*) and octopus (*Octopus vulgaris*), sourced from the Okesiri Coastal water body in Ondo State, Nigeria. Despite the known nutritional benefits of periwinkle and octopus, there is no available information on their cholesterol content in Okesiri or how it varies with environmental factors and seasonal changes. This gap in knowledge poses a challenge for local communities that rely on these species for food and for managing their health risks associated with dietary cholesterol. Furthermore, understanding the proximate composition and how it fluctuates over time can inform better harvesting practices and conservation strategies. Mollusks, including periwinkles and octopuses, are known to be excellent sources of protein, essential amino acids, and various micronutrients

Over the course of the study, samples were collected each month, totaling 120 octopus and 240 periwinkle for the entire experiment (using 20 octopus and 40 periwinkle monthly). The research aimed to evaluate the cholesterol and nutrient content of these species. Samples were gathered during both the rainy and transition seasons. The cholesterol content was analyzed using the CHOD-PAP enzymatic colorimetric method, while proximate composition analyses were conducted to determine the fat, ash, protein, moisture, and carbohydrate levels. The results showed that octopus had a moisture content of 72.47 ± 1.3%, a fat content of 3.73 ± 0.2%, and a protein content of 9.23 ± 1.1%. In comparison, periwinkle exhibited a moisture content of 72.06 ± 1.2%, a higher ash content of 1.56 ± 1.3%, a fat content of 2.42 ± 0.2%, a protein content of 9.08 ± 0.6%, and a notably higher fiber content of 0.61 ± 0.4%. Carbohydrate content was also higher in periwinkle at 15.26 ± 0.8%. Cholesterol analysis showed that octopus contained a higher cholesterol level than periwinkle.

The cholesterol levels in octopus were consistently higher than those in periwinkle, which may be relevant for individuals monitoring cholesterol intake. Nonetheless, the moderate cholesterol levels in both species suggest they can be safely included in a balanced diet. In conclusion, both periwinkle and octopus offer valuable nutritional benefits, with octopus providing higher protein and fat, while periwinkle is a better source of essential minerals and fiber. Sustainable harvesting and careful environmental management are necessary to preserve and maximize these nutritional qualities. These findings offer insights into the nutritional and cholesterol profiles of these mollusks, highlighting their potential for dietary and aquaculture applications.

GENETIC POPULATION STRUCTURES AND PRESENCE-ABSENCE VARIATION AMONG MUSSELS (*Mytilus* spp.) ACROSS THE NORTH ATLANTIC

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An incomplete mussel (*Mytilus* complex) distribution map, particularly in eastern Canada, makes it difficult to discern between phenotypically and genetically similar species. To address this, mussel DNA samples from across Québec (QC), New Brunswick (NB), Prince Edward Island (PEI), Nova Scotia (NS) and Newfoundland (NF) in eastern Canada, as well as Norway (NO), the Netherlands (NL) and Spain (ES) in Europe, were genotyped using a panel of 69 single nucleotide polymorphisms (SNPs) that originated from recent research on the population structures of *Mytilus* spp. in other parts of the world. These findings helped delineate *M. edulis*, *M. trossulus, M. galloprovincialis* and hybrid zones across the North Atlantic, which is valuable in understanding population dynamics within current and potential future aquaculture sites. Additionally, HiFi-sequenced samples from PEI, NS, NF and NL were consecutively mapped to the phased genome to recursively assemble a *M. edulis* pangenome. Subsequently, 24 resequencing samples from PEI were mapped to the pangenome to assess individual gene presence-absence variation (PAV). A high degree of PAV was identified across samples, with core and dispensable genes assessed by gene ontology to infer putative function. Ultimately, this work will help inform breeding strategies as PEI develops the first *M. edulis* breeding program in Canada.

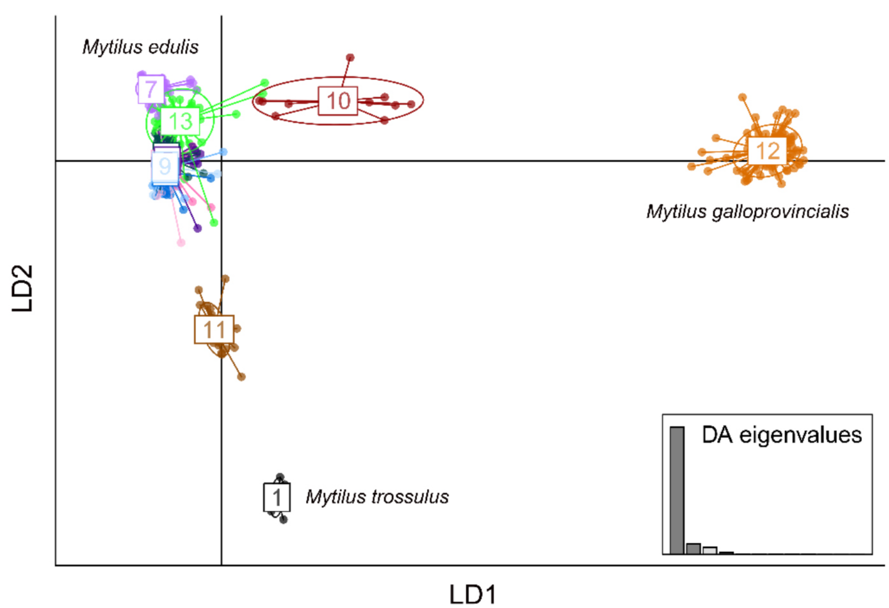

FIGURE 1. Discriminant analysis of principal components (DAPC) of 69 SNP genotypes across 1053 samples from populations across the North Atlantic plotted on the first two linear discriminant (LD) axes.

ESTABLISHING AQUACULTURE EDUCATION IN NORTHERN CALIFORNIA AT COLLEGE OF THE REDWOODS

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Across the state, community colleges have been developing aquaculture programs offering either two-year degrees or specialized certificates to meet the industry's growing needs. In recent years there has been an increase in aquaculture development in Northern California. This includes California State Polytechnic University, Humboldt establishing California's first commercial open-water seaweed farm, the expansion of local oyster farmers, new inland seaweed production, and a proposed Yellowtail Kingfish farming project by Norwegian company Nordic Aquafarms. Amongst these developments, College of the Redwoods (CR), located in the heart of Humboldt County, is among the few institutions in the state offering an Associates of Science degree in Aquaculture Technology. CR's program emphasizes practical farming practices, real-world applications, and transfer readiness, ensuring students are prepared for both immediate entry into the workforce and continued education.

This presentation explores the development journey of CR's aquaculture program, highlighting each step from initial planning, implementation, and future goals. Topics will include curriculum development, program requirements, creation of outreach materials, social media engagement, and strategies for student recruitment. Additionally, the presentation will showcase CR's efforts and process for an on-campus wet lab. To enhance practical learning, CR has explored potential collaborations with industry leaders like Nordic Aquafarms, with discussions around setting up temporary facilities to provide students with hands on experience even before the wet lab is fully operational. CR's relationship with the local university, Cal Poly Humboldt, has provided students with valuable hands-on experience in their full operating hatchery, furthering strengthening practical training. Collaborative efforts between education institutions and industry stakeholders continue to play a significant role in the development of CR's aquaculture program.

THERMAL TOLERANCE OF OYSTERS *Crassostrea virginica* SELECTED FOR INCREASED GROWTH AND TOLERANCE TO DERMO DISEASE

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The eastern oyster (*Crassostrea virginica*) is a species that holds strong ecological and economic importance. Dermo disease, caused by the protozoan parasite *Perkinsus marinus*, is a prominent cause of mortality in the eastern oyster, and is predicted to increase in prevalence with climate change. Thus, there is interest in using genomic and phenotypic selection to increase growth and resilience to dermo in oysters produced for restoration efforts. However, selection for some traits may result in unintentional selection for adverse traits. To that end, the purpose of this study was to determine if selection for growth and dermo resilience impacts thermal tolerance in the eastern oyster.

The oyster lines used in this study included: 1) an unselected wild control, FLWC; 2) a genomic control mated from oysters with average genomic estimated breeding values (GEBVs) for dermo resilience and whole oyster weight, FLC; 3) phenotypically selected oysters based on survival from dermo challenge in laboratory, FLP; and 4) genomically selected oysters based on high GEBVs for dermo resilience and whole oyster weight, FLGS. A chronic LT_{50} (lethal time that causes 50% mortality rate) assay was conducted, with each line of oysters exposed to 23°C, 28°C, 36°C, and 38°C for 20 days. For each temperature, three replicate recirculating aquaculture systems held 50 animals from each line.

Mortality exceeded 50% only in the 38°C trial. Preliminary analysis shows that the LT_{50} of the FLP and FLGS groups were significantly higher than that of the FLWC group (Figure 1). Additionally, the LT_{50} of the FLGS group was significantly higher than the FLC group. Selection for dermo tolerance did not have an adverse effect on thermal tolerance. Rather, genomic and phenotypic selection for resistance to dermo disease significantly increased thermal tolerance when compared to a wild control, and genomic selection significantly increased thermal tolerance in comparison to a genomic control.

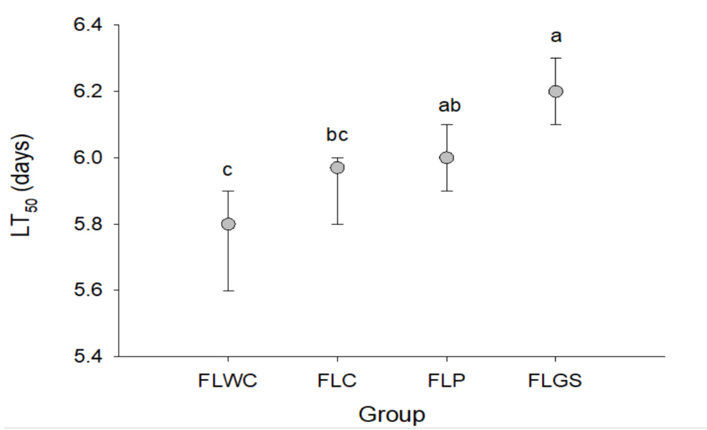

Figure 1. Lethal times that caused 50% mortality (LT_{50}) in the wild control (FLWC), genomic control (FLC), phenotypically selected (FLP), and genomically selected (FLGS) lines. Vertical lines represent upper and lower 95% confidence intervals.

THE PHYTOBIOTIC EFFECTS OF *Spirulina platensis* ON GROWTH PERFORMANCE, IMMUNE RESPONSE, AND SKIN COLOR OF RAINBOW TROUT, *Oncorhynchus mykiss*

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Spirulina is a fast-growing cyanobacterium receiving much attention because of its high levels of proteins, vitamins, antioxidant-quality pigment fatty acids, and antimicrobial properties as a supplemental protein and other nutritional sources. *Spirulina platensis* and *S. maxima* are two major species used in aqua feed. The spirulina meal might be useful to enhance the growth and also improve the immune response in rainbow trout.

The aim of the study was to evaluate the effects of *Spirulina platensis* on the growth performance, feed utilization, immune response, and, skin pigmentation of rainbow trout. A total of 120 fish with an average weight of 17 ± 2 g were divided into four groups (3 experimental groups with diets containing 2.5%, 5%, and 7.5% spirulina, and a control group). The four diets were fed for 12 weeks at satiation level. All the data were subjected to one-way ANOVA using R programming.

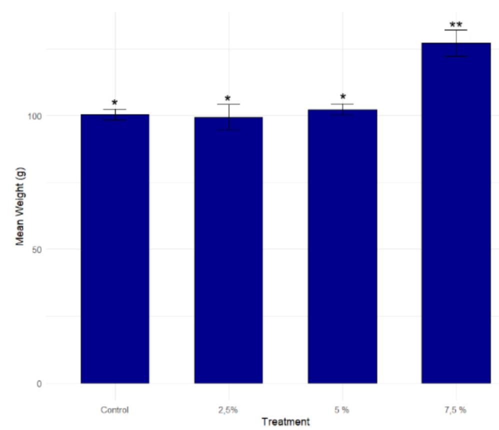

Figure 1: Effect of spirulina supplementation on growth of trout

Results revealed significantly the highest growth performance was found in in the group fed 7.5% Spirulinacompared to other groups. Immune markers showed that C3, IgM, and lysozyme levels were significantly higher in the 7.5% group, whereas C4 levels remained unaffected. Skin pigmentation, measured by β-carotene concentration, increased significantly in fish fed 7.5% and 5% Spirulina, enhancing skin coloration.

The study highlighted Spirulina's potential as a growth enhancer and immune stimulator, attributing its effects to β-carotene and other pigments such as C-phycocyanin. Additionally, Spirulina promoted better carotenoid absorption in fish skin, improving pigmentation. These results suggest that including Spirulina at 7.5% in fish diets can maximize benefits in aquaculture by enhancing fish health, growth, and marketability.

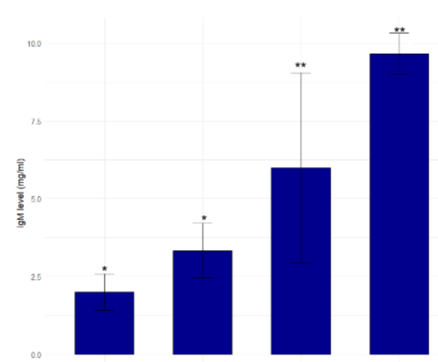

Figure 1: Effect of spirulina supplementation on the level of IgM

OPTIMAL GOLDFISH DENSITY FOR PLANT GROWTH IN AQUAPONIC SYSTEMS

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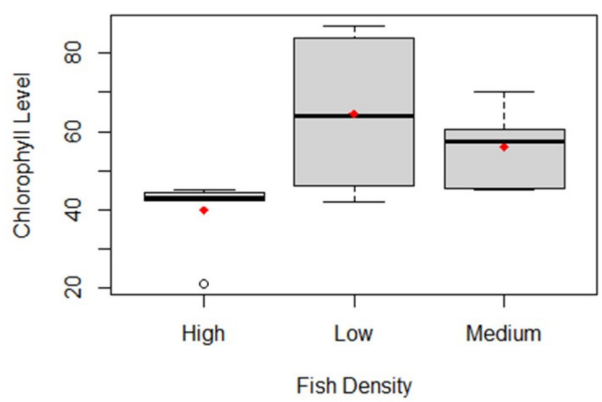
Aquaponics is an innovative sustainable farming method that integrates aquaculture with hydroponics. In an aquaponic system, the nutrient rich water from fish is used to grow plants which in turn purify water for fish. It is an environmentally friendly option for fish and plant production, addressing the increasing demand for food while being a great solution in arid and semi-arid regions where water resources are limited. The success of aquaponics systems depends on achieving a balance between fish density, water quality, and nutrient availability for plant growth. This study aimed to determine the optimal stocking density of goldfish (*Carassius auratus*) to maintain water quality while promoting growth performance in both goldfish and basil (*Ocimum basilicum*) in a flood-and-drain aquaponic system. Three goldfish densities, 0.5 kgm^{-3}, 1.5 kgm^{-3}, 2.5 kgm^{-3} (low, medium, and high, respectively) were examined to assess their effects on water quality and growth performance of goldfish and basil. Water quality parameters such as dissolved oxygen, pH, temperature, conductivity, salinity, Total Ammonia Nitrogen (TAN), Nitrate-N, Nitrite-N were tested while the growth performances were observed based on the initial and final weights of fish and plants. At the end of the experiment, the plant nutrient biomass was analyzed to assess plant health.

Our findings highlighted that medium stocking density achieved an optimal equilibrium, maintaining water quality within safe thresholds for fish while providing adequate nutrients for plant growth. Goldfish displayed improved health metrics in low and medium densities while plants exhibited a better growth in medium densities compared to low and high densities. This study illustrates the importance of optimal stocking density of fish in aquaponic systems to maximize productivity and sustainability, providing insights to improve the efficiency of aquaponic farming.



DIFFERENTIATION OF HUMAN PATHOGENIC *Vibrio* spp. ON COMMERCIALLY AVAILABLE CHROMOGENIC AGARS

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Vibrio spp. are naturally occurring marine bacteria that cause vibriosis, often through raw or undercooked seafood consumption. Current methods to isolate food and environmental *Vibrio* spp. focus on *Vibrio parahaemolyticus* (*Vp*) and *V. vulnificus* (*Vv*), however, the recent increase in prevalence of vibriosis caused by *V. cholerae* (*Vc*), *V. alginolyticus* (*Va*), *V. fluvialis* (*Vf*), and *V. mimicus* (*Vm*) highlights the need to better understand their ecology and prevalence.

This study evaluated the use of commercially available chromogenic agars to isolate and differentiate *Vp, Vv, Vc, Va, Vf,* and *Vm*. Up to 50 isolates of each species from both clinical and environmental origin were streaked to CHROMagar™ Vibrio, HardyCHROM™ Vibrio, and Vibrio *ChromoSelect* Agar. They were also streaked to TCBS, the current standard for vibrio isolation. The phenotypes of well-isolated colonies were observed, and the percentage of isolates with a typical phenotype for each species determined (Table 1).

On TCBS, *Vp*, *Vv*, and *Vm* colonies appeared green; *Va* appeared large yellow; *Vc* appeared yellow; and *Vf* was partially-fully inhibited, but grew yellow-green when present. On CHROMagar™, *Vp* colonies appeared mauve; *Vv*, *Vc*, and *Vm* appeared green-blue; *Va* grew creamy; and *Vf* colonies ranged from white to light purple. On *ChromoSelect*, *Vp* grew blueish-green; *Vv*, *Vc*, and *Vm* grew purple, although *Vv* was partially-fully inhibited; *Va* appeared large, blueish-green, and spread; and *Vf* varied to include both blueish-green and purple pigments. On HardyCHROM™, *Vp* grew teal; *Vv*, *Vc*, and *Vm* grew magenta to purple, but the agar around *Vv* fluoresced under UV light; *Va* ranged from clear, to clear with teal centers, to completely teal; and *Vf* grew variably, ranging from white, to colonies with both teal and/or purple pigment.

Vibrio spp. in this study grew a broader range of phenotypes on the chromogenic agars compared to TCBS, which may allow researchers to better differentiate and target species of interest in environmental samples. Preliminary results indicate that the chromogenic agars are also less inhibitory than TCBS (data not shown), and work is currently underway to compare recovery rates among them.

TABLE 1. Percentage of Vibrio spp. presenting with a typical phenotype on different Vibrio-specific agars.

Species	n	TCBS	CHROMagar	ChromoSelect	HardyCHROM
Vp	22	91%	86%	91%	95%
Vv	24	88%*	83%	92%*	83%
Va	25	100%	96%	92%	96%
Vm	32	100%*	100%*	100%*	100%*
Vc	23	100%	100%	100%	100%
Vf	50	100%*	100%*	96%*	88%*

* Indicates that the typical phenotype was based upon most common observations in this study and not manufacturer's recommendations.

INVESTIGATION OF THE POTENTIAL FOR CANNIBALISM OF FINGERLING AND STOCKER SIZE CATFISH BY OVERSIZED HOLDOVER FISH IN COMMERCIAL CATFISH PRODUCTION SETTINGS

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Oversized fish has been a significant problem for the Alabama catfish industry in recent years. These leftover fish, described as “big fish” by the catfish industry, remain in ponds following harvest and often grow beyond premium market sizes (0.45 – 1.81 kg; 1.25 – 4 lbs.). Big fish in excess of 18 kg have been routinely reported by commercial farmers and catfish processing plants. This issue is especially exacerbated under production scenarios involving hybrid catfish, which can grow faster and to larger sizes than channel catfish. These leftover fish consume feed intended for the new stock, receive lower prices at harvest, and could potentially have a negative impact on the new stock through competition for feed, disease transmission, stress, or even cannibalism. Over the years there have been several anecdotal observations by farmers and researchers that would suggest these big fish engage in cannibalism, the question is to what extent, if any, these actions impact the overall survival of fingerling and stocker size fish. To investigate this production concern, we plan to conduct a series of electrofishing samplings following stocking of commercial ponds that are known to have issues with oversized fish. Shortly after new fingerlings are added to the pond (~1wk), oversize fish will be collected. The stomach contents of big fish will be analyzed to determine if they have been feeding on the newly stocked fish. The second portion of this investigation will consist of small-scale research pond-based experiments. Treatment A will represent the control group in which ponds will be stocked with fingerlings only and will be fed an appropriate feed ration to mimic normal production conditions. Treatment B will consist of ponds stocked with a small number of oversized fish (~ 3.6 – 18.1 kg; 8 - 40 lbs.) collected from west Alabama catfish farms. These fish will be allowed to acclimate to their new conditions for a minimum of 1 week prior to the stocking of fingerlings. Once the fingerlings are stocked, fish will be fed a ration that accounts for the presence of both the over-sized fish as well as the juveniles. Treatment C will be stocked in the same manner as treatment B, however, treatment C will receive a feed ration that only accounts for the fingerlings. After a pre-determined period, ponds will be harvested. During harvest all fish will be weighed and counted to determine the survival, and FCR. The large fish will be weighed, measured, and sampled to examine stomach contents. Fish otoliths will be collected to determine age. Data generated from this project should help gauge the impact oversized holdover fish have on performance and survival of newly stocked fingerlings. We plan to carry out controlled pond studies with both hybrid catfish and channel catfish. The findings of this study could further highlight the need for improvements in harvest and pond cleanout techniques not only to reduce losses due to missed market size fish and reduced prices, but to improve production performance and profitability of subsequent crops.

MASS CULTURE OF THE ALGA, *Dunaliella salina* IN CLOSED PHOTOBIOREACTORS FOR THE PRODUCTION OF BETA CAROTENE AND BIODIESEL

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Mass culture of alga in round photobioreactors of 500 liter capacity with internal illumination tubes were used for the study. These photobioreactors had 5 tubes each having a length of 120 cm. in each tube 200 light emitting diodes of white light were placed which had illumination control. Three 60 watt flood LED (red) lights were also placed on the roof of the tank. The resultant illumination was 170μmol/m^{-2}.The illuminations for the tanks was fixed for 16 hours daily with low illumination during start and shut down time of a cycle. The tanks were filled with 0.5M Sea water and a marine micro-algae *Dunaliella salina* was used as an organism in this study. The tanks were fed with 10% CO_2 and 90% N2 mixture of gas for a period of 30 minutes a day with a flow rate of 3 L/min, for every hour 1.25minutes was set for dosing with micro-bubbles through a ceramic diffuser. The tanks were fed with three different modified *D. salina* media, Nitrate based media, Ammonia based media and fish paste based media for making highest biomass during the period of 10 days. The highest biomass was recorded in fish paste based media with a 18.76x10^6 cells/ml, a count of 15.2x 10^6 cells/ml in nitrate based media and 14.89x10^6 cells/ml in ammonia media. The total wet biomass generated were in the range of 1.97g/L, 1.6g/L, 1.56g/L for the three media respectively. The algae produced will be filtered, dried and used for biodiesel production.

QUANTIFYING eDNA OF SEA SCALLOP *Placopecten magellanicus* LARVAE AND ADULTS IN A LABORATORY SETTING

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Environmental DNA (eDNA) can offer a non-invasive, cost-effective and efficient method for monitoring aquaculture and commercial fisheries populations to inform sustainable fisheries management practices. eDNA tools must be thoroughly ground truthed to determine best practices for their appropriate application. While quantitative eDNA assays for sea scallops (*Placopecten magellanicus*) have been developed and calibrated for sperm and dockside conditions, we lack quantification rates of scallop eDNA generation and degradation, and calibration for other life stages. Here we applied qPCR methods to quantify the eDNA signals from different life stages and densities of scallops in laboratory settings. We conducted multiple larval dilution experiments to establish a linear relationship between larval numbers and resultant gene copy numbers, establishing an average estimate 3.09 x 10^7 gene copies individual^{-1}. We also conducted a controlled mesocosm experiment to quantify eDNA shedding rates of scallops and relate these rates to different biomasses of non-spawning scallops in mesocosms. There is a significant relationship between biomass and peak gene copy values as determined by biexponential five parameter (5p) modeling. This is the first experiment to our knowledge that evaluates DNA shedding rates and identifies relationships to biomass and larval concentration in sea scallops. These relationships will help to inform field sampling efforts and interpreting data from natural experiments.

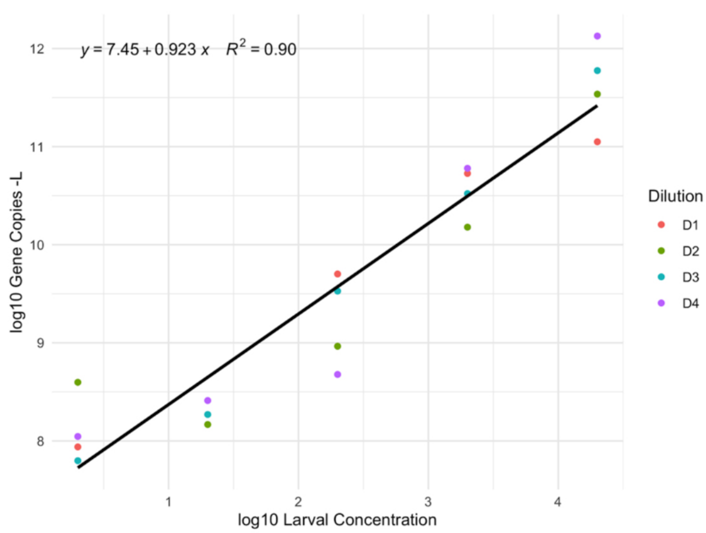

Figure 1: Relationship between larval concentration and gene copies. Larval concentration and gene copy number are linearly related, as demonstrated by this linear regression of scallop larvae concentrations and gene copies L^{-1}. The four replicate dilutions (D1-D4) are annotated in different colors. All values were log-10 transformed.

eDNA METHODS CAN DETECT TEMPORAL AND SPATIAL VARIABILITY OF SEA SCALLOP *Placopecten magellanicus* DNA IN FARMED AND WILD POPULATIONS

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Environmental DNA (eDNA) is potentially a non-invasive, cost-effective and efficient method for monitoring aquaculture and commercial fisheries populations to inform sustainable fisheries management practices. eDNA tools must be thoroughly ground truthed to determine best practices for their appropriate application. Using sea scallop aquaculture farms and a vertically-stratified sampling design above a wild sea scallop bed in Penobscot Bay, Maine, we evaluated the temporal and spatial variability in scallop eDNA signal. The available scallop qPCR probe and primers successfully detected scallop eDNA on scallop aquaculture farms, above a wild well-characterized, deeper scallop bed, and at a site lacking sea scallops and established high temporal and spatial variation in this signal. Seasonal gene copy number per liter seawater maxima on sea scallop farms did not occur after peak scallop spawning, as indicated by GSI values, and did not occur in tandem with maximum counts of bivalve larvae with one exception of one farm site. Sea scallop eDNA was detected at all depths above a wild scallop bed and at a site lacking scallops, indicating that transport of eDNA and quantifying stochasticity in 'background' signals is an important consideration in future studies. The scallop eDNA signal increased at both wild population sites and across depths after maximum GSI were observed during the time of assumed peak larval presence from 30-45 days after spawning. The high spatial and temporal variability in scallop DNA detection supports the need for carefully constructed sampling designs that are informed by organismal life history traits and patterns and the physical oceanographic characteristics of local waters to best apply eDNA tools to monitoring commercially important species.

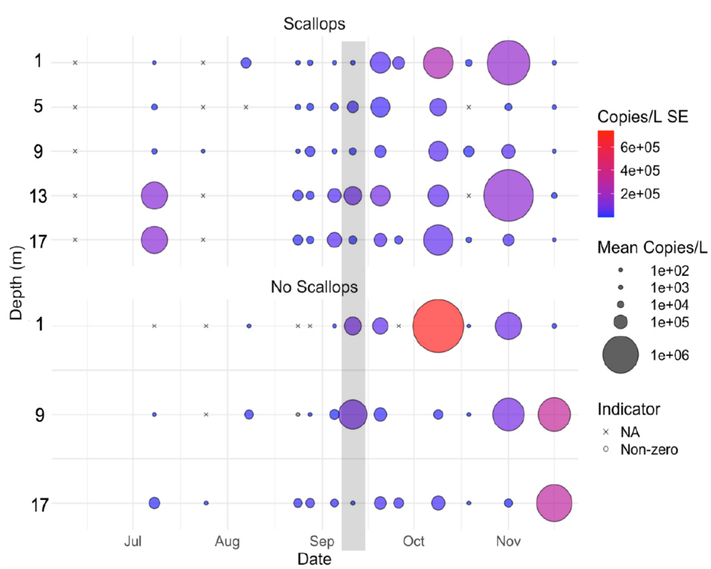

Figure 22: Bubble plot of vertical sampling above a wild sea scallop bed. Bubble plot of gene copy values at different depths at the wild bed and distant sites in 2023. Gene copy L^{-1} values are indicated by circle size and variability (standard error) is indicated by circle color. Xs indicate undetectable quantities of eDNA at a sampling event. The gray box indicates the sampling week in which peak GSI occurred. The wild bed site estimated scallop density is 0.86 scallops m^{-2}.

IMPROVING AQUACULTURE LITERACY FROM GREAT LAKES TO GREAT SEAS

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Indonesian aquaculture is bountiful. Indonesia is the world's second-largest producer of aquaculture products with a total annual production of 14.6 million tonnes. Indonesia embraces this bounty; they view aquaculture as a positive job or activity, as exciting and forward thinking, and necessary in order to access food security. Indonesia is also the home to over 11,000 fisheries extension officers. In comparison, the U.S. is the 20th-largest aquaculture producer in the world, with only 0.48 million tonnes. Additionally, U.S. residents only consume 11.0 pounds per person each year; this rate is less than the recommended intake of 26.0 pounds per year. U.S.'s states also typically have only 0.5-1 extension agents that focus on aquaculture for the entire state. This lack of aquaculture literacy in the U.S. often creates a lack of social license for the expansion of U.S. aquaculture. However, the U.S. is heavily regulated which promotes safe and sustainable farming practices. Therefore, partnerships in Indonesia could provide optimal case studies of high production aquaculture systems and an aquaculture-literate society accepting of farming in the water; and, in return, we in the U.S. could provide examples and training of sustainable practices and seafood consumer safety programs, thus bringing together a very unique partnership - especially for those in Extension and/or Sea Grant programs.

In June 2024, Dr. Lauren Jescovitch and Mr. Elliot Nelson traveled to Java, Indonesia to continue to develop collaborations and key relationships in the aquaculture community. During their travels, they had discussions with the Ministry of Marine Affairs, visited three college campuses and one fishery high school, delivered four workshops to students, lecturers, and extension officers, interviewed five high school students, and presented aquaculture educational presentations at the World Aquaculture Society's Asian Pacific Chapter's annual meeting. They gathered 360- virtual reality footage to create farm tours that will be shared as an experiential-based program to improve U.S. aquaculture literacy as well as obtained verbal agreement of at least one high school from Indonesia to join the Aquaculture Challenge program. Although many outcomes were produced during these travels, the greatest impact of all was the development of a new collaborative idea to focus on implementation of backwards design with the logic model framework for fisheries and aquaculture extension agents. Join us as we showcase our travels to Indonesia, and the teachings we learned in both the similarities and differences in U.S. and Indonesia aquaculture.

THE STRUCTURAL VARIATION LANDSCAPE IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*) GENOME AND ITS POTENTIAL ROLE IN DISEASE RESISTANCE

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Structural variants (SVs) are typically defined as genetic polymorphisms that affect >50bp of sequence, including deletions, insertions, inversions, duplications, and translocations. While SVs are an important source of genetic variation and an important cause of inter-individual differences, they have been neglected in genetics studies compared with SNPs. Here, we defined the SV landscape in European seabass (*Dicentrarchus labrax*), a high value European aquaculture species. We used whole genome sequencing (WGS) in 90 animals to identify 21,428 high-confidence SVs using an established pipeline, with rigorous filtering and manual curation of every SV. These SVs were annotated to estimate potential effects on genes. Integrating SVs and SNP data generated previously, we imputed the SVs for 990 fish with phenotype data for viral nervous necrosis (VNN), one of the main infectious diseases in European seabass, allowing a GWAS analysis using the SVs. In GWAS, 108 (BS, binary survival) and 122 (DD, days to death) SVs exceeded genome-wide significance in a single QTL region matching previous work based on SNPs. The results will improve our understanding of the role of SVs in genetic architecture of traits relevant to aquaculture.

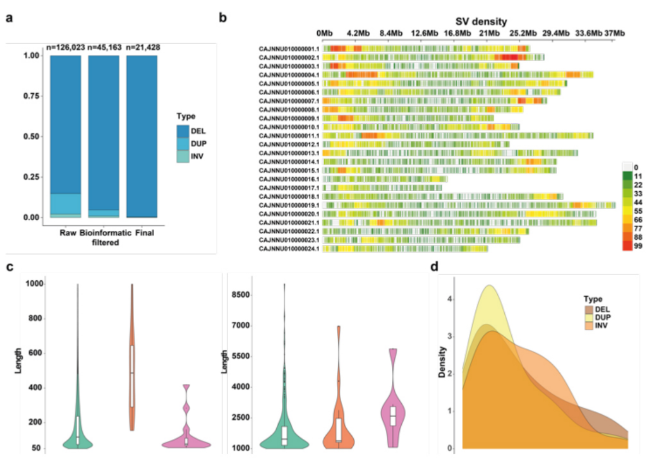

Figure 1. Landscape of SVs detected in the European Seabass. **a.** Illustration of SV counts before and after various filtering steps. **b.** SV density along different chromosomes (window size 1Mb). **c.** Violin plot for deletions (blue), duplications (orange), and inversions (pink), split into two length ranges: 50 to 1,000bp and 1,000 to 10,000 bp. **d.** SV minor allele frequency plot.

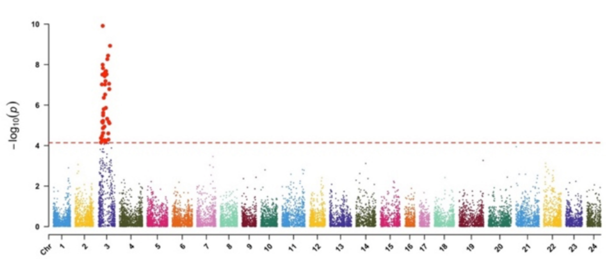

Figure 2. Manhattan plot about the GWAS with GCTA software for the trait of disease resistance. The values on the y-axis represent the −log10 of the P value and the x-axis the positions on the chromosomes. The red line is the 5% chromosome-wide significance threshold (Bonferroni correction) based on binary survival data.

REGENERATING COAST OF PUJADA BAY: A COMMUNITY-BASED MANGROVE REHABILITATION AND ENHANCEMENT IN THE CITY OF MATI, DAVAO ORIENTAL, PHILIPPINES

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Mangrove ecosystems play an integral role in coastal communities. They are crucial in mitigating climate change, supporting coastal ecosystems, and providing habitat for marine fauna, making them both ecologically and economically significant. The Province of Davao Oriental has the longest coastline in the country with a stretch of 513.2 km. In addition, the City of Mati, the Capital City of the province of Davao Oriental is home to diverse floral and faunal species within its coastal ecosystems, including 18 estimated rare and endangered mangrove species. Despite their importance, mangrove populations are deteriorating due to anthropogenic factors such as the booming tourism industry, increasing population, mining, overfishing, fishpond and beach resort conversion. This alarming decline has spurred increased efforts in mangrove planting and conservation.

In response, the Regional Integrated Coastal Resource Management Center XI (RIC XI) of the Davao Oriental State University, in collaboration with Mama Earth Foundation Inc., Department of Environment and Natural Resources (DENR), Bureau of Fisheries and Aquatic Resources (BFAR), and the local government unit, has spearheaded the Community-based Mangrove Rehabilitation and Enhancement Project in Pujada Bay. This initiative aims to restore mangroves through reforestation while also providing livelihoods for indigenous people and members of non-governmental organizations like the Women's Association of the City of Mati.

This report presents the community-based efforts focused on mangrove reforestation by the Women's Association on its economic and environmental opportunities, and challenges at the mangrove sites of the Malizia Mangrove Park within Pujada Bay. The Mangrove Park, initiated by the Mama Earth Foundation, is a reforestation project that encourages active community participation in planning and implementing mangrove rehabilitation and development. The project goal is to plant 1,084,000 of local species of mangroves present in their sites like *Rhizophora stylosa, R. mucronata, R. apiculata, Avicennia marina, A. officinalis, Ceriops tagal, Sonneratia alba,* and *Bruguiera gymnorrhiza* seedlings within coastal barangays of the City of Mati. Approximately 380-400 women participated from the Women's Association of nine coastal barangays. About 854,500 mangrove seedlings were planted as reported in 2023. Results indicated widespread recognition among the women's stakeholders and other local people of the social and economic benefits of the reforestation and enhancement of the mangrove park. Despite there being economic and organizational challenges, there is a strong desire to develop the area for mangrove ecotourism in the City of Mati. Empowerment and strengthening community involvement in planning and decision-making are recommended for sustainable socio-economic development for the coastal communities.

EVALUATION OF ALGAL TURF SCRUBBER BIOMASS AS A FOOD SOURCE FOR JUVENILE EASTERN OYSTERS (*Crassostrea virginica*)

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Eastern oyster aquaculture relies heavily on the generation of microalgae, which accounts for 30–40% of total operating expenses. Oysters mainly consume algae, bits of detrital matter, fragments of seagrasses and numerous other non-algae cells including small animals such as copepods and rotifers, as well as eggs and larvae of various species if they are taken in with the algae. This live-food requirement can be a constraint to economic bivalve production due to the cost and production challenges. To overcome this restriction and decrease the use of microalgae in these facilities, several research studies have concentrated on assessing alternate diets and replacing microalgae in bivalve hatcheries. Algal Turf Scrubber (ATS) biofilms represent a promising approach to wastewater treatment, leveraging the natural nutrient uptake capabilities of various algal species. These ATS biofilms are complex matrices that house algae species such as (microalgae, macroalgae, ciliates, and bacteria) capable of utilizing a wide array of mechanisms for macro- and micronutrient uptake and turnover. Previous studies have demonstrated ATS biomass can be produced at a fraction of the cost of microalgae. The primary objective of this research is to reduce the cost of oyster production by integrating ATS biomass as a feed source. Algal turf scrubber biomass was harvested daily and used in feeding trials to replace 0-100% of the standard oyster diet of microalgae. The juvenile oysters were tagged and measured weekly for 6 weeks to determine the growth performance. At the termination of the experiment, protein, energy, ash, aweight, and shell length were determined. Water quality (ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and phosphorus were measure weekly. Growth performance data and algal turf scrubber biomass compositional data will be presented.

GENOME EDITING AND TRANSPLANTATION OF INDUCED PRIMORDIAL GERM CELLS IN NILE TILAPIA *Oreochromis niloticus*

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Genome editing and broodstock technologies offer significant potential to accelerate genetic improvement in aquaculture species. However, several challenges persist, including low germline transmission rates of desired edits, limited multiplication rates of donor-to-recipients for certain gene modifications, and the extended waiting periods required for donor fish to grow when gonial cells are used as donor. Among germ cell types, gonial cells like spermatogonia or oogonia are commonly used as donors due to their relative abundance and plasticity which allow them to develop into either sperm or eggs depending on the phenotypic sex of the recipient gonads. While primordial germ cells (PGCs) represent an alternative donor cell type, their use is constrained by the limited quantity available per fish. Unlike in mammals, PGC specification in fish is directed by maternally deposited germ plasm, comprising mRNAs such as *vasa, dnd1, nanos3, piwi-like, bucky ball, daz-like, tdrd*.

Recent advancements by Wang et al (2023) demonstrated that PGC can be induced by injecting germ plasm mRNA cocktails into zebrafish zygotes. These induced PGCs (iPGCs) successfully migrated to the gonadal anlagen of recipients and matured into functional gametes. Building on this foundation, we aimed to validate and optimize this technique in Nile tilapia. We tested various combinations of germ plasm cocktail to induce PGCs and evaluated their colonization rates. In addition, we investigated the timing of PGC induction and harvesting, genome editing and genotyping of iPGCs, and their transplantation efficiencies.

iPGC technology offers numerous advantages, including the ability to achieve 100% germline editing albeit still mosaic, elimination of the need to grow donor fish, easier harvesting and transplantation into recipients, and increased multiplication rates for edits impacting gonial cell quantity. These advancements hold promise for accelerating the commercial application of genome editing and broodstock technologies in aquaculture, facilitating more efficient and precise genetic improvements.

CHARACTERIZATION, EXPRESSION, AND IMMUNE FUNCTION ANALYSIS OF SUPPRESSOR OF CYTOKINE SIGNALING 3a FROM CHUB MACKEREL *Scomber japonicus*

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The suppressor of cytokine signaling (SOCS) family plays a pivotal role in regulating diverse biological processes, including cytokines expression, nitric oxide (NO), and reactive oxygen species (ROS) production, primarily through modulation of the JAK-STAT pathway. This study identified and characterized a homolog of SOCS3 from *Scomber japonicus* (designated SjSOCS3a). The coding sequence of *SjSOCS3a* is composed of 618 bp, encoding 205 amino acids containing SH2 domain (39-137 residues) and SOCS3 box domain (165-205 residues). Pairwise sequence alignment discovered that SjSOCS3a had the highest homology with the ortholog from *Thunnus maccoyii* and the lowest homology with *Bos taurus* SOCS3. Multiple alignments of SOCS3 homolog revealed that both SH2 and SOCS box domains were conserved across all selected species. Phylogenetic analysis showed diverged distinct taxonomic clusters of SOCS3, including fish, amphibia, leaves, reptiles, and mammals.

SjSOCS3a mRNA was ubiquitously expressed in almost all of the collected tissues with the highest level in the heart and the lowest level in the liver. The mRNA expression of *SjSOCS3a* in blood was significantly increased at 12 hours upon poly I:C, LPS, *Vibrio harveyi,* and *Streptococcus iniae* challenges. *In vitro* assays revealed that *SjSOCS3a* transfection into RAW 264.7 cells significantly reduced NO and ROS production under LPS treatment (Fig 1). In conclusion, these findings indicate that SjSOCS3a may regulate the innate immune response of *Scomber japonicus*.

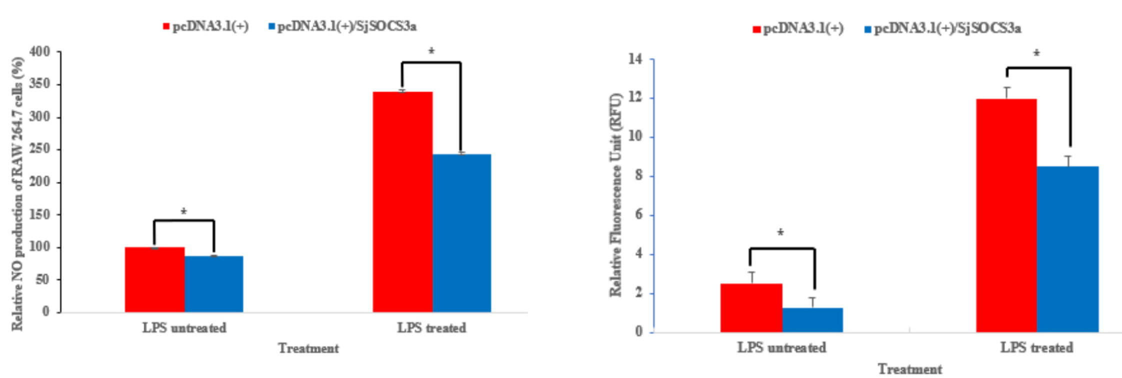

Fig 1. Griess assay (left) and DCFH-DA staining (right) revealed that relative production of NO and ROS was significantly decreased in SjSOCS3a overexpressed RAW 264.7 cells during LPS treatment.

OBSERVATION ON GROWTH OF MALE AND FEMALE LARGEMOUTH BASS IN PONDS AND FLOATING RACEWAYS

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There is an expectation that female largemouth bass (LMB) will grow faster than male LMB in aquaculture. In the sport fishery, trophy largemouth bass are often observed to be female. It has also been documented that female LMB tend to live longer than male LMB and that females are larger than males among older fish of the same age. Whether there is significant difference in growth rate among male and female largemouth bass in commercial aquaculture over a two-year production cycle is not clear. If so, culture of a single sex may be a useful strategy. In this study, observations were made on growth rate of feed trained male and female largemouth bass (*Micropterus nigricans*) during the second year of growth in small experimental ponds (P) and floating raceways (RW).

One-year-old stocker size largemouth bass were grown out in two formats (P and RW) in 2022 and in a single RW in 2023 and 2024. In all three seasons, fish were fed a 45% protein 20% lipid diet once daily to apparent satiation. In 2022, fish were stocked into three 0.04ha ponds and two 41m^3 floating pond raceways. 50 fish in each pond and 100 fish in each raceway were inserted with a Passive Integrated Transponder (PIT) tag. In 2023 and 2024, fish were stocked into a single 41m^3 raceway. In all seasons, weekly water quality was performed to determine total ammonia nitrogen, nitrite, nitrate, alkalinity, and pH, in addition to daily water parameters including dissolved oxygen and temperature. At stocking fish tag number, weight, and length were recorded. At harvest recovered tagged fish were euthanized and measured for individual weight and length. Visual examination of internal gonads was the basis for determination of sex at harvest.

No difference in growth rate with regard to sex was observed among LMB in any format or season.

No difference in growth rate with regard to sex was observed among LMB in any format or season.

Year/format	Interval in Days	Number of tagged fish	Specific Growth Rate Male (Mean±SE)	Specific Growth Rate Female (Mean±SE)
2022 Raceways	158	170	0.59±0.01	0.57±0.01
2022 Ponds	162	127	082±0.02	0.84±0.02
2023 Raceway	196	106	0.69±0.02	0.67±0.01
2024 Raceway	188	152	0.40±0.01	0.41±0.01

EAST VS WEST COAST: A GROWER'S PERSPECTIVE OF SIMILARITIES, DIFFERENCES AND TRENDS OF OYSTER AQUACULTURE IN THE USA

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When describing oyster aquaculture, it is tempting to think 'there are a thousand ways to grow an oyster'. As the industry evolves, farmers are using their personal experiences to innovate on the go, and thus have come to a variety of gear types and husbandry tactics that best suit their needs and environments. Having spent time managing large-scale oyster farms on both the East and West coasts, my goal is to provide insights for farmers to help their operations succeed.

In the Northeast, annual farming patterns are heavily influenced by the weather. Particularly in the Damariscotta River, ME, the farming cycle consists of raising gear from the river bed in spring, thinning, planting seed, tumbling seed, then sinking gear; all while harvesting and maintaining the gear. Floating cages must be sunk in the fall to prevent winter ice and winds from destroying it. On the West coast, especially in California, ice is much less of an issue. The yearly husbandry involves planting seed from fall through spring; tipping racks, flipping bottom bags and harvesting occur year round. There is much less seasonality to oyster grow out on the West coast, and many farms plant seed at harvest density, typically in intertidal gear. Intertidal gear is growing in popularity on the East coast, due to the ability to 'set it and forget it'. This gear type self-regulates biofouling, however on the West coast only for around 10 months; after that, barnacles and seaweed start to take over. On the East coast, intertidal gear is only used for about 4 months as a nursery in the summertime.

The differences between East and West Coast cultures that I see make the biggest impacts are the culture types of subtidal vs intertidal gear. While subtidal gear requires more year round effort to control biofouling, mortality can be as low as 4%. While intertidal gear requires less maintenance during grow out, proximity to the mud causes an average mortality of 50% in Tomales Bay, CA. Both coasts are adapting to the changing climate, where waters are warmer than ever before. The East coast is adopting more intertidal gear while the West coast is adopting more subtidal gear. Both gear types have their advantages, and will help growers succeed as this industry flourishes.

EXPANDING THE ROLE OF AQUACULTURE TO ADDRESS SOCIETY'S GRAND CHALLENGES

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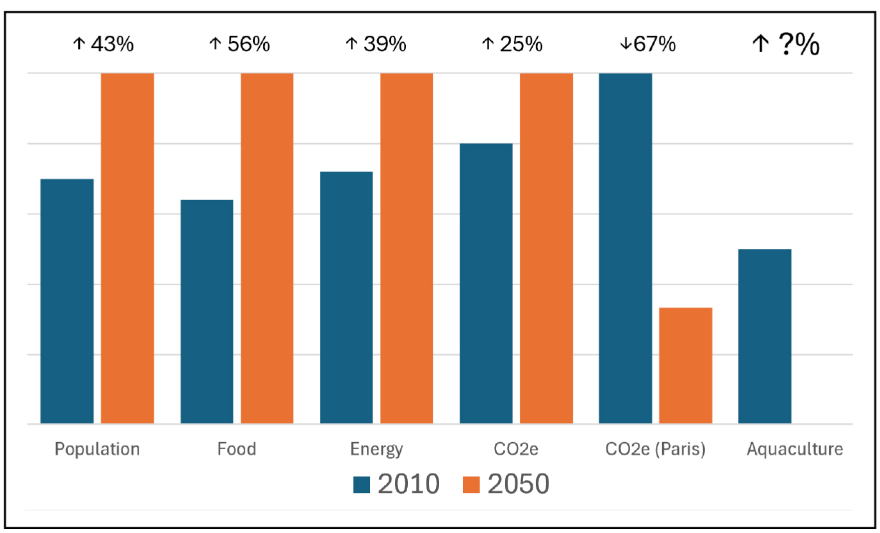
As populations grow, demand for resources expands and climate changes, there is increasing urgency to enhance existing or develop new approaches to help societies address some of their most pressing challenges. For example, to meet the Paris climate goals to hold "the increase in the global average temperature to well below 2°C above pre-industrial levels", CO_2 will need to be removed from the atmosphere at rates of up to 10 $GtCO_2$/y. By 2050 it is projected that global food production will need to increase by >50%. And global energy demands are expected to continue to rise including a doubling of electricity needs by 2050. Current trends in global aquaculture show that across many sectors, there is dramatic and impressive growth in production that exceeds the growth of nearly all other major energy or food systems. However, most of the current production in aquaculture is focused on the food sector even though aquaculture can play a direct or indirect role in addressing other grand challenges. For example, several forms of aquaculture are emerging as part of integrated technologies for carbon sequestration or other negative carbon industries. Aquaculture across a variety of species is also being advanced to develop low carbon biofuels. And aquaculture is being used to grow coral reefs or enhance coastal salt marshes or oyster reefs as a form of green infrastructure towards habitat restoration and buffering against coastal erosion through wave energy absorption and land accretion. These and other approaches expand the portfolio of applications for aquaculture and present the opportunity to grow the industry beyond its current focus on food production. Here I review the major drivers of society's most pressing challenges, how the global aquaculture industry is currently (and positively) responding to these needs, and areas of opportunity for the industry to expand into other sectors. I show that beyond its important and expanding role in feeding the globe, aquaculture also has the potential to positively impact other areas of global importance towards growing a more sustainable future.



EXPLORING THE POTENTIAL IMPACTS OF COMPOSITE ROPE AND POLYETHYLENE NETRON ON BAY SCALLOP *Argopecten irradians* SURVIVAL AND GROWTH

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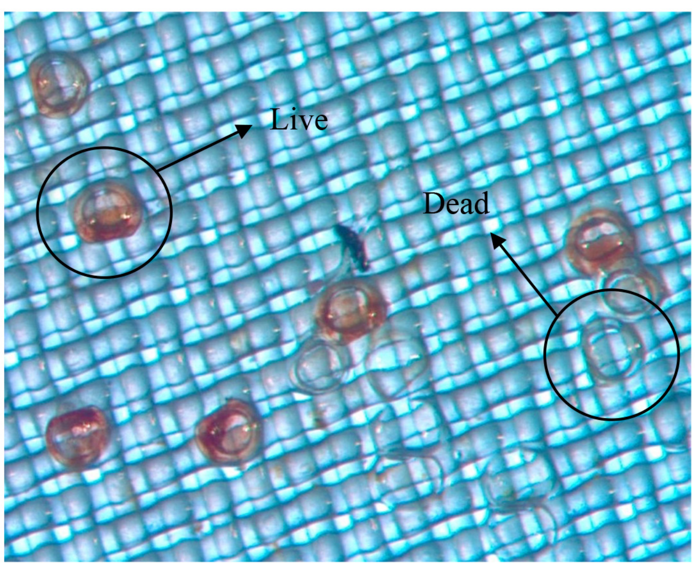
The bay scallop, *Argopecten irradians*, is a coastal shallow-water marine bivalve native to the Eastern coast of the United States. They range from Cape Cod, Massachusetts to the Gulf of Mexico. Populations of bay scallops faced largescale decline in the early 1900s yet continued to support a substantial fishery until populations crashed in the 1980s; populations have never recovered in part due to the loss of eelgrass coverage and degraded water quality. Concurrently, mass-production of plastic products began in the mid-1900s, and are now widely used in maritime industries. Globally, plastics comprise 80% of marine debris. Not only can plastics fragment into smaller micro- and nano- sized particles, they can leach chemicals into the surrounding water, potentially contributing to water quality issues. In New England, aquaculture and fishing industries are vital components of the economy, contributing significantly to employment, revenue generation, and overall economic prosperity in the region. Nantucket, Massachusetts is home to the last commercial wild-caught *A. irradians* fishery. Natural populations of the bay scallop are supported by release of cultivated spat by the town's hatchery.

The aim of this exploratory study was to determine if leachates from two common fishery and maritime plastic products would impact the survival or growth of *A. irradians* larvae. Experiments were conducted during June and July of 2023 at the Nantucket Shellfish Hatchery. Leachates were produced from composite rope and polyethylene Netron. The rope and Netron were cut into pieces weighing 0.5, 1, and 5 grams and placed into separate 1 liter glass jars to be incubated at ambient water temperatures in the dark for 10 days to produce the leachate. The water was then filtered through a combusted GF/C filter to obtain the leachate for exposure testing. D-stage larvae ranging from 2-4 days old were used in the experiments.

The image above shows both live and dead larvae from the experiments. Preliminary findings suggest a potential relationship between plastic leachate exposure and decreased survival rates among the larvae, highlighting the urgent need for further investigation into the toxicological impacts of plastic pollution on marine life.



ACHEIVEMENTS AND CHALLENGES TO THE ADAPTATION OF WALLEYE *Sander vitreus* CULTURE TECHNIQUES TO RECIRCULATION AQUACULTURE SYSTEMS

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Iowa's Dept of Natural Resources (IDNR) Rathbun Fish Culture Research Facility has developed pilot scale recirculation aquaculture systems (RAS) for egg incubation, larviculture, and growout to produce 225 mm fingerlings. Since 2019 over 1.1 million >40 mm fingerlings have been produced in larviculture and since 2015 over 150,000 >225 mm fingerlings have been produced in these systems. Adapting existing walleye culture techniques to RAS technology has required additional fine tuning to maintain a quality product for fisheries enhancement stocking. Larviculture techniques of clay turbidity was found compatible with biofiltration and surface spray was effective in clearing surface films to facilitate gas bladder inflation. Use of Cornell dual drain style tanks and constant water velocity had to be managed to prevent caudal fin erosion. Growth performance and survival rate from larvae to 180 days of age are acceptable however the incidence of deformity is challenging. The presence cranial deformity at rates sometimes of over 50% are a concern and studies are being conducted to elucidate the factors causing these deformities.

AIR PRIMING AS A MANAGEMENT TOOL FOR TRANSITIONING HATCHERY PRODUCED OYSTERS TO THE INTERTIDAL ZONE

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Aquaculture is the fastest growing food production sector globally and investing in research that has the potential to improve farmed species' resilience to climate change will be critical for industry growth. Stress priming, also called stress hardening or conditioning, can induce short or long-term stress 'memory' that can enhance ability to cope with environmental challenges. Priming has been well-studied in crop production and has growing recognition in aquaculture as the environments that aquacultured organisms are outplanted into are becoming increasingly unpredictable and extreme. One step in the aquaculture process that is particularly vulnerable is when oysters are shipped from a hatchery, where they are kept in a relatively homogeneous subtidal environment, to a grow-out farm, where they are exposed to air for several hours each day (low tide).

We exposed year-old hatchery-produced *Crassostrea gigas* that were ready for transport to a grow-out farm to a 6-hour air exposure (priming event). After 1, 2, or 3 days of recovery, both control and primed oysters were subjected to a two-hour low tide. We found that primed oysters maintain a stable rate before and after low tide while the control oysters had a significant increase after the low tide (See Figure 1). This elevated 'oxygen debt' in control oysters indicates a heightened metabolic cost of restoring cellular homeostasis. We hypothesize that the primed individuals had previously upregulated antioxidant defenses during recovery from priming, which reduced the amount of oxidative stress experienced during the low tide.

The results of our study suggest that air priming may have a positive influence on the oysters' ability to smoothly transition to the intertidal zone by reducing the amount of oxygen debt they 'owe' after a low tide.

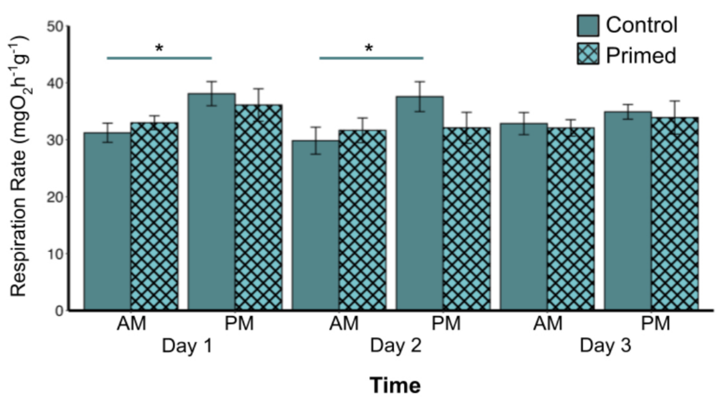

Figure 1. Respiration rate of control and air primed *C. gigas* 1, 2, and 3 days after the primed oysters experienced a 6hr air exposure. Asterisks indicate significant differences (Welch Two Sample t-test, $P < 0.05$) between the AM and PM respiration rates for only the control oysters on Day 1 and Day 2.

BUILDING ALASKA'S MARICULTURE WORKFORCE: STRATEGIC DEVELOPMENT AND LOCAL INITIATIVES FOR SEAWEED AND SHELLFISH CULTIVATION

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As Alaska's mariculture sector continues to grow, workforce development plays a crucial role in supporting the cultivation and processing of seaweed and shellfish across the state. This presentation will highlight two key initiatives: the Alaska Mariculture Workforce Development Plan and a collaborative project between Alaska Sea Grant and the Kodiak Archipelago Leadership Institute (KALI), both aimed at addressing workforce needs in the evolving mariculture industry.

The Alaska Mariculture Workforce Development Plan serves as a strategic roadmap to meet the workforce demands of Alaska's emerging mariculture sector, focusing on shellfish and seaweed farming. The plan emphasizes expanding public awareness, creating training opportunities, and promoting sustainable industry involvement. Workforce development is essential to scaling the industry, particularly in processing, product development, and marketing. By leveraging the University of Alaska's resources and coordinating with industry partners, the goal is to grow mariculture into a $100 million industry over the next 20 years.

Complementing this statewide effort is the collaboration between Alaska Sea Grant and KALI, which focuses on developing a workforce specifically for seaweed cultivation and processing in the Kodiak Archipelago. This program addresses the unique challenges of rural and coastal communities, delivering tailored training on seeding, harvesting, handling, and processing to improve product quality and shelf-life. The initiative emphasizes sustainable practices, aiming to integrate seaweed farming with existing community activities and foster economic diversification.

Together, these initiatives aim to build a resilient, skilled workforce capable of driving mariculture growth in Alaska, while enhancing economic opportunities and environmental stewardship in rural coastal communities.

THE CHANGING SEASONAL ECOLOGY OF *Vibrio parahaemolyticus* POPULATIONS IN THE NORTHEAST US

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Shellfish-borne illnesses from pathogenic *V. parahaemolyticus* (Vp) in the Northeast US have significantly increased over the past 15 years. Tracking and forecasting the population dynamics of this environmentally transmitted pathogen is key to informing risk assessment and providing for a better understanding of ecological mechanisms underlying human illness potential and expansion.

Previous studies in the Great Bay Estuary (GBE) of New Hampshire and Maine suggested a range of ecosystem targets (water temperature, pH, ecosystem conditions, plankton communities, nutrients) as drivers of *V. parahaemolyticus* concentration variation in estuarine ecosystems. Tracking these variables can complement management strategies as tools that can provide for improved pre-harvest risk management. The current database for Vp and ecosystem drivers in GBE now spans 18 continuous years at two sites. A combination of descriptive and predictive modeling and multivariate community analysis representing sites and the harvest-area were used to re-analyze Vp concentrations in oysters in relation to water quality and meteorological factors.

The long-term surveillance of *V. parahaemolyticus* populations revealed a steady increase in concentration from 2007-2017, followed by a decline in more recent years. This 6-year decline occurred despite record high sea surface temperatures, a primary ecosystem driver, in the Gulf of Maine and GBE. The recent trend strays from previously identified and predicted trends (Hartwick et al 2019), thus, predictive models aimed at fitting the data to a trend where increases in *V. parahaemolyticus* concentrations continue in recent years, are not consistent with the data observed. *V. parahaemolyticus* pathogenicity markers were detected in aquaculture and wild-caught oysters with increasing frequency from 2014-2020 and have also since declined, which coincides with total *V. parahaemolyticus* concentration trends and regional vibriosis trends. Continued analysis of the full Vp database, along with continuous databases for *Vibrio vulnificus* and *Vibrio cholerae* is underway to provide for expanded risk assessment and ecological comparisons of these pathogenic Vibrio species in the GBE as a model approach for shellfish harvesting areas in the Northeast US.

RESTORATION OF *Totoaba macdonaldi*: INTEGRATING CONSERVATION AND COMMERCIAL AQUACULTURE TO ENSURE SUSTAINABILITY

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Totoaba is a sciaenid fish endemic to the Sea of Cortez. It is listed under the Convention on International Trade in Endangered Species (CITES) and under the US Endangered Species Act (ESA). After the collapse of its commercial fishery in 1974 all fishing and trading of totoaba have been banned in Mexico.

In 2013 private company Santomar (previously known as Earth Ocean Farms, or EOF) started operating an offshore totoaba farm under an Environmental Management Unit (UMA) permit from the Mexican Ministry of Environment and Natural Resources (SEMARNAT). As required by this permit, Santomar complies with strict requirements for traceability and restocking. This model supports local communities and offers a productive alternative to illegal fishing. Through the UMA process it is possible to differentiate legal from illegal products, and to combat the illicit trafficking of totoaba while allowing restoration efforts and commercial farming. A special one-time permit was obtained by EOF in 2014 to capture 60 totoaba individuals to serve as founders of a captive population and to start a breeding program geared towards preserving genetic diversity to be used for restoration efforts, and a separate program to select for fast growth for commercial fish production. The project has grown over the past 12 years to production of over 500 tons of totoaba in 2024, in a 340-ha ocean concession, a research and a commercial hatchery, and a processing facility near La Paz, in Baja California Sur.

As part of its restoration efforts Santomar releases genetically diverse totoaba fingerlings to the Sea of Cortez. Releases have full genetic traceability via 21 genetic markers identified for the broodstock. The genetic program and molecular markers are outsourced to the US-based Center for Aquaculture Technologies (CAT). To date approximately 250,000 fingerlings averaging 9g have been released in 8 separate liberation events attended by the authorities and aided by coastal communities. These events are used as opportunities to educate the public about conservation of the seas, on-site and via social media. There is no data on the impact of restoration efforts but anecdotally, fishermen report incidental catches of totoaba in areas of the Gulf where it had not been present for many years. In 2022 EOF obtained an aquaculture registration of its UMA from CITES which allows the trade of totoaba meat internationally, with full traceability to the production unit. In 2024 the Mexican Congress approved an initiative to modify the Law on General Import and Export Taxes to allow farmed totoaba to be exported, while continuing to ban all trade of wild caught totoaba. The opening of the export market promotes a legal alternative to the illegal totoaba market and creates a sustainable economic model that does not depend on the destructive exploitation of the environment. Santomar has shown that it is possible to balance aquaculture production with the conservation of marine ecosystems, ensuring both food security and sustainable development of coastal regions.

PROPRIETARY FORMULATION OF LACTIC ACID BACTERIA IMPROVES GROWTH, FEED CONVERSION, AND TOLERANCE TO INFECTION BY *Enterocytozoon hepatopenaei* AND *Vibrio alginolyticus* IN WHITE SHRIMP *Penaeus vannamei*: INSIGHTS INTO IMMUNE AND METABOLIC PATHWAY MODULATION

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This study explores the impact of SYNLAC Prime, a proprietary formulation of lactic acid bacteria probiotics on the growth performance, health status, and metabolic profile of white shrimp *Penaeus vannamei* fed with experimental diets, including a control diet without probiotic supplementation, and diets supplemented with SYNLAC Prime at concentrations of 10^5 CFU (g diet)$^{-1}$ (P5) and 10^6 CFU (g diet)$^{-1}$ (P6) for 56 days. Results indicated a significant enhancement in growth performance in probiotic-treated shrimp relative to the control group, attributed to structural improvements in the digestive tract, particularly the increased abundances of B cells in the hepatopancreas. The administration of dietary probiotics markedly reduced the severity of *Enterocytozoon hepatopenaei* (EHP) infection and decreased cumulative mortalities following *Vibrio alginolyticus* challenges (Fig 1).

Shrimp in the P6 group exhibited significant elevations in phenoloxidase activity, respiratory burst, lysozyme activity and phagocytic activity compared to control group. Furthermore, there was an upregulation of several immune-related genes in the hepatopancreas, including serine protease (SP), prophenoloxidase (proPO) I, proPO II, and penaeidin 3a. Additionally, the expression of β-1, 3-glucan binding protein and SP mRNA was significantly increased in hemocytes. Untargeted metabolomics analysis using LC-MS/MS revealed significant changes in the hepatopancreas metabolic profile, highlighting alterations in energy metabolisms pathways, such as citrate cycle and nicotinate and nicotinamide metabolism, as well as amino acid metabolisms pathways, including arginine and proline metabolism, taurine and hypotaurine metabolism, and histidine metabolism. These findings underscore the potential of SYNLAC Prime probiotics in enhancing shrimp growth, immune function, and metabolic pathways, offering valuable insights for advancing health management strategies in shrimp aquaculture.

DIETS OF MORMYRIDAE (TELEOSTEI : OSTEOGLOSSIFORMES) IN TWO RIVERS OF CONGO BASIN (BOUMBA AND KADEI) IN EASTERN CAMEROON

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As part of a larger research program on the diversity and role of the forest in maintaining the local ichthyofauna of the Congo Basin, a study focused on the diets of Mormyridae, a family of many fishes represented in the Boumba and Kadei rivers in eastern Cameroon. Indeed know the diet of savage specie fish can help to make their conservation by aquaculture processes.

The collection of different fish samples took place monthly from December 2018 to May 2019 on the landing stages of Boumba and Kadei rivers independently of the fishing gear (dormant gillnets, cast nets, hooks and creels) used by the fishermen.

The Captured fish were identified using specific keys, then were labeled, photographed, measured (total and standard lengths), weighed and dissected in order to remove their stomachs and intestine which were preserved in tubes containing alcohol at 70%. These stomachs and intestine were then emptied separately and the contents rinsed in Petri dishes, then filtered through a sieve and the retained fractions were sorted, separated, identified, counted and weighed in order to evaluate the rate of food from the riparian forests.

A total of 22 specimens of Mormyridae, divided into seven genera and 10 species were collected at Boumba compared to a total of 87 specimens divided into 11 genera and 24 species at Kadei.

The qualitative analysis of stomach and intestine contents reveals that, independently of the seasons, the diet of Mormyridae of Boumba and Kadei oscillates around three food categories: macroinvertebrates, macrophytes, and other various particles; however the most represented food item in the diet of Mormyridae are macroinvertebrates, which still identifiable until the intestine level (see table 1) .

Table 1: Presence-absence of prey identified in the intestine contents of dissected individuals.

		Foods items in the intestin						
		Macroinvertebrates				Others		
	Species	Co	Di	Le	BI	Det	VN	CB
Boumba	*Campylomormyrus tamadua*			+				
	Marcusenius mento		+					
Kadei	*Campylomormyrus* sp.1					+		
	Campylomormyrus sp.2					+		
	Cyphomyrus psittacus					+		+
	Marcusenius greshoffi					+		
	Marcusenius mento					+		
	Momyrops attenuatus	+				+		
	Mormyrops anguilloides						+	
	Mormyrus caballus					+		
	Mormyrus cashive					+		
	Mormyrus iriodes	+				+		
	Petrocephalus simus				+			

The Mormyridae are highly prized by the local population due to their taste and their fairly large body mass, thus depending on their strong preference for animal food origin, the Mormyridae have good aquaculture potential. This is for example the case of *C. tamadua* which has a good ability to exploit the different invertebrates present in its living environment; it can easily adapt to feeding by Diptera larvae in pond. It is also the case of *M. anguilloides* which due to its fish-eating predatory nature, could be tested in aquaculture to regulate the populations of very fertile and invasive species (*Oreochromis nilitucus* etc.) Furthermore, the success of these species in ponds must also take into account the physico-chemical parameters of the water suitable for the proper development of each of them.

However, the forest of the Congo Basin is home to numerous insects (macroinvertebrates) which are important source of food for the ichthyofauna of his different rivers. Consequently, deforestation would negatively impact certain taxa of fish such as the Mormyridae which depend on them and would inevitably lead to their loss.

ASSESSMENT OF NATURAL OYSTER POPULATION HEALTH AND DYNAMICS TO INFORM RESTORATION AND AQUACULTURE PLANNING IN LONG ISLAND SOUND

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Oysters provide essential economic and ecosystem services to coastal communities including Long Island Sound (LIS) where natural, self-sustaining populations supply seed for the Connecticut aquaculture industry. Both regionally and nationally, shellfish restoration efforts continue to increase and expand existing natural beds, presenting an opportunity to increase oyster-related ecosystem services. Little is known, however, about how expansion may affect the proliferation and transmission of oyster parasites between restored and cultured populations. To better understand the host-parasite-environment interactions in a rapidly changing environment, we completed monthly disease (*Perkinsus marinus* (Dermo), *Haplosporidium nelsoni* (MSX), *Haplosporidium costale* (SSO)) and reproduction assessments at two unmanaged, self-sustaining natural oyster beds in Connecticut over two years. Continuous water monitoring (temperature, salinity, dissolved oxygen, chlorophyll, and pH), combined with oyster biometrics, provides a quantitative understanding of the seasonal dynamics and will help to identify relevant water quality variables.

Preliminary analysis shows that the presence of Dermo disease follows historic trends. Body condition scores and gonad development were correlated with temperature. Adult mortality rates of 24-34% were observed during summer months when Dermo infection was high, but overall population densities remained stable as a result of high natural recruitment in the late summer and early fall. Water variables deviated from historical trends, with temperatures frequently exceeding 25°C and pH ranging from 7.0 to 7.7. Further disease analysis using qPCR and histology are underway and will improve our understanding of how changing environmental factors (eg., temperature and pH) are affecting oyster population health and disease progression. Using this comprehensive approach, we aim to fill critical information gaps and develop a hazard analysis to guide restoration planning in a way that promotes the success of natural, restored, and cultivated oysters and in turn supports healthy, resilient ecosystems and coastal communities.

DIETARY BENTONITE MITIGATES HIGH-IRON WATERBORNE TOXICITY IN CHANNEL CATFISH (*Ictalurus punctatus*)

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Iron (Fe) is an essential micronutrient for fish, playing a crucial role in various physiological processes, including oxygen transport, enzymatic activity, and overall metabolic function. However, excessive concentrations of iron in aquatic environments can have harmful effects on fish health, leading to oxidative stress, impaired organ function, reduced growth, and even mortality. Currently, there are no widely accepted or effective therapeutic agents available to mitigate the toxic effects of elevated iron in catfish production systems. This lack of intervention strategies highlights the urgent need for alternative solutions to protect fish from iron-induced toxicity.

A promising strategy to mitigate waterborne iron toxicity in aquaculture is the incorporation of dietary supplements such as bentonite, a natural clay known for its strong adsorption properties. Given its ability to bind excess metals and prevent their systemic accumulation, the present study aimed to evaluate the protective effects of dietary bentonite in channel catfish (*Ictalurus punctatus*) exposed to elevated environmental iron levels. To assess this, three experimental diets were formulated: a control diet containing 0% bentonite, a diet supplemented with 2.5% bentonite, and another supplemented with 5.0% bentonite. Catfish from each dietary group were subjected to a high iron concentration of 9.5 mg/L (as Fe^{3}+), representing 25% of the determined 96-hour LC_{50}. Additionally, a non-exposed control group was maintained in parallel for each dietary treatment. The experiment was conducted in triplicate over a period of eight weeks to determine the long-term effects of dietary bentonite supplementation. Following exposure to Fe, significantly lower growth was observed for the fish group fed with the control diet in comparison to bentonite-supplemented (at either dose) fish groups. During iron exposure, Fe accumulation in the liver and plasma was significantly higher in the control diet group compared to bentonite-supplemented dietary groups. Likewise, Fe exposed group fed with the control diet showed oxidative stress in the liver based on significantly higher malondialdehyde (MDA) content as well as reduced catalase (CAT) and superoxide dismutase (SOD) activities, whereas activities of these enzymes increased significantly in dietary bentonite treatments. Overall, these findings demonstrate that dietary bentonite supplementation effectively mitigates waterborne iron toxicity in channel catfish by improving growth performance, reducing iron accumulation in tissues, and enhancing antioxidant enzyme activity. The results suggest that bentonite can serve as a practical and sustainable dietary intervention in catfish farming to counteract the adverse effects of elevated environmental iron levels.

IMPACT OF ANTIBIOTIC USE IN *Litopenaeus vannamei* culture: RISKS AND SUSTAINABLE ALTERNATIVES

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The use of antibiotics in *Litopenaeus vannamei* aquaculture is a common practice for managing bacterial diseases for enhancing shrimp growth and survival. However, the indiscriminate and prolonged use of antibiotics may be hazardous to human health and aquatic ecosystem as well as the well-being of the shrimp. Antibiotic resistance develops in bacterial populations, with resistant genes potentially transferring to human pathogens through the food chain, reducing treatment effectiveness and endangering public health. Sub-lethal doses also disrupt shrimp gut microbiota, impairing immunity, digestion and nutrient absorption. The environment is also seriously impacted because the pond water and sediments hold antibiotic residues and disrupt microbial diversity and develops resistant strains to grow. The residues can be spread in nearby ecosystems harmful to non-target organisms and destabilizing the ecological balance. Residual antibiotics in shrimp create food safety problems which cause trade restrictions in international markets. To alleviate these risks, the sustainable practices such as biosecurity measures, immunostimulants, and probiotics are the most effective alternatives to antibiotics. These approaches serve to safeguard shrimp health, while also preventing damage to the environment and ensuring public health. Government has to be responsible for setting standards for the controlled use of antibiotics. The integration of these sustainable practices will guarantee the long-term viability of shrimp farming, protect aquatic ecosystems, and maintain food safety standards.

EFFECTS OF MICROALGAE WITH NORMAL AND HIGH LIPIDS ON GONADAL DEVELOPMENT OF NORTHERN QUAHOGS *Mercenaria mercenaria*

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Effort to increase broodstock gametes development with a high microalgae lipids cultures monitored with flow cytometry and BODIPY 505/515 was done for a period of 11 weeks. From two diet groups, control with a commercial f/2 algal food and treated groups with 0% nitrogen algal food, an observation to gonad development of adult quahogs was done with five stages of gonad development were able to be identified (early active, late active, ripe, partially spawn and spent/inactivate stages to spent/inactivate). Before the feeding trial started, the broodstocks were majorly in the late active and ripe stage and after 11 weeks of feeding trial, they were at Stage 4 and 5 for both control and treated groups. This decline was due to insufficient number of the live feed (microalgae cultures) fed to the adults. Quality and quantity of microalgae as live feed for broodstocks must be equal in order to provide a better growth and survival. In terms of quality, the treated feed had a higher amount of total lipids content, but their cell concentration was reduced. Applying feeding strategy by combining the high lipid microalgae cultures with regular ones can be applied to meet what lack.

AGRICULTURE TECHNOLOGY RESEARCH THROUGH INTERDISCIPLINARY APPROACHES AT KENTUCKY

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Agriculture technology (AgTech) refers to soilless plant production encompassing hydroponics (using synthetic fertilizer) and aquaponics (using fish waste fertilizer) that aims to improve yield, efficacy, and profitability by incorporating high-tech applications. Soilless agriculture has several advantages over soil-based agriculture such as efficient nutrient regulation and higher planting density resulting in higher yield per hectare along with better quality produce. In this project, we focus on utilizing fish feed as well as synthetic fertilizer (for comparison) as nutrients for growing bib lettuce (*Lactuca sativa var. capitata*), tomato etc. Nutrient uptake and distribution of these nutrients through fish, bacteria, and plants will be investigated for identifying important nutrients and their ratios for higher crop productivity. Epigenetic changes due to nutrient source (synthetic vs. biofertilizers) and different hydroponic system types, such as deep-water culture, etc. will be evaluated by identifying methylated and non-methylated regions in the whole genome sequence of the plants, such as lettuce and tomato through a bioinformatics approach. A functional profile of the bacterial species responsible for nutrient recovery in mineralization will be generated using metagenomics and meta-transcriptomics tools. Phylogenetic distribution will be identified for the top abundant aerobic/anaerobic bacterial species responsible for the mineralization of the nutrients. Various changes in top 10 aerobic/anaerobic bacterial species for 2 types of media will be studied through metagenomics, functional annotation by identifying various genes to explore mechanisms of adaptation, identifying various biological pathways responsible, changes in protein/proteome responsible for stress response, antibiotic resistance, changes in metabolism, post-translational modifications, etc.

Campylobacter coli ILLNESS OUTBREAK ASSOCIATED WITH RAW OYSTER CONSUMPTION IN MAINE

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An outbreak of Campylobacter illness involving two cases and implicating consumption of raw oysters, was first reported to the Maine Center for Disease Control and Prevention (ME CDC) in early July 2024. The ME CDC, Maine Department of Marine Resources (ME DMR) and the Maine Department of Health and Human Services Health Inspection Program initiated an investigation that eventually linked six confirmed cases to the consumption of raw oysters from two aquaculture lease sites in the same harvest area in Freeport, ME. The ME CDC Health and Environmental Testing Laboratory identified the causative agent as *Campylobacter coli* in two clinical isolates from the first and third reported illnesses. Whole genome sequencing was utilized to compare the two clinical isolates revealing a 0 single nucleotide polymorphism (SNP) difference and definitively linking the cases.

The illness investigation included six case interviews by field epidemiologists, facility inspections at a dozen retail locations and shellfish dealers, four in-field inspections of aquaculture operations and one in-field inspection of an open water wet storage site. After a review of the known pollution sources and water quality data for the shellfish growing area, it was hypothesized that the likely source of contamination was wild birds which regularly perched on the floating surface bags used to grow oysters at both implicated aquaculture lease sites. A recall of oysters from the two implicated aquaculture lease sites was initiated on July 17th for product harvested between June 19th and July 16th.

ME DMR instructed the aquaculture lease holders to modify their operation plans and incorporate submergence of their product for 14 days prior to harvest. To support reopening the lease sites, the DMR public health laboratory collected oyster samples from the floating surface bags and from the submerged cages and tested them for fecal coliform, an indicator species routinely used in the National Shellfish Sanitation Program. Results indicated that levels of fecal coliform in oyster meats collected from the floating surface bags were sporadically high (2400 MPN/100g) and also showed that submerging the oysters for 14 days consistently decreased fecal coliform levels. The investigation was concluded and both aquaculture lease sites were reopened almost a month after the first illnesses were reported. No additional cases were linked to this outbreak.

ASSESSING EQUITY IN THE EMERGING SHELLFISH AQUACULTURE INDUSTRY

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As shellfish aquaculture becomes a key component of climate-resilient, low-impact food systems, the allocation of public trust waters for private use through state-granted leases raises questions about equity. While shellfish farming promises environmental and economic benefits, little is known about who gains access to these resources and how. This project addresses this by asking three critical questions:

1. What information is collected about shellfish aquaculture lease applicants?
2. How and why do states differ in how they collect this data?
3. What do we know about the demographic profile of lease and/or license holders?
4. How equitable are the procedures used to allocate leases and licenses?

To address these questions, we examined shellfish lease application materials, and conducted interviews with state officials. Our results reveal that data collection varies widely across the states. North Carolina, for example, is the only coastal state to collect data on race and gender in its shellfish leasing applications while Maryland, in contrast, conducted its first demographic survey in 2024 as part of a needs assessment, targeting current and potential leaseholders. All other coastal states do not have systematic demographic data efforts..

Initial findings highlight significant gaps in data availability, which will impede efforts to assess the fairness of leasing processes or distributional outcomes. Our ongoing work is investigating the reasons for variation in state practices and evaluate the equity of current leasing procedures. This research will inform recommendations for more inclusive policies, ultimately aiming to ensure that the benefits of shellfish aquaculture are equitably distributed across all communities.This includes the need for standardized demographic data collection and equitable resource allocation policies in the shellfish aquaculture industry to promote fairness in the management of public waters and support the broader goals of sustainable and just food systems.

DEVELOPMENT OF AN ENERGY HARNESSING DEVICE FROM FISH MOVEMENT AND WATER CIRCULATION IN RAS USING PIEZOELCTRIC MATERIAL

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Recirculating Aquaculture Systems (RAS) industry seeks sustainable and energy-efficient to maintain water quality, aeration, and circulation as well as operation of different low power sensors for monitoring. My research explores an innovative approach to reduce external power demands for low power operating sensors or MEMS in RAS by designing and integrating an energy harnessing device using piezoelectric materials to harvest mechanical energy from fluid dynamics within the system.

When piezoelectric materials are subjected to mechanical stress generates electrical energy. Research has been by me in 2020 to development of a piezoelectric device using ocean wave. A mathematical model has been developed to calculate harnessed power by different wave heights. In my current research I am using PVDF, a flexible polymer-based piezoelectric material to harness energy from fish tanks, where the natural movement of fish can generate mechanical energy. Further, these flexible installations do not interfere with the flow of water or the fish's natural behavior results ensuring smooth operation of the RAS. PVDF can also be installed in inside of pipes, tank walls or grates, allowing it to capture kinetic energy from water flows. Experiments have been in hydrodynamics lab of Florida Atlantic University; Figure 1 shows the experimental setup with PVDF piezo material and four wave gauges to measure various data from the wave gauges and PVDF were recorded.

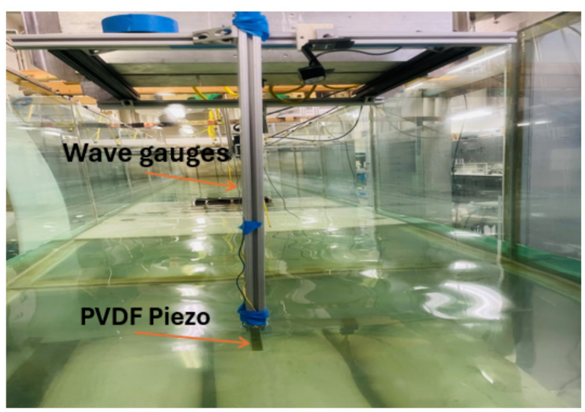

Fig. 1. Experiment Setup in Wave Tank

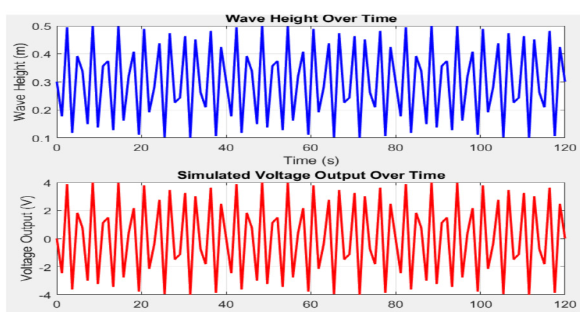

Fig. 2. Data analysis Using MATLAB Soft

EFFECT OF REARING TEMPERATURE, GENDER, PRESENCE OF SKIN, FILLET LOCATION, FREEZING AND STORAGE ON THE VOLATILE PROFILE OF ATLANTIC SALMON *Salmo salar* USING SIFT-MS

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Atlantic salmon (*Salmo salar*) is one of the most widely consumed seafood products in the United States, with flavor being a critical factor in consumer preference and acceptance. The volatile compounds present in salmon play a significant role in shaping its flavor profile and are often indicators of product quality. Salmon is a fatty fish so it is more susceptible to spoilage and has a short shelf life. The spoilage is due to physical, microbiological and chemical processes such as lipid oxidation and proteolysis. These chemical processes affect salmon due to either its protein and high-fat content, causing off-flavors, rancidity, and loss of nutritional quality, making the fish unmarketable which results in food waste and economic loss. The purpose of this study is to investigate the effect of rearing temperatures, gender, presence of skin, fillet location, freezing and storage on the volatile profile of Atlantic salmon.

Fish reared at different temperatures (13^{o}C and 23^{o}C) were killed by a blow to the head, bled and filleted. Fillets were frozen at -80^{o}C until analysis. Samples were thawed on ice for 1 hour before analysis. Raw and frozen samples of skin-on, skin-off and skin alone from the same location on the fillets from each fish were compared. The head, centre and tail of fillet was tested for variation of volatiles in fillet. Storage was compared from 0, 3, 5, 7 and 12 days in refrigerator at 4^{o}C. 2g samples cut into small pieces (1mm) mixed in 20ml of 0.5% ethanol were processed for analysis. The samples were homogenized and held at 42^{o}C in a shaking water bath for 30 minutes. Six replicates were conducted. Measurements were done using SIFT-MS Headspace method. Statistical analysis was conducted using 3-way ANOVA with Fisher's least significance difference posthoc analysis (p value < 0.05).

The fish reared at 23^{o}C had higher concentrations of 38 volatiles as compared to the fish reared at 13^{o}C. The higher concentration is probably due to greater growth rate and metabolism. The fish at 23^{o}C were 328 ± 23.9g and fish at 13^{o}C were 184 ± 9.62g in weight and the fish at 23^{o}C were 30.9 ± 0.17cm and fish at 13^{o}C were 27.3 ± 1.04cm. Female salmon had higher concentrations of 20 volatiles suggesting that male and female fish produce distinct volatile profiles at the same growth temperature. Muscle and skin individually showed higher concentrations of all the volatiles than Skin on muscle. The Head of the fillet showed the highest concentration of volatiles followed by centre and then tail. Freezing fillets at -80^{o}C maintained the volatile concentration at the same level as it was in the fresh salmon. The volatile concentration increased as the time of storage increased when stored at 4^{o}C. This research establish how the factors like rearing temperature, gender, skin on vs off, fillet location and freezing influence volatile profile of salmon and also identify the volatiles that results in off-odors.

ENDOGENOUS NIMAVIRUSES: INHABITANTS OF CRUSTACEAN REPETITIVE DNA

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The genomes of penaeid shrimps and other decapods harbor endogenous nimaviruses, relatives of the devastating pathogen white spot syndrome virus (WSSV). Endogenous nimaviruses exclusively insert into specific nucleotide motifs occurring in repetitive sequences, e.g., microsatellites and 28S rDNA, most likely by using virus-encoded DNA recombination enzymes, the integrase. This strongly suggests that endogenous nimaviruses are actively colonizing sequence "niches" within the host genome. Repeat-specific integration, which targets redundant parts of the host genome, can be seen as an adaptive tactic that minimizes the cost on the fitness of the host.

To investigate the prevalence and diversity of endogenous nimaviruses in publicly available crustacean genome data, we queried WSSV proteins against the NCBI whole-genome shotgun contigs (wgs) database. Majanivirus genomes were identified from all penaeid genomes analyzed, demonstrating the prevalence of endogenous nimaviruses in Penaeidae. Nimaviral sequences were absent from Astacidea and Palaemonidae, suggesting that nimaviral colonization is subject to lineage-specific biases within Decapoda. Genomes of clopoviruses, a group of divergent nimavirus-like endogenous viruses, were identified from isopod genomes, indicating that *Nimaviridae* and its close relatives represent an important part of the crustacean virome.

Discovery of endogenous nimaviruses has redefined *Nimaviridae* as a diverse viral family with many representatives hiding in the host genome. The current ICTV Taxonomy designates WSSV as the only member of *Nimaviridae*. Given the abundance and diversity of endogenous nimavirus genomes in the sequence database, we envision that there will be a need to formalize a sequence-based taxonomy and description of nimaviruses, as has already been implemented for other viral families.

THE SHRIMP GENOME IS HOME TO BENIGN RELATIVES OF WHITE SPOT SYNDROME VIRUS (WSSV)

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The genomes of penaeid shrimps and other crustaceans harbor endogenous nimaviruses, relatives of the devastating pathogen white spot syndrome virus (WSSV). Endogenous nimaviruses do not seem to cause disease in shrimp, and their gene sequences are so divergent that they do not cause false positives in PCR or antigen tests targeting WSSV. The discovery of endogenous nimaviruses proved extremely useful to investigate the evolution of WSSV, as it was previously thought to be an “orphan” virus whose relatives were not discovered at all. However, the reason why they are present remained unknown.

Genomic analyses revealed that endogenous nimaviruses insert exclusively into specific nucleotide motifs occurring in repetitive sequences, especially microsatellites. Penaeid endogenous nimaviruses target (TAACC/GGTTA)n repeats, which typically occur in the telomeres of arthropod chromosomes but are also dispersed throughout the chromosomes in penaeid shrimps. Motif-specific integration is enabled most likely by virus-encoded DNA recombination enzymes, the integrases. These observations suggest that endogenous nimaviruses are genomic parasites colonizing “niches” defined by repetitive sequences in the shrimp genome. Repeat-specific integration, which targets redundant parts of the host genome, can be seen as an adaptive tactic to minimize the possibility of destroying host genes, which could be lethal.

Endogenous nimaviruses challenge our conventional understanding of what viruses are and how they behave. With the discovery of diverse, benign relatives of WSSV, it appears that viruses may have the potential to coexist with, rather than harm, the host. The next challenge will be to uncover the mechanisms and evolutionary drivers that lead some viruses to become lethal pathogens, while others evolve into genomic “free-loaders”.

PRECAUTIONARY PRINCIPLE, THE PERMIT PROCESS, PERSISTENCE AND PIVOTS – AN UPDATE ON OCEAN ERA'S OFFSHORE INITIATIVES

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Offshore aquaculture offers abundant opportunities to expand global food production and grow biomass for industrial use (feeds, fertilizers, industrial materials), with minimal impacts. In the U.S. the primary constraint to growth of offshore operations – either research, demonstration or commercial – is the 'precautionary principle' approach to the permit process, under both Federal and State jurisdictions. This presentation will review Ocean Era's ongoing offshore aquaculture initiatives, and provide updates on permit status, anticipated deployment schedules, and recent pivots in project priorities. We then ask: what, really, is the most precautionary approach?

Our Blue Fields project – a deep-cycling macroalgae culture array at a site 8 nm offshore of Kona – is funded by ARPA-E, and is currently in design and permit stages. This array was originally proposed to be closer to shore, using a deep-seawater upwelling system, but an LCA showed that this would be very C-intensive. The current plan is for the array to descend to 200 m depth at night, to allow macroalgae to absorb nutrients, and to then rise to the surface during the day.

Our Velella Epsilon project is pioneering the permitting process in U.S. Federal waters for a single cohort of only 20,000 fish in a demonstration net pen array off Sarasota, FL, in the Gulf of Mexico. This demonstration project was initiated in 2017, under National SeaGrant funding, but permitting has been slowed by anti-aquaculture activists intervening at every opportunity. A recent mooring reconfiguration, and change of target species (from a single-point mooring for kanpachi, to grid-mooring for red drum), will further delay deployment to an unknowable extent.

Ocean Era is also applying for requisite Federal and State permits in Hawai'i State waters for a commercial fish and macroalgae operation off 'Ewa Beach, Oahu, at a site further offshore from the previous moi farm operation. This project proposes to culture kyphosids (<u>nenue</u>: chubs or drummer - *Kyphosus vaigiensis*), Pacific threadfin (<u>moi</u>: *Polydactylus sexifilis*), and amberjack (<u>kahala</u>: kanpachi - *Seriola rivoliana*) in submersible Polar-Cirkel-style net pens, along with a range of macroalgae (<u>limu</u>) species. Community consultations have been in process since 2019.

The US is heavily reliant on imported seafood that is either fished or farmed under regulatory jurisdictions and food safety standards over which we have no control. Terrestrial animal proteins have significantly greater impacts on consumer health, land- and fresh water-use, and exacerbate the global climate crisis. Offshore aquaculture in Kona, Mexico and Panama has proven to have minimal impact on local ecosystems. The precautionary principle – i.e. the action with least likely negative impacts – therefore compels us all – pioneers, eNGOs, the public and regulators – to pursue research, demonstration and commercial production in US waters with greater alacrity.

BREAKING THE BOTTLENECK OF THE INTENSIVE CULTURE PRODUCTION OF WALLEYE *Sander vitreum* FINGERLINGS UTILIZING RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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The Vermont Fish and Wildlife Department has been utilizing recirculating aquaculture systems (RAS) to rear walleye (*Sander virteus*) to produce fingerlings for stocking into Lake Champlain since 2011. Proof of concept techniques have been applied with successive production years to refine production procedures relating to feeding rates, the use of greenwater (algae) for turbidity, low light intensity throughout the larval production cycle and the utilization of self-cleaning tanks.

Fingerlings are reaching 50mm in length within 33-35dph. Analysis of oxytetracycline (OTC) otolith marking of stocked fingerlings is documenting that intensively cultured fingerlings have consistently outperformed fingerlings reared extensively using pond culture and that the recruitment of intensively reared fingerlings is making a significant contribution to the fishery. Reliable production output and elevated performance and recruitment resulting from intensive culture techniques using RAS will positively impact sport fish enhancement programs as well as forwarding potential commercial development of this species.

LESSONS FROM 25 YEARS OF SEAFOOD RATINGS: LIMITS AND OPPORTUNITIES

Jennifer Dianto Kemmerly, Vice President, Global Ocean Conservation

Monterey Bay Aquarium

Seafood is a key element in global food systems, necessary to the billions of people who depend on fisheries and aquaculture for nutrition and livelihoods, yet unsustainable practices continue as a changing climate further exacerbates environmental and social concerns. For 25 years Monterey Bay Aquarium has assessed the environmental impact of fisheries and aquaculture supplying the US market, and through our Seafood Watch program we have established a consumer and business demand for sustainable sources. This presentation will review our learnings to date, including persistent limiting factors among the aquaculture systems we have assessed, understanding the role of governance, social and other underlying issues impeding sustainability, and the role of collaboration and engagement along the entire supply chain.

DEVELOPMENT OF AN ENVIRONMENTAL REVIEW FOR A SOUTHERN CALIFORNIA OFFSHORE FARM FOR CALIFORNIA YELLOWTAIL *Seriola dorsalis*

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Because the U.S. maintains a trade deficit of over $20 billion annually from importing seafood and that more than half of that product is farmed, it is well-accepted that the U.S. must increase its domestic production. The greatest opportunity to realize this goal is to use the open ocean where conflicts with other users are minimized, water quality is high and the impacts of operations to the environment can be mitigated. In 2013 the FAO published *A global assessment of offshore mariculture potential from a spatial perspective* which provided measures of the status and potential for offshore mariculture development. The primary deciding factors were depth (25 to 100 meters), current speed (0.35 to 3.5 km/h) and distance from port (<25 NM), and within those ranges, the report concluded that the U.S. has more open ocean space available for marine farming than any other country. In response to this necessity, Hubbs-SeaWorld Research Institute has worked over the past two-and-half decades with several investment groups, most recently Pacific6 based in Long Beach, California, to obtain permits to grow a local species, CA yellowtail (*Seriola dorsalis*) for the expanding U.S. market. The proposed farm, Pacific Ocean AquaFarms, represents an important and timely review of the opportunities for and limitations to the advancement of marine aquaculture in the U.S. Exclusive Economic Zone and for the need for new tools to advance that goal.

The farm needs specific permits from the U.S. Army Corps of Engineers (USACE) under the Rivers and Harbors Act and from the U.S. Environmental Protection Agency (EPA) under the Clean Water Act, and farming operations have to be conducted under existing authorities for the production of food and protection of the environment. Since the project will require permits and approvals from several federal agencies, it is subject to environmental review under the National Environmental Policy Act (NEPA) through consultation with all agencies responsible for management of natural resources. NEPA is a highly transparent and complicated review process that is structured around existing regulatory constraints and by the concerns of citizens that assists in the scoping of the review.

Although many of the concerns voiced about marine fish farming are generalize (e.g., use of fishmeal), most are highly site specific. Accordingly, site selection is critical to the permitting process as is evaluation of the site(s) being considered for localized impacts. Marine spatial planning tools developed by NOAA afford front-end assessments of promising farming sites that can meet operational criteria as well as mitigate conflicts with other user groups and protected resources. Advancements in effluent and depositional modelling can be used to select sites that avoid negative impacts from farm operations and escapement models are available to assess how the escapement of farmed fish (considered to be a pollutant) might impact the fitness of the wild stocks of fish. Use of these tools on the POA project can be precedent setting for subsequent permitting efforts, but the costs can be prohibitive to the applicant. For marine farming to advance, the use of these tools should be normalized and underwritten by the agencies.

PREPARING THE SEA GRANT WORKFORCE FOR THE FUTURE: THE SEA GRANT AQUACULTURE ACADEMY

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Aquaculture has been the fastest growing sector of global food production for decades with average annual growth rates reported as high as 8% and total estimated farmgate value of $263.6 billion in 2018 (FAO 2018; 2020). The United States (US) is an exception to this trend. We rank 3rd in terms of population but 16th in aquaculture production despite substantial coastal and inland resources for farming and an unrivaled seafood demand where we import ~90% of the products consumed. In the case of the US, slower aquaculture sector growth suggests that factors beyond population or demand exist and there are additional obstacles to industry expansion. These include but are not limited to the high production costs, prioritization of other coastal uses (e.g., fishing, tourism, development), social acceptance, policy decisions at the federal and state levels, and the current legal and regulatory environment (Garlock et al. 2020).

The advancement of a US domestic aquaculture industry is a complex topic and highly interdisciplinary issue. Sea Grant programs around the country have been serving the aquaculture community and more broadly the US working waterfronts for ~50 years through research, extension, education, and outreach. As the domestic aquaculture landscape continues to rapidly evolve and continuous innovations strive for sustainable production, it is critical that we ensure that the next generation of Sea Grant professionals are well-rounded experts in this field. Given Sea Grant's history in sustainable fisheries and aquaculture, staff throughout the network are also keenly positioned to help usher in the next phase of the seafood economy that enhances and expands aquaculture production while also helping to address on-going challenges facing fishing communities and working waterfronts. The *Sea Grant Aquaculture Academy* was developed to provide an interdisciplinary curriculum tailored to the needs of Sea Grant staff that is informed by network needs and designed with input from a range of partners. The first cohort of 23 Sea Grant associated aquaculture professionals completed the Academy in 2024 with a second cohort planned for 2025. We will share a detailed overview of the recent program including design, implementation, and evaluation highlights in hopes of informing other professional development programs at all levels.

HUMANE KILLING MEETS INNOVATION: ASSESSING IMPACTS OF SLAUGHTER METHODS ON FISH WELFARE AND PRODUCT QUALITY

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Fish play a crucial role in human nutrition, and their importance in aquaculture will only grow as our global population increases. However, with an estimated 78 to 171 billion farmed fish produced annually, concerns about humane slaughter practices are increasingly in focus as fish welfare gains attention. Despite international guidelines promoting humane killing methods, inhumane techniques like asphyxiation and exsanguination without stunning remain prevalent across industries. These practices negatively affect public perceptions about farmed fish and may also have detrimental effects on product quality, both of which are critical for responsible industry advancement. The current study assessed the effectiveness of ike-jime (pithing), percussive stunning, and asphyxiation to humanely kill rainbow trout (*Oncorhynchus mykiss*) and hybrid striped bass (white bass, *Morone chrysops* ♀ × striped bass, *Morone saxatilis* ♂), as well as the subsequent impacts on product quality. The effectiveness of each stunning and/or killing method was assessed using a robust neurophysiological indicator of consciousness - that is, the presence or absence of visually evoked responses (VERs) in the electroencephalogram (EEG). Product quality was measured across a suite of parameters including evolution of rigor mortis, muscle pH, drip loss, flesh color, and muscle fiber microstructure. Results will be shared that demonstrate correlations between humanely slaughtered fish and subsequent product quality in hopes of promoting innovation and gaining support at all steps of the seafood supply chain.

TARGETING DELTA-6 DESATURASE rich *Arthrospira platensis* Sp-6 AS POTENTIAL FEED TO DRIVE SUSTAINABLE GROWTH OF AQUACULTURE INDUSTRY: AN *in-silico* STUDY

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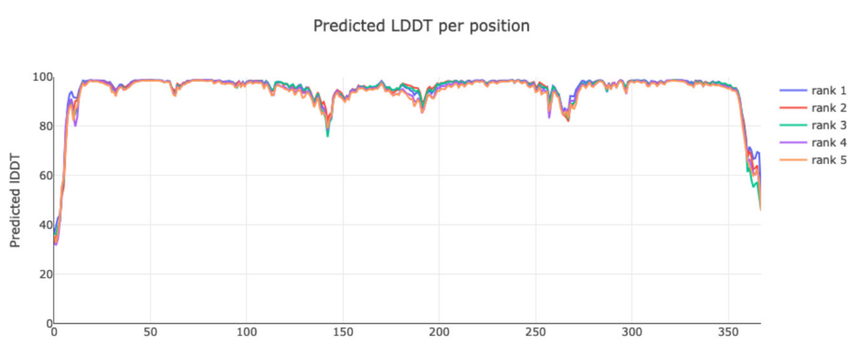
From time immemorial food chain has been relying on microalgal species as a primary source of polyunsaturated fatty acids (PUFA). Delta-6 desaturase plays a crucial role in the synthesis of important PUFA like EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). In fish, PUFA play pivotal roles in growth, development, reproductive health and immune systems being major constituents of cellular membrane and acting as precursors of physiologically active molecules including hormones.

Fishes are incapable of synthesizing all the necessary PUFA by themselves. Therefore, most fishes are dependent on microalgal species in the food chain to satiate their PUFA requirements. Delta-6 desaturases catalyze conversion of linoleic acid (LA) into gamma-linolenic acid (GLA) leading to the formation of EPA (eicosapentaenoic acid) and DHA which is considered as a rate-limiting step. Therefore, we posit to target Delta-6 desaturase rich *Arthrospira platensis* Sp-6 as a potential feed. Delta-6 desaturase protein with accession no. ACN65115.1 in *Arthrospira platensis* Sp-6 and the full-length protein containing 368 amino acids was retrieved from NCBI. *In silico* prediction of physiological properties by protparam such as isoelectric point, molecular weight, instability index, aliphatic index and GRAVY was observed to be 6.48, 42154.73 Da, 40.69, 98.26 and 0.181 respectively. Interpro Scan results illustrated the presence of Delta6-FADS-like (cd03506) domain spanning from amino acid position no. 70 to 332. The 3D model of the protein was generated using Alphafold2, mean LDDT score of the model 1 (out of 5 models) was scored 94.89 validating this as highly accurate model. The NJ phylogenetic tree revealed that among 20 homologous proteins, homologous protein in *Oscillatoria sp*. SIO1A7 was the closest evolutionary homolog being evident by strong bootstrap value. Consurf results investigated all the evolutionary conserved residues retrieved from multiple sequence alignments of all the homologous proteins which were marked by 9 -different color codes. Thus, envisioning delta-6 desaturase rich *Arthrospira platensis* Sp-6 as a potential feed might be beneficial for overall growth and development of fishes thereby driving sustainable growth of aquaculture industry.



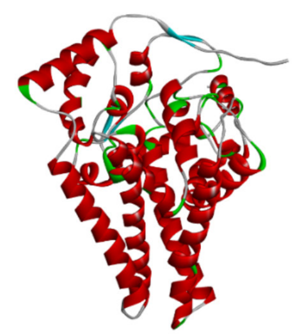


Fig.1: LDDT score for Delta-6 desaturase models; **Fig.2:** Delta-6 desaturase Model by Alphafold2

PACKING AND TRANSPORT OF ADULT ZEBRAFISH CAUSE BOTH ACUTE AND LONG-TERM ELEVATED WATER CORTISOL LEVELS

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Live fish are often transported both short and long distances, for example, as farmed fish from hatcheries to on-grow fish farms, as ornamental fish that are being collected in the wild, or when experimental fish species are shipped between laboratories. The zebrafish (*Danio rerio*) is a popular research animal used globally in the fields of life science, such as toxicology and neuroscience. Despite the fact that zebrafish are commonly transported both nationally and internationally, it is still not fully known how transporting zebrafish affects their welfare. To better understand the short- and long-term physiological and welfare effects of transporting zebrafish, we performed a series of experiments transporting adult zebrafish.

Fish transport experiments were repeated five times. Adult wildtype zebrafish were raised at a zebrafish core facility in Stockholm, Sweden and transported to our research facility in Gothenburg, Sweden, a journey that took around 6 hours by car. Animals were packed for the transport in plastic bags (20 fish per bag) according to commonly used standard operations procedures. Upon arrival, the transport water was collected for analysis of water quality and content of the stress hormone cortisol that was measured using a radioimmunoassay. After being acclimatized to the new facility the fish were transferred to tanks with fresh aquarium water, and kept up to nine days during which the water was collected for analysis.

Our experiments showed that all the fish survived the journeys and the acclimatization period. However, water cortisol levels in the transport bags after the transports were on average 0.1 ng/ml, which is higher than cortisol levels normally measured in their aquaria. One day after the transport, the cortisol levels showed large variations between the home tanks, but had dropped to about a tenth of the transport concentrations (Figure 1). After the transport experiment, the water cortisol levels remained elevated until five days, when they were significantly lowered. This indicates that packing and transporting of adult zebrafish induce an elevated secretion of cortisol in the water, which could be a sign of both acute and long-term stress.

More research is needed to better understand the welfare implications of the elevation of secreted cortisol, but we foresee that our findings could have implications for the aquaculture and transport of other fish species, for example, farmed or ornamental fish.

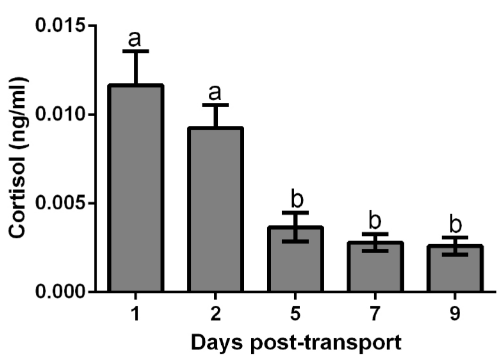

Figure 1. Water cortisol data from one of the transport experiments. Water was collected from the home tanks during nine days following the transport.

TRANSOVARIAL TRANSMISSION: TOWARDS SUSTAINABLE SHRIMP AQUACULTURE THROUGH INTERGENERATIONAL MANIPULATION OF GENE EXPRESSION

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Transovarial transmission is a fascinating biological phenomenon observed in many invertebrates, it involves the transmission of various agents, such as pathogens, symbiotic microorganisms, or genetic material, from the parent generation to their offspring via the eggs during vitellogenesis and oocytes development. OSDel is a crustacean transovarian transmission tool developed in our lab, were proven to piggyback dsRNA and silence specific genes in *M. rosenbergii*. Moreover, the tool has been proved to piggyback a chimeric CAS9-OSDel into oocytes and edit genes in the black soldier flies. Herein, OSDel tool was utilized for transovarial transmission of the white spot syndrome virus (WSSV) dsRNA (dsWSSV) in *L. vannamei* females. The intergenerational effect of OSDel piggybacked dsWSSV on RNAi immune priming and resistance against WSSV of the offspring of treated females was tested. Indeed, a systemic RNAi response was found in *L. vannamei* larvae and postlarvae taken from OSDel-dsWSSV, treated females. We found that offsprings born to mothers treated with OSDel-dsWSSV were surprisingly resistant to a major viral threat, WSSV. This breakthrough represents a novel approach to shrimp disease control, potentially paving the way for broader protection of broodstocks and their offsprings toward sustainable crustacean farming applicable to WSSV and various crustacean viruses, among other pathogens.

IMPACT OF PHYTOCHEMICAL SUPPLEMENTS ON THE MODULATION OF STRESS FACTORS IN FISH CULTURED IN FARMING CONDITIONS

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The constant growing demand for food supply to sustain the human population and its everlasting conflict with the supply in demand illustrates the necessity of an alternate option for nutrition supply which has been recently recognized through the usage of farming fish. Major nutrition supplements for meat have been mostly found through the common farming fish including Tilapia, Bass and Catfish. These fish are being commercially mass produced causing the fish to undergo several stress factors including crowding, handling, transfer, diseases, and treatment with chemicals. These stress factors are causing lower quality meat production in fish by affecting their growth, blood glucose levels, blood cortisol levels and other physiological disturbances. To alleviate the effects of stress in these fish, we are trying to imply natural methods for stress modulation using natural phytochemicals and nutraceuticals which are found in plants and are known for their therapeutic effects. For this experiment, the fish will be distributed into four groups of nutritional implementations: a) the control feed with no implied stress (CCF), b) feed provided with phytochemicals with no induced stress (CP), c) commercial feed with stress induced by cortisol (SCF), d) stress induced through cortisol and phytochemical supplements (SP). For over an 8-week period these fish will be observed over 4 different groups for their stress response for analyzing different outcomes of the stress parameters and their mitigation through phytochemicals and nutraceuticals. Our expected outcomes are increased growth parameters, high immunity and improved hepatosomatic index in fish treated with phytochemicals within a shorter dosage use of these phytochemicals.

THE EFFECTS OF DIFFERENT SOYBEAN MEAL SOURCES ON THE GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* REARED IN THE CLEARWATER SYSTEM

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Soybean meal (SBM) is one of the essential plant-protein sources in aquaculture feed. Several methods have been used to reduce oligosaccharides and other antinutrients to improve the quality of the SBM. In this work, we used low oligosaccharide (LO-SBM) and enzyme-treated soybean (ET-SBM) meals as the primary protein source; this study sought to assess the effects of varying replacement percentages of SBM with LO-SBM or ET-SBM on the growth and feed utilization efficiency of Pacific white shrimp. Nine isonitrogenous (35% protein) and isolpidic (8% lipid) diets were formulated. The basal diet utilized solvent-extracted soybean meal as the primary protein source and was incrementally replaced (40%,60%, 80%, and 100%) using LO-SBM or ET-SBM. Fifteen juvenile *L. vannamei* were stocked in each of the 44 indoor recirculating aquaculture system tanks. Diets were randomly assigned to five replicate tanks. The experiment was performed for 5 weeks, during which the shrimp were fed four times throughout the day, and the feed ration was adjusted on a weekly basis. At termination, shrimp were counted, and the group was weighed to assess survival, growth, and FCR. Subsequently, two shrimp guts were collected per tank of the basal, LO-SBM100%, and ET-SBM-100% treatments. The remaining shrimps from each tank were preserved and later analyzed for whole-body proximate composition. All growth metrics in the trial showed better performance or shrimp feed LO-SBM diets ($p<0.05$). Except for phosphorus retention ($p<0.001$), we observed no significant differences in whole-body proximate composition ($p>0.05$). The outcome showed the potential of replacing the solvent-extracted soybean meal with a new variety of low oligosaccharide and enzyme-treated meals when needed, as well as survival or feed conversion ratio (FCR). Additional results on gut microbiomes will be presented. Low oligosaccharides and enzyme-treated SBM supported good shrimp growth and are suitable protein sources.

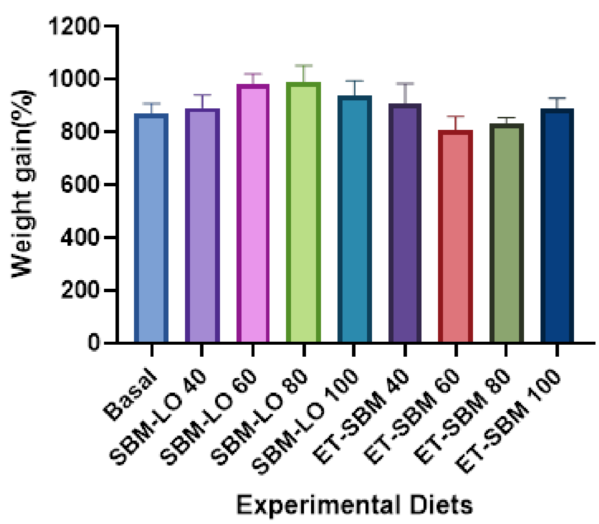

Figure1: Weight gain (%) of Pacific white shrimp offered various SBM diets. Bars represent weight gain (%), and the error bar represents the standard error of the mean.

SUPPORTING INDUSTRY AND RESEARCH: EXPERIMENTAL FARMING OF KELP AND SHELLFISH AT THE UNIVERSITY OF MAINE'S DARLING MARINE CENTER

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Maine's aquaculture industry, both for kelp and shellfish, has been expanding both in number of producers, scale, and farm-gate value over the last decade. With the goal of supporting industry growth and sustainability via applied research, the Aquaculture Research Institute (ARI) at the University of Maine (UM) has made key investments through a cooperative agreement with the USDA-ARS to develop and manage a full scale, diversified aquaculture research farm located at the UM Darling Marine Center (DMC). The aquaculture experimental farm is a 1.7-acre farm located in the Damariscotta River in Midcoast Maine permitted for a wide variety of low-trophic level species and gear types with both intertidal and subtidal tracts.

At the DMC we are able to bring multiple species of macroalgae and shellfish from the hatchery through harvest. Our kelp research focuses on increasing the resilience and sustainability of nursery culture and its downstream effects on production. The key innovation in the nursery is the use of seaweed photobioreactors to develop a kelp strain library for preserving genetic diversity, strain selection, and reducing the need for wild harvest for sorus tissue. Our shellfish research program centers on genetic selection of oysters, ecophysiology, and novel gear/species exploration. An onsite research hatchery at the DMC supports oyster breeding and seed production for novel species such as razor clams, while the farm itself provides a platform for testing novel gear and grow out techniques.

FIGURE 1. (*top left*) Seaweed photobioreactors (*top right*) Mid-season kelp longline (*bottom left*) Rigid trays for sea scallop nursery production (*bottom right*) Students measuring oyster growth

CULTURE PERFORMANCE AND PHYSIOLOGY OF TRIPLOID EASTERN OYSTERS IN MAINE

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Triploid oysters have become a cornerstone of oyster aquaculture due to their faster growth and reduced reproductive effort compared to diploids. While extensively used in the southern and mid-Atlantic U.S., their performance in the colder waters of the northeast remains under-investigated since their development in Maine ~40 years ago. This study evaluated both the culture performance and oxygen consumption of triploid and diploid oysters in Maine's Damariscotta River Estuary to inform farmer seed choices and improve bioenergetic models of triploid growth.

Two-millimeter triploid and diploid seed oysters were deployed at two sites along a natural temperature gradient with a warmer, upper estuary farm site, Pemaquid Oyster Company (POC), and a cooler middle estuary site, the Darling Marine Center's (DMC) experimental farm. Shell and tissue growth was monitored over two summer seasons. Environmental variables such as temperature, chlorophyll, and particulate organic matter were recorded. In the winter between growing seasons, we conducted a 60-day starvation experiment coupled with oxygen consumption trials to explore if basal metabolism was different between ploidy after other contributions to oxygen demand were eliminated.

Triploids had significantly faster growth than diploids at both sites, reaching market size up to three months earlier at POC. At POC, nearly 100% of triploids reached market size by the end of the study compared to only 50% of diploids. Almost no DMC diploids reached market. Triploids starved significantly slower than diploids. However, oxygen consumption rates between triploids and diploids were not significantly different at any time point. These results indicate a strong advantage of triploid oysters, particularly at colder NE sites where product could reach market size before another season of overwintering. Additionally, triploids may handle starvation better than diploids, however it is unlikely due to metabolic needs.

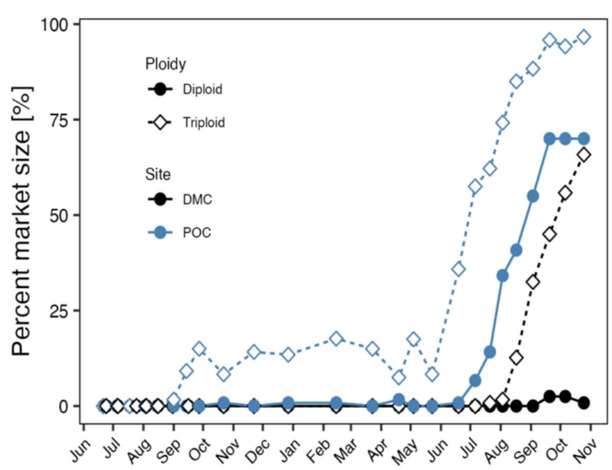

FIGURE 1. Percent of oysters above marked size: 76 mm (~3 inches) in shell height. Measurements start in 2022.

FARM SCALE INSIGHTS: PREDICTING OYSTER GROWTH IN NEARSHORE ENVIRONMENTS

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Matching the right species to the right location is critical for success of new aquaculture farms, particularly for non-fed species, such as oysters, where the farmer relies on the site for both appropriate temperatures and naturally occurring food. These conditions vary in both time and space and the equipment and means to measure conditions in several potential locations over the necessary timescales is out of reach of most farmers. Remote sensing from earth observing satellites can provide the necessary spatial-explicit time series data to reduce the risk in site selection. Thermal sensors can retrieve sea surface temperature (SST). Proxies for shellfish food, such as chlorophyll *a,* can be retrieved from ocean color sensors. However, typical ocean observing satellites with 1 km resolution cannot reliably retrieve data from narrow estuaries, and small bays where oyster culture typical happens. High-resolution satellite (<100 m) such as the Landsat suite and Sentinel 2 can provide this data at the scale of nearshore oyster farm.

To help reduce risks of site selection we (1) estimated and validated a regional Dynamic Energy Budget model for eastern oysters, (2) generated daily climatologies from a decade of Landsat 8 & 9 SST as well as yearly medians of chlorophyll and POM, and (3) coupled the DEB model and satellite products to predict time to market estimates across southern and midcoast Maine. Satellite forced predicted growth was validated on eight datasets of shell height and six data sets of dry tissue weight spanning four separate estuary systems and five years. Only ~8% of the region reached market size in the second growing season (1-1.5 years), while the majority of the region took 2-5 years. This method shows potential of using high resolution satellites to derisk nearshore shellfish site selection.

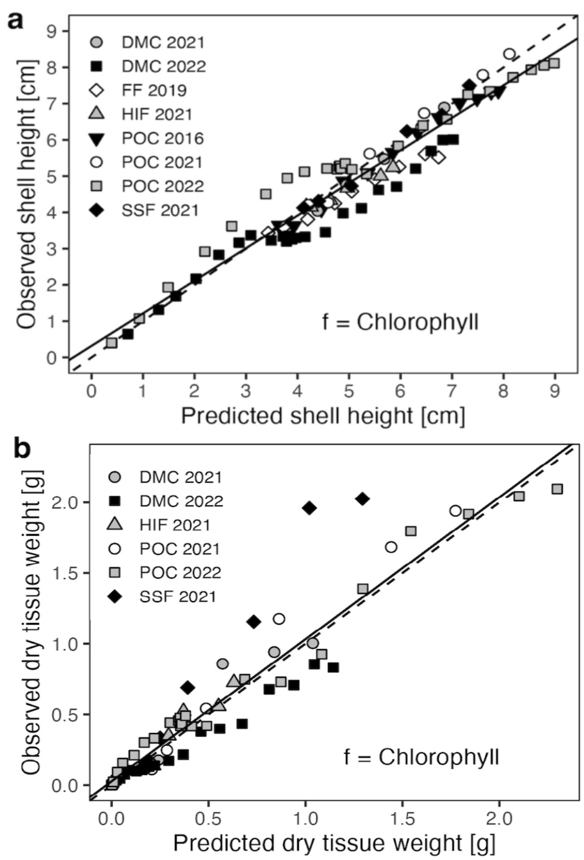

FIGURE 1. Observed vs predicted shell height (*a*) and dry tissue weight (*b*) forced with Landsat 8 & 9 SST and Sentinel 2A/2B chlorophyll

EVALUATION OF WATER QUALITY ACCORDING TO THE FEEDING AMOUNT IN A LOW-WATER EXCHANGE FLOW-THROUGH AQUACULTURE SYSTEM

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In this study, we aimed to develop a novel modified flow-through aquaculture system to reduce excessive water exchange rates and address uncontrollable water quality issues associated with traditional flow-through aquaculture systems. The new system was designed to reduce the water exchange rate from 24 to 9 cycles per day by equipping it with CO_2 degassing towers containing biological media. Additionally, this system was designed to reuse approximately 70% of the wastewater, discharging the remaining 30% into the sea. By discharging wastewater, the system can recover heat, thereby increasing the water temperature in the aquaculture tanks. The system consists of water treatment devices, CO_2 degassing towers, filters containing silver nanoparticle, heat exchange devices, cooling and heating units, and medium-pressure UV lamps.

To verify the performance of the low-water flow-through aquaculture system based on feed supply amounts for olive flounder, various preliminary experiments were performed over 43 days, from June 2 to July 13, 2024, with a total of 3,180 flounder (body weight: 75.1 ± 5.7 g) placed in four tanks. The stocking density was 50 fish/m^3, and the water exchange rate in the tank was 3.1 m^3/h (6.7 cycles/day). Water temperature and salinity were 21.53 ± 0.38°C and 31.37 ± 0.43 psu, respectively. The feed supply amounts were set at 1.33% (800 g), 1.67% (1,000 g), and 2% (1,200 g) of the flounder's body weight. Changes in the dissolved oxygen (DO), pH, turbidity, and total ammonia nitrogen (TAN) in breeding water were measured every 4 days according to the feed supply amount. DO, pH, and turbidity were measured using the YSI-ProDSS sensor, and TAN was analyzed using Nessler's reagent with the DR3900 spectrophotometer (HACH®). As a result, when the feed supply was 1.33% of body weight, DO and pH decreased from 7.05 to 6.80 mg/L and from 7.63 to 7.60, respectively, while turbidity increased from 0.57 to 0.83 NTU. When the feed supply was 1.67% of body weight, DO and pH decreased from 6.56 to 6.20 mg/L and from 7.62 to 7.59, respectively, with turbidity increasing from 0.81 to 1.07 NTU. Additionally, when 2% of body weight feed was supplied, DO and pH decreased from 6.7 to 5.98 mg/L and from 7.69 to 7.53, respectively, while turbidity more than doubled, increasing from 0.90 to 1.84 NTU. Meanwhile, TAN remained between 0.03 and 0.3 mg/L, well below the design threshold of 2 mg/L in all experiments. Further studies will be conducted to investigate the water quality characteristics of low-water flow-through aquaculture systems.

CLASH (CORE-LIT APPARATUS FOR SEAWEED HATCHERIES): AN ALL-IN-ONE MODULAR SEAWEED HATCHERY SYSTEM

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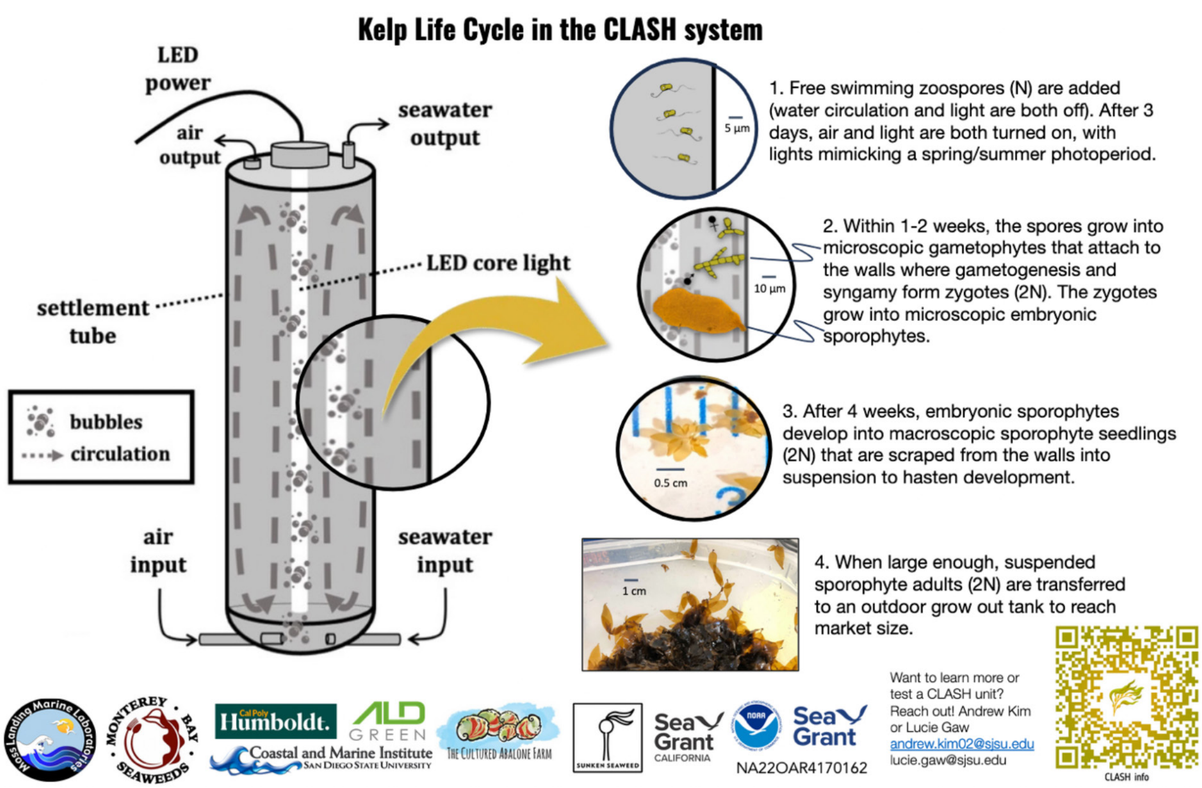
Seaweed farming in the US remains a relatively nascent industry with the vast majority of current production focused on offshore kelp farming. Land-based seaweed aquaculture has the potential to supplement production for the seafood market while expanding the suite of species cultured and reducing regulatory and permitting challenges. In order to expand the land-based seaweed aquaculture industry, there is a need for low-cost and scalable hatchery methodologies aimed for use in land-based culture systems. Here we present a novel hatchery technology, CLASH (Core-Lit Apparatus for Seaweed Hatcheries), an all-in-one affordable and modular system that can be applicable for culturing a diverse array of species in varying conditions. We have successfully utilized the CLASH system to produce cultures of bull kelp (*Nereocystis luetkeana*), giant kelp (*Macrocystis pyrifera*) and winged kelp (*Alaria marginata).* Future studies will determine ideal stocking densities, nutrient supplementation, and light exposure to maximize productivity for each species. Utilizing the CLASH system, we aim to work with our research and industry partners to introduce new commercially relevant species for aquaculture, create new seaweed strains for land-based farming, generate biomass for restoration projects, and scale-up for use in industry.



ONLINE CURRICULUM DEVELOPMENT AND VALIDATION FOR PARENT-FOCUSED NUTRITION EDUCATION PROGRAM "ABOUT TROUT! POND TO PLATE"

Hannah Kindelspire*, Annie Roe, Shelly Johnson, Siew Guan Lee, Jolene Kendall, Jacob Bledsoe, Carolyn Ross, and Matt Powell

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This curriculum addresses the need for increased awareness and understanding of consuming trout as a nutritious food option. It aims to fill the gap in nutrition education by focusing on the health benefits of trout and equipping families with practical skills to incorporate trout into their weekly meals. Designed as a 12-module, video-based course, the curriculum is intended for parents or guardians with children aged 4-9 years. Each module guides families through preparing a trout-centered recipe that promotes hands-on learning and family participation. The course is structured to be flexible, allowing participants to complete one module per week over 12 weeks at their own pace.

Curriculum topics and content include nutritional benefits of trout, food safety guidelines, and strategies for creating a positive mealtime environment using child-centered nutrition phrases (Figure 1). Content also features a special "DIY Pond to Plate" module, connecting families to Idaho's aquaculture industry through the expertise of University of Idaho aquaculture scientists. Each module has knowledge checks to gauge comprehension and reinforce key learning points. The curriculum will go through an evaluation process including a peer-review and content validation. Expert reviewers were chosen to create a diverse panel to provide expertise in each area including food safety, aquaculture, nutrition and child education. Expert reviewers will complete a content validation form which then will be scored to create content validity index (CVI). All 12 modules will receive an individual CVI score based on content and scientific accuracy. The overall course will receive a CVI score based on learning outcomes, assessments, course modules and social cognitive theory constructs. The CVI score shows the agreement rate of reviewers for each scoring criteria and ranges from 0-1. An accepted item should have a CVI of 0.8 or higher and 0.79 or lower will indicate a need for revision. By doing so, we aim to offer a comprehensive, research based educational resource that encourages healthy eating habits and empowers families to integrate trout into their weekly diet. The curriculum will be available through University of Idaho Extension publishing in English and eventually translated to Spanish for anyone to access.

Figure 1. Curriculum Module Outline

About Trout! Pond to Plate Modules	
Module 1: Introduction to Trout and Its Nutrition Benefits	Module 2: Understanding Seafood Safety
Module 3: Talking About Food	Module 4: Purchasing Seafood
Module 5: Cooking with Kids	Module 6: DIY Pond to Plate
Module 7: Exploring Flavors and Cooking Methods	Module 8: Creative and Colorful Trout
Module 9: Understanding Different Types of Fats	Module 10: Healthy Eating Patterns and Seafood Recommendations
Module 11: Meal Planning and Leftovers	Module 12: Course Review

A NEEDLE IN A HAYSTACK - UNDERSTANDING YESSOTOXINS AND THEIR ROLE IN SHELLFISH MORTALITY.

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For nearly a century, shellfish in Washington State have experienced ‘summer’ mortality events and these shellfish losses have been difficult to understand. In the literature phytoplankton have been noted but not implicated in these mortality events because spawning stress and bacteria were thought to be the drivers of mortality.

A bloom of P*rotoceratium reticulatum* associated with a massive shellfish mortality event opened the door to earnestly evaluating the role of phytoplankton in summer mortality events in Washington State. The SoundToxins phytoplankton research and monitoring partnership had been documenting increasing numbers of *P. reticulatum* and *Akashiwo sanguinea* in summer months, which had been associated with declining shellfish health or mortality at various sites in Puget Sound. However, the door was flung wide open in July 2019, as a dramatic scene of surfacing stressed, gaping Manila clams brought to everyone’s attention that something was wrong. Everything was aligning, pathology reports, observations of the bay, and analytical testing suggesting that yessotoxins (YTX) could be the cause. Subsequent YTX concentrations in clam tissues were measured at a maximum of 0.28 mg/kg and their histology demonstrated damage to digestive glands. A culture of *P. reticulatum*, isolated from North Bay during this massive bloom and shellfish mortality event, showed YTX reaching 26.6 pg/cell, the highest recorded toxin quota measured in the U.S. to date. This grand mortality event led to the uncovering of previously collected data which further demonstrated YTX and other phytoplankton involvement in shellfish mortality events in Washington State over the last century. Why the needle in the haystack? YTX produced by *P. reticulatum* were first isolated from scallops in Japan in 1986, so it is a relatively ‘new’ toxin and something not commonly on the radar of researchers.

A DOG'S TALE OF BIRDS AND BIVALVES

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Birds are common around marine waters especially where delectable shellfish are grown. Canadian geese are particularly troublesome in some embayments in Washington State where they dine on the plentiful fish and shellfish in bays and rest on the shoreline after a full meal. Such was the case in a Puget Sound inlet where Canadian geese and other waterfowl were becoming a problem for shoreline homeowners who also wanted to enjoy the clams and oysters on their beach. It was more than just navigating the way through the droppings, it was what the droppings were doing to their shellfish. Through testing of their shellfish tissues and trying out various techniques for waterfowl management on land, the homeowners were able to drop their clam and oyster shellfish meat fecal coliform levels of over 4,000 fc/100g of tissue to undetectable levels year after year. In this presentation, I will share simple, effective techniques for appropriately discouraging waterfowl on shorelines and overwater structures.

SECOND GENERATION SELECTION RESPONSE OF GULF OF MEXICO EASTERN OYSTER (*Crassostrea virginica*) BRED FOR PERFORMANCE IN LOW AND HIGH SALINITY ENVIRONMENTS

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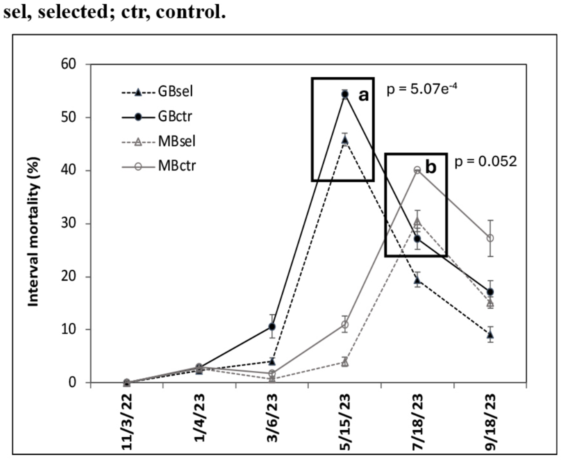
The decline of natural reefs of the eastern oyster *(Crassostrea virginica*) in the northern Gulf of Mexico (Gulf) has stimulated the development of aquaculture to support the market demand and restoration programs. Gulf habitats feature diverse salinity environments that may require oysters bred for habitat-specific optimal genetic characteristics. Here, we report the performance of eastern oyster bred in low-salinity environments after one generation of selection.

Selective breeding employed a common-garden approach where families were pooled at fertilization for communal rearing and molecular pedigrees were used a posteriori to determine parentage and estimate breeding values in a walk-back selection process. The F1 generation pool was bred in 2020 and included 202 full and half-sib families that were deployed on three low salinity sites and two high salinity sites for growth challenge. Breeding values (BV) for height were estimated at harvest size in fall 2021.

Parents with highest BVs were bred in 2022 with a selection differential of 5.4% within low salinity and a selection differential of 2.61% from two high salinity sites. A control pool was also generated using 25 full sib families with BV's deviating -0.086% from the high select and +0.15% from the low select.

Offspring were deployed at 6 growout testing sites in fall of 2022, 4 low salinity sites received oysters selected for performance at low salinity and 2 high salinity sites received oysters bred for performance at high salinity. Selected oysters at two low salinity test sites had significantly greater survival at peak mortality (Fig. 1) and shell height 10-months post deployment than control oysters, indicating positive response on growth and correlated increase in survival. There were no significant differences in growth between the selected and control oysters at high salinities sites, potentially reflecting the lower selection differential within the high salinity line.

FIGURE 1. Interval mortality in selected and control groups at two culture sites. Error bars denote standard error over replicate bags. GB, Grand Bay; MB, Mobile Bay; sel, selected; ctr, control.



WATER GATEKEEPING IN FLORIDA AND OBSERVABLE IMPACTS ON OYSTER REEFS IN THE SOUTH FORK OF THE LOXAHATCHEE ESTUARY

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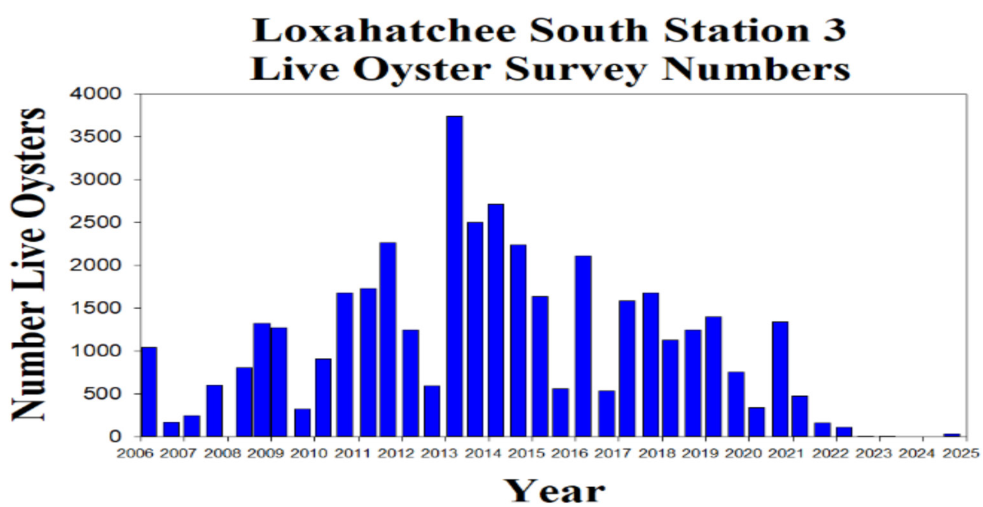
Florida has a long history of water management and control. The initial plan to drain the Everglades for development and agriculture originates in 1881, and in 1907 the Everglades Drainage District was created. This diversion of water flow, in some instances through the use of locks and dams, has had observable impacts on many Florida estuaries including the Saint Lucie, Loxahatchee, and Caloosahatchee Rivers and all of the Everglades drainage basins. One species of concern that lives in these estuaries that is directly affected by this water control is the eastern oyster (*Crassostrea virginica*). Attempts to restore the natural water flow to Florida and also reduce impacts on *C. virginica* were initiated with the Water Resources Development Act in 1996 and finalized with the Comprehensive Everglades Restoration Plan (CERP) in 2000.

This diversion of water flow has had impacts on oyster reefs that can be observed over both short-term and long-term periods. One estuary being monitored as part of CERP is the Loxahatchee River Estuary. Monitoring includes reef survey counts and monthly recordings of water salinity. The North and South Forks of this estuary are areas of focus and have two drastically different freshwater inputs and saltwater tidal influence. The North Fork has more natural water input and the South Fork has more controlled input through the S-46 lock on the C-18 canal. When water control locks are open for short periods the freshwater release may result in distress to eastern oysters that may ultimately result in death of oysters on that reef, however oysters typically recover in less than a year. In contrast, withholding freshwater over long periods may result in elevated estuarine salinity over oyster reefs leading to chronic disease and increased densities of reef pests, ultimately deteriorating the reef structure, which is leading to permanent loss of reef habitat. A better understanding and awareness of when reefs are showing signs of decline due to chronic hyper-salinity can be red flags used to notify water control managers when water releases may be necessary to save these reefs.



COULD ATLANTIC CROAKER *(Micropogonias undulatus)* BE THE NEXT BIG MARINE FINFISH SUCCESS STORY?

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Atlantic Croaker, a drum species native to the Atlantic and Gulf State regions, has a high potential for multi-purpose aquaculture development, including live baitfish, food fish, and restoration. Demand for Atlantic Croaker has soared in recent years, with live bait prices exceeding $3-$5.50 each in Florida and a foodfish market expanding regionally, initiating fisheries management measures. Aquaculture, not increased wild catch, is, therefore, the best method to increase market value and fulfill demand in this fishery without threatening wild stocks. Live Advantage Bait in collaboration with several research institutions such as MOTE Marine Lab and Texas A&M have demonstrated commercial quantities of Atlantic Croaker can be spawned year round, reared with high survival through larval rearing and can reach market size within 4-5 months depending on season and market. We have also demonstrated a huge market demand with the first fully comprehensive economic survey in Florida. Responding to a potential $156 million live bait market in the SE United States, and an unquantified yet significant food fish market, could Atlantic Croaker be the next big marine fish species for aquaculture production? We will make an argument for and against the potential of this new species to U.S. aquaculture.

SURROGATE BROODSTOCK TECHNOLOGY: OPTIMIZATION OF PROTOCOLS FOR ATLANTIC SALMON

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Surrogate broodstock technology (SBT) enables production of donor-derived gametes, and subsequently, offspring *via* surrogates. Germline stem cells (GSCs) can be harvested from donor fish and transplanted into multiple surrogate fish. Subsequently, the transplanted GSCs proliferate, differentiate and develop into functional eggs or sperm, depending on the sex of the surrogate. This allows upscaled production of salmon strains of interest, for instance threatened strains (for conservation purposes) or elite individuals with high robustness (in aquaculture). Furthermore, using cryopreserved GSCs as donor cells opens the possibility to utilize and propagate (long-term) valuable, stored genetic material. In addition, surrogates with shorter generation intervals (early maturing strains or species closely related to the GSC donors) can be used to shorten the time required to produce donor-derived offspring. If implemented in conservation or breeding programs, SBT may become an important tool to achieve more efficient and targeted production of valuable salmon strains.

So far, SBT has been successfully applied to several fish species for the preservation of genetic resources. However, in the case of commercial application technical and operational challenges require attention, for instance the unpredictable success rates of GSC transplantation. Several factors may influence GSC transplantation success, such as the age of donors and surrogates, endogenous germ cells competing with transplanted GSCs, and GSC quantity and purity. The use of immature donors, germ cell-free surrogates, and *in vitro* propagated GSCs, may contribute to increased transplantation success. Nevertheless, each protocol needs to be optimized for each fish species of interest. SBT protocols remain to be developed for Atlantic salmon, an endangered and at the same time highly valuable commercial species. In the "CELLS4TRAITS" project, we aim to optimize protocols for cultivation, propagation, mass-production and preservation of GSCs carrying desired genetic traits (disease resistance and sterility), suitable for mitigating sustainability issues in Atlantic salmon aquaculture. As part of the project, we are currently testing protocols for enrichment and propagation of salmon GSCs *in vitro*, either through 3D or 2D cell culture systems, to obtain a higher supply of GSCs and improved transplantation success.

BEYOND BRINE SHRIMP: FEED ATTRACTANTS AND LIQUID *ARTEMIA* REPLACEMENT DIETS FOR THE CULTURE OF THE ALBINO RAINBOW SHARKMINNOW *Epalzeorhynchos frenatum*

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Brine shrimp *Artemia spp*. nauplii are a popular first-feed used in ornamental aquaculture due to their convenience, excellent digestibility, and appropriate nutritional profile for larval fish, while also provoking an active feeding response with their rapid movements. Major drawbacks are the variability in cost and nutritional profile. Alternatives include inert formulations, such as commercially available microparticulate and liquid microdiets; however, these diets lack intrinsic movement, and thus, larval ingestion rates may not be enough to support the rapid growth and development necessary during this early life stage.

Three amino acid feed attractants (L-alanine, betaine, and L-tryptophan) top-dressed onto commercially available microparticulate diets and two brands of liquid *Artemia* replacement diets (Liqualife® and EZ-Artemia®) were tested in separate investigations to determine if such alternatives could be cost-effective options. Experimental systems consisted of 3L tanks with flow-through filtered water each containing 75 Albino Rainbow Sharkminnow *Epalzeorhynchos frenatum* larvae fed twice daily for 7 to 21-days depending on the experiment. Feed attractants were evaluated in a series of inclusion rate experiments to identify which attractants, and in what amounts, increased survival and growth. For the liquid *Artemia* replacement diets, *Artemia* nauplii were replaced at 50% and 100% with the goal of identifying which brand performed better and if the live *Artemia* nauplii diet could be entirely replaced by a more cost-effective and convenient alternative. Survival and standard length were used to gauge diet success.

Feed attractant enhanced diets showed increases in survival by as much as 10% (Figure 1); however, neither brand of liquid *Artemia* replacement diet showed any increase in survial or growth when compared to the live *Artemia* nauplii reference diet.

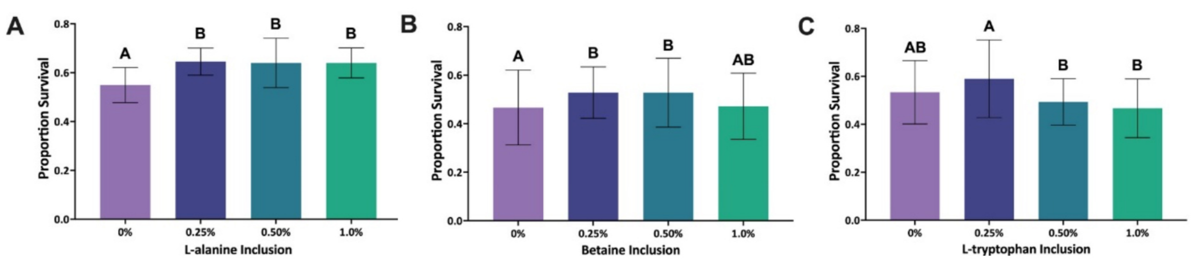

Figure 1. Mean proportion survival (±SEp) of larval *E. frenatum* fed experimental microparticulate diets top-dressed with 0%, 0.25%, 0.50%, or 1.0% L-alanine (A), betaine (B), or L-tryptophan (C) after 7 days of feeding. Different letters above bars indicate statistical significance (P≤0.05, generalized linear mixed model with Tukey's post-hoc test).

CHANGING UP THE PROTOCOL: CAN ALTERNATIVE FIRST FEEDS IMPROVE THE CULTURE EFFICIENCY OF THE CHERRY BARB *Puntius titteya*

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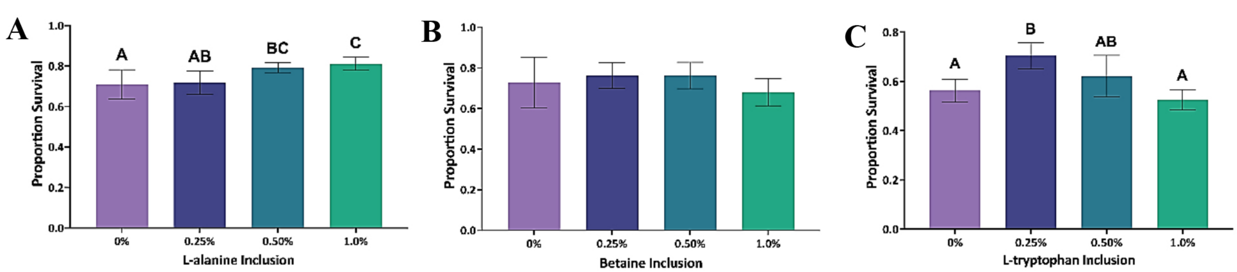
The cherry barb, *Puntius titteya*, is an extensively cultured ornamental fish species in the United States, with a monthly production of approximately 60,000 individuals satisfying current market demands. Although existing culture protocols are reliable, refining these methods could reduce production costs without diminishing yield. A primary expense of *P. titteya* culture arises from the reliance on live *Artemia* spp. nauplii as a first feed, which, despite supporting acceptable larval survival and growth rates, can incur costs related to the purchase of cysts, decapsulation, and feed administration. Inert feeds, such as microparticulate diets (MDs) and liquid *Artemia* substitutes, present viable options with appropriate nutritional profiles and particle sizes. However, the absence of motility in these diets may reduce larval feeding response, risking feed waste, degraded water quality, and wasted money. This issue may be mitigated by enhancing ingestion rates of microparticulate diets with feed attractants—powdered amino acids that stimulate larval olfactory senses to theoretically boost larval feeding and survival. Additionally, liquid diets such as Liqualife® and EZ-Artemia® are designed to mimic the smell, taste, and size of *Artemia* spp. nauplii, thus presumably maintaining the larval feeding response.

Three feed attractants (L-alanine, betaine, L-tryptophan) were evaluated in a series of larviculture trials where attractants were top-dressed onto commercially available diets and fed to larval *P. titteya* for 7 days. Results show L-alanine and L-tryptophan both increased survival with inclusion rates of 0.5% and 0.25%, respectively (Fig 1A, C). Inclusion of betaine did not affect larval survival after 7 days (Fig 1B). Two brands of liquid diets, LiquaLife® and EZ-Artemia®, were tested in a 14-day trial to investigate the potential of replacing either 50% or 100% of *Artemia* spp. nauplii. Neither larval survival nor growth were improved when including liquid diets, however EZ-Artemia® did render better survival than LiquaLife® at 100% inclusion. These results indicate that *Artemia* spp. can be reduced during larviculture of *P. titteya*, however further investigation into weaning practices and feeding density is warranted.

Figure 1. Mean proportion survival (±SEp) of larval *P. titteya* fed experimental microparticulate diets top-dressed with 0%, 0.25%, 0.50%, or 1.0% L-alanine (A), betaine (B), or L-tryptophan (C) after 7 days of feeding. Different letters above bars indicate statistical significance (P≤0.05, generalized linear mixed model with Tukey's post-hoc test).



"WHAT'S IN THE WATER?" Part II: A NEX-GEN STUDY OF SHRIMP POND MICROBIOMES AND THEIR RELATIONSHIP TO SHRIMP HEALTH

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Shrimp health is largely impacted by water quality and the diversity of the microbial populations in the rearing water. Microbes that colonize the shrimp come from the rearing water and sediment. Evidence suggests that environmental factors affect these microbial communities. The shrimp microbiota impacts the development and growth of the organism, the immune system, nutrition and metabolic processes, and the inhibition of pathogenic bacteria. Pathobiome describes the concept that microbial communities interact with the host with the result being either beneficial or harmful.

We have initiated long-term studies of the microbiomes of shrimp pond water with and without disease and with and without treatment. The water microbiome evolves with the changes in the water quality and the microbial species diversity responds to environmental pressures. Our goal is to collect microbiome data from infected ponds, to study the microbial diversity throughout the disease, and to determine triggers for dysbiosis from changes in the microbial content in the rearing pond. This knowledge would allow the development of treatment regimens to reinstate microbial diversity in the pond and the shrimp.

Previously we confirmed WFS infection in two ponds that were at different stages of disease. Microbes from the pond water were collected on filters, DNA was isolated from each pond sample and sent to Charles River Microbial Solution Services for their TAS NGS-based bacterial Identification by targeted amplicon 16S/ITS sequencing (NGS-TAS-16S-20). In 2024, we reported the top ten microbes identified from each pond with conclusions from this data limited.

Subsequently we obtained the full data set with 186,572 reads for Pond A2 and 217,302 reads for Pond A4 with 57% of the reads classified for both samples. The ponds exhibited a similar background of microbes; Pond A2 had 15 Phyla and Pond A4 had 21 Phyla represented in their bacteria populations. The similarity in microbes is not surprising because the ponds were from the same farm, shared the same water supply, contained the same shrimp species, and had the same farming practices. The Phyla that represented over 1% of the microbial population in both ponds were Bacteroidota, Chloroflexota, Cyanobacteriota, Planctomycetota, Pseudomonadota (synonym Proteobacteria), and Actinomycetota. Actinomycetota and Proteobacteria had a high percentage of reads from both ponds. However, Pond A2 with the severe infection had about half the number of Actinomycetota as Pond A4, (21% and 45.60% respectively) while Pond A2 had almost twice the number of Proteobacteria (45.725% and 25.95%).

IMPACTS OF DOUBLE-CRESTED CORMORANT *Nannopterum auritum* ON CATFISH *Ictalurus* sp. AQUACULTURE IN THE BLACK BELT REGION OF ALABAMA AND MISSISSIPPI

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With over 12,000 water hectares in catfish (*Ictalurus* sp.) aquaculture production, and annual production greater than $112 million, the Black Belt region of eastern Mississippi and western Alabama is one of the largest regions of catfish aquaculture production in the U.S. Recent regional declines in farmed water acres highlight the importance of understanding challenges to production, such as fish losses, due to avian predators, especially double-crested cormorants (*Nannopterum auritum*: hereafter cormorant). While considerable research has documented and quantified losses due to cormorants in the Mississippi Delta, little has been done in the Black Belt. Therefore, the objectives of this study were to: 1) assess the distribution and relative abundance of cormorants on catfish farms in the Black Belt, 2) quantify the diet of these cormorants, and 3) evaluate the economic impacts of cormorant predation on catfish production.

During the 2023-2024 field season, we conducted surveys from a fixed-wing aircraft every 2-4 weeks between November-April to estimate the relative abundance of cormorants on a subset of 37 farms in the region. Following each survey, we collected cormorants with firearms for diet analyses from a subset of farms where cormorants were observed during the preceding survey. Collected birds were immediately gavaged with 60cc phosphate buffered saline to halt digestion, placed on ice, and then transported to a lab for necropsy. We then removed the esophagus and proventriculus from collected birds and catalogued prey items.

A total of 74 cormorants were collected during the first field season, with 29 consuming ≥1 catfish, and 33 consuming ≥1 shad (*Dorosoma* sp.). Of cormorants that consumed catfish, most were collected in April (n=12). The average weight of catfish consumed was 80.3 g.

We will use non-linear mixed models to assess spatial and temporal variability among diets. Occupancy and n-mixture models will be used to predict cormorant distribution and abundance, and we will combine this data with bioenergetics models to determine regional catfish consumption and develop enterprise budgets reflecting farm characteristics with and without depredation by cormorants. Results will be used to inform the timing and implementation of management.

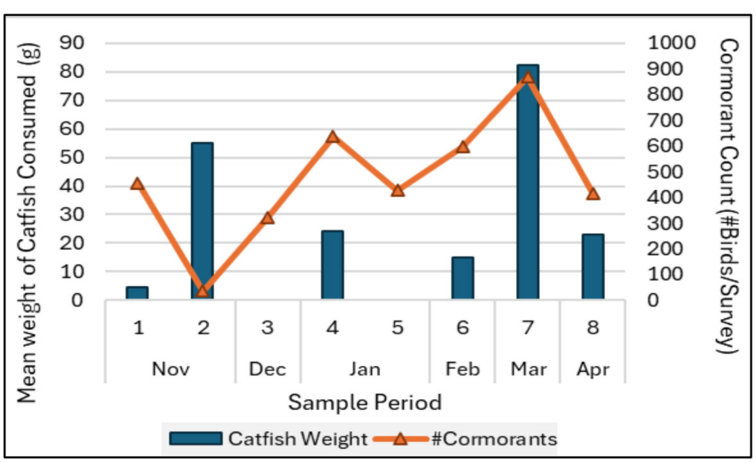

Figure 1: Mean weight of catfish consumed, and number of cormorants counted during each survey period.

INTERACTIVE WORKSHOP: COMMUNITY-LEVEL OPEN CAPABILITY KITS FOR PROTECTING AQUACULTURE GENETIC RESOURCES

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Safeguarding economically important agricultural species has developed into multi-billion-dollar global industries driven by storage, evaluation, and distribution of genetic resources as cryopreserved germplasm maintained in repositories. Aquaculture is following in these footsteps. As the tremendous potential in germplasm repositories is being realized by the broader aquaculture community, a window of opportunity has emerged to shape the future of genetic management and to avoid problems that emerged during the growth and development of modern agriculture. Communities are reevaluating how they move valuable genetic resources around the world. The lack of repository capabilities endangers advances across many industries including aquaculture, conservation programs, natural fisheries, biomedical models, and addressing food security and poverty alleviation. Restricting repository development to individual species or groups will result in disparate and incompatible processes, and therefore better outcomes will be achieved by working at the community and network levels. Open hardware will be critical in supporting this development as devices that are customizable and made available as digital files will accelerate community development, drive innovation, and ensure access to diverse communities. In addition, the generalization of open hardware across organisms and biological levels of organization can provide a foundation for developing repositories and a means for addressing cross-taxa challenges. This will allow leveraging of existing resources and information to bring much-needed scalability and application. In this workshop we will explore the culmination of almost a decade worth of open hardware development demonstrated in the form of an open-capability package (Figure 1). Such packages will combine open-hardware technologies and provide an initial foundation for community members to access cryopreservation in the laboratory or field. These diverse technologies provide a powerful alternative to traditional research and proprietary development by enabling combined efforts across multiple communities to establish and operate germplasm repositories and manage and eventually commercialize genetic resources of aquatic species.

FIGURE 1. Community-level kits will provide capability to enable generalization across species, drive harmonization of outputs, and ensure standardization of processes.

EVALUATION OF THE SURVIVAL AND GROWTH OF GREEN SEA URCHIN *Strongylocentrotus droebachiensis* JUVENILES IN TWO LEASED SITES IN MAINE: LPA/ LEASE IN THE JORDAN RIVER IN FRENCHMAN BAY AND IN AN OYSTER FARM IN THE NEW MEADOWS RIVER IN WEST BATH UNDER DIFFERENT DENSITIES, HOLDING INFRASTRUCTURE DESIGN, AND MACROALGAE DIETS

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The University of Maine – Center for Cooperative Aquaculture Research has been providing sea urchin juveniles *Strongylocentrotus droebachiensis* to growers along the Gulf of Maine. The farmers are developing systems combining their main crops (oysters, scallops, and seaweed) with them which provides an extra income using the infrastructure already established and/or experimenting with the sea urchins to mitigate and control biofouling, especially in the oysters and scallops' gear. Another way the farmers are growing green sea urchin is in modified lobster cages, known as bottom cages.

We designed a project to evaluate the survival and growth of green sea urchin *Strongylocentrotus droebachiensis* juveniles in two leased sites in Maine: LPA/lease in the Jordan river in Frenchman Bay and in an oyster farm in the New Meadows River in West Bath.

We evaluated the effectiveness of two settlement substrates (bio-barrels and wavy polycarbonate plates), the survival and growth of the juvenile in bottom cages at three different stocking densities, and the survival and growth of the juvenile sea urchins using lab seeded sugar kelp and wild-set fouling organisms (macro-alga and invertebrates) in an oyster farm.

Ocean resources implemented the use of bottom cages with three stocking densities (100, 150, and 200 juveniles per cage), and Winnegance Oyster Farm was seeking to add urchins and their feed into an existing oyster farm and test two readily available food sources for suitability as urchin feed: lab seeded sugar kelp and Wild-set fouling organisms (macro-algae and invertebrates).

The survivorship per density at 150 and 200 sea urchin per cage in the Jordan River site was 100%, and 87% for the 100 sea urchin per cage. We attributed this finding to eventual escapes due to the size of the sea urchins at out planting or when the cage was open to add food. The correlations observed in all the cages sampled in the Jordan River site were negative independent of the density. This finding suggested that there was no difference in growth rate amount the different densities, leading to the conclusion that 34-sea urchin per cubic foot could yield a better growth and a more efficient use of the space in the cage. This project in the Jordan River site confirmed that the initial minimum test diameter at out planting is 15 mm.

SET IT AND FORGET IT! A RED ABALONE *(Haliotis rufescens)* AND DULSE *(Devaleraea mollis mollis)* IMTA COMPARISON OF A DUAL AND SINGLE TANK DESIGN.

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Integrated Multi-Trophic Aquaculture (IMTA) with abalone and seaweed offers promise as a climate-resilient system that may mitigate negative effects from ocean acidification, while simultaneously increasing production. This study tested new IMTA innovations for the co-culture of red abalone (*Haliotis rufescens*) and dulse (*Devaleraea mollis*) in traditional dual tanks vs. a single tank system. We built upon a previous study where a 65% recirculation rate in a dual tank system significantly buffered pH and resulted in greater abalone growth and compared it to a single tank design, with flow rates of 0.7, 1.4, and 4.0 gallons per minute. After 6 months, there were no significant differences in total length and weight of abalone in any of the treatments, though on average, abalone grown in the 0.7 GPM treatment exhibited the poorest body condition (g/mm²). Abalone were sacrificed, and their shell strength was quantified with a materials testing device that measured the force required to fracture their shell. There were no differences between the treatments, but there was a trend towards individuals in the paired design having stronger shells. The growth of dulse was positively correlated with flow, where low-flow tanks grew significantly less. Given the lack of significant differences between the dual tank and the single tank designs in the medium and high flow tanks, single tank systems may offer a viable cost-savings by removing pumps, significantly reducing maintenance needs and labor, and by doubling abalone production in the same footprint.

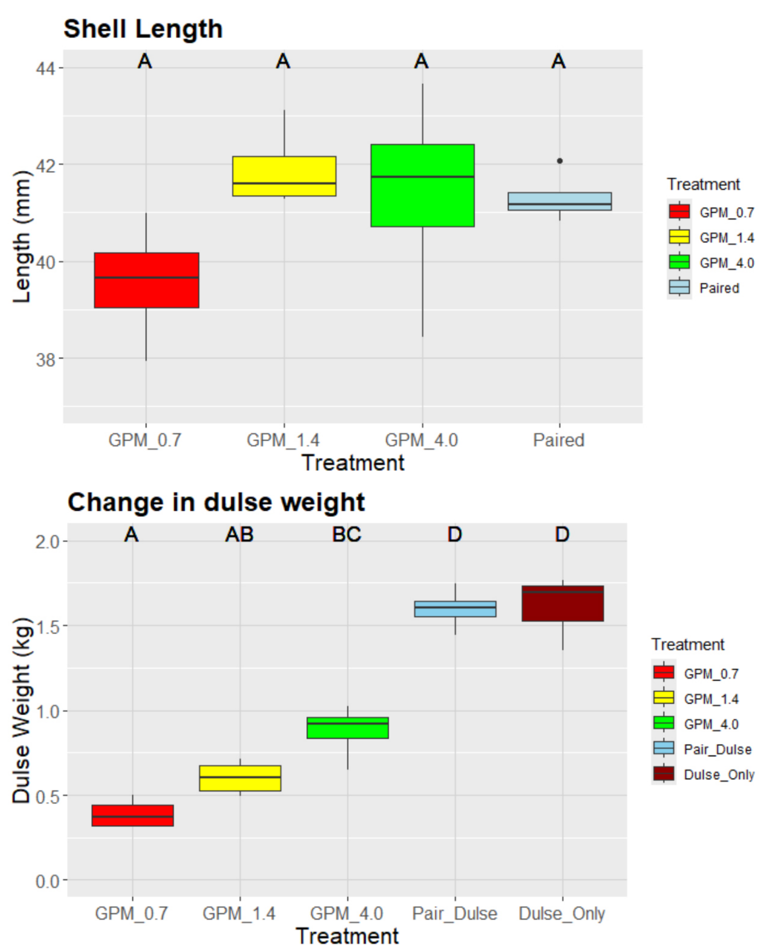

Figure 1. Total shell length after 6 months in treatments (top) and average weekly growth of dulse in each treatment (bottom). Shared lettering indicates statistical similarities (ANOVA p-value >0.05).

ANNOTATION OF FOUR RAINBOW TROUT GENOMES IN SUPPORT OF A PAN-GENOME REFERENCE

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Rainbow trout (*Oncorhynchus mykiss*) are a widespread aquaculture species and a model organism for fish research. Currently, there are four chromosome-level rainbow trout genome assemblies available. The first genome (Omyk_1.0) was assembled from the Swanson line, a line of rainbow trout from Alaska that has been in hatcheries for two generations and is thus classified as "semi-domesticated". The second rainbow trout genome assembly, USDA_OmykA_1.1, came from a different clonal line, Arlee, which is fully domesticated and originated in northern California. The other two genome assemblies are from wild fish that were captured in the Whale Rock Reservoir in southern California (USDA_OmykWR_1.0) or Keithly Creek in Idaho (USDA_OmykKC_1.0). Annotation of protein coding genes from the four genomes is needed to enrich the reference transcriptome and to enable pan-gene comparative analyses. However, a high-quality RefSeq annotation from NCBI is currently only available for the Arlee reference genome assembly.

Here, we developed a bioinformatic annotation pipeline to generate a reference transcriptome for each of the four genome assemblies using the Comparative Annotation Toolkit, with the Arlee RefSeq gene set as the reference, along with the BRAKER3 pipeline for the incorporation of novel gene predictions. Input for gene models came from public rainbow trout RNA-seq data and the OrthoDB database. New long-read transcriptome (Iso-Seq) data that we generated from a disease resistance study was used for discovery of novel genes and transcript isoforms in all four genomes. For functional annotation of the predicted gene models, we leveraged the Arlee RefSeq gene set, as well as the InterPro database to predict protein domains, gene ontology and pathways.

Additionally, to better understand the impact of rainbow trout genome structural variation on gene structure and content, we used the program MCScanX to identify syntenic blocks based on gene order collinearity among the four genomes. The synteny information will enable us to identify gene differences that may be associated with differences in the life history and evolution of the four genetic lines.

Overall, the annotated rainbow trout genomes and synteny dataset provide vital resources for the aquaculture research community and for basic research on the physiology, genetics and evolution of rainbow trout.

EFFECT OF SEASON AND STRESS ON PREVALENCE OF WHITE SPOT SYNDROME VIRUS IN *Procambarus clarkii*

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White spot syndrome virus (WSSV) is a significant pathogen impacting crustaceans globally, posing a threat to aquaculture industries. In the United States, WSSV has been documented in Louisiana crayfish farms since 2007. In 2022, *Procambarus clarkii* housed in laboratory raceways at the E.W. Shell Fisheries Center in Auburn, Alabama, exhibited lethargy and mortality patterns consistent with WSSV. qPCR assays later confirmed the presence of WSSV, indicating the first documented case of this disease in Alabama.

From 2022 to 2023, crayfish surveys conducted across two watersheds revealed that WSSV was both widespread and seasonal, affecting *P. clarkii* and other crayfish species. The highest WSSV detections occurred when water temperatures ranged between 18 and 30 □. Given that pathogens often become more virulent under stress, we hypothesized that laboratory stressors could activate WSSV in otherwise healthy carriers. To test this, crayfish suspected of carrying WSSV but testing negative were subjected to PIT-tagging, a stressful procedure, while a control group was left untagged. Both groups experienced 100% survival over 12 weeks, with only two borderline positive WSSV detections in the control group at the end of the study.

This study demonstrates that WSSV is now endemic in parts of Alabama, affecting multiple crayfish species during warmer months. However, our results suggest neither warm temperatures nor laboratory-induced stress reliably trigger virulence or detectable WSSV levels at the study site. Further research is necessary to identify the specific conditions under which WSSV becomes pathogenic, leading to high mortality rates in crayfish populations.

THE SUCCESSES AND CHALLENGES OF GROWING BULL KELP IN ALASKA

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Bull kelp (*Nereocystis luetkeana*), found along the North American west coast, is a species of kelp highly prized for both its ecological and its unique palatability for human consumption. Currently, all of the bull kelp on the market is sourced from wild harvest, but with permit-based supply limitations and climate change threats to the persistence of bull kelp forests, farmers and producers are looking to develop bull kelp farming techniques to source this desirable species. However, due to the unique morphology of bull kelp (i.e. hollow stipe, highly buoyant), traditional kelp farm arrays have not been successful in producing a desirable product. This past year, the Bull Kelp Research Squad (BKRS), a working group of bull kelp farmers and researchers in Alaska, was formed to address the successes and challenges of farming bull kelp and collaborate to develop a proof of concept that bull kelp can be farmed. Over the past year, we have shared innovations in farm and experimental design, discussed the successes and challenges of growing bull kelp, compiled a list of prioritized questions for future research, written an SOP for monitoring across different experimental and farm designs, and submitted proposals for further collaborative research. By collaborating and sharing designs for innovative array designs and seeding methods, BKRS is establishing the basis of an industry that could be unique to Alaska.

EVALUATION OF A RAPID DISEASE DETECTION METHOD FOR VIBRIO IN MARINE WARM WATER FINFISH AQUACULTURE

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Rapid and accurate detection of bacterial diseases is critical to maintaining healthy stock in commercial warmwater marine finfish aquaculture. Intensive rearing conditions and environmental factors (i.e., suboptimal stocking density, water temperature, salinity) can induce stress and increase fish susceptibility to infection by ubiquitous Vibrio species that are opportunistically pathogenic. This study focuses on developing molecular techniques for use in an aquaculture facility to detect the presence of *Vibrio harveyi.* We compare the effectiveness of two different screening methods for *V. harveyi*: 1) a portable PCR system, miniPCR® mini16 thermal cycler (Cambridge, MA) under farm conditions, and 2) a benchtop PCR system, EDVOTEK EdvoCycler Jr.® (Washington, DC) in a laboratory setting. Additionally, we assess the direct input of unpurified samples versus purified DNA as template for PCR.

Biofilm samples were collected from aquaculture tanks housing red drum (*Sciaenops ocellatus*) at FAU Harbor Branch Oceanographic Institute. Both crude swab samples and extracted DNA samples were tested on the mini16 in the field and the Edvotek thermocycler in the lab. PCR was conducted with Platinum™ Direct PCR Universal Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA) to amplify a 601 bp DNA fragment of the *rpoB* gene in *Vibrio harveyi*. A pure *V. harveyi* culture (ATCC 14126) served as the positive control. PCR products were visualized via gel electrophoresis to compare the DNA band size and intensity among samples. We expect to successfully employ both methods for *Vibrio harveyi* detection, with potential differences in the quality of PCR results between the direct input of unpurified samples and purified DNA.

This study aims to provide insights into the feasibility of rapid, field-based disease detection methods in aquaculture, offering a comparison of the efficacy between field-deployable and traditional lab-based PCR techniques. These results could streamline diagnostic workflows and support better management practices in warmwater marine finfish aquaculture. Our overall goal is to develop rapid and early disease diagnostic tools that can be easily accessible to producers and veterinarians to assess fish health at a commercial production level.

EFFECTS OF HYPOXIA ON EARLY DEVELOPMENT AND LIPID UTILIZATION IN AQUACULTURE SPECIES: FLORIDA POMPANO *Trachinotus carolinus* and RED DRUM *Sciaenops ocellatus*

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The increasing distribution and intensity of hypoxia (low dissolved oxygen, DO), exacerbated by cold weather-induced stratification, eutrophication, and algal blooms, poses a significant challenge for aquaculture in regions such as the Gulf of Mexico and Florida's Indian River Lagoon. This study investigates how low DO impacts early development and lipid utilization in two commercially important aquaculture species: the Florida pompano (*Trachinotus carolinus*) and red drum (*Sciaenops ocellatus*).

Fertilized eggs from each species were individually incubated under three DO conditions: severe hypoxia (20% saturation, 1.6 mg/L), moderate hypoxia (50% saturation, 3.9 mg/L), and normoxia (100% saturation, 7.6 mg/L). At 24 hours post-fertilization, larvae were sampled to assess hatch survival, development, and fatty acid (FA) lipid utilization. Severe hypoxia led to complete mortality in Florida pompano within 24 hours, while moderate hypoxia altered body structure and oil droplet length, with significant changes in polar FAs. In red drum, moderate hypoxia affected body and yolk length, with neutral FA utilization most impacted. Specific relative percentages of FAs between each species will also be discussed. These findings highlight species-specific differences in lipid metabolism under hypoxic stress, offering critical insights for optimizing conditions in aquaculture hatcheries to improve survival and development under suboptimal oxygen levels.

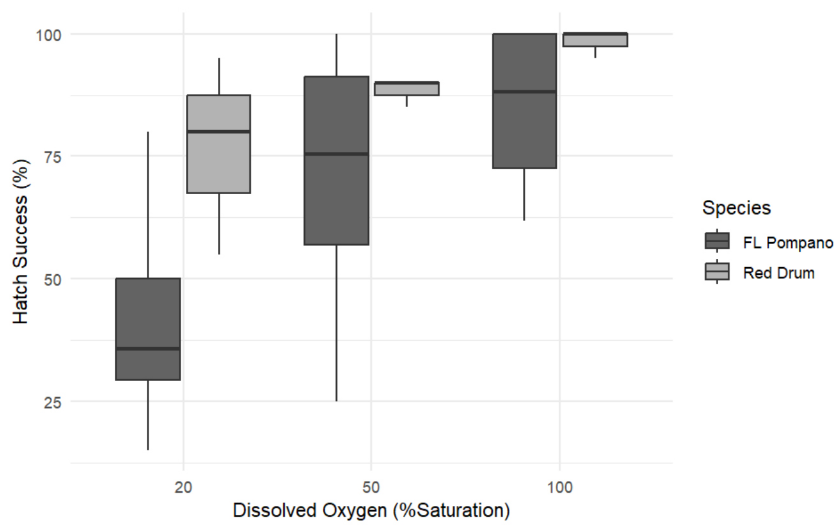

Figure 1. Distribution of percent hatch success across dissolved oxygen saturations (100, 50, 20%) for Florida pompano (*Trachinotus carolinus*) and red drum (*Sciaenops ocellatus*).

THE DYNAMICS OF WATER QUALITY, MICROBIAL DIVERSITY IN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) OF SEA URCHINS, MULLET FISH, AND GREEN SEAWEED FOR SUSTAINABLE MARICULTURE

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A vital goal of sustainable aquaculture is understanding the microbial community assembly and dynamics under different settings, thus providing insights for more holistic management and manipulation. Regarding this, we explored the microbial communities in an Integrated Multi-Trophic Aquaculture (IMTA) system composed of sea urchins (*Paracentrotus lividus*), mullet fish (*Mugil cephalus*), and green seaweed (*Ulva fasciata*) as a biofilter. Over twelve weeks, the system was monitored for water quality parameters, and samples were taken for 16S rRNA amplicon sequencing to characterize and determine the influence of water quality parameters on the microbial community in the system. The study highlights the essential role of microbial communities in sustaining the ecological balance and functionality of IMTA systems. Water samples exhibited higher microbial diversity, driven by dynamic environmental conditions, while host-associated microbiomes showed niche specialization, reflecting their roles in nutrient assimilation, immune support, and detoxification. *Ulva fasciata* demonstrated its effectiveness as a biofilter, maintaining stable nutrient loads and microbial composition. The presence of beneficial microbes, such as Pseudoalteromonas, Rubidimonas, Granulosicoccus, Nonlabens, and Alteromonas, and the lower abundance of *Vibrio spp*. underscore the potential of *Ulva* in pathogen control and nutrient cycling. Nutrient availability, particularly nitrogen and phosphorus, influenced microbial interactions, which shaped community structure. Temporal shifts in microbial composition due to water quality changes were buffered by the system's resilience, particularly through the stabilizing effects of *Ulva*. These findings reinforce the importance of microbial diversity and interactions in improving sustainability and productivity in IMTA systems. The study provides a foundation for understanding the microbial community in IMTA in enhancing nutrient recycling, pathogen control (using *Ulva* as a biofilter), and system stability, paving the way for more sustainable aquaculture practices.

FRESHWATER MUSSELS: GLOBAL BELLWETHERS AND ENHANCERS OF AQUATIC ECOSYSTEM HEALTH

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Freshwater mussels (Bivalvia: Unionoida) inhabit many tidal and non-tidal freshwater settings, including natural systems (streams, rivers, ponds and lakes) and novel, man-made systems (stormwater ponds, living shorelines). Globally, there are more than 1,000 species, but they are one of the most imperiled animal groups due to their complex life histories and sensitivity to habitat and water quality degradation. This is concerning because freshwater mussels often exist in dense "mussel beds" that furnish diverse ecosystem services, much like their marine counterparts. For example, mussel beds help stabilize erosion, enrich benthic habitats for other plants and animals, and offset some types of water quality impairments (e.g. excess nutrients, pathogens). Expanding the aquaculture toolkit to include freshwater mussels can unlock opportunities to promote resilience and ecosystem services in a greater array of niches along the river-to-sea continuum, including impaired urban areas that need the greatest attention.

Projects to conserve, restore, or enhance freshwater mussels have been hampered because the causes of declines still exist in many areas, and wild populations are too depleted to supply animals for restoration projects. Fortunately, this problem is being solved by recent advances in hatchery propagation and aquaculture. Hatchery-sourced mussels have been successfully reintroduced and used to augment extant populations. In recent pilot studies, mussels also survived and grew quickly in altered and man-made habitats, such as living shorelines and stormwater ponds, respectively. While promising, some questions remain regarding the magnitude and geospatial extent of the potential ecosystem service uplift from mussel investments. Like their marine counterparts, freshwater mussels tend to aggregate and therefore do not provide universal benefits everywhere. Causes of past mussel declines persist in many areas. Genetic and biosecurity risks need to be carefully managed. Nevertheless, there appears to be no shortage of locations in most watersheds where mussel investments could be viable, thereby expanding the toolkit of nature-based tactics aimed at improving aquatic ecosystem health. Freshwater mussels are also excellent subjects to stimulate community-based science and educational interest in aquatic ecology because they are sessile, long-lived, and can be seeded and monitored in accessible aquatic habitats along the urban-rural spectrum.

Mussel investments for ecosystem services have been largely unexplored because managers, scientists and funders are still mainly focused on rare species conservation in places where they still exist. Regarding ecosystem services, the greatest return on investment will result from working with foundation species in impaired habitats, typically near underserved communities. Once established, mussel beds can be augmented with rarer species, many of which are usually found only within a protective mussel bed. Hence, extending "shellfish aquaculture" to include noncommercial organisms such as freshwater mussels can help promote environmental awareness, water quality, and climate resilience in places that need it the most.

SPONGE RESTORATION AQUACULTURE: REPRODUCTIVE VIABILITY FOLLOWING *IN SITU* PROPAGATION FOR 4 NEARSHORE SPONGIIDAE SPECIES IN THE FLORIDA KEYS

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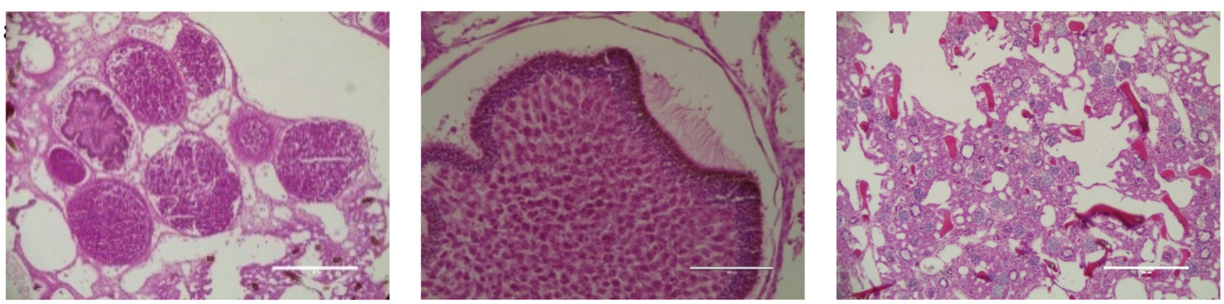
Since 2010, a coalition of natural resource managers and scientists have engaged in sponge restoration to mitigate a series of sponge die-offs in the nearshore hardbottom habitats of the Florida Keys. Sponge restoration aquaculture is a viable tool to accelerate the natural progression of sponge recolonization following ecological disturbances (e.g., algal blooms and hurricanes). For sponge restoration aquaculture to be successful, it is important that aquacultured propagules become reproductive to sustain future populations. There is a need to survey the reproductive capacity of restored sponges to evaluate recruitment dynamics as natural resource managers work to re-establish nearshore sponge communities associated with seagrasses. Using histology and light microscopy, gametogenesis was documented for 4 Spongiidae species (sheepswool sponge *Hippospongia lachne,* yellow sponge *Spongia barbara,* glove sponge *Spongia graminea,* and grass sponge *Spongia (S.) tampa*) in a Florida Fish and Wildlife Conservation Commission-permitted sponge nursery near Key West, Florida. Preliminary results reveal gametogenesis in 88% of the sampled Spongiidae propagules (n=95 histology samples from 4 species) 4-7 years following *in-situ* asexual aquaculture propagation. During April, May, and June, 69% (n=66) of the sponges were spermatogenic and 19% (n=18) were in oogenesis. This study will answer key questions about sponge reproductive biology in support of the current state of Florida sponge restoration and management efforts.



A B C

A Sheepswool sponge *Hippospongia lachne* oogenesis (scale bar 400 microns). B *H. lachne* ciliated pre-larva (scale bar 100 microns). C Grass sponge *Spongia (S.) tampa* spermatogenesis: individual spermatozoa visible within spermatic cysts (scale bar 400 microns). Photo credits: Shelly Krueger

DIFFERENT MANAGEMENT STRATEGIES FOR ARTIFICIAL SUBSTRATES ON NITRIFICATION PROCESSES OF *Penaeus vannamei* IN A SUPER-INTENSIVE BIOFLOC SYSTEM

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The aim of this study was to test the effect of different management strategies for artificial substrates on the nitrification process, microbial composition of water and biofilm, and growth of *Penaeus vannamei* in a super-intensive biofloc system.

The research was conducted over 60 days using experimental units of 200L and had the following treatments: T1 – artificial substrate + biofloc + shrimp + aeration (control), T2 - artificial substrate + water + aeration, T3 - artificial substrate + water, and T4 - only artificial substrate. The experiment was divided into two phases. Phase 1 involved maintaining the artificial substrates under the management strategy for 30 days. Phase 2 involved the use of substrates from phase 1 in a shrimp nursery for 30 days. Pre colonized artificial substrates (Needlona®) were employed at a ratio of 200% of the tank's lateral area to promote biofilm growth. In phase 1, T1 used a stocking density of 500 shrimp m^{-3} (9.72 ± 0.50g).

In phase 2, shrimp weighing 0.10 ± 0.05g were stocked at a density of 1750 shrimp m^{-3} and all treatments included artificial substrates from phase 1, aeration, water, and shrimp. Molasses were the carbon source organic used. In phase 2, ammonia control was observed in treatments T2, T3, and T4 from the 10th day onwards. T4 had a nitrite spike, controlled from day 14 onwards, suggesting recovery of the nitrifying bacteria community. At the end of the phase 2, T2 and T4 showed higher abundance of coccoid bacteria in the biofilm compared to T1 and T3. T4 also had more bacillus.

The shrimp final weight was higher in T2 compared to the other treatments. These findings suggest that maintaining the substrate submerged in water (T3) can be considered practical management for artificial substrates and that it does not limit the nitrification process between culture cycles. Furthermore, exposure of artificial substrates to air (T4) also did not affect the nitrification process, leading to the recovery of the bacterial community, and the proliferation of various bacterial groups.

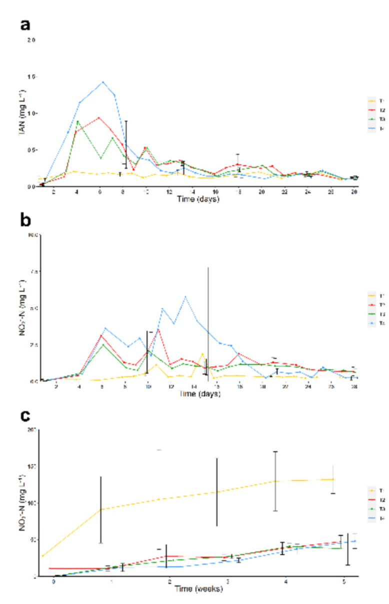

Figure 1. Mean values of nitrogen compounds (mg L^{-1})

THE EFFECTS OF PROTEIN COATED MICROPARTICULATE DIETS (PCMD) ON THE GROWTH, SURVIVAL, WHOLE-BODY NUTRIENT CONCENTRATIONS AND PROTEIN REGULATION OF CALIFORNIA YELLOWTAIL *Seriola dorsalis* LARVAE

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In marine finfish aquaculture, one of the greatest obstacles faced by producers, is the rearing of larvae and propagation of healthy juveniles. Providing adequate nutrition to early-stage marine finfish has proven difficult due to a number of technical challenges associated with the small artificial feeds (*i.e* microdiets) used to feed them. Microdiets tend to leach their water-soluble nutrients at very high rates when suspended in water, largely due to the high surface area-to-volume ratios of these small (100-1500 µm) particles. Leaching results in reduced payload delivery to target organisms as well as nutrient loading in the surrounding water. Nutritional deficiencies result in lower growth and survival and can have delayed effects on later life stages. In the present study, we evaluated novel coating technologies as a means of reducing the leaching of water-soluble nutrients from commercial microdiets to improve nutrient delivery to larval finfish.

Scanning electron microscope (SEM) images indicated that the protein coatings were effectively applied to the surface of commercial-type microdiets resulting in smooth surfaces with reduced porosity (Fig. 1). One of these specialized coatings was then applied to a modified open-formula microextruded marumerized (MEM) particle, designed for marine finfish larvae; hereafter, referred to as the "Protein Coated Microparticulate Diet" (PCMD). This diet was compared with similarly formulated uncoated microdiets and a commercial microdiet (Otohime) in benchtop, acceptability, and growth trials using larval *Seriola dorsalis* (California yellowtail). Protein coatings decreased the leaching of water-soluble nutrients while increasing particle stability of the PCMD's when suspended in water. The results of the acceptability trial indicated that coated microdiets were consumed at equal rates when compared to non-coated microdiets. No significant differences in larval growth were observed at the end of the growth trial (Tukey's HSD, $p<0.05$), however, larvae fed PCMD's trended higher than those fed uncoated diets.

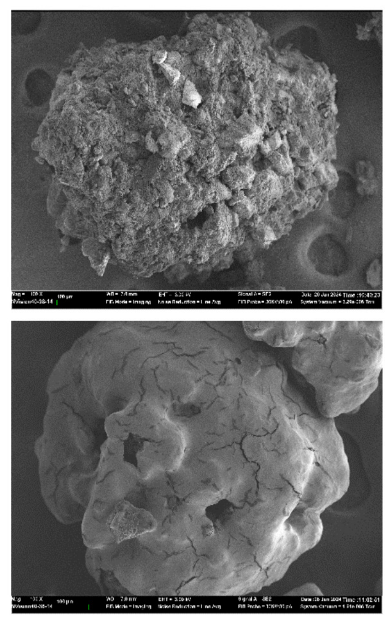

Figure 1. SEM images of uncoated (top) and protein coated (bottom) commercial diet. Taken on NVision 40 SEM at 100x magnification.

The reduced leaching of water-soluble nutrients by PCMD's resulted in improved nutrient delivery and should reduce nutrient loading of larval culture systems. These coating technologies appear to be applicable to a wide range of commercial-type diets and may provide an opportunity to greatly improve the early life nutrition of marine finfish larvae.

AQUACULTURAL ENGINEERING SOCIETY - RAS FUNDAMENTALS: OXYGENATION

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When it comes to running a successful and profitable aquaculture farm it is critical to avoid fish loss while maximizing growth and feed efficiency. There are many factors that can contribute to the successful production of fish and shrimp, however, if you do not have suitable dissolved oxygen in the water, the farm will be guaranteed to fail. Oxygen is as critical to aquatic animals as it is to human life.

Managing suitable dissolved oxygen levels is accomplished through two processes: aeration and oxygenation. Aeration is the process of mixing ambient air with water. Oxygenation is the process of delivering pure oxygen to water. While these two technologies deliver oxygen to the water, they are different processes that have their own benefits and drawbacks. This module will cover topics such as oxygen transfer rates, how environmental factors affect oxygen transfer and saturation, differences among aquatic species, oxygen demands of a system, how to design aeration and oxygenation systems, and an overview of the novel technologies the supplier industry has to offer.

FACTORS INFLUENCING U.S. CATFISH SUPPLY

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As one of the most successful and established U.S. aquaculture sectors, the U.S. catfish industry has experienced notable fluctuations over the years. While there has been an overall upward trend in the farmer's price and production efficiency due to technological advancements and effective production strategies, the farming area has witnessed a noticeable decline over the last two decades. Yet the production volumes have stabilized over the last decade. This study investigated the factors influencing domestic catfish supply from farms. Using 25 years of supply data (Figure 1), this study focused on establishing a relationship between the round weight processed and various factors including feed prices, the area under farming, and macroeconomic indicators like soybean and corn mill prices, imports, population, inflation, and unemployment rates. This paradoxical situation called for a detailed study. A Trans-log generalized regression model indicate d an inverse relationship between feed prices and the quantity of catfish supplied. Conversely, a positive relationship exists between the area under production and the quantity of catfish supplied, suggesting that increased production area boosted catfish production. Macroeconomic factors played a significant role as well; with population growth and inflation positively influencing the quantity supplied, as producers increase output to meet rising demand and counter inflationary pressures. The findings highlighted the influence of various micro and macro-economic factors that shaped this vital U.S. aquaculture sector.

DIETARY INCLUSION OF WHOLE INSECT LARVAL MEAL AND ORGANIC ACID (SODIUM BUTYRATE) IMPROVE THE SOYBEAN MEAL UTILIZATION IN RAINBOW TROUT, *Oncorhynchus mykiss*

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High inclusion of soybean meal (SBM) in aquafeed induces enteritis (SBMIE) and ultimately reduces nutrient utilization and decreases the growth performance of carnivore species, including rainbow trout (*Oncorhynchus mykiss*). Based on the available literature, insect meal (black soldier fly larvae, BSFL) and organic acid (sodium butyrate, SB) exhibited positive effects in terms of improving the growth performance and health of fish. This study aimed to investigate the effects of supplementation of BSFL meal and SB in SBM-based diets on growth performance, feed conversion ratio, and distal intestinal structure of trout.

A total of 855 fish (30 g) were distributed into 9 treatments with five replicates (19 fish/tank). Nine experimental diets (42% crude protein and 20% lipid): fish meal-based diets (FM), SBM-based diets (SBM_{30}, 30%SBM and SBM_{40}, 40%SBM), $SBM_{30+BSFL}$, 30%SBM+5%BSFL, $SBM_{40+BSFL,}$ 40%SBM+5%BSFL, $SBM_{30+SB,}$ 30%SBM+0.2%SB, $SBM_{40+SB,}$ 40%SBM+0.2%SB, $SBM_{30+BSFL+SB,}$ 30%SBM+5% BSFL+0.2%SB, and $SBM_{40+BSFL+SB,}$ 40%SBM+5% BSFL+0.2%SB are being fed twice at satiation level for 16 weeks. Fish were sampled every four weeks for 16 weeks. All the data were subjected to one-way ANOVA using R programming.

At the end of the feeding trial, the growth performance, feed utilization, and distal intestinal histology of the trout were measured. The results showed that there was no significant difference in average weight (AVG) and weight gain (%) at the 4th, 8th, and 16 weeks among the dietary groups ($p > 0.05$). However, in the 12th week, the trout fed SBM40 registered a significantly lower value, while those fed SBM_{30+SB} recorded a higher response ($p<0.05$). The feed conversion ratio (FCR), protein efficiency (PER), and HSI value did not differ ($p > 0.05$) among the dietary groups. The histological results did show that the trout fed soy diets appeared to have a loss of vacuolization in the villi and increased lamina propria thickness within these folds. Shortening and thickening of the folds were most apparent in the $SBM_{40+BSFL}$, the SBM_{40+SB}, and the $SBM_{30+BSFL+SB}$, but the $SBM_{30+BSFL}$ appeared similar to the control diet. Genes associated with growth performance, oxidative stress, gut barrier integrity and acute inflammatory-related cytokines and chemokines, NF-kB and TNF-α-related genes, and regulators of B and T lymphocytes function are being analyzed. Additionally, gut microbiome data are being analyzed.

Based on the results obtained in the present study, it is suggested that 5% whole larval insect meal or 0.2% sodium butyrate may be used as a complementary additive in high soybean meal diets for rainbow trout.

A NUTRIGENOMIC STRATEGY TO DETERMINE THE OPTIMUM LEVELS OF SOYBEAN MEAL AND SOY PROTEIN CONCENTRATE FOR THE ENHANCEMENT OF NON-SPECIFIC PATHOGEN RESISTANCE IN RAINBOW TROUT

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Soy-based proteins are core ingredients in rainbow trout (*Oncorhynchus mykiss*) diets. The goal of the study was to increase the usage of soybean meal (SBM), decrease the soy protein concentrate (SPC) in trout feeds and determine the strain-specific resistance against bacterial and viral pathogens. The study was composed of two phases, phase I: pre-challenge study, fish feeding trial for growth performance followed by phase II, a disease challenge trial – viral (Infectious hematopoietic necrosis virus, IHNV) and bacterial (*Flavobacterium psychrophilum*, Fp) pathogens.

Phase I: In 8 weeks feeding trial, 1500 fish (4.2 g) were distributed into 10 treatments in triplicates (50 fish/tank) following a 2 × 5 factorial design, where two types of rainbow trout strains, i.e., commercial strain 1 (CT1) vs. commercial strain 2 (CT2) were fed with 5 different isonitrogenous (44% crude protein) diets with varying ratios of SBM to SPC: Diet 1 (control), 0% SBM/SPC + 30% fishmeal; Diet 2, 11% SBM + 18% SPC; Diet 3, 22% SBM + 13% SPC; Diet 4, 33% SBM + 6% SPC; and Diet 5, 44% SBM + 0% SPC. Phase II: After 8 weeks of feeding trial, fish from their respective treatments were combined and split into two groups randomly. One group was challenged with IHNV 220-90 by i.p. with 4000 pfu/fish, the second group was challenged with Fp by i.m. with 1.21 x 10^7 cfu/fish.

Based on the growth performance, increasing the SBM up to 33% with a reduced SPC of 6% (Diet 4) showed no significant ($p > 0.05$) difference with the control (Diet 1). For the CT1 with IHNV challenge, fish fed diet 3 had the highest Cumulative Percent Mortality (CPM) at 17.78%±0.12, while the fish with diet 5 had the lowest CPM at 13.33%±0.07, but the IHNV CPMs did not show significant difference among treatments ($p<0.05$). For the CT1 with *Fp* challenge, fish with diet 3 had the highest CPM at 46.67%±0.12, while the fish with diet 1 had the lowest CPM at 22.22%±0.21, but the Fp CPMs did not show a significant difference among treatments ($p<0.05$). Similar results were observed in the CT2 challenges, no significant difference among treatments. When comparing the same diet between the two strains, a significant difference was observed between CT1-Fp-C (46.67%±0.12) and CT2-Fp-C (15.56%±0.04) ($p=0.0326$). A significant difference was also observed between CT1-Fp-D (28.89%±0.04) and CT2-Fp-D (11.11%±0.04) ($p=0.0048$). No significant difference was found for the other diet between the two strains. Antibody responses to IHNV or *Fp* were detected at 7 and 28 dpi in fish exposed to the respective pathogens; however, there were no significant differences among different diet treatments for both strains CT1 and CT2. The results revealed that different fish strains fed different diets did not show a significant difference in lysozyme activity.

The distal intestinal histology is being analyzed. Genes associated with gut barrier integrity, acute inflammatory-related cytokines and chemokines, NF-kB and TNF-α-related genes, and regulators of B and T lymphocyte function are being analyzed. Overall, CT2 strain performed better than CT1 strain in terms of growth performance and disease resistance against pathogens.

IRRIGATION AND INFRASTRUCTURE DEVELOPMENT ON RIVER AND THEIR IMPACTS ON ECOSYSTEM AND MIGRATORY FISH POPULATIONS

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River development for irrigation and water supply profoundly impacts global aquatic resources. The expansion of irrigation infrastructure is crucial to meet the increasing food demands of a growing global population, and it is expected to grow substantially in the next two decades. However, it is important to acknowledge that this infrastructure can adversely affect aquatic fauna, particularly fish. Fish are highly vulnerable to changes in flow patterns, obstructions in migration pathways, and limited access to vital habitats. Failure to consider and protect the migration needs of fish populations during the design and installation of infrastructure can result in significant environmental, social, and financial consequences. This issue is prevalent worldwide, as evidenced by numerous examples. Two key points to highlight are the shared challenges faced by many countries about fisheries impacted by river infrastructure development, and the potential for substantial environmental, social, and financial costs if the migration needs of fish populations are not considered and safeguarded during the design and installation of infrastructure. The expansion of irrigation infrastructure poses a threat to the diversity of freshwater fish on a global scale, as it creates barriers that impede their access to crucial nursery, feeding, and spawning habitats.

Fish passage involves movement in both directions, not just upstream migration. While there is typically a focus on helping fish move upstream past barriers, it is equally crucial to facilitate downstream movement. This is especially critical for diadromous species like eels, which must migrate downstream to reach their spawning grounds. It is essential to comprehend the impact of hydropower and irrigation systems on downstream migration to devise successful solutions.

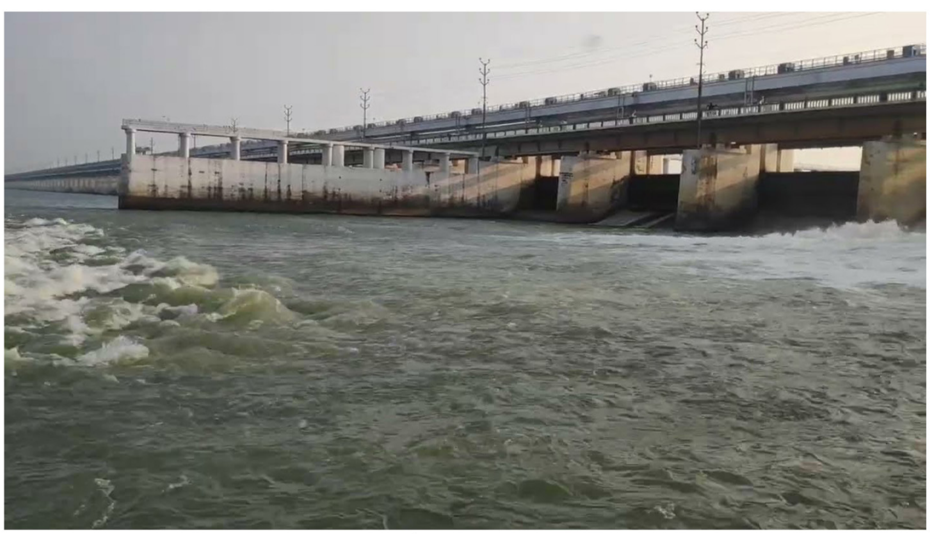

Fig: Barrage

THE ALMASI PROJECT: ENVIRONMENT AND SUSTAINABILITY MESSAGES FOR CHILDREN IN AFRICAN DIGITAL ANIMATED FORM

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Almasi meets a magic butterfly who becomes her spirit guide to the wilderness. She demonstrates a way of being; to her everyone is a person, including trees, fish, and beetles, to whose needs we can pay attention. As humanity leaves our patrimony of privation and poverty behind, the next generations have the opportunity to grow up loving the wilderness, not fearing it. We now have the technology to develop in ways that support wild ecosystems to flourish alongside us. Almasi & her cousins start their adventures among the mangroves of Mida Creek because these represent the most crucial and delicate environments on Earth, as fish nurseries and preventing erosion.

We present an animation that is high-tech and labor intensive, in 52x10-minute episodes. Artificial intelligence (AI) is driving animation costs down around the world, but we are still at a lower price point than AI. Soft power – African animation has the power to preserve our culture and present our stories to children globally for years. We aim to transform development and conservation, to give animals the freedom of the land; to cause an interconnected network of wildlife conservation areas. Across Kenya, and one day across Africa. Multimedia University of Kenya has proven competence and the certainty of completion. Episodes 1 and 2 of Almasi are available online on YouTube in full. Completed entirely by the animation graduates in Nairobi. Our animation costs are $2,000 a minute compared to $4,000 in the Philippines, the world's principal outsourcing destination for animation. Artists work within a profit-sharing model with long-term ownership of the show. Project is de risked by being housed at Multimedia University of Kenya, employing graduates. We have prepared more episodes and plan to secure additional funding for our teaching efforts.

HOW PROBIOTIC SUPPLEMENTATION IMPACTS MICROBIAL DIVERSITY AND LARVAL PERFORMANCE IN CULTURED WHITE SEABASS *Atractoscion nobilis*

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Larval white seabass (*Atractoscion nobilis*) culture at the Hubbs-SeaWorld Research Institute utilizes *Artemia* as a live feed, but is sometimes hindered by pathogenic bacterial overgrowth in the live feed production process. A commercially available probiotic, Sanolife® MIC (INVE Aquaculture), was tested for its ability to modify microbial populations in both live feeds and white seabass culture tanks. Sanolife® MIC was administered to either *Artemia* storage tanks or directly to larval rearing tanks. Outcomes of microbial diversity, microbial composition, larval growth and performance were compared across four distinct treatment groups: *Artemia* with Sanolife® MIC, larval culture water with Sanolife® MIC, non-probiotic control, and addition of Rotifers to standard feeding protocol. Growth and survival of fish in each treatment was monitored weekly. Samples for microbiome analysis were collected from *Artemia* storage tanks, larval fish culture water and larval white seabass at various time points during the first two months of life. Culturing on TCBS (Thiosulfate–citrate–bile salts–sucrose) media was also conducted in conjunction with microbiome sampling to evaluate growth of potentially harmful bacteria over time across the different treatments. To achieve a broader understanding of total bacterial community characteristics across treatments, *Artemia*, larval fish and larval culture water were sequenced using 16S rRNA amplicon sequencing. The primary goal of this study is to assess correlation between microbiome characteristics and white seabass larval performance in aquaculture. Secondarily, this study assesses the relative contribution of live feeds and culture environment to the larval white seabass microbiome over time. Probiotics are a promising solution to increase the success of larval fish rearing, but fundamental knowledge on the interaction between probiotics and the microbiome is still being developed.

RED DRUM (*Sciaenops ocellatus*) AQUACULTURE IN THE UNITED STATES – CHALLENGES AND OPPORTUNITIES

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The first red drum farm was constructed circa 1990, in Palacios, Texas. Currently, there are six red drum farms operating in the United States, and they are all in Texas within a 50 mile radius of each other. All six farms have hatcheries for spawning. The farms range in size from 125 acres to 500 acres. Farming methods have not really changed since the first farm was built. Broodfish are still spawned using essentially the same photothermal regimes developed in the 1980's. Fingerling are produced in outdoor earthen ponds, much as they were 30 years ago.

The primary constraint to entering the sector is land. A prospective red drum farm needs a suitable water supply for fingerling production and growout. Coastal land with access to salt/brackish water is increasingly scarce. The farm needs to have a requisite amount of land to produce enough fish for weekly sales, ideally without gaps in production. Recirculating technologies do not need the amount of land necessary for outdoor pond production, but they cannot compete at present.

The challenges to bringing a redfish to market are many. Toxic algal blooms are a seasonal threat. Extreme weather events, such as hurricanes and freezing weather, are always a potential threat in any given year. As of 2021, the USDA has included foodfish in the Emergency Assistance for Livestock, Honey Bees, and Farm Raised Fish program (ELAP). This program covers losses associated with declared natural disasters, and it helped the redfish industry to survive the February 2021 freeze event in Texas.

MAXIMUM THERMAL ADAPTABILITY OF THE SOUTHERN BLACK DRUM *Pogonias courbina*: AN EMERGING SPECIES FOR AQUACULTURE

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The Southern black drum *Pogonias courbina* was an important marine fisheries resource, but due to overexploitation, it is now considered vulnerable by the IUCN. They are widely distributed in the Southwestern Atlantic Ocean, and as such, are exposed to a wide range of temperatures. Despite this, their temperature tolerance range is still unknown. Critical thermal maximum (CT_{max}) has been widely used to explore fish acute thermal warming tolerance. Nevertheless, these results are directly influenced by the temperature at which fish are reared before the trial. The present study aims to determine the influence of short-term acclimation temperatures (T_{acc}) on the CT_{max} of juvenile *P. courbina*.

In the experiment, 180 fish per tank (3.1±0.6g) were randomly distributed in four recirculating aquaculture systems (RAS) with different temperatures (29.2±0.3; 31.3±0.3; 33.1±0.2; 35.0±0.4). Each RAS had temperature control (heater or chiller), constant aeration, mechanical and biological filtration to ensure good water quality. During the trial, the fish were fed four times a day with commercial feed. After 12 days, nine individuals (three at a time) were randomly sampled with a net and transferred to 16 L glass tanks with a heater, thermometer, and aeration to avoid stratification. The aquarium was previously filled with water from the 'original system. The temperature ramping-up rate was set at 0.3 °C min^{-1}. Once each fish lost equilibrium, the temperature was recorded.

The general CT_{max} of *P. courbina* was 40.3±0,9°C. Fish CT_{max} was linearly correlated with temperature ($p<0.01$). All CT_{max} were statistically different ($p<0.01$) and ranged from 39.2°C for fish acclimated at 29°C to 41.4°C for fish kept at 35°C (Fig 1.). These findings provide useful information on the upper water temperature limit for juvenile *P. courbina*. This report can help fish farmers establish appropriate conditions to produce this emergent aquaculture species.

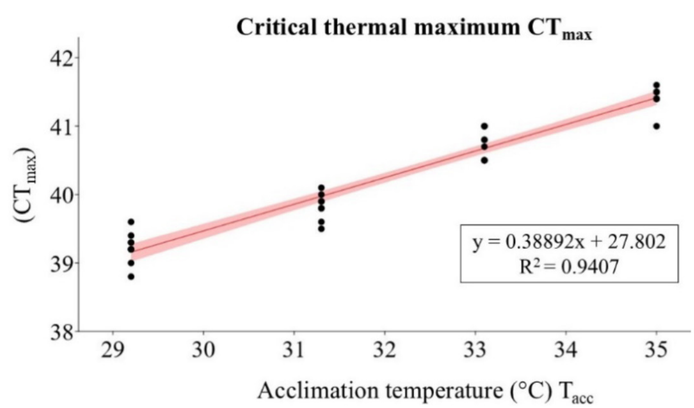

Figure 1 *Pogonias courbina* CT_{max} under different T_{acc}.

EXPLORING THE IMPACT OF REEF COMPOSITION ON THE RECOVERY OF A DECLINING *Crassostrea virginica* REEF IN THE MISSISSIPPI SOUND

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The eastern oyster, *Crassostrea virginica*, provides many ecosystem services including but not limited to building reef structures that serve as a major contributor to local, regional, and global carbonate budgets. To fill the role as a major contributor despite being a rapidly degrading resource, an oyster reef must maintain a positive feedback loop by the addition of shell from living oysters that, in death, create habitat that promotes larval recruitment and thus reef growth. If shell is broken down or removed at a rate higher than it is added, then a reef will enter a negative feedback loop and ultimately cease to exist. In 2016 a mortality event occurred in the western Mississippi Sound that provided the opportunity to monitor the status of reef recovery over an eight-year time frame. Here, we explore the changes in composition of one reef over time as a case study of a reef recovering from mass mortality and failed recruitment events. The presence of living oysters and the resultant boxes on a reef is a crucial component for reef recovery. Ultimately restoration and management efforts will need to include a shell budget in order to have long-term success measured by reef growth and addition of adult oysters to the population.

CHALLENGES FACED BY AQUACULTURE FARMERS IN MALAYSIA

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Aquaculture in Malaysia faces multiple challenges that hinder the sector's efficiency and long-term sustainability. This study aims to identify and analyse key obstacles faced by aquaculture farmers, focusing on labour, feed costs, and water quality. A survey was conducted with 25 local farmers from various culture practices between January to June 2024. This study showed 100% of the farmers relied on foreign labour for groundwork. However, this led to issues of human error, often exacerbated by a lack of training and language barriers, despite the cost benefits compared to local labour. Most farmers were affected by the rising feed cost and uncertain market price of the fish, causing them to switch culture species from time to time. The cost of feed has surged by 20-25% in the past year, driven by the high reliance on imported fishmeal, fish oil, and soybean meal. While alternative feed options like algae meal and black soldier fly larvae show promise, their cost and environmental impacts require careful consideration. Water quality degradation, largely caused by poor resource management and pollution, has emerged as a critical concern, contributing to disease outbreaks and increased operational costs, particularly in shrimp farming. Ten out of 25 farmers are spending 5-12% of operational cost on water treatment before use for farming. Addressing these challenges will require a combination of technological solutions, regulatory improvements, and sustainable resource management practices.

TWO-EYED SEEING AND ITS BENEFITS IN AQUACULTURE AND FISHERIES YOUTH DEVELOPMENT AND OUTREACH

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Advancing sustainable aquaculture requires collaboration between Indigenous knowledge keepers and western scientists. There are several programs in Maine that aim to engage Indigenous learners in sciences while also supporting cultural heritage. Two-eyed seeing, the weaving of Indigenous knowledge and Western science, not only makes science education further accessible to Indigenous students, it also results in higher rates of engagement for all involved. The Kinap Mentorship Program is a peer support, culture building, and outreach program at the University of Maine at Machias that allows Indigenous college students to connect with local Wabanaki communities in their science and cultural areas of interest. The Wabanaki Youth in Science (WaYS) program provides mentoring and training opportunities in the life sciences for Native American youth in Maine. The WaYS program, which was motivated by a shortage of young natural resource professionals on tribal lands, uses a multifaceted approach such as community outreach and internships to recruit and retain native youth in STEM fields. The AquEOUS (Aquaculture Experiential Opportunities for Undergraduate Students) Fellowship at UMaine provides paid fellowships for undergraduate students to work with programs like Kinap and WaYS to co-create research projects that support the goals of Wabanaki communities in fisheries, conservation, and food sovereignty. These programs provide learning experiences and support to Wabanaki youth, such as touch tanks, tide pooling, youth fishing and harvesting, aquaculture development, aquaponics, while centering the values of reciprocity, kinship with nature, Wabanaki homelands knowledge, and spiritual connection to earth.

MICROBIOME RESPONSE TO ANTIMICROBIAL DISRUPTION IN LONG TERM *Saccharina latissima* GAMETOPHYTE CULTURES

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Germplasm banking of temperate kelp species will be an important tool for temperate macroalgae aquaculture to establish resiliency, and scaling, and as a conservation tool for maintaining genetic diversity in wild populations struggling under pressures stemming from global climate change. Gametophyte culture has been practiced in other kelp production regions, principally for *Saccharina japonica*, and has allowed for a fully closed lifecycle and the advent of selective breeding programs that have greatly improved product quality and yields. Long term maintenance and scaling of gametophyte cultures for *Saccharina latissima* hold similar promise, however their applications to at-scale production of seed and selectively bred lines are only just beginning. There are many challenges to overcome in order to operationalize commercial scale gametophyte-based nurseries and seed production.

The Aquaculture Research Institute at the University of Maine has established SMART: the Sustainable Macroalgae Aquaculture Research and Technology program, with the goal of optimizing techniques for gametophyte-based nurseries and germplasm banking. Early challenges have highlighted the need to understand the dynamics of contaminants within GP cultures, specifically those relating to the microbiome of the cultures, and the results and consequences of corrective interventions. Little is known about the microbiome of gametophytes in culture conditions, how these communities shift over time, and how communities shift following perturbations.

Here, we present an initial microbiome challenge experiment utilizing a mixed female *S. latissima* gametophyte culture (a blend of six clonal cultures) exposed to varying levels of the antibiotic kanamycin. Kanamycin is aminoglycoside bacteriocidal antibiotic commonly used in research and human medicine to treat severe bacterial infections. Replicate 1g cultures were distributed across three treatments; High Kanamycin (10ml/l), Low Kanamycin (5ml/l) from a 1g/100ml stock solution, and Control, with 5 replicates each. Prior to treatment, replicates were sampled (both water and tissue) for microbiome characterization via Amplicon sequencing 16s rRNA, as well as water samples for metabolomic analysis. Treatments were resampled at 1 week, post treatment representing cessation of treatment, and 2, 4, and 6 weeks after the conclusion of treatment. Initial insights on microbiome and metabolomic response are discussed.

VISUALIZING SKELETAL DEFORMITIES OF *Amphiprion ocellaris* USING DIFFUSIBLE IODINE-BASED CONTRAST-ENHANCED COMPUTED TOMOGRAPHY

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Ocellaris clownfish (*Amphiprion ocellaris*) are a popular marine fish in the aquarium trade and are a major contributor to the profits of marine ornamental fish farms globally. Although this species has been aquacultured since the 1970s, many research gaps in the biology and culture of these fish remain. A major hurdle facing culture of this species is the inadvertent production of deformed, unmarketable fish that must be culled prior to distribution, thus reducing profits for producers. Skeletal deformities appear to be one of the most prevalent types of deformities among many cultured species, including *A. ocellaris*. Deformities of this type have rarely been categorized and empirically described with large knowledge gaps in the etiologies for morphological features as well other processes influencing their development. This study aims to characterize common deformities observed within clownfish aquaculture through diffusible iodine-based contrast-enhanced computed tomography (or dice-CT). Juvenile and adult *A. ocellaris* specimens with gross deformities and apparently normal specimens (n=39) were scanned utilizing this technique and the resulting projections were reconstructed to visualize and describe the morphological differences occurring in deformed fishes (Figure 1). Morphometric shape/statistical analyses will be utilized to further delineate differences between deformed and normal specimens (i.e. partial procrustes superimposition, principal components analysis, canonical variates analysis). Both undesirable and desirable deformities (i.e. stubby clownfish) were accessed to help inform research on the causative factors for deformity development. With a better morphological understanding of these fish, more precise manipulation of their physical traits should be possible.

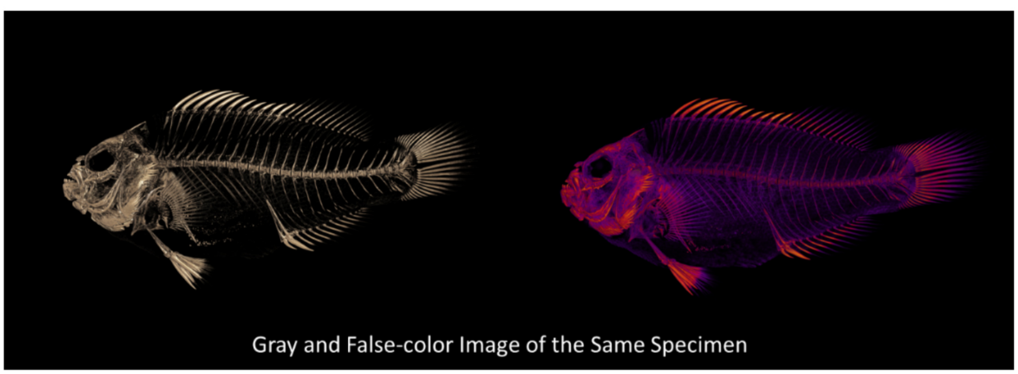

Figure 1. Grayscale and false-color image of an unstained *A. ocellaris* specimen. Data captured on the GE V|TOME|X M 240 CT. Reconstruction of the specimen utilized GE phoenix reconstruction software, and 3D rendering used VG Studio Max, and 3D Slicer.

EFFECTS OF LARVICULTURE PROTOCOL MANIPULATIONON THE SURVIVAL AND GROWTH OF OCELLARIS CLOWNFISH *Amphiprion ocellaris*

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Although ocellaris clownfish (*Amphiprion ocellaris*) are acknowledged as the first and most popular marine ornamental fish bred in captivity, aquaculture practices for this species remain affected by knowledge gaps and inconsistencies in basic culture parameters. Due to their popularity, ocellaris clownfish represent a significant profit source for marine ornamental aquaculture farms worldwide; therefore, there is a vested interest in improving culture practices to increase production efficiency and reduce losses. Green-water larviculture introduces microalgae to the rearing tank to darken the water, presumably increasing contrast and prey visualization, as well as providing continuous enrichment to live-feeds. Unfortunately, rearing live microalgae is labor-intensive, costly, and requires specialized equipment, thus many ornamental fish producers rely on commercially available algae concentrates. Additionally, larval nutrition can also be crucial to the success of marine fish rearing, as they are unable to synthesize certain nutrients, like highly unsaturated fatty acids (HUFAs) and need to ingest these through diet. It is well established that nutritional deficiencies contribute to deformity rate within several aquacultured species. This may also be the case in the ocellaris clownfish, as issues with undesirable deformities continue to impact commercial producers. In the first part of this study, *A. ocellaris* larvae were reared for ten days using three different algae concentrates containing *Tisochrysis sp.* (T1, RotiGreen® ISO 1800) or *Nannochloropsis* sp. (T2, RotiGreen® Nanno and T3, RotiGreen® Omega) to determine which protocol yielded the highest survival and best growth compared against a no-algae control. Of these, T3 produced significantly higher survival than other treatments, and thus was selected for a subsequent experiment to assess effects of different densities of algae concentrates (0.015 mL/L, 0.03 mL/L, and 0.06 mL/L) on larval survival and growth. There were no significant differences in any of the algal densities investigated. Survival was recorded at approximately 60% in all treatments. Moreover, another experiment will be conducted to evaluate the inclusion of copepods within the diet, to mimic natural forage. For this study, ocellaris clownfish larvae will be reared for ten days with a diet of *Parvocalanus crassirostris*, *Oithona colcarva,* or a 50/50 mixture of the two at a rate of 5 nauplii/mL twice daily. Based on these results, further studies will be conducted comparing copepod inclusion against a standard larval reference diet to assess survival, growth, and rate of deformity development in post-metamorphic juveniles. Together, results of these studies will help to create new larviculture parameters for these valuable fish to help ornamental producers thrive.

TRADEOFFS OF SPLITTING OYSTERS AT THREE TRIGGER DENSITIES IN OFF-BOTTOM OYSTER AQUACULTURE

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Labor and grow-out gear are major expenses for commercial off-bottom oyster farms, making their efficient use critical for staying in business. Routine husbandry practices, such as splitting or thinning oysters into lower densities, must balance labor demands with effective gear utilization. While low stocking densities are shown to improve growth, tradeoffs with labor and gear are often overlooked. Additionally, research has not considered the potentially differing effects of initial stocking density from the density that triggers splitting. This farm-scale study standardized initial stocking density (10%) and evaluated the impacts of three different trigger densities (25%, 50%, and 75%) on time-to-market, growth metrics, survival, labor requirements, and gear utilization.

In April 2023, 27 FlipFarm baskets were stocked with ~25 mm oysters and deployed across three lines on the VIMS Research Farm in the York River, Chesapeake Bay (9 baskets per line). Baskets were haphazardly assigned to one of three trigger densities and monitored for growth, split once a treatment level reached the trigger volume, and restocked at 10% by size class, creating additional baskets as needed. By June 2024, the 25% trigger density had a 15.1% (± 3.95%) higher mean percent-to-market than the 75% trigger density (Fig 1). Surprisingly, percent survival did not differ between treatments. Market-sized oysters from the 75% trigger density had a 2.8mm (± 1.04) greater shell height but a 1.5% (± 0.06%) smaller cup ratio than those from the 25% density. In contrast, condition index and dry tissue weight did not differ between treatments. Regarding labor needed and gear use throughout the study, the 25% trigger density used 224 baskets and required 133.5 hours of labor, the 50% density used 185 baskets and 112 hours, and the 75% density used 63 baskets and 58 hours (Fig 2). For farmers, a lower trigger density could offer faster returns but comes at a higher labor cost, while a higher trigger density could reduce labor expenses but delay market-ready yields. These findings highlight the critical tradeoffs in strategies for this ubiquitous husbandry practice, encouraging off-bottom farmers to reevaluate their splitting techniques to maximize profitability and ensure long-term financial stability.

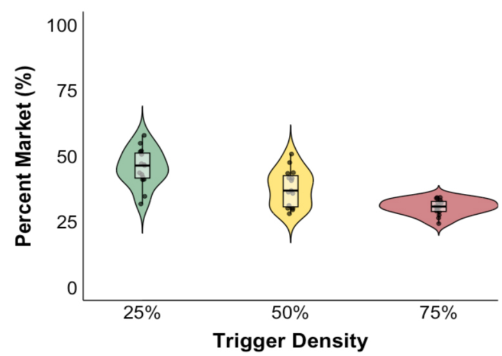

Figure 1. Percent that made it to market by trigger density.

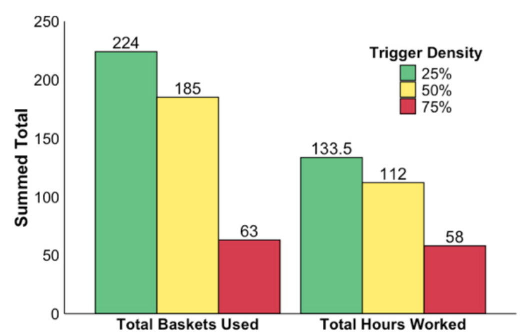

Figure 2. Baskets used and hours worked by trigger density.

BIOMARKERS IDENTIFICATION IN MUCUS OF *Genypterus chilensis* AND *Seriola lalandi* ASSOCIATED TO GONADAL MATURATION AS A NON-INVASIVE DIAGNOSTIC TOOL FOR REPRODUCTIVE MANAGEMENT OF MARINE FISH

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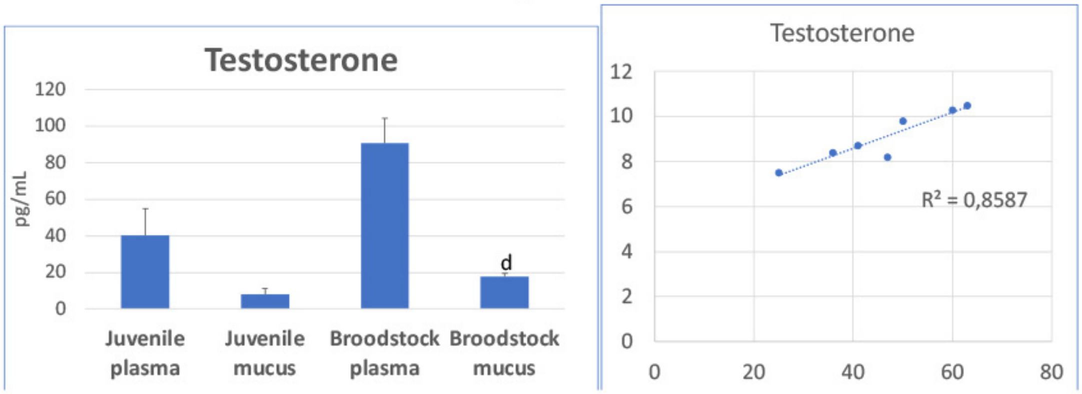
S. lalandi and *G. chilensis* are native species that present the greatest expectations for Chilean aquaculture diversification. However, the success of its production depends on efficient reproductive management, where minimally invasive methods of estimating the gonadal maturity are required to contributed to the fish welfare. To evaluate the use of epidermal mucus as an easily accessible matrix for the analysis related to ovarian maturation, in this work we show the pattern of different types of biomarkers in the epidermal mucus of *S. lalandi* and *G. chilensis.*

To achieve this objective, we compared mucus and plasma samples from adult females in different states of gonadal maturation of *S. lalandi* (5 to 10 years-15 to 25kg) acclimated to captivity and from wild adult females of *G. chilensis* (1.8 to 3 kg). Markers such as vitellogenin and estradiol were measured in both matrices. In general terms, it was only possible to detect estradiol in the plasma of some individuals with concentrations that did not exceed 2 ng/mL. However, vitellogenin could be detected in mucus (0.1 to 4 ng/mL), but with values tenfold less plasma level (1 to 40 ng/mL).

Testosterone was detected in plasma and mucus. In juveniles and mature individuals Testosterone was higher in plasma compared to mucus. A positive correlation (Pearson Coef. 0.85) could be observed between mucus and plasma.

In addition, considering the available knowledge related to the impacts of oxidative stress on the reproduction in different fish species, we evaluated the oxidative status in the mucus of both species by analyzing the antioxidant capacity using ORAC and DPPH. The results show that it is possible to measure antioxidant capacity in mucus and shows the antioxidant capacity of *S.lalandi* is higher than the antioxidant capacity of *G. chilensis*, (2800 µmol ET/mL and 140 µmol ET/mL, respectively) via ORAC and DPPH (1500 and 400 µmol ET/mL, respectively).

Our results indicate that the mucus of *S. lalandi* and *G. chilensis* adults is a matrix that can be used for the evaluation of gonadal development and the oxidative status of native species, and emerging species of Chilean aquaculture.



FEED TRAINING EVALUATION OF PHASE ONE LARGEMOUTH BASS PRODUCED IN MANAGED NURSERY PONDS AND FLOATING IN-POND RACEWAYS

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Feed training is a crucial aspect of largemouth bass (LMB) production, particularly in commercial aquaculture systems where artificial pelleted feeds are used to minimize cannibalism and optimize growth. Traditionally, LMB fingerlings are produced in managed nursery ponds; however, floating in-pond raceways represent a potential alternative rearing system that could enhance production efficiency by reducing space and operational costs. This study compares the feed training efficiency, survival rates, and growth performance of Phase I LMB fingerlings produced in managed nursery ponds and floating in-pond raceways.

This 3-week study evaluates feed training efficiency among fish grown in a managed nursery pond (control) and a floating raceway (treatment). Treatments were randomly assigned to aquariums (132L) supplied with a flow of water at 27°C and aeration. They were stocked with fish harvested from managed nursery ponds (2.68 ±.03 g/fish) and floating raceways (0.72 ± .02 g/fish). Each aquarium was stocked with 200 fish and fed to satiation with a pelleted feed (50% protein 10% fat). Amount fed was recorded. Temperature, dissolved oxygen, total ammonia nitrogen and nitrite were monitored. The number of fish in each aquarium and batch weight of each aquarium was recorded at stocking and harvest.

The results showed that both groups of fish successfully adapted to the pelleted feed, with high survival rates observed for both treatments: 95.5% for pond-reared fish and 96.5% for raceway-reared fish. However, significant differences were noted in specific growth rates (SGR) and final harvest densities. Raceway-reared fish had a higher SGR (8.3 ± 0.26 %BW/day) compared to pond-reared fish (4.8 ± 0.07 %BW/day), likely due to their smaller initial size. Feed efficiency for pond and raceway treatments was 119% and 109%, respectively. It was not significantly different.

The findings of this study suggest that floating in-pond raceways can produce LMB fingerlings that feed train as effectively as those reared in managed nursery ponds. Otohime feed was well accepted and highly efficient.

A DEVIL AND AN ANGEL, HEMOLYMPH BACTERIA IN *Procambarus clarkii*

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Unlike mammals, crustaceans have bacteria colonization in their hemolymph, the origin of hemolymph bacteria and their role in crayfish survival aroused our interest. We observed that *Vibrio* significantly enhanced the load of hemolymph bacteria in *Procambarus clarkii*. *Vibrio* specifically degrades peritrophins-44 in crustaceans via the secreted protease of C1 esterase (StcE). Crayfish expression of the protease inhibitor (inter-α-trypsin inhibitor heavy chain 4 , ITIH4), inhibiting the enzymatic activity of StcE. Interestingly, the crayfish exhibits a relatively high tolerance to heavy metals. The *Exiguobacterium,* the symbiotic bacteria in the hemolymph of *P. clarkii,* were proved to be primary contributor to Cr(VI) tolerance. Further investigation suggested that it resists Cr(VI) through the activation of the ABC transporter system and the reduction of Cr(VI) via the reductase gene *nfsA*. Bacteria colonize the crayfish's hemolymph during its development. And the bacteria in hemolymph achieve a dynamic balance with the crayfish, helping the crayfish adapt to the water environment.

DEVELOPMENT OF A NEW KUMAMOTO OYSTER BREEDING POPULATION BASED ON INDIVIDUALS COLLECTED FROM THE ARIAKE SEA, JAPAN, IN 2006

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Kumamoto oysters (*Crassostrea sikamea*) are highly regarded on the US Pacific Coast due to their deep-cupped shells (Figure 1) and firm summer meats. Previously, it had been reported that some commercial Kumamoto stocks were highly inbred and were contaminated by accidental hybridization with Pacific oysters. Consequently, in 2006, Kumamoto oysters were collected from the Ariake Sea, Japan, to supplement existing commercial Kumamoto stocks and to develop a new breeding population for the Molluscan Broodstock Program (MBP) – a breeding program to improve US Pacific Coast oyster broodstock.

The G_1 generation and early life stages of the G_2 generation of the new Japanese Kumamoto stock were maintained in quarantine conditions and subjected to numerous histological exams and genetic tests to ensure the absence of detectable diseases of concern, before release and planting of G_2 spat at farm test sites.

Harvest traits (yield, survival, growth, shell dimensions and shape) of families of the G_2 and G_3 generations were determined at a sub-tidal farm site and compared to those of established Pacific Coast commercial stocks. A single MBP Kumamoto breeding population was produced in the G_5 generation by crossing individuals from Japanese families and Pacific Coast stocks. Analysis of the ITS1/2 genomic regions of samples from the G_5 and previous generations indicated no genetic hybridization with Pacific oysters.

Mean individual weights and shell lengths of the G_3 Japanese families were significantly less (Tukey-Kramer; $p<0.05$) than those of families produced from established Pacific Coast commercial stocks; however, other harvest traits (yield, survival, average individual weight, shell dimensions and shape) were not significantly different. Harvest traits of crosses between the Japanese and Pacific Coast populations were intermediate in value compared with those of the two parental populations. Heritability values for G_5 harvest traits were high, ranging from 0.83 for shell depth to 0.97 for survival, indicating strong potential for genetic improvement through selection. Genetic correlations were generally positive among harvest traits, except for shell shape (depth/(width + length) which showed negative (but non-significant) correlations with individual weight and meat content, indicating that selection for deep shell cupping may have a negative effect on these other traits.

Figure 1. Kumamoto oysters (C. sikamea) collected from the Ariake Sea, Japan

EFFICACY OF A RECOMBINANT PROTEIN VACCINE AGAINST LARGEMOUTH BASS VIRUS IN LARGEMOUTH BASS (*Micropterus salmoides*)

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Aquaculture is considered one of the fastest growing food-producing industries worldwide and accounts for nearly 50% of the world's food fish. Worldwide aquaculture annual economic losses due to fish diseases are estimated to be around $6 billion dollars. Largemouth bass (*Micropterus salmoides*) are widely considered the most popular sport fish in the United States, however a growing demand for a steady supply of adult largemouth bass as food fish has increased in large cities (Chicago, New York, Philadelphia) and the most recent USDA Census of Aquaculture (2018) has shown yearly sales of $30 million dollars. Largemouth Bass Virus (LMBV), a member of the genus Ranavirus in the family Iridoviridae, is a cytoplasmic dsDNA virus that was originally identified in the Southeast U.S. in the early 1990s. Other isolates would later be identified throughout the world (Canada, China, Germany, India, Thailand). In the natural environment, the incidence of LMBV disease usually occurs during the summer, but largemouth bass infected with LMBV generally have no lesions or obvious symptoms, which makes diagnosis difficult. The lethality of LMBV is also problematic to establish as natural infections have a protracted acute phase and assessing mortalities is challenging in bodies of water, however laboratory challenges have indicated up to 100% mortality within a two-week period. Although significant progress has been achieved in the treatment of other diseases, no effective treatment for LMBV is available. Vaccination is one potential strategy to reduce the impact of LMBV; however, very little work has been done on the adaptive immune system of largemouth bass. We developed a recombinant LMBV protein (rLMBV) and evaluated its immunogenicity *in vivo.* Largemouth were injected with ISA 763AVG adjuvant only or a 70:30 adjuvant to antigen ratio using rLMBV and ISA 763AVG. All largemouth bass that received the rLMBV protein generated a rLMBV specific antibody response and there was a very statistically significant response between the vaccinated and adjuvant control. Most recently, a vaccine trial began to establish whether largemouth bass immunized with the rLMBV protein confer protection against an LMBV laboratory challenge. The outcome of this vaccine trial will be discussed.

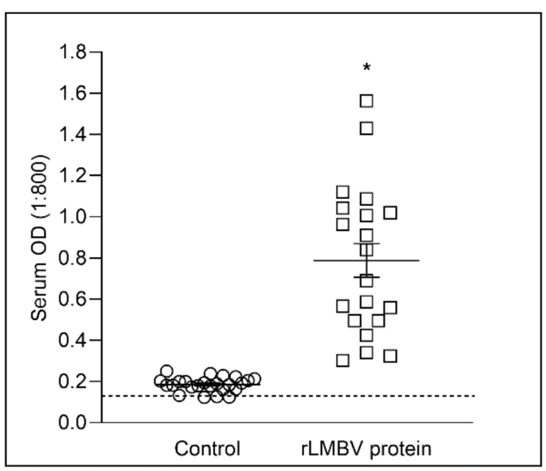

Figure 1. Evaluation of largemouth bass IgM antibodies generated against a recombinant LMBV protein. The mean absorbance ± SD for each group is shown as a horizontal line. An asterisk denotes a significant difference, P <0.0001. The dashed line represents the mean background absorbance value.

INVESTIGATING THE PHYSIOLOGICAL EFFECTS OF HARMFUL ALGAL BLOOM TOXINS ON JUVENILE PACIFIC OYSTERS *Magallana gigas*

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Climate change, specifically increased atmospheric carbon dioxide (CO_2), has profoundly altered global ecosystems, leading, among other things, to increased sea surface temperatures and levels, ocean acidification, and greater prevalence of waterborne diseases. In the past two decades, this shift in ocean conditions has contributed to more frequent harmful algal blooms (HABs) along coastal areas, raising concerns about the threats that HABs pose to shellfish aquaculture.

Shellfish aquaculture is valuable ecologically for its role in sustaining healthy marine ecosystems, and economically for being a source of food. In the United States, the Pacific oyster *Magallana gigas* is a commercially valuable shellfish species by weight but is threatened by recurring summer mortality events. These events have primarily been attributed to infectious pathogens, such as *Ostreid herpesvirus*-1 and *Vibrio* spp., as well as to elevated seawater temperatures. Recently, HABs have gained attention for their potential, yet less known, role in these mortality events. Toxins produced by HAB species can adversely affect aquatic and terrestrial animals. Research indicates that HAB toxins can impact shellfish by affecting their feeding, morphology, growth, reproduction, and survival at both juvenile and adult stages. While there are indicators suggesting a correlation between HABs and summer mortalities in Pacific oysters, a definitive link has not yet been established.

We are investigating the physiological effects of a toxin-producing algae (*Alexandrium catenella*) on juvenile Pacific oysters. Our objectives include assessing survival, growth, and gene expression patterns in juvenile Pacific oysters upon laboratory exposure to *A. catenella*. The goal is to determine whether these exposures increase Pacific oysters' susceptibility to summer mortality through physiological changes.

Juvenile oysters (n = 25) will be placed in 3L tanks, each fitted with a magnetic stirrer, a mesh barrier, and artificial seawater at 22°C. Following starvation for 24 h, oysters will be treated with either toxic *Alexandrium sp.*, non-toxic *Alexandrium* sp., a dietary algal mix of *Nannochloropsis* spp., or no algae for 72 h. Each treatment will be replicated in three separate tanks, totaling 12 tanks. Mortalities will be monitored daily, and oysters (n = 5) and water samples will be collected every 24 h. Toxin concentration in both oyster and water samples will be measured with a Saxitoxin (PSP) Enzyme-Linked Immunosorbent Assay (ELISA) kit. Oyster RNA will be evaluated for quality and submitted for sequencing. Preliminary results will be presented.

OFFSHORE FARM PERMITTING – THE ULTIMATE COLLABORATION

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Without a defined path to permitting offshore aquaculture, U.S. industry development has been languishing – essentially not happening at all.

This is the greatest challenge of our lifetime – cultivating proteins to feed our growing world population, without harming our environment or depleting our natural resources. It's about Balance – Balance with the environment, Balance with the economic markets, and balance with our natural resources.

We the Farmers here at Aquauclutre America know that we must farm seafood to feed the world. Famed ocean explorer Jacques Cousteau knew in 1970 and we are certain now more than ever.

The United Nations knows – and they advise us that we must double aquaculture production by 2030 to meet our global demand for seafood. Seafood currently garners an average of 17% of proteins consumed in the world. Ocean farming can double US wild capture of seafood, utilizing one tenth of 1% of the U.S. EEZ.

The Universities and researchers know – as they have been researching for years and now there is scientific proof that done right, seafood farming is sustainable – finfish, shellfish and seaweeds, on shore, near shore and off shore.

It is about using the tools for proper siting, modeling, farm planning and operations. It is about the integration of robotics, environmental monitoring and AI. We have the necessary tools at our disposal. It is about transparency and collaboration, responsibility and social license.

Industry perspective will be provided, as Manna Fish Farms, Inc. navigates the path to permitting one of the first ocean farms in the United States!

FLORIDA SEA GRANT'S HARVEST PROGRAM: HELPING AQUACULTURE REAP VALUE AND ENHANCE STUDENT TRAINING

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In support of a growing Blue Economy, Florida Sea Grant launched its HARVEST student internship program to foster the development of a burgeoning aquaculture industry and provide valuable real-world experience to students interested in aquaculture.

HARVEST, Helping Aquaculture Reap Value and Enhance Student Training, is an internship program that offers university students paid internships with partnering aquaculture businesses in Florida. Students are provided with valuable workforce development skills to solve 'real-world' problems that impede the productivity of aquaculture businesses. During their internship, students aid in a variety of needs denoted by the aquaculture business such as efforts to increase survivability, productivity, efficiency, sustainability, public outreach, and education. The duties of each internship include but are not limited to: on the farm/hatchery work (animal husbandry, harvesting, processing, culling, data collection); outreach, communications and marketing; equipment maintenance and engineering; development/expansion of new culture methods; and research project assistance.

Florida Sea Grant supports up five student-business internships per year and have supported 17 to date. Florida Sea Grant extension agents and faculty specialists partner with aquaculture business to co-mentor the HARVEST intern during their 2-9 month internship experience. Each HARVEST internship's duties and projects are based on the needs of the aquaculture business partner and have broader implication to the industry.

ASSESSING IMPACT OF CONTAMINANTS OF EMERGING CONCERN ON OYSTER HEMOCYTES

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Contaminants of emerging concern including pharmaceuticals and persistent organic pollutants are increasingly detected in estuaries and waterways. Although there are growing number of studies on the toxicity of contaminants of emerging concern, its impact on marine invertebrates and specifically on hemocytes is less well studied. The eastern oyster *Crassostrea virginica* is a filter feeder that is exposed to a variety of compounds in the seawater within its natural habitat. Hemocytes, the main immune effectors of *C. virginica*, are potentially exposed to the various bioaccumulated compounds. The purpose of this study is to identify the impact of a few of these contaminants of emerging concern such as the beta-blocker atenolol on oyster hemocyte function. Globally, atenolol has been found in wastewater at concentrations as high as approximately 1uM. In this study, hemolymph was collected from *C. virginica* and collected hemocytes were exposed in-vitro to environmentally relevant concentrations of atenolol over a 24-hour period. Flow cytometry and function assays including viability, phagocytosis, reactive oxygen species, lysosomes and apoptosis were utilized to measure the impact of atenolol on hemocytes. Results suggest that in-vitro exposure of hemocytes to atenolol (0.04 nm to 1uM) did not significantly impact oyster hemocyte function. This study utilized *C. virginica* hemocytes to assess the impact of atenolol on hemocyte function and can be utilized as an in-vitro model for understanding the impacts of contaminants of emerging concern on bivalves. Further investigation on other types of contaminants of emerging concern are needed.

INCLUDING OCEAN ACIDIFICATION EFFECTS ON BIOCALCIFICATION IN A DYNAMIC ENERGY BUDGET MODEL

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Climate change impacts on ocean biogeochemistry are expected to alter calcium carbonate formation by organisms, necessitating accurate predictive models based on physiological mechanisms. Dynamic Energy Budget (DEB) theory offers a mechanistic and integrative framework to model the metabolism of organisms under changing environmental conditions. Here, we propose a generalized approach to include effects of ocean acidification in DEB modeling by formulating the impacts of changes in saturation state (SS) on the bioenergetics of calcification. While applicable to any species capable of biogenic calcification (microalgae, shellfish, fish, or corals), the model was tested on five bivalve species for which extensive tissue and shell data were available. The model was successfully applied to larval, juvenile, and adult life stages compared to published data. It reproduced typical tissue and shell growth patterns under favourable SS with more accuracy than a typical DEB model. We explored the effects of more detrimental SS values for biocalcification on shell and tissue dynamics and identified missing data and experiments that should help calibrate model parameters. This work represents a necessary step to predict the physiological response of biocalcifiers to changes in ocean acidification. It may also provide a needed mechanistic tool for shell dynamics to be integrated in nutrient cycling models.

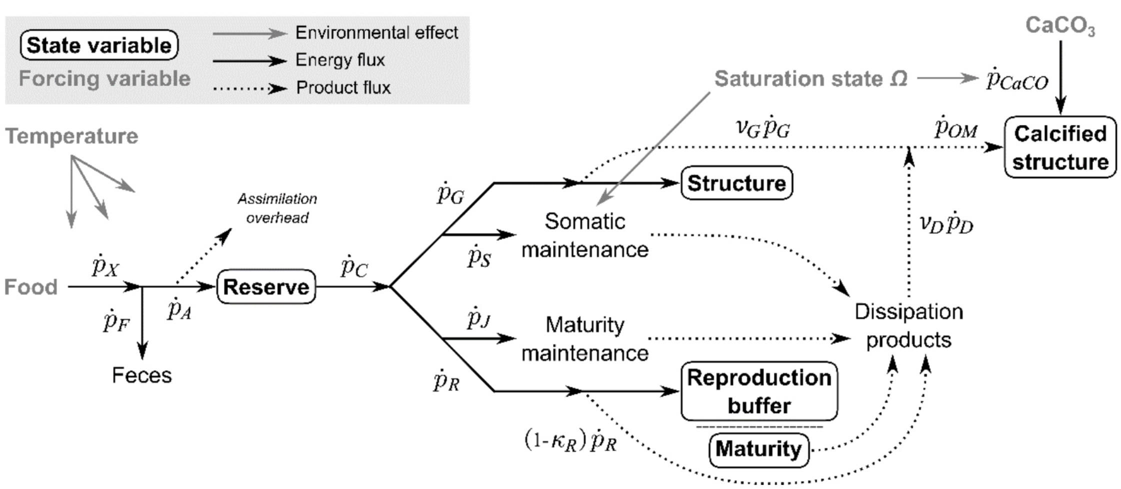

Fig. 1. Conceptual scheme for the DEB model including the formation of biogenic carbonates. State variables (clear boxes) are linked via energy fluxes (plain arrows). The forcing variables and their influence in the model appear in grey (text and arrows) and DEB product formation are shown as dotted lines.

USING METAPOPULATION MODELING TO SUPPORT OYSTER RESTORATION AND AQUACULTURE IN LOUISIANA'S COASTAL BASINS

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Process-based models are critical to guide restoration and management efforts, particularly in coastal zones where conditions are ever changing. Modeling oyster reefs represents a challenge in that they are constituted in metapopulations, which can be sustainable as a whole, yet extremely dynamic at a local scale. In this project, we are developing a metapopulation model coupling hydrodynamic/water quality modeling (Hydro) with larval transport modeling (Transport) and on-reef individual based modeling (Reef) to support decision making in Louisiana estuaries (Fig. 1). Environmental variables generated by the Hydro model force the Transport model, which simulates oyster larval settlement over space and time. The Reef model also uses environmental variables from the Hydro model to compute individual oyster bioenergetics on the reef and uses larval settlement from the Transport model to determine population dynamics. Finally, the Reef model provides larvae through spawning to the Transport model, which simulates their dispersal. We present an overview of this model and its application to one major Louisiana estuary for natural oyster reefs and oyster aquaculture. Model outputs include reef connectivity matrices, single reef spawning contribution and available settlement area, on-reef oyster density and individual oyster growth among other metrics. This study provides decision makers with a process-based tool to aid in managing wild populations under current and projected future conditions. It should also provide valuable information for site selection in a developing aquaculture industry in Louisiana.

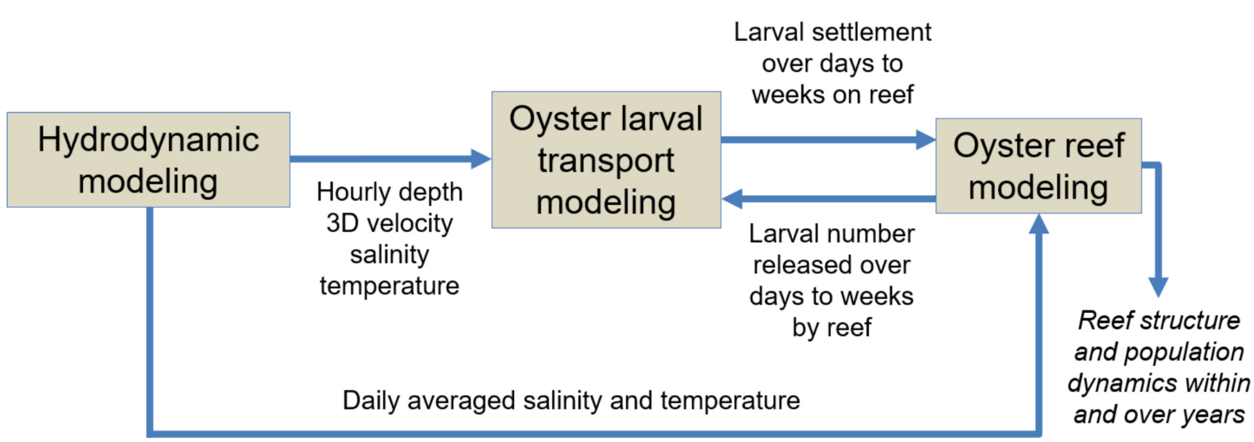

Fig. 1. Conceptual diagram shows the metapopulation model outputs and coupling.

SIMPLIFIED WORKFLOW FOR FASTER GENOTYPING-BY-SEQUENCING USING PARTIAL COMBINATORIAL DUAL BARCODES AND OPTIMIZED ANALYSIS PIPELINE

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Targeted Genotyping by Sequencing (GBS) is a robust and cost-effective method for marker-assisted breeding and selection. Targeted GBS provides a scalable workflow, allowing for thousands of markers to be genotyped in up to thousands of samples per day. As GBS use expands, demand increases for higher throughput, faster turnaround times, and increased multiplexing capability.

To achieve higher throughput with less material and greater ease of use, we propose a new library prep workflow using partial combinatorial dual barcodes for use with the Ion GeneStudio™ S5 System that not only increases available barcodes, but also achieves complete, normalized libraries for up to 3072 samples in less than a day. Additionally, we propose an analysis workflow optimized for more than 6000 samples to be sequenced and genotyped in less than 24 hours using a single Ion GeneStudio™ S5 System. These optimizations enable completion of genotyping of >30,000 samples within a 5-day week. Here we report early testing results showing high quality results achieved using this novel workflow and analysis pipeline.

For Research Use Only. Not for use in diagnostic procedures.

DIETARY SUPPLEMENTATION WITH Β-GLUCAN, MANNAN-OLIGOSACCHARIDES, AND NUCLEOTIDES: EFFECTS ON INTESTINAL HISTOLOGY OF NILE TILAPIA *Oreochromis niloticus*

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Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed fish species globally and holds economic importance, particularly in Brazil. Enhancing the health and performance of tilapia is crucial for sustainable production, and dietary additives emerged as a strategy. Investigating the effects of these additives on intestinal histology is essential, as the intestine plays a vital role in nutrient absorption and overall fish health. In this context, the present research evaluates the effects of three commercial dietary additives: Mannan-oligosaccharides (MOS)+ β-glucan, and nucleotides from two different sources on the intestinal histology of Nile tilapia.

The analyses were conducted at the Laboratory of the Aquaculture and Genetics Research Center (NEPAG) of the Londrina State University (UEL), in partnership with the Laboratory of Animal Pathology of UEL. The fish were distributed in a completely randomized design with five treatments (T2: MOS/β-glucan: 2 g/kg, T3: Nucleotides 15% (From *Saccharomyces cerevisae*): 2.1 g/kg, T4: MOS/beta-glucan + Nucleotides 15%: 1.05 g/kg from each, T5: Free Nucleotides: 2.1 g/kg, and T6: Free Nucleotides + MOS/β-glucan) and a control treatment, each with three replicates. 12 fish were allocated to each of the 18 aquariums (60 L), amounting to 36 fish per treatment and 216 fish in total. The experimental period lasted 46 days, and the fish were fed to apparent satiety.

For the sample collection, 6 fish from each treatment were anesthetized and euthanized by spinal section. Then, the collection of the proximal intestine was performed. An adapted system of semiquantitative scoring was used to analyze intestinal morphology. Six independent parameters were employed for classification: Villi height (A), Lamina propria width (B), Abundance of goblet cells- GC (C), Degree of eosinophil granulocyte infiltration in LP (D), Abundance of intraepithelial lymphocytes (E), and Apoptotic enterocytes (McKnight cells) (F). A scale of 1 to 3 was used for each classification, with the lowest score representing the best normal condition and greater integrity of the villi and intestinal cells. Histological slides stained with Alcian Blue were analyzed to count the absolute number of goblet cells. No effect of the treatments was observed for any of the histological parameters evaluated through the score. However, an effect was observed in the absolute count of goblet cells, with treatments T2 and T4 standing out compared to the others. That said, T4 showed a good synergy between additives, yielding better results than the additives containing only MOS/β-glucan (T2) and Nucleotides 15%(T3).

Table 1: Absolute count (mean± standard deviation) GC in Nile tilapia fed with MOS/β-glucan, Nucleotides 15% and Free Nucleotides.

Treatment	Absolute count of GC
T1	279.50±156.86 b
T2	385.50±61.69 a
T3	275.00±55.20 b
T4	437.33±129.60 a
T5	275.00±29.89 b
T6	283.40±38.14 b
p-valor	0.009

Different letters indicate statistical differences ($p \leq 0.05$)

PRODUCTION PERFORMANCE AND HEPATOSOMATIC INDEX OF TWO NILE TILAPIA *Oreochromis niloticus* STRAINS FED DIETS SUPPLEMENTED WITH β-GLUCAN AND MANNAN OLIGOSACCHARIDES

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The increased demand for food production drives the intensification of Nile tilapia (*Oreochromis niloticus*) production, making breeding programs essential to achieve this goal. When managing these programs, new genetic material should be introduced after multiple generations to reduce inbreeding and enhance genetic diversity. Evaluating production performance when including new genetics and focusing on the role of feed additives, are crucial to understanding their impact on breeding programs. Therefore, this study aimed to evaluate the growth performance and hepatosomatic index of two genetic strains of Nile tilapia from the genetic improvement program fed with β-Glucan + Mannan oligosaccharide (βG+MOS). The experimental design was a 2 × 2 factorial with two genetic groups and two treatments: diets with and without βG+MOS.

The groups consisted of the 12th generation of the Tilamax genetic improvement program (TILAMAX) and a cross between Tilamax and an introduced test strain (CBTILAMAX). A total of 96 tilapias (31.1 ± 5.7 g) were weighed, measured and distributed into 16 aquariums (6 fish/aquarium; 24 fish/group; n=4). The initial total length and initial standard length were 11.40 ± 0.83 cm and 9.42 ± 0.69 cm respectively. The 41-day experimental period maintained optimal water conditions by daily monitoring. At the end of the feeding trial, the K factor (CF_{FTL} and CF_{FSL}, hepatosomatic index (HSI), final weight (FW), total length (FTL), and standard length (FSL) were measured, and other production performance parameters were computed based on the final data. The condition factor showed that all the groups exhibited adequate health and growth during the experiment, with no differences in strain or use of additives. The hepatosomatic index also did not differ between groups. Similarly, no significant differences were observed for standard lengths, total weight gain (TWG), condition factors, and specific growth rate (SGR). Based on these results, it is concluded that there were no differences between TILAMAX and CBTILAMAX related to performance under the effect of feeding with βG+MOS. These results support the successful integration of the test strain into the Tilamax genetic improvement program and provide a foundation for future studies with this strain.

Table 1: Effects of β-Glucan + Mannan oligosaccharide (βG+MOS; additive) and genetic strain on K factor, hepatosomatic index and production performance (mean ± standard error) in Nile tilapia juveniles

Variable[I]	Additive[II]	Strain		Mean	*P*-value		
		TILAMAX	CBTILAMAX		Additive	Strain	A x S
CF_{TL} (%)	Control	2.20 ± 0.12	2.12 ± 0.03	2.16 ± 0.06	0.338	0.915	0.288
	0.2%	2.07 ± 0.03	2.15 ± 0.02	2.11 ± 0.02			
	Mean	2.14 ± 0.23	2.13 ± 0.02				
CF_{SL} (%)	Control	3.72 ± 0.21	3.63 ± 0.09	3.68 ± 0.11	0.845	0.757	0.276
	0.2%	3.57 ± 0.05	3.74 ± 0.05	3.66 ± 0.04			
	Mean	3.65 ± 0.11	3.69 ± 0.05				
FTL (cm)	Control	15.9 ± 0.31	16.5 ± 0.27	16.2 ± 0.21	0.855	0.491	0.154
	0.2%	16.4 ± 0.28	16.2 ± 0.26	16.3 ± 0.17			
	Mean	16.21 ± 0.21	16.4 ± 0.19				
FSL (cm)	Control	13.4 ± 0.27	13.9 ± 0.25	13.6 ± 0.19	0.795	0.573	0.158
	0.2%	13.7 ± 0.27	13.5 ± 0.23	13.6 ± 0.17			
	Mean	13.5 ± 0.19	13.7± 0.17				
FW (g)	Control	89.1 ± 4.01	99.1 ± 4.82	94.10 ±	0.942	0.373	0.219
	0.2%	94.5 ± 5.72	92.9 ± 3.94	93.76 ±			
	Mean	91.84 ± 3.48	96.02 ± 3.11				
TWG (g)	Control	58.1 ± 3.01	64.28 ± 4.35	61.91 ±	0.743	0.847	0.217
	0.2%	64.8 ± 5.70	60.3 ± 3.69	62.60 ±			
	Mean	61.4 ± 3.22	62.3 ± 2.84				
SGR (% g day^{-1})	Control	2.64 ± 0.07	2.59 ± 0.11	2.61 ± 0.06	0.308	0.244	0.439
	0.2%	2.86 ±0.18	2.62 ± 0.12	2.74 ± 0.11			
	Mean	2.75 ± 0.10	2.60 ± 0.08				
HSI (%)	Control	2.45 ± 0.10	2.36 ± 0.16	2.41 ± 0.09	0.932	0.472	0.913
	0.2%	2.48 ± 0.15	2.35 ± 0.20	2.42 ± 0.12			
	Mean	2.47 ± 0.09	2.36 ± 0.13				

Table 1: Effects of β-Glucan + Mannan oligosaccharide (βG+MOS; additive) and genetic strain on K factor, hepatosomatic index and production performance (mean ± standard error) in Nile tilapia juveniles.

DIETARY SUPPLEMENTATION OF β-GLUCAN, NUCLEOTIDES, AND THEIR COMBINATION ON THE PRODUCTIVE PERFORMANCE OF CHANNEL CATFISH *Ictalurus punctatus*

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Ictalurus sp. are one of the most important fish in global aquaculture due to their fast growth, and efficient feed conversion. In the United States, they are the most farmed food fish nationwide, supporting rural economies and providing affordable protein for human consumption. To enhance the sustainable production of those fish, dietary additives are essential to ensure the long-term success of catfish farming.

In this scenario β-glucans could act as immunostimulants, making fish more resistant to pathogens. Nucleotides, on the other hand, serve as building blocks for DNA and RNA, supporting cell replication, tissue regeneration, and immune response. Combining both additives may promote a synergistic effect to improve the positive outcomes even further. In this study, three treatments were tested: β-glucan derived from microalgae *Euglena gracilis* (0.2%), Nucleotide (0.2%), and a combination of both (0.2% of each additive) while the control group received no additives. A total of 20 aquariums (110 L; n=5) were used, with 30 fish in each (initial weight 6.77±0.12). Fish were fed rations corresponding to the tank biomass (~3.5-5%), and water quality parameters, were measured three times per week to ensure optimal environmental conditions.

After 70 days of feeding, no statistical differences were observed in production performance parameters, including final weight, weight gain, daily weight gain (DWG), apparent feed conversion ratio (FCR), and survival rate. The data were analyzed using a 2×2 factorial design. Normality and homogeneity were assessed with the Shapiro-Wilk and Bartlett tests. If these assumptions were not met, a non-parametric Kruskal-Wallis test was performed.

Table 1. Productive performance parameters (mean ± standard deviation) of channel catfish supplemented with additives and their combination.

Variable	Additive	Mean	*P*-value		
			β-glucan	Nucleotide	β * N
Final weight (g)	Control	42.67±0.79	0.819	0.498	0.304
	β-glucan	41.09±1.97			
	Nucleotide	40.52±3.75			
	β + N	41.54±2.32			
Weight gain (g)	Control	35.89±0.79	0.803	0.478	0.306
	β-glucan	34.34±1.88			
	Nucleotide	33.78±3.62			
	β + N	34.73±2.28			
DWG (g)	Control	0.52±0.01	0.803	0.478	0.306
	β-glucan	0.49±0.02			
	Nucleotide	0.48±0.05			
	β + N	0.50±0.03			
FCR (kg of feed/ kg of weight gain)	Control	1.33±0.01	0.375	0.330	0.350
	β-glucan	1.37±0.03			
	Nucleotide	1.37±0.05			
	β + N	1.37±0.04			
Survival rate (%)	Control	100±0.00	0.488	0.488	0.777
	β-glucan	99.33±1.33			
	Nucleotide	99.33±1.33			
	ß + N	98.66±2.66			

Complementary analyses, such as blood parameters, *in vivo* and *in vitro* immune assays, histological morphology of the intestine, and results after bacterial challenge tests will be evaluated to determine the full impact of the additives on their health. In addition, a feeding trial with Nile tilapia (*Oreochromis niloticus*) evaluated similar feed additives. This collaborative effort will provide valuable insights for best feeding managements for both fish species.

SUSTAINABLE TECHNOLOGY AND MATERIALS AT MAINE OCEAN FARMS

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Maine Ocean Farms is an aquaculture business based in Freeport Maine, focused on the cultivation of oysters. Since its founding in 2017 the operation has grown from four initial LPA licenses to a ten-acre standard lease with over two million oysters in cultivation. Steady annual growth in scale paired with a constant focus on refining processes and finding efficiencies has built a solid foundation of business development.

Through the growth of the business over the past eight years, the Maine Ocean Farm team has consistently sought solutions for operational sustainability. Two focal points of this effort have been the reduction in single use plastics and the reduction of direct carbon emissions. This talk will cover key moments of success, lessons learned, and next steps as both initiatives are in continual progress.

OCEAN
FARM SUPPLY

In looking to reduce the use of single use plastics, an internal audit of our operations highlighted the use of plastic harvest bags as the greatest occurance. Through the initial effort to find a plastic free solution, we partnered with a manufacturer in Austria, Packnatur, to develop a cellulose based mesh specifically designed for shellfish packaging. This beechwood fiber mesh is home compostable, biodegradable, and sourced from certified sustainable forests. Seeing an opportunity to diversify revenue streams, we founded Ocean Farm Supply to sell this Ocean Harvest Bag mesh and deliver a sustainable packaging solution to the market.

In the effort to reduce direct carbon emissions on the farm we have undertaken a pilot program to implement a custom-built workboat with a fully electric power train. The project incorporates the installation of two shoreside charging stations to achieve operational usability and range. In partnership with the power train manufacturer, charging station developer, vessel designer, and municipal advocacy group, this pilot project is intended to offer a case study of power transition for the industry and real time data collection.

JUVENILE LARGEMOUTH BASS *Micropterus salmoides* DISEASE SUSCEPTIBILITY, GUT MICROBIOTA, AND IMMUNE RESPONSE TO *Aeromonas* SPP. INFECTION

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Large-scale culture of largemouth bass (LMB, *Micropterus salmoides*) has increased in recent years, and it remains a critical species for sportfish restoration and has shown promise as a food fish species. LMB are susceptible to numerous diseases (bacterial, viral, and parasitic), which can lead to massive economic losses for production facilities. Various bacterial diseases are responsible for LMB mortality, but *Aeromonas* spp. infections, including *Aeromonas veronii* and *Aeromonas hydrophila*, are associated with rapid, high-level mortality rates. These pathogens may be linked to increased rearing stressors in aquaculture systems, whether it be poor water quality parameters, increased temperature, or previous infections, since *Aeromonas* spp. are often considered opportunistic pathogens.

Due to limited information on the ontogeny of the LMB immune response and susceptibility to bacterial infections, developing new disease management techniques is critical. To elucidate these aspects of LMB health, we conducted an experiment to evaluate the long-term disease susceptibility and characterize LMB immune parameters during the critical juvenile rearing period. We hypothesized that the susceptibility to experimental *Aeromonas* spp. infection and immune response would be dynamic over this period.

To complete this study, *A. veronii* (ARS-LMB-32-2018) and *A. hydrophila* (ARS-LMB-9-2022) strains have been used to conduct monthly immersion pathogens challenge using juvenile LMB (~1 to 15 g) for six months. In addition to the challenge trials, the LMB will be sampled monthly (pre- and post-challenge) for spleen, kidney and distal intestine tissue to examine targeted immune gene expression and characterize microbial communities in the gut. Results from the month 1 challenge indicate that LMB are susceptible to *Aeromonas* spp., mortality rates observed at month 1 followed a similar trend with preliminary virulence assessments of the isolates. *A. veronii* produced a higher cumulative percent mortality (CPM) compared to *A. hydrophila* (27% and 10%; respectively). This long-term experiment will discern a more complete understanding of LMB health and disease susceptibility. An increased understanding of opportune times for treatment interventions will also be elucidated through this increased understanding of pathogen and host immune dynamics.

MECHANISMS UNDERLYING PROBIOTIC EFFECTS ON NEUROTRANSMISSION AND STRESS RESILIENCE IN FISH VIA TRANSCRIPTOMIC PROFILING

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Probiotic administration provides beneficial effects to cultured fish species, such as improved nutrient utilization, changes to the immune system, and disease resistance. Recent studies have highlighted the significant impact of probiotic treatments on the central nervous system (CNS) and stress regulation via the microbiota-gut-brain axis. However, the available knowledge regarding this important axis in fish remains limited. Therefore, a study was conducted using transcriptomic profiling to further our understanding of the mechanisms underlying probiotic effects in an important aquaculture species, the olive flounder (*Paralichthys olivaceus*).

Juvenile olive flounder (average weight, ~8 g) were incorporated into two diet trial setups: a 1-month lab-scale trial and a 6-month field-scale trial, each with and without the probiotic strain *Lactococcus lactis* WFLU12. In both trials, RNA-Seq was performed using liver samples collected from fish at 1 month post-feeding (mpf). In the field-scale experiment, growth performance was evaluated monthly, and serological parameters were measured at 1 mpf.

The results of the lab-scale trial showed that probiotic administration upregulated genes related to neurotransmission (i.e., *htr3a*, *mao*, *ddc*, *ntsr1*, and *gfra2*) and highlight the ability of probiotics to modulate neurotransmission via the microbiota-gut-brain axis. In the field-scale experiment, fish growth was significantly promoted, and the sera levels of AST, LDH, and cortisol were higher in the control group than in the probiotics group. Furthermore, genes involved in stress responses (i.e., *hsp70*, *hsp90B1*, *hspE1*, *prdx1,* and *gss*) and transcriptional regulators (i.e., *fos*, *dusp1,* and *dusp2*) exhibited upregulation in the control fish compared to the probiotic-fed group, indicating that probiotic administration can alleviate stress levels in fish. Overall, this study provides valuable insight into the neurotransmission and stress reduction mechanisms underlying the beneficial effects of administering probiotics to cultured fish.

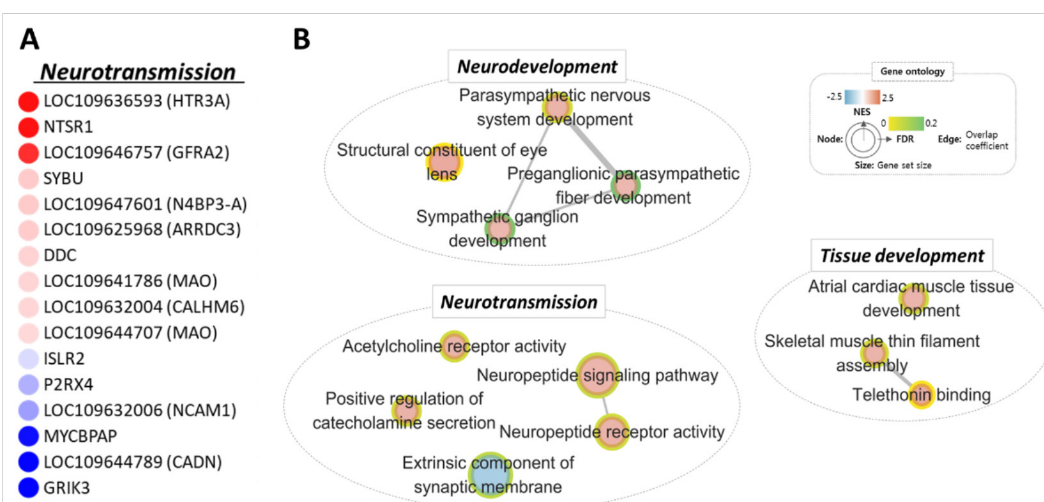

Figure 1. Transcriptome analysis of fish fed with probiotics.
(**A**) List of DEGs related to neurotransmission. (**B**) Gene set enrichment analysis.

GENOME-WIDE ASSOCIATION MAPPING AND GENOMIC PREDICTION OF SCUTICOCILIATOSIS DISEASE RESISTANCE TRAITS IN VACCINATED POPULATION OF OLIVE FLOUNDER *Paralichthys olivaceus* USING A 70K SNP GENOTYPING ARRAY

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Scuticociliatosis, a devastating parasitic disease caused by *Miamiensis avidus*, poses significant challenges in olive flounder (*Paralichthys olivaceus*) aquaculture due to high morbidity and mortality rates. While vaccination offers a promising disease management approach, it does not ensure complete efficacy across all fish, likely due to genetic variation in immune responses. Genomic selection and prediction are essential for identifying individuals with strong immune responses, enabling breeders to select disease-resistant candidates more effectively than the traditional pedigree-based methods. This study investigated the genetic basis of resistance to scuticociliatosis after vaccination, aiming to support selective breeding for disease-resistant olive flounder.

A cohort of 474 fish from 141 full-sib families received a formalin-killed vaccine followed by a challenge test with *M. avidus*. A custom high-density 70K SNP genotyping array was used to estimate genetic parameters, perform a Genome-wide Association (GWAS), predict genomic breeding values, and estimate its accuracy for host resistance to scuticociliatosis.

The results revealed significant genetic variation in post-vaccination resistance, with heritability estimated at 0.10. We identified 16 significant SNPs across several chromosomes using GWAS, with candidate genes linked to immune response pathways. Various prediction models were constructed to estimate host resistance traits, with Bayesian Lasso (BL), Bayesian B (BB), and Bayesian C (BC) achieving the highest accuracy. Prediction ability increased with the number of SNP markers and population size. Furthermore, by selecting significant SNPs based on GWAS to build a prediction model, the prediction ability increased significantly to 0.545, compared to 0.091 with randomly selected markers.

These findings offer valuable insights into host resistance to scuticociliatosis, facilitating improved selective breeding strategies in olive flounder aquaculture.

DEVELOPMENT OF OPTIMIZED GENOMIC PREDICTION MODELS TO ENHANCE GROWTH PERFORMANCE OF OLIVE FLOUNDER *Paralichthys olivaceus* UNDER EXTRUDED PELLET FEEDING REGIME

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Olive flounder (*Paralichthys olivaceus*) is a prominent aquaculture species in South Korea, with over 50% of the national production originating from Jeju Island. Currently, approximately 90% of Korean olive flounder farms utilize moisture pellets (MP), processed from small fish. However, the instability of MP supply has increased due to challenges in securing fishery resources, driven by resource depletion and marine pollution. Additionally, the high moisture content of MP complicates storage and results in rapid disintegration in water, increasing the risk of disease. In response, the Korean government is pursuing a policy mandating the use of extruded pellet feeds (EP). Despite this, farmers hesitate to adopt EP due to the observed lower growth rates in fish fed with EP compared to MP. Thus, there is a critical need for breeding efforts to enhance growth rates under EP feeding regimes to maximize production efficiency. This transition is also essential for the future automation of the olive flounder aquaculture industry.

To track growth, 1,200 fish were tagged with passive integrated transponders, and the body weight of each individual was measured at two-month intervals from June to October 2023 under a commercial EP feeding regime. Additionally, weight gain rate and specific growth rate, calculated from body weight data, were also used as phenotypic data. Genotyping was performed using a custom 70K SNP chip designed by our laboratory. The collected data were then used to conduct a genome-wide association study (GWAS) and to develop an optimized genomic prediction model.

Heritability estimates ranged from low to moderate, with the highest value of 0.38 observed for final body weight. GWAS results revealed no significant SNP markers based on the Bonferroni cutoff, indicating a polygenic architecture for growth traits. Among the 10 algorithms used to develop the prediction models, the highest predictive ability (53.8%) was achieved with GBLUP using final body weight as the target phenotype. Furthermore, we observed an increase in prediction ability of up to 79.5% by utilizing the top SNP markers identified through GWAS.

These findings provide valuable insights for selecting superior broodstock to produce high-growth progeny, supporting the development of new olive flounder lines with enhanced growth performance under EP feeding regimes. This research can thus contribute to a successful shift from MP to EP diets within the Korean aquaculture industry.

INTEGRATIVE STRATEGIES FOR ENHANCING GUT AND LIVER HEALTH IN SALMONIDS: ADDRESSING CHALLENGES OF SOYBEAN MEAL AND FUNCTIONAL FEED SOLUTIONS

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Aquaculture is an important industry for sustainable protein production, supplying nearly half of global seafood demand. However, the industry's reliance on fishmeal (FM) as a primary protein source presents sustainability and economic challenges. To address these issues, plant-based protein alternatives, particularly soybean meal (SBM), have been widely explored due to their availability and cost-effectiveness. While SBM is cost-effective and widely available, its antinutritional factors (ANFs) impair nutrient absorption, induce soybean meal-induced enteritis (SBMIE), and compromise fish growth and health. SBMIE, characterized by intestinal inflammation and disrupted barrier integrity, mirrors human inflammatory bowel diseases, where pro-inflammatory cytokines like TNF-α activate pathways such as NF-κB, exacerbating inflammation. The liver, another vital organ, is also affected, with SBM diets causing lipid metabolism disruptions, bile acid imbalances, and hepatic vacuolization.

Functional feeds incorporating prebiotics, probiotics, synbiotics, and postbiotics present a promising solution. These additives enhance gut integrity, modulate immune responses, and promote a beneficial microbiota. For instance, beta-glucans and mannan oligosaccharides restore intestinal morphology, boost immune activity, and mitigate SBMIE, while probiotics like *Pediococcus acidilactici* improve intestinal health and disease resistance. Liver health can also benefit from functional additives such as amino acids, bile acid emulsifiers, and vitamins, which alleviate SBM-induced hepatic damage and improve nutrient utilization.

This review integrates advances in dietary strategies to enhance gut and liver health in salmonids, proposing functional feeds and microbiome engineering as transformative solutions. Improving fish welfare, growth performance, and sustainability addresses key challenges in modern aquaculture. Future research should focus on refining additive formulations, unraveling molecular mechanisms, and conducting long-term evaluations to optimize the benefits of these strategies.

STEROIDOGENESIS IN FEMALE ATLANTIC SALMON (*Salmo salar*) TISSUE AT TIME OF OVULATION

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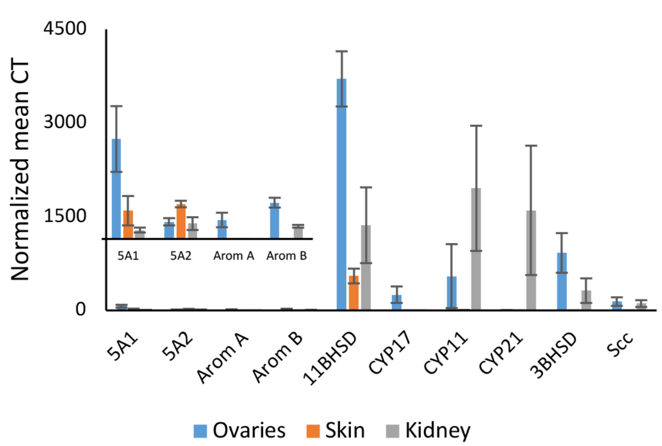
INTRODUCTION: The ability to control reproductive outcomes is key to successful Atlantic salmon aquaculture production. Steroidogenic enzymes shape circulating steroid hormone profiles and metabolites needed to induce changes in fish physiology that ultimately lead to spawning. This study reports on the steroidogenic capabilities of ovary, interrenal plasma and ovarian fluid in female Atlantic salmon at the time of spawning.

METHODS AND RESULTS: Ovary, interrenal, plasma, ovarian fluid and skin samples were collected from female (n = 15) Atlantic salmon at the time of spawning. These tissues were analyzed for steroidogenic enzyme transcript abundance and enzyme activity as well as steroid hormone concentrations. The abundance of mRNA for steroid hormone enzymes CYP11A, STAR, HSD3B1, CYP19A1, CYP19A2, HSD11B, CYP11, CYP17, CYP21, SRD5A1 and SRD5A2 were quantified by real-time quantitative PCR. Enzyme activity was analyzed for 3β-HSD, 5α-DHP, and the Cytochrome P450 enzyme group from isolated microsomes. Hormone concentrations from the enzyme activity assays and tissue concentrations were determined using LC-MS/MS to measure 26 hormones from all steroid classes. Transcripts of HSD11B and both isoforms of SDR5A were detected in salmon skin. CYP17 was only measurable in testis and ovary tissues and CYP19A1 was only measurable in ovary. CYP19A2 was detected in all tissues except skin. Hormones of all classes were detectable in interrenal, ovarian tissues and ovarian fluid. Glucocorticoids were primarily measured in the interrenal tissue and pregnanes were mainly measured in ovarian tissues. Interestingly, high concentration of 5α reduced testosterone (5αDHT) was measured in the ovarian tissue and slightly less was measured in the interrenal tissue. Additionally, high levels of 17 hydroxyallopregnanolone, an identified pheromone, was measured in the interrenal tissue.

DISCUSSION: This study was the first to measure steroidogenic enzymes in the skin of Atlantic salmon suggesting that skin is a site of steroid metabolism in reproductively mature Atlantic salmon. This study was also the first to identify tissue-specific synthesis of steroid hormone enzyme (via mRNA expression) coupled with steroid hormone concentrations to indicate where key hormones are produced in salmon. This information can potentially be used to better understand spawning in Atlantic salmon.



SHIFTS IN OYSTER SPAWNING PATTERNS IN DELAWARE BAY: A COMPARATIVE STUDY (2014 VS 2024)

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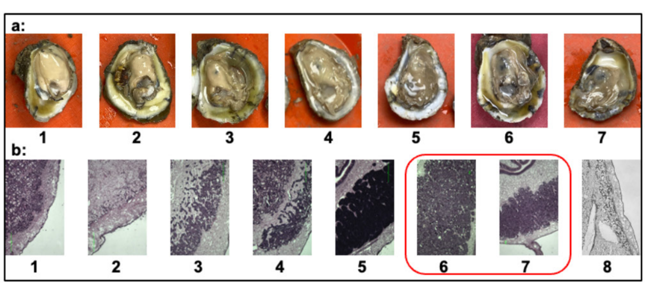
Understanding the timing of oyster spawning in Delaware Bay is critical for effective oyster restoration, enhancement, and management. This knowledge is essential for optimizing shell planting, shellfish transfers, and harvest strategies. A deeper insight into local spawning patterns and their interaction with environmental factors can improve both the conservation of oyster populations and support the shellfish industry while benefiting ecosystem health.

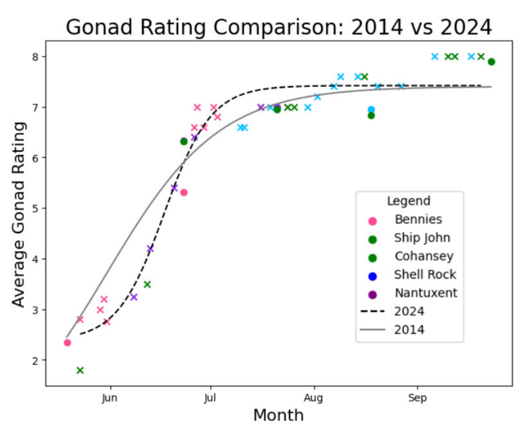
Oyster spawning in Delaware Bay occurs from late spring to early summer. Timing is largely influenced by the seasonal temperature cycle and food availability. Like other regions, the Delaware Bay is warming, which could alter the timing of oyster reproduction.

This study examined the use of gross observations of oyster condition to provide real-time feedback to the industry stakeholders and resource managers, followed by histological analysis to gain detailed insights into gonadal tissue development and the timing of spawning. Weekly samples of ten to fifteen oysters were collected from commercial landings during the 2024 season and compared to archived monthly samples from 2014. Histological gonad ratings were correlated with gross observations and used to determine the timeline of gonad development and spawning during 2024, then compared with a histological timeline from 2014 to assess change over the last decade. Corresponding temperature and salinity data were analyzed to understand environmental influence on the observed spawning periods.

Despite an average annual temperature increase of 0.91°C since 2014, our findings indicate that the spawning period remained mid to late June, but was shorter in 2024. This pattern indicates later season shellplants may fail to obtain set, informing management strategies in the face of climate change.



Rating scales for condition (a) and gametogenesis (b). (Kim et al. 2006).
Circled are ratings for oysters that are actively spawning.



LOKO IʻA TO LAWAIʻA: SAFEGUARDING CULTURAL CONNECTIONS TO CORAL REEF SEAFOOD SYSTEMS IN HAWAIʻI

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There is growing interest in developing social and cultural indicators for marine management, yet ecosystems are typically articulated using mainly ecological concepts. Further, the resilience of coastal communities to climate change impacts and related social-ecological uncertainties depends on social and cultural considerations that are underrepresented in management by agencies such as NOAA Fisheries. While NOAA Fisheries has initiated some efforts to identify social and cultural priorities for lawaiʻa (fishing), loko iʻa (Hawaiian fish ponds) have not been a focus of integrated agency management. Yet, they were traditionally part of an interconnected seafood system that local communities are working to restore. Therefore, this project is developing collaborations with coastal communities – particularly those centered around loko iʻa and with linkages to conventional aquaculture – to identify their priority concerns given uncertain futures due to climate change and other anthropogenic factors that affect cultural connections with coral reefs. Through this collaboration, this project will develop biocultural indicators and thresholds that can be used to monitor impacts and evaluate effectiveness of NOAA management, thereby improving NOAA's contributions to the resilience of coastal communities and their well-being. It also contributes to the work of the USDA multistate committee focused on Marketing, Trade, and Management of Aquaculture and Fishery Resources. In this presentation, we outline a community-centered process that identified this area of research, potential partners, and products that will benefit communities as well as management agencies. Leading with community rather than agency needs is paramount for designing authentic engagement processes that benefit rather than overburden the people they are designed to serve.

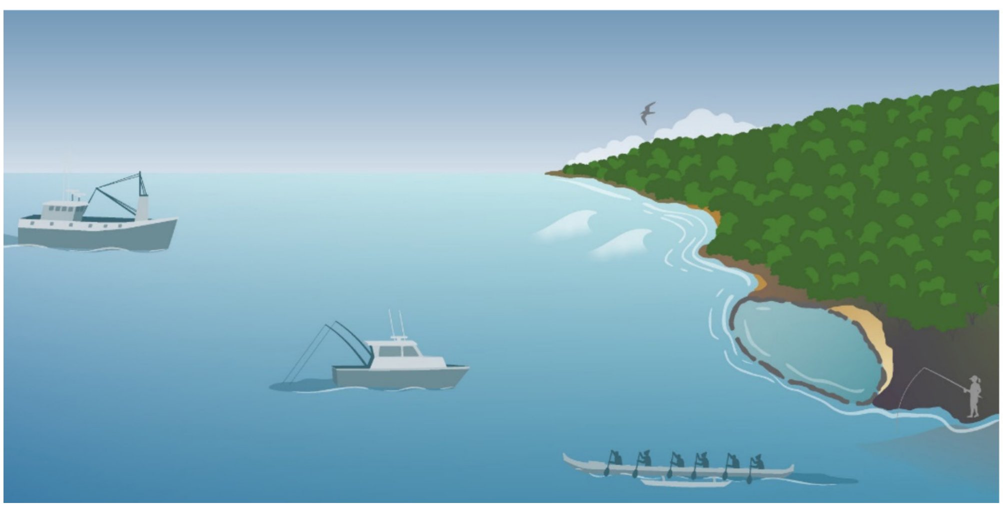

Figure 1. Loko i'a (Hawaiian fish ponds), depicted as the semi-circular kuapā (rockwall) on the right, are part of an interconnected seafood system that includes lawaiʻa (fishing).

INCREASED STOCKING DENSITY REDUCES THERMAL RISK DURING EMERSION: A WIND TUNNEL STUDY USING ROBO-OYSTER BIOMIMETIC LOGGERS

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Intertidally cultured oysters encounter high aerial body temperatures that pose physiological challenges, including reduced growth, disease-resistance and survival. Husbandry practices, such as a farm's chosen stocking density, may impact these body temperatures, potentially leading to differences in growth and survival. Accurate monitoring of body temperature, with which most temperature loggers struggle, could allow growers to assess their stock's thermal risk. Synthetic mimics of organisms with embedded temperature loggers, or "robo" loggers, have accurately mimicked body temperature and could effectively measure thermal risk in intertidal oyster culture.

We conducted wind tunnel experiments to evaluate the effect of stocking density on oyster body temperature. Adult Pacific oysters (*Magallana gigas*) fitted with thermocouples were placed in bags at the top and bottom of an oyster group at ten densities from 50 to 500 and then heated for 3 hours at "calm" and "windy" wind speeds (0.3 and 1.4 m/s). We hypothesized that higher stocking densities would reduce oyster body temperature. Oysters in densely stocked bags (≥ 250/bag) were ~5°C cooler than those in sparse ones, but temperatures of oysters positioned on top were not density-dependent and 5-15 °C warmer (to 26-37 °C).

Concurrently, we evaluated "robo-oyster" loggers' ability to mimic oyster body temperature by collocating them with live oysters, hypothesizing that the loggers would accurately approximate oyster body temperature. Robo-oysters respond like real oysters to changes in density, wind speed, and bag position, but generally overestimate body temperature.

Our findings suggest stocking densities ≥ 250/bag may protect some stock from acute heat stress, but may increase thermal heterogeneity, which may lead to differences in growth and survival. We find biomimetic "robo-oysters" to be a useful tool for monitoring oyster body temperature because they can approximate real oysters while being more robust and user friendly for long term field deployment. These results emphasize the importance of studying thermal risk on a micro-scale in intertidal culture environments, particularly as the climate warms.

A B

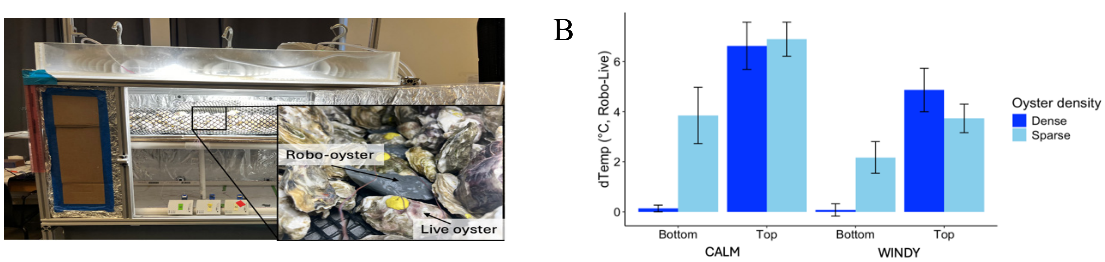

Figure 1. A) Wind tunnel experimental setup with pop-out of live oysters with thermocouples and robo-oysters. B) Differences between the final hour average temperatures of collocated live and robo-oysters. Bars are standard errors.

A COMPARISON OF GROWTH AND MORTALITY OF EASTERN OYSTERS *Crassostrea virginica* IN SOUTH FLORIDA ESTUARIES

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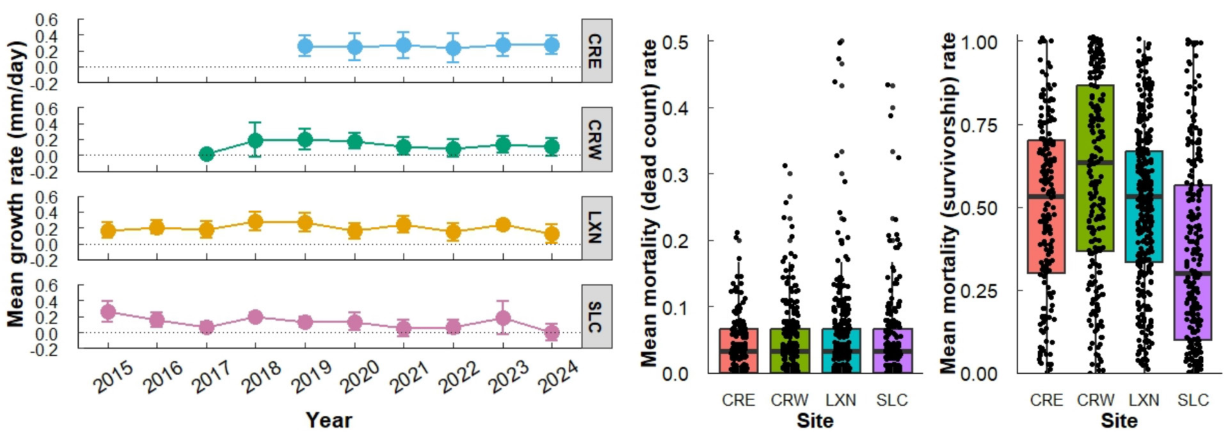
Growth and mortality rates are key components to understanding population health. Changes in growth or mortality of estuarine species, including eastern oysters (*Crassostrea virginica*), can indicate alterations in water conditions or habitat suitability. Comparing rates of growth and mortality over various timeframes can be used to track the natural cycle of estuaries and to identify when conditions may be negatively impacting oyster populations.

In south Florida, oyster growth and mortality rates have been monitored as part of the Comprehensive Everglades Restoration Plan (CERP). Beginning in 2015 (St. Lucie estuary and Loxahatchee River estuary) or 2018 (Caloosahatchee River estuary), wild oysters were planted in open cages at representative reefs. Metrics included monthly measurements of shell heights and live and dead counts. From that data, the average oyster size, mortality, and mean growth rate was determined for each estuary.

Shifts in oyster health can be assessed through the degree and direction of fluctuations in mean oyster size, growth rates, or mortality of oysters. Assessing growth rates and mortality rates may help to evaluate the relative effect of water conditions versus predators or disturbance events. Evaluating how fluctuations in size, growth, and mortality align with changes in water conditions, such as salinity and temperature, or predator populations can be used to understand changes in estuary conditions and to provide guidance for future restoration of oyster habitat. The metrics of oyster growth and mortality will serve as baseline conditions used to evaluate system-wide change in hydrology as the full Everglades Restoration Plan is implemented.



COMMERCIAL USE OF BIO-ACOUSTIC TECHNOLOGY FOR DETERRING BIRDS FROM ROOSTING AND CONCGREGATING ON FLOATING GEAR: NON-LETHAL SOUNDSCAPES DESIGNED TO JAM THE BIRDS ABILITY TO COMMUNICATE

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Intro: The increased use of floating gear in commercial shellfish operations has led to a greater presence of birds, resulting in significant guano accumulation on the floats. Since 2015, health authorities in several states have tested water samples around these operations, occasionally detecting elevated coliform levels. In 2021, eight consumers in Rhode Island were hospitalized with *Campylobacter* infections, prompting the closure of two shellfish farms. As a result, new regulations are requiring the farmers to have a written operational plan outlining bird deterrence measures. Traditional deterrents, such as kites, cannons, spikes, and zips, have often proven ineffective and costly over time.

Purpose: Over the past 6 years of direct R&D, we have developed a non-lethal, sound-based technology to deter birds from congregating in commercial agriculture, aquaculture, power substations, food processing plants, and dairy operations.

Method: Our technology is unique in its ability to disrupt and "jam" bird communication. Unlike traditional deterrents that rely on fear, our system targets instinctual behaviors crucial for survival. Birds feel unsafe when they cannot effectively communicate and will then naturally leave the area. The technology includes three setting allowing the ability to focus on specific bird species based on their communication patterns. Our technology offers a deterrent technique birds do not habituate to.

Key Findings: Our technology has been successfully deployed across a variety of commercial agricultural settings, reducing, or eliminating bird-related damage and contributing to increased yields, from cherries and grapes to walleye and koi. Additionally, it has demonstrated effectiveness in reducing bird presence and the associated health risks in power stations, food processing facilities, and dairy farms. At the time of this submission, we are working with several oyster farms to prevent birds from roosting on equipment, thereby minimizing contamination risks and supporting regulatory compliance regarding active bird deterrence.

Future Implications: Our non-lethal bird deterrent system is a massive step forward in helping commercial shellfish operations meet and exceed health related regulatory obligations, protect workers, be "good" neighbors and increase operational efficiencies.

Fig1: GTG-700 Trailer AC power with directional array, Dafter MI

Fig 2: GTG-700 Trailer with Solar and 360-degree speaker array Sutton Bay, MI

MODELING METAPOPULATION DYNAMICS AND CONNECTIVITY IN OYSTER RESTORATION

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Restoration of native oyster populations relies on an understanding of inter-population connectivity and metapopulation dynamics. This study modelled the effects of connectivity on recruitment and metapopulation dynamics of the eastern oyster *Crassostrea virginica* for a planned restoration by the US Army Corps of Engineers in the Tangier Sound-Pocomoke Sound (TSPS) system, an area of about 70,000 ha encompassing 56 potential restoration sites in lower Chesapeake Bay. Modeling integrated (i) historical and recent data on habitat suitability, (ii) analysis of multiple connectivity matrices representing diverse environmental conditions, and (iii) a stage-structured metapopulation model linking individual population models for each of the populations through the connectivity matrices. Biophysical modeling involved release of virtual larvae from multiple potential reef patches in the metapopulation, advection for 2 weeks, larval mortality, and settlement on reef patches for 1 week.

Connectivity patterns were diverse and varied significantly among the metapopulations, but could be classified according to the construct of source-sink dynamics. Populations were characterized by their value to restoration, 'bet-hedging' strategies, resilience to climate change, and as linked harvest grounds subsidized by protected source populations. Populations in a metapopulation could be sources, sinks, pseudo-sinks and stepping stones, among others. Modeling indicated that the TSPS system was comprised of three loosely connected metapopulations, which made it difficult to optimize connectivity of simulated restored populations and metapopulation recruitment concurrently. Consequently, a tradeoff exists between restoration of populations across the whole of the TSPS system and maximizing metapopulation recruitment.

THE USE OF CAPTIVE BROODSTOCK TO ACCELERATE THE REINTRODUCTION OF AN ENDANGERED SACRAMENTO RIVER WINTER RUN CHINOOK SALMON *Oncorhynchus tshawystcha* TO BATTLE CREEK, CALIFORNIA

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California's Central Valley is home to four salmon runs, including fall, late-fall, spring, and the endangered winter-run Chinook Salmon *Oncorhynchus tshawytscha*. The development of California's water delivery infrastructure affected all Central Valley salmonids, particularly the Sacramento River Winter-run Chinook Salmon (winter-run). Winter-run now exist as a single population, which is constrained to spawn outside of their historic range in the mainstem Sacramento River below Shasta and Keswick Dams. While the historic distribution of winter-run included Battle Creek, a tributary to the Sacramento River downstream of Shasta Dam, they were locally extirpated from this tributary due to migration barriers and habitat degradation associated with hydropower development. An ongoing, multifaceted recovery effort, which includes habitat restoration, fish passage, and reintroduction through captive propagation, has resulted in multiple years of winter-run Chinook Salmon returning to Battle Creek beginning in 2019.

In the current study, we use an Ensemble Random Forest approach to evaluate the relative impacts of several factors on return success and precocious maturation in adult winter-run returning to Battle Creek using genetic tagging and associated hatchery pedigree data. Among the covariates in the model, number of eyed eggs per mm female body length, spawn year and relative fecundity were the most predictive factors (Figure 1). While sperm storage method (fresh versus cryogenic storage) was less predictive, cryogenic storage of sperm had the highest nominal impact and was negatively correlated with return success and positively correlated with precocious maturation. Collectively, the results of this study highlight the demographic success of the Battle Creek reintroduction effort, as well as illuminate critical factors to consider in the adaptive management of winter-run in the Central Valley. More broadly, these conclusions will serve to inform the responsible use of conservation aquaculture as a tool for the reintroduction of extirpated fish populations across the globe.

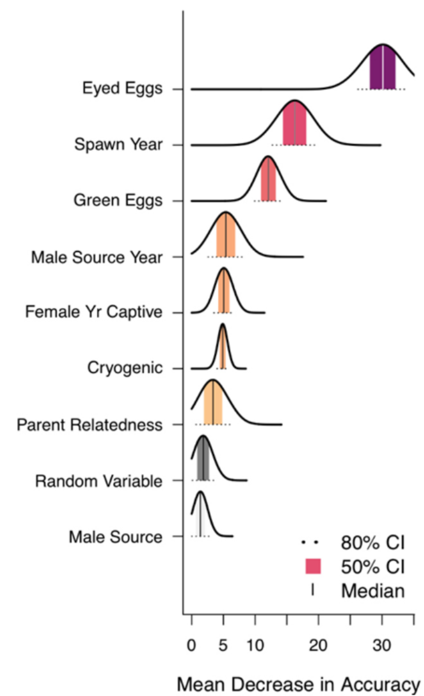

Figure 1: The variable importance metric, measured by mean decrease in accuracy, of covariates used in the Ensemble Random Forest of return success and time at large. The vertical bar indicates the median variable importance, the shaded region indicates the 50% confidence interval, the underline dotted region indicates the 80% confidence interval, and the solid line indicates the full range of variable importance across 200 Random Forests in the ensemble; dark colors indicate higher overall importance while light grey shaded regions are variables that fell below the median random variable importance.

BRIDGING THE KNOWLEDGE GAP BETWEEN CORAL CONSERVATION AND THE REEF KEEPING HOBBY: AN EXPERIMENT WITH HIGH SCHOOL STUDENTS IN GROTON, CT

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The overall health of the world's coral reefs are in decline. Overfishing, rising temperatures and pollution are all negatively affecting one of the oldest and most stable ecosystems on our planet. Although many organizations are focused on rebuilding the reefs, there is an untapped potential available. The global reef aquarium market is currently valued at over $5 billion dollars annually. The Covid-19 pandemic brought a rise in companion owners and increased the number of hobbyists maintaining their own saltwater aquariums at home. These hobbyists have been successful at maintaining and caring for a coral reef biome in their own homes for years. They spend thousands of dollars purchasing the right salt, life support, lighting systems and more. These hobbyists are part of online communities that share their successes, support each other's failures and push the limits of what is possible in a closed aquarium system.

At the Marine Science Magnet High School (MSMHS), located in Groton, CT, teachers work to connect the ideas of coral reef conservation and the business of the saltwater industry. Students work in the school's 20,000 gallon aquaculture facility where they gain skills in all aspects of aquaculture. Specific to reef keeping, students learn how to propagate corals, maintain a reef aquarium, and photograph various aquatic organisms. Additionally, MSMHS students operate a wholesale business by propagating specific species of coral, speaking with stores throughout the Northeast and providing organisms for the aquarium trade market.

Funds raised from the sale of coral are used to support the school's aquaculture facility, provide financial scholarships for undergraduate studies, and hire alumni to return to MSMHS for work. This model can be used for a number of other educational institutions and can be scaled appropriately. The work allows for significant opportunities to unlock environmental secrets that have been out of reach by the scientific community. Educating students on both coral aquaculture and business, bridges the gap between conservation and profitability, improves the educational experience and ultimately creates a more sustainable environment.

FIGURE 1: *Acropora sp.* fragment being grown for the aquarium hobby.

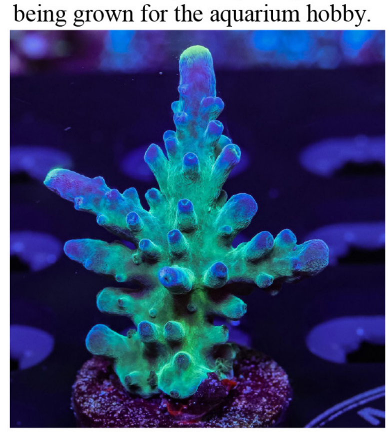
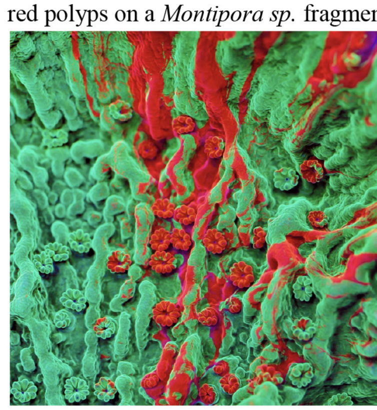


FIGURE 2: Macro image of green and red polyps on a *Montipora sp.* fragment



BEYOND THE CATCH: THE ROLE OF CLIMATE RISKS IN FISH TRADE

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Fish and fishery products are the most widely traded food commodity globally. The production and trade of these products are influenced by the climate risks posed by extreme weather events. Meanwhile, trade can also soothe the impact of climate risks occurring in a country and increase its resilience to those risks. This study analyzes the impacts of extreme weather events, such as hurricanes, droughts, and heatwaves, on fish trade trends in the past decade at the country level. We utilize the yearly net export change in value and volume as indicators of fish trade trends and the global climate risk index as a measure of the impacts of weather-related loss events. We also include the Worldwide Governance Indicators and the Index of Trade Freedom as measures of the country's governance quality and level of involvement in open markets, respectively. This research aims to identify the potential pressures and opportunities of fish trade facing climate changes and highlights the regions with a high level of vulnerability.

COMPARISON OF ACID INDOLUBLE ASH WITH YTTRIUM OXIDE FOR ESTIMATING APPARENT NUTRIENT DIGESTIBILITY IN TROUT

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Acid insoluble ash (AIA) has been used as a marker for nutrient digestibility studies in various animals for a long time. In aquaculture, it has been used as an internal or external marker for apparent nutrient digestibility. However, there have been conflicting results among studies regarding reliability of AIA as the marker in aquaculture. One major reason identified is that the method for AIA measurement is rudimentary and lacks sensitivity and reliability. To address this matter, a few years ago, we developed a significantly improved method for measuring AIA. Therefore, it is necessary to compare AIA with other known markers for apparent nutrient digestibility studies with fish, using the new method.

The present study with rainbow trout involved spiking a reference diet mixture with Celite (a pure commercial AIA) at 0, 0.3 and 0.6%, respectively, and yttrium oxide at 0.1%, mixing each of the three spiked references with fish meal, soybean meal, and DDGS, respectively, at 7:3 ratio, and making 12 diets (including the original three reference ones) by extrusion. Digestibility trials involved 36 tanks, three tanks per diet and 25 fish per tank. Each diet was randomly assigned to a tank of fish and fed to apparent satiation twice daily for 7 days prior to fecal collection.

There were drastic differences between the two markers in apparent digestibility coefficients (ADC) determined for several nutritional attributes in diets fed to rainbow trout, containing each of the three protein ingredients. For example, using yttrium oxide as a marker, average ADC of proteins in diets containing fish meal, soymeal or DDGS were 89.2, 87.8, 85.6%, respectively. In contrast, when AIA was used as a marker, mean ADC of proteins in the same diets containing the fish meal, soymeal or DDGS changed to 76.4, 41.2, 17.3%, respectively. The variation in ADC of a specific attribute among three tanks was also drastically larger for AIA than yttrium oxide. Careful examination of data revealed a huge difference in changes from diets to feces between yttrium and AIA composition within total ash. The percentages of yttrium in total ash in feces increased to 25.8-84.6% of those in diets, with an average of 53.1%. In contrast, the percentages of AIA in total ash in feces decreased to 27.6-67.7% of those in diets, with an average of 53.8%. Consequently, AIA content in feces was significantly reduced for most diets, indicating substantial loss as it went through the biological process within trout guts and under an aqueous environment. It is concluded that comparing to yttrium oxide, AIA was a much less reliable marker for digestibility study with trout.

AGE RELATED REPRODUCTIVE PERFORMANCE IN MALE BLUE CATFISH *Ictalurus furcatus*

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Hybrid catfish, the progeny of channel catfish (*Ictalurus punctatus*) females by blue catfish (*I. furcatus*) males, are in high demand by the U.S. aquaculture sector due to their superiority for pond and raceway culture. Typically, older blue catfish (≥5 years of age) are selected for artificial fertilization. Males are sacrificed, their testes are removed, macerated (cut), and sperm is released in an immobilizing media and quantified. It is only once the male has been sacrificed that sperm quantity can be assessed. Observations at our facilities and reports from hatcheries indicate a high degree of variation for testes size, sex hormone profiles, and sperm traits. It is essential to establish links between paternal age and reproductive success, as it is apparent that advanced paternal age causes changes to sperm that can have consequences for offspring health and performance through molecular changes and DNA mutations. Thus, a better understanding of interlinked physiological and molecular processes associated with paternal ageing will advance our male diagnostic toolbox.

This study investigated reproduction of blue catfish males aged 2 to 10 years, focusing on key reproductive parameters. A total of 103 males were sampled, representing 9 age classes. Fish morphometric data were collected, and blood was drawn to quantify testosterone (T), 11-Ketotestosterone (11-KT), osmolality, and ions. Histological images of testes assessed stages of spermatogenesis. Sperm were activated and kinematics analyzed. Testes transcriptome was also profiled across immature and mature individuals. Preliminary results showed that sperm were detected in 13%, 64%, and 100% of males at 2, 3, and 4+ years of age, respectively. Sperm kinematic traits increased at 4 years of age with a high degree of variation among males (Fig. 1AB). To identify differentially expressed genes (DEGs), pairwise differential gene expression analysis was performed between 2 vs. 4-year-old males (Fig. 1C). The numbers of upregulated DEGs and downregulated DEGs were 2,088 and 981, respectively. A similar comparison was conducted between 4 vs. 7-year-old fish, showing 18 downregulated DEGs and 19 upregulated DEGs (Fig. 1D). These data are paramount for designing studies to manipulate and control testes development to enhance production efficiency.

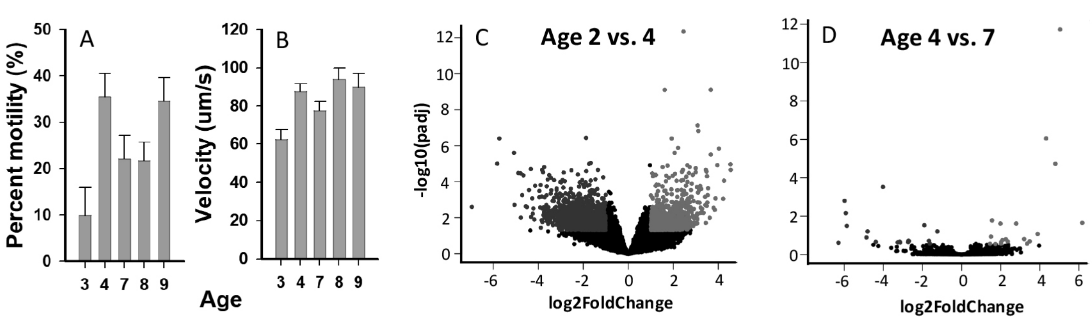

Fig 1. Impact of paternal age on sperm motility, sperm velocity, and pairwise differential gene expression between 2 vs. 4-year and 4 vs. 7-year-old blue catfish males.

NUTRITION AND FEEDING PRACTICES FOR LARGEMOUTH BASS – A REVIEW

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Largemouth bass *Micropterus salmoides* is a high-value sportfish and foodfish that is increasingly cultured in the US and other countries. Considerable research has been conducted to determine the nutrient requirements of largemouth bass and facilitate development of practical feeds to optimize fish performance and production economics. Formulation of feeds with environmentally benign ingredients (including many plant products) is increasingly preferred, though this can be challenging with carnivorous species. Minimal use of marine fish meal and oil in feeds is often required to attain certifications that can enhance marketability and long-term production sustainability of cultured fish. Plant ingredients modified to increase protein and decrease anti-nutritional factors have shown promise in bass feeds, as well as alternative animal ingredients such as insect meals. In addition, inclusion of feed additives such as enzymes, prebiotics, probiotics, palatants and others can increase feed intake and nutrient utilization of feeds to improve the overall nutrient utilization, cost-effectiveness and environmental impact of feeds for largemouth bass.

This presentation will provide an overview of the nutrient requirements and feeding practices for largemouth bass and suggest additional research needs to address industry-relevant knowledge gaps for scientists and producers.

SCALING EMERGING MARKETS: HOW THE VOLUNTARY CARBON MARKET AND THE MARKETS FOR NEXT GENERATION FEEDSTOCKS CAN EVOLVE AND CATALYZE CONCURRENT EXPANSION

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The unregulated Carbon Markets hold an unexploited potential to integrate environmental policy instruments with market solutions for rapid expansion of sustainable systems. The Carbon Markets have deployed a growing list of instruments to transition our high-polluting industries from unmeasured, unrestricted emissions to capped and regulated emissions with the long-term objective of zero-emissions. Newer, more innovative instruments will be needed to complement the current capacity of the Compulsory Market if the sustainable investments are ever to outpace the growth and necessity of unsustainable industry.

Next generation feedstocks represent an emerging market of sustainable, renewable biomasses that calculate efficient use of natural resources, solve difficult problems of unsustainable industry, and offer financial opportunity in sustainable industry. Seaweed is already on the cusp of commodity status. A novel financial instrument could dynamically engineer the expansion of the North American seaweed sector by providing the liquidity and financial activity inherent in tradeable instruments. Anchored to a carbon value a seaweed instrument could be one of a select few in a new asset class of climate finance instruments that populate the Voluntary Carbon Market (VCM) introducing robustness and integrity to the VCM and simultaneously generating the trading activity to substantiate market growth and commercial production in the US seaweed sector.

PRODUCTION PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF CHANNEL CATFISH *Ictalurus punctatus* FED DIETS SUPPLEMENTATED WITH SOY LECITHIN

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Soy lecithin (SOL) is extracted from crude soybean oil during the degumming process and is a rich source of phospholipids. This ingredient has been used as an aquafeed supplement to improve metabolic and physiological responses of farmed fish. Two separate feeding trials were conducted to evaluate SOL supplementation in channel catfish fingerling feeds. The first feeding trial determined the optimal SOL inclusion level through a dose-response feeding trial, followed by assessment of fish physiological responses after a stress challenge, and cumulative survival following *Edwardsiella ictaluri* challenge. This trial used experimental feeds formulated with plant-based ingredients, and SOL was included at 0, 0.5, 1.0, 1.5, and 2% at the expense of soybean oil. The second feeding trial compared production performance, stress response, and survival upon bacterial challenge in fish offered a diet containing SOL at the optimal level as determined in Trial 1 (1%) or diets containing soybean or catfish oil. In both feeding trials channel catfish juveniles (~4.5g) were stocked in a recirculating aquaculture system with 30 fish/tank and fed for 70 days. At the end of the first feeding trial, production performance and condition indices were assessed and blood and digesta were sampled. Fish offered the diet containing 1.5% SOL had higher weight gain and better feed efficiency when compared to the control group. No statistical differences were observed in whole-body proximate composition, however, the catfish fed diets supplemented with 0.5% SOL had a higher protein conversion efficiency than the unsupplemented group. The intestinal microbiota did not present differences, except for a mild effect on alpha diversity (Pielou's evenness index). Blood samples were collected 0, 0.5, 1, 2, and 6 hours after a 2-minute acute air emersion stressor. Post-stressor changes were observed in plasma cortisol, glucose, lactate, and osmolality over time, with an interaction observed between experimental diet and time for cortisol. Upon termination of the second study, blood and tissue samples were collected to assess production performance and welfare. The impacts of these diets on the innate immune response was assessed by qPCR targeting select genes associated with innate immunity. Catfish fed diets supplemented with SOL presented higher weight gain and feed efficiency when compared to catfish fed the soybean oil only diet. However, no differences were observed between SOL and catfish oil for the same parameters. No differences were observed for condition indices, survival, or blood parameters. Results of blood chemistry, gene expression, histology, and bacterial challenge for the second feeding trial are currently being evaluated. Collectively, these data suggest dietary SOL supplementation can enhance growth performance of channel catfish juveniles, and possibly affect physiological responses associated with acute stress and disease resistance.

INTEGRATING IN-SITU AND SATELLITE DATA TO PREDICT BIOTOXIN CONTAMINATION IN SHELLFISH USING ARTIFICIAL NEURAL NETWORKS

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Temporary closures of shellfish harvesting due to contamination by marine biotoxins remain a critical obstacle to the sustainable growth of shellfish production in Europe, particularly in Portugal. This industry plays a vital role in the economy of Portuguese coastal regions, yet biotoxin contamination presents substantial economic risks. As a result, the ability to predict contamination could greatly improve production management and minimize disruptions, offering a pathway to mitigate the economic impacts of such contamination events.

One difficulty in such predictions is the heterogeneity and high dimensionality of the data. In our case, biotoxin contamination values in shellfish, acquired by the Portuguese Institute for Sea and Atmosphere (IPMA) through the routine analysis for toxins quantification in shellfish, form irregular time series since samples are not taken at precise and fixed intervals. Sentinel-3 satellite image data, which provides data on sea surface temperature (SST), chlorophyll-a (chl-a) and multi-spectral images obtained from the Copernicus program, can provide thousands of values at each instant around each point of interest.

In this work we propose a two-step iterative process using the southern coast of Portugal as a case study. In a first step, correlation analyses and self-supervised learning is used to identify relevant regions and correlated sample locations contributing to the shellfish contamination in a target area and then extract compact representations that can feed predictive models. This is done by measuring Pearson correlations between previous contamination and satellite information across different production areas and current contamination with different time lags and then using artificial neural networks (ANN) to obtain signatures of the relevant vectors. These ANN are trained in a self-supervised manner as autoencoders and can be applied both to satellite data and contamination time series. In the second step, these signatures are then used in prediction models to predict the variation in contamination values at a target location in different time frames. This is an iterative process because the performance of the prediction models can then be used to fine-tune the input selection and signature generation to improve the final encoding and predictions.

Although we use the southern Portuguese coast as a case study to illustrate our approach, the present methodology can be applied to other cases where spatial-temporal patterns of SST and chl-a correlated with biotoxin shellfish contamination are available and, with suitable adaptations, potentially to other data sources depending on each particular case. The identification of the most informative data from neighboring production areas, time lags and variables (e.g., past contamination, SST, and chl-a) helps optimize the subsequent ANN model to predict contamination.

INSIGHTS INTO A NOVEL AQUACULTURE INDUSTRY IN NORWAY: CULTIVATING JUVENILE RED KING CRAB (*Paralithodes camtschaticus*) TO COMMERCIAL SIZE IN CAPTIVITY

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Red king crab (RKC) (*Paralithodes camtschaticus*) is an exclusive and highly valued decapod species that appeals to markets worldwide. In 2023, Norway exported 2447 tonnes of RKC, amounting to $ 80 million. The Norwegian RKC fishery is two-folded: a quota-regulated (QR) and a non-regulated (NR) fishery. The QR fishery is designated east of North Cape in northern Norway at 26°E and south of 71°30'N, while the area west of 26°E is the NR fishery. In Norway, the RKC is, by definition, an invasive species; hence, its migration west of this demarcation line is to be limited. Thus, there is an open fishery in the NR area. The RKCs caught in this area are often small, below commercial size, and usually discarded. Due to intensive fishing over the years in the QR area, the quota has decreased. In 2024, the quota is 60% less than in 2023.

At Nofima, feeding trials of juvenile RKCs with an initial weight of approx. 500 g have been performed. The trials lasted up to three years, and the juveniles molted three times. Marine by-products such as shrimp, blue mussels, sea urchins, capelin, whitefish, and lumpfish were used as feed.

The RKC weight increased successively (Fig. 1). The overall mortality rate was 45%, peaking at the last period of the trial.

Today, pilot studies are being performed on feeding juvenile RKCs. Success would potentially create a new industry and thereby supplement the RKC fishery in Norway.

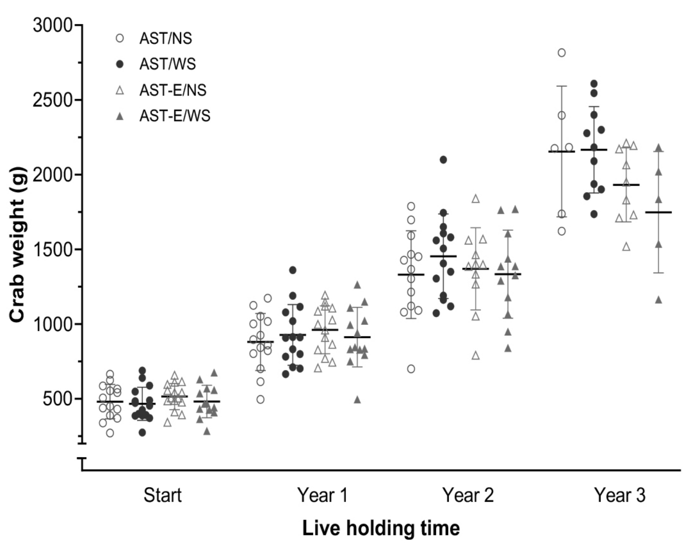

FIGURE 1. Weight increase after moultings (1–3) for juveniles kept at ambient seawater temperature (AST) and elevated seawater temperature (AST-E) either without (NS) or with (WS) access to sand.

THE 'ROUNDTABLE ON AQUACULTURE-AIDED FISHERIES ENHANCEMENT, CONSERVATION, AND RESTORATION': DEFUSING AND ADVANCING THE DIALOGUE ON HATCHERIES AND STOCKING PROGRAMS THROUGH INNOVATIVE ENGAGEMENT AND COMMUNITY-BUILDING

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Hatcheries and stocking programs have long been subject to controversy, sometimes with ferocious vigor, with some arguing against these techno-fallacies based on a large body of research demonstrating a lack of efficacy and even harm to wild fish genetics and ecosystems, and others finding new promise in how they might address rapidly changing ecologies and social needs for fisheries or conservation. Scientific advances and guiding principles such as the Responsible Approach provide a means for approaching such issues in a responsible and science-based manner but have not been widely and effectively implemented. Here we report on an innovative initiative to break the deadlock and advance the dialogue on hatcheries and stocking programs through innovative engagement and community-building. A Roundtable session at the 2024 World Fisheries Congress was convened with a diverse panel of 20 subject matter experts and attracted over 80 participants to discuss challenges and opportunities to better use aquaculture-aided approaches. Common themes included a sense that hatcheries and stocking are human endeavors but that their human dimensions have been neglected in science and policy; the diversity of approaches and contexts (one size does not fit all); and a strong sense among participants that a more open and constructive dialogue is overdue. In this presentation, we will discuss key insights from the Roundtable, and ongoing activities aimed at building an increasingly broad-based, trans-disciplinary community of practice around the use of aquaculture-aided approaches.

UNLOCKING HUFA DEGRADATION PATHWAYS: THE ROLE OF DHA, EPA, AND ARA IN GUT-EVACUATED *Artemia franciscana* FOR MARINE LARVAE DEVELOPMENT

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While advances in live feed manipulation have been made, no artificial feed can fully replace zooplankton, making live feed indispensable for many species in marine hatcheries. Despite lacking essential fatty acids (FA) for marine larvae development, particularly EPA (eicosapentaenoic acid – 20:5(n-3)), DHA (docosahexaenoic acid – 22:6(n-3)) and ARA (arachidonic acid – 20:4 (n-6)), *Artemia*, is widely favored due to its broad acceptance.

Cysts of three strains – Vinh Chau (VC), Great Salt Lake (GSL), and San Francisco Bay (SFB) – were hatched (2 gL^{-1}) under standardized conditions. After a 24-hour hatching period, the nauplii (approximately 263±14 animals ml^{-1}) were collected and either enriched with INVE Aquaculture's Selco® emulsion or left unenriched (Control- NE). Following the enrichment, the animal's gut was cleared using Silicon Dioxide (SD) at a density of 1gL^{-1} for 2h, rinsed, and stored in clean seawater at either 27ºC (E27) or 16ºC (E16). Samples from each treatment were collected at specific intervals post-hatching (24, 48, 50, 53, 56, 62, and 74h) for Fatty Acid Methyl Ester (FAME) analysis. Data was analyzed by three factors: *Artemia* strain (GSL, SFB, VC), time after hatching (48h, 50h, 53h, 56h, 62h, 74h), and treatment (NE, E27, and E16). Differences in FA (area %) were compared within strains to evaluate enrichment (NE vs. E27) and temperature effects (E27 vs. E16), and across strains to assess strain influence.

FAME analysis identified 42 fatty acids (FAs) across strains, with only seven showing no significant differences throughout the experiment. The remaining 35 FAs were further analyzed and results showed an inconsistent FA behavior across treatments (Table 1), with significant degradation differences observed within each strain (Table 2). The difference in FA composition across strains, suggests that strains use different FA degradation pathways under identical conditions. Animals from the E27 treatment showed the highest number of significantly different FAs across all strains and those from the E16 the greatest discrepancies emphasizing temperature's effect on FA composition. Spaghetti plots confirmed linear time progression and strain-specific differences in FA levels. Enrichment affected the FA levels across strains differently, with EPA, increasing significantly in SFB but less so in GSL and VC *Artemia*. DHA, initially minimal or absent across strains, showed the greatest increase after enrichment. Its degradation afterwards is likely linked to synthesis of EPA as literature suggests a natural conversion of DHA into EPA.

Table 1 Statistical different FAs per treatment (NE, E27 and E16) within each strain (VC, SFB and GSL).

	NE	E27	E16
VC	19	24	22
SFB	15	23	7
GSL	17	23	17

Table 2 Common FAs to treatments (NE, E27 and E16) within each strain (VC, SFB and GSL).

Strain	VC	SFB	GSL
FA	31	28	27

A NEW FISH TO FRY: THE BRASSY CHUB *Kyphosus vaigiensis* IS READY FOR COMMERCIALIZATION

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Kyphosis vaigiensis is a warmwater marine finfish, found throughout the central and western Pacific. *K. vaigiensis* and its congeners are a cosmopolitan group of reef associated herbivores known as chubs, drummers, or nenue (US Atlantic, Australia, and Hawai'i respectively). In Hawaii, nenue are highly favored, and often used in poke (traditional raw fish salad). Their herbivorous nature makes them an appealing candidate for aquaculture, because fishmeal and fish oil do not need to be a large percentage of their diet. Further, they can tolerate a wide variety of agricultural grains, making their feed less expensive, the price less volatile, more scalable globally, and less impactful (being closer to the base of the food chain). Because of these characteristics Ocean Era began a concerted effort to investigate *K vaigiensis*' potential for commercial culture in 2015.

Since 2015, Ocean Era (and predecessor Kampachi Farms) has performed almost a dozen feed trials with nenue. We have completed five hatchery production runs, and we have shared fish with other producers and research institutions around Hawaii. The chubs have been raised in a variety of systems including tanks, fishponds, and net-pens. Recently local chefs have been working with the fish, and initial seafood market acceptance has been very positive.

This presentation will describe our results, methods, and observations for all stages of production and marketing. Additionally, behaviors of note, and experiences with parasites and pathogens will be discussed. We will make the case that - after almost 10 years of research - this robust, herbivorous marine fish could be the "tilapia of the seas".

THE AFFECT OF NUTRIENT SOURCE ON GROWTH RATE, PROXIMATE COMPOSITION, AND BACTERIAL COMMUNITY IN SEVERAL SPECIES OF TROPICAL MACROALGAE GROWN IN LAND-BASED CULTURE SYSTEMS

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Nutrient source, light availability, and a host of other environmental parameters will affect the physical and chemical characteristics of macroalgae. When macroalgae is being grown for a specific purpose (e.g. uptake nutrients in a settling pond, or making carrageenan), the environmental parameters of the culture system should be controlled, or at least monitored, as they affect the seaweed's ability to provide the service or make the compound of interest.

To better understand how several species of tropical macroalgae react to different culture conditions, Ocean Era, Inc (OEI) undertook a series of experiments exposing four types of tropical macroalgae (*Caulerpa lentilifera, Ulva lactuca, Halymenia hawaiiana, Gracilaria parvispora*) to different sources of nutrients. The nutrient sources were either effluent water from a marine finfish aquaculture system (high in ammonia), or deep seawater (high in nitrite + nitrate). Growth rate, proximate analysis, and C:N ratios were analyzed to understand the effects of the conditions.

Macroalgae's ability to "clean up" finfish effluent is well documented, but there is little information about the safety of that macroalgae for human consumption. To that end, OEI, and partners at the University of Hawai'i Mānoa explored the microbiome associated with seaweed grown in different conditions. Additionally, we examined how fish effluent water might affect levels of potentially pathogenic bacteria.

This presentation will provide an overview of the work that took place over 18 months, covering 10 separate trials, conducted at OEI's facility in Kona, HI. Basic culture system information, performance metrics, resultant nutritional data, and microbial species data will be discussed.

OPTIMIZING NEOMALE BROODSTOCK PRODUCTION FOR ALL-FEMALE SABLEFISH AQUACULTURE

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Sablefish (or black cod, *Anoplopoma fimbria*) is a burgeoning aquaculture species in Canada and the U.S., with established and emerging markets worldwide. Neomale (XX-genotype male) broodstock are key to the production of faster growing, more profitable, all-female sablefish stocks. Current industry protocols involve dietary treatment with a synthetic androgen, 17-alpha methyltestosterone (MT), during a critical period of early development to induce XX-genotype fish to develop as phenotypic males. At maturity, sperm collected from neomales is used to fertilize eggs from normal female broodstock to produce all-female offspring. However, MT-induced neomales often exhibit gonadal anomalies, including partial female development, abnormal morphology, and non-functional sperm ducts, limiting their utility as broodstock. These anomalies are likely associated with competing androgenic and estrogenic signals during sexual differentiation, as MT overrides but does not suppress estrogen production in XX fish.

Aromatase inhibitors (AIs), which directly suppress estrogen synthesis, may induce more 'natural' testicular differentiation in XX fish. The combination of an AI with MT has also been demonstrated in some cases to be more effective than either factor applied alone. We tested two AIs: a non-steroidal (AI-1) and steroidal AI (AI-2), alone or combined with MT, for neomale production. Treated diets were fed to all-XX genotype post-larval sablefish and gonad tissues were sampled for histology immediately post-treatment and at 4 and 12 months. Control males (XY) were reared and sampled in parallel for comparisons to normal testicular differentiation.

Aromatase inhibitor treatments yielded gonads with two lobes, resembling those of XY controls, while MT-treated gonads retained single-lobed gonads, similar to those of XX controls. Combined AI+MT treatments produced mixed phenotypes with one or two lobes. Histologically, gonads of AI-treated XX fish resembled those of XY controls, showing non-meiotic spermatogonia, whereas MT and AI+MT treatments advanced to meiosis (i.e. spermatogenesis) and exhibited spermatocytes and spermatids. Intersex gonads (ovotestes) were commonly observed across treatments, except the AI-2 and control groups.

This study highlights a promising AI-based dietary treatment for inducing more natural testicular differentiation in neomales, potentially overcoming androgen-induced anomalies associated with the use of MT. Future work will refine AI dosage and evaluate the reproductive success of AI-derived neomales for more scalable all-female production of sablefish for aquaculture. This research was supported by NOAA Office of Aquaculture through Internal Competitive Aquaculture Funds (ICAF).

EFFICACY OF A NEW GONADOTROPIN-RELEASING HORMONE PRODUCT FOR SPAWNING INDUCTION AND SYNCHRONIZATION IN SABLEFISH BROODSTOCK

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Aquaculture production of sablefish (*Anoplopoma fimbria*) and many other finfishes relies on treating broodstock with synthetic gonadotropin-releasing hormone analogs (GnRHa) for induction and synchronization of spawning. These peptides trigger the release of gonadotropins from the pituitary gland, stimulating gonadal sex steroid (e.g., estrogen and androgen) production and development. Ovaplant (Syndel Laboratories), a synthetic salmon GnRHa pellet has been shown to effectively induce maturation and synchronize spawning in captive sablefish broodstock. However, Ovaplant pellets were recently discontinued and fully replaced with a liquid product, Ovaplant-L (Ov-L). While the same hormone is used for both products, the matrices are quite different, resulting in very different diffusion characteristics. Ovaplant pellets use a cholesterol-based matrix that releases the GnRHa more slowly compared to the sucrose gel-based (liquid) matrix in Ov-L. The faster release and shorter duration of hormone exposure using Ov-L may not be as effective in multiple-batch spawners like sablefish.

This study investigated different Ov-L injection protocols to determine if the new product could successfully induce spawning in sablefish and if a particular protocol is more effective. We tested a single-dose Ov-L protocol outlined in the Ovaplant-L INAD (# 13-298) for the first time in sablefish along with a modified (two-dose) protocol that better mimicked the previous slow-release pellets through inclusion of a priming Ov-L dose, followed by a full or half dose to prolong the GnRHa exposure. We conducted two separate trials either using freshly caught sablefish broodstock (held captive ~3 months) or older reconditioned broodstock (held captive > 3 years). Fish were initially ultrasounded to identify broodstock at the appropriate gonadal stage then randomly divided into treatment groups (n = 4–5 fish/group). Ultrasound images and blood were collected prior to treatment, followed by weekly ultrasounds to track gonadal development and blood draws at 1, 2, and 6 weeks to determine sex steroid levels in the plasma. For each trial we evaluated the percentage of fish that spawned, days to first spawn, number of spawns, the number of eggs produced and egg quality (based on fertilization and symmetry of cell divisions).

In the freshly caught broodstock group, spawning rates were 100% for fish treated with a single or two-dose regimen of Ov-L. However, in the reconditioned broodstock group, only the two-dose treatment successfully induced spawning. Injection(s) with Ov-L induced increases in blood estradiol levels, with the two-dose regimen eliciting a greater response than the single-dose regimen. No fish spawned in the sham-treated control group from either trial and sex steroid levels were low. Overall, these trials demonstrated that Ov-L treatment can successfully induce spawning in sablefish, and that different injection protocols may be required for freshly caught versus reconditioned wild-origin broodstocks. This research was supported by NOAA Office of Aquaculture through Internal Competitive Aquaculture Funds (ICAF).

EXPLORING SINGLE-STAGE OXIC PROCESS FOR SIMULTANEOUS RAPID RECOVERY OF PHOSPHATE AND NITRATE VIA BIOFLOCS TO PROMOTE CIRCULAR ECONOMIC

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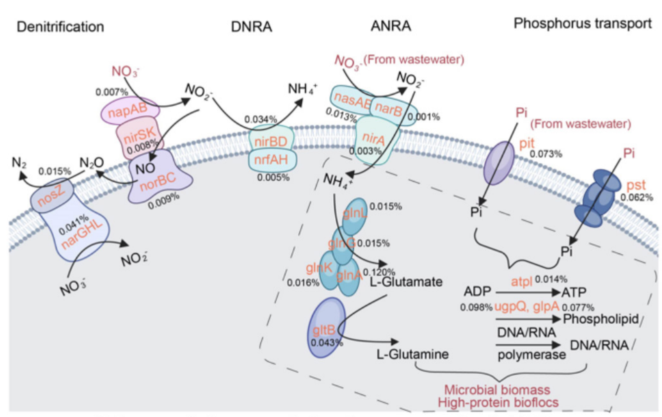
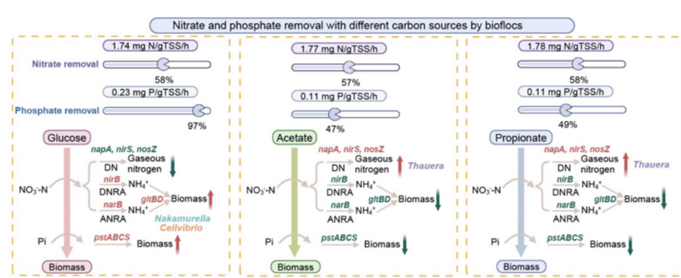
Intensive aquaculture systems often contain high concentrations of nitrate and phosphate, leading to environmental and economic burdens. Bioflocs technology, a novel approach in aquaculture, addresses these issues by removing these nutrients and producing protein rich microbial biomass. This study explored a novel single stage oxic process for simultaneous rapid recovery of phosphate and nitrate using different carbon addition strategies in bioflocs growth reactors.

Our findings revealed that bioflocs rapidly and effectively assimilated nutrients from high concentration environments. The phosphate removal mechanism involved biomass formation, achieving simultaneous removal efficiencies of 5.5 ± 0.2 mgP/gTSS/d for phosphate and 41.8 ± 2.0 mgN/gTSS/d for nitrate, harvesting biofloc concentrations of 3599 ± 33 mg/L. The crude protein content of the bioflocs exceeded 50%, with essential amino acid indices over 0.9, indicating potential for high quality aquafeed. Actinobacteriota and Bacteroidota were dominant during the phosphorus removal process, with significant proliferation of Nakamurella. Additionally, gene amplification related to assimilation, aerobic denitrification and inorganic phosphate transport was observed, suggesting biofloc technology is a promising method for efficient phosphate and nitrate removal.



Pathways of nitrogen and phosphorus conversion in bioflocs

This study also investigated the effects of different carbon sources on this process. Results revealed that phosphate removal rate in the glucose group was 0.61± 0.02 mg/L/h, significantly higher than those in the acetate (0.28± 0.01 mg/L/h) and propionate (0.29 ±0.03 mg/L/h) groups ($p < 0.05$). However, the three groups observed no significant differences in nitrate removal rates ($p > 0.05$). The superior performance of the glucose group in simultaneous nitrogen and phosphorus removal is likely due to the higher biomass synthesis. In contrast, nitrate removal in the acetate and propionate groups was primarily driven by denitrification, resulting in lower sludge production and reduced phosphate uptake. For practical application of bioflocs in simultaneous nitrogen and phosphorus removal, glucose is recommended as the optimal carbon source. This research promotes the circular economy by recovering nutrients, reducing reliance on traditional feed sources, and minimizing environmental contamination.



Carbon source on the performance of single-stage aerobic simultaneous nitrogen and phosphorus removal by bioflocs

BOOM-BUST DYNAMICS IN NATIVE AND NON-NATIVE MUSSEL SPECIES AND THEIR HYBRID CROSSES IN IRISH NEAR SHORE ENVIRONMENTS OVER TWO DECADES

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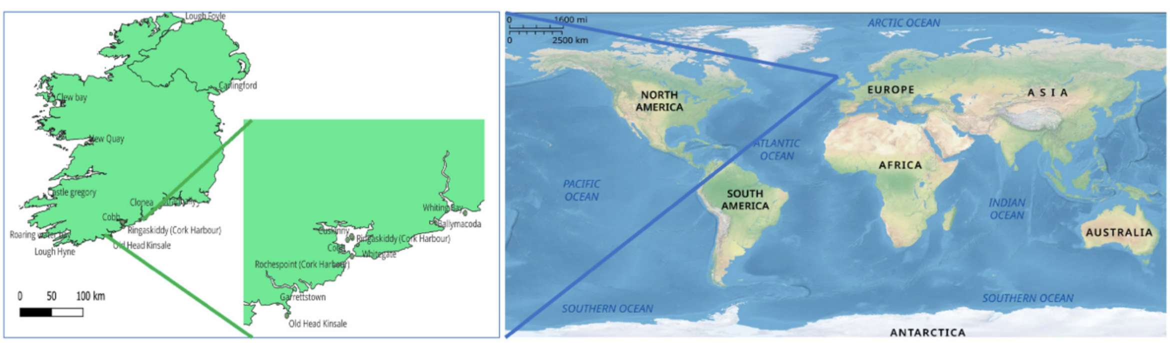
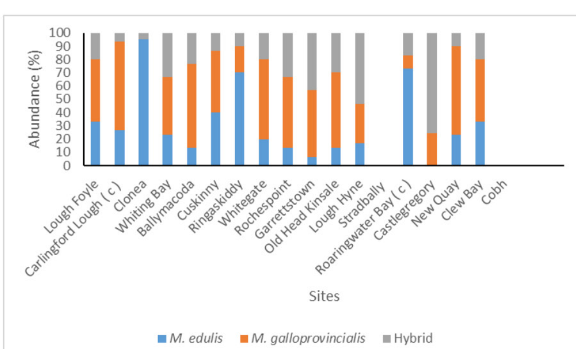
Mussels belonging to the Genus *Mytilus* are a significant ecological and commercial species of nearshore marine habitats. Along the south, west and north coasts of Ireland, intertidal mussel populations consist of the native blue mussel *Mytilus edulis*, non-native Mediterranean mussel *Mytilus galloprovincialis* and hybrids of both while *M. edulis* is exclusive to the east coast. *M. galloprovincialis* is listed in the ''World's worst 100 invasive species'' as it has outcompeted native mussel and bivalve species worldwide. The objectives of this study were to sample 16 mussel populations (n=471), historically investigated over two decades as well as new populations, and determine species and hybrid distribution and abundance (%) using PCR. The length and weight frequency distributions were also investigated to provide information on size patterns across different coastal locations and wave exposure levels. Mussels were absent from two sites where they were present in a past study. Overall, *M. galloprovincialis* was dominant at most sites, similar to two decades earlier. At a marine nature reserve, hybrids remained dominant. Mussel species/hybrid diversity and abundance remained stable at two Irish sites, while a "Boom-Bust" dynamic existed at eight sites between *M. edulis* and *M. galloprovincialis*. This instability of species composition and abundance may be due to natural phenomena, such as the Atlantic Multidecadal Oscillation (AMO), or natural fluctuations and adaptation to localized environmental parameters. What is evident is that although *M. galloprovincialis* may "disappear" during particular years and for prolonged periods of time, hybrids still remain and the non-native species reemerge to dominate.





THE ROLE OF NATIVE AND INVASIVE NON-NATIVE MARINE INVERTEBRATE SPECIES AS CARRIERS FOR BIVALVE PATHOGENS *Vibrio spp.* AND OSTREID HERPESVIRUS-1 MICROVAR.

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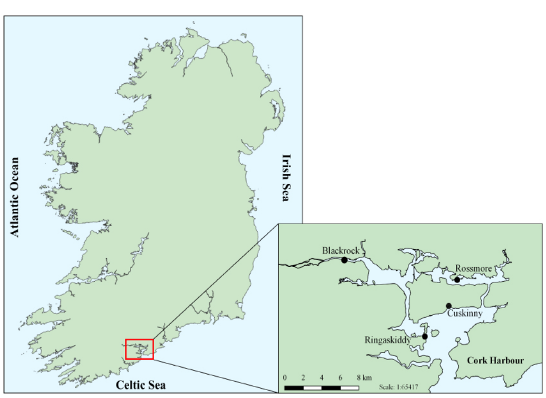
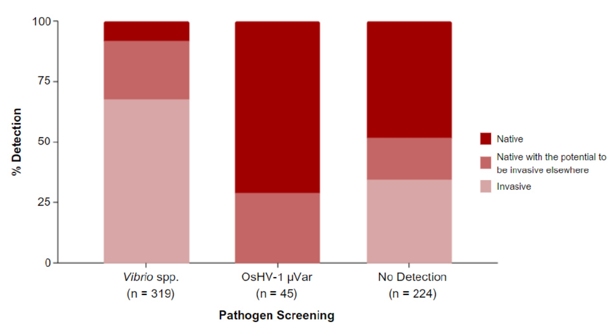
Invasive non-native species (INNS) are expanding their geographic range due to climate change, maritime traffic (primary route) and aquaculture (secondary route), resulting in the potential spread of microbes associated with them. Few studies have investigated the INNS-Pathogen phenomenon. In this study, marine invertebrate species (native and INNS) were sampled monthly over three months and screened by PCR for ostreid herpesvirus-1 microVar (OsHV-1 µVar) and *Vibrio* bacteria. Both pathogens are negatively associated with bivalve aquaculture globally. Samples sites included a shipping port, an oyster farm, a marsh nature reserve and a riverine site.

Crustacea, Mollusca, Polychaeta, Tunicata and Porifera were sampled. 54.3% (n = 319/588) including all taxa and sample sites were positive for *Vibrio* spp. The first detection of *Vibrio salmonicida* associated with Atlantic salmon *Salmo salar* was detected in the INNS beaked barnacle *Austrominius modestus*. OsHV-1 µVar (7.7 %, 45/588) was detected in Crustacea, Mollusca and Polychaeta at non-culture sites and in mussels *Mytilus* spp. at a much lower temperature (average SST 11.25°C) than previously recorded. The shipping port had the highest diversity of *Vibrio* spp. and detection of OsHV-1 µVar. Over half (51.1%) of "recently dead" shore crabs *Carcinus maenas* had either pathogen detected compared to 29.4% of living crabs. OsHV-1 µVar detection was significantly higher in dead crabs (24.4%) compared to living crabs (5.9%). Findings from this study contribute a better understanding of the role of estuarine native and INNS as vectors/carriers of pathogens, and how the spread of INNS might facilitate the spread of pathogens.

Sample Site	Taxonomic Group	Common Name	Latin Name	Native or Invasive
Rossmore (oyster farm)	Tunicata	Carpet sea squirt	*Didemnum vexillum**	INNS
		Leathery sea squirt	*Styela clava*	INNS
	Porifera	Breadcrumb sponge	*Halichondria panicea**	N
	Mollusca	Pacific oyster	*Crassostrea gigas*	N
		European flat oyster	*Ostrea edulis*	N
Total	**3**		**5**	
Ringaskiddy (shipping port)	Mollusca	Common whelk	*Buccinum undatum*	N
		Common cockle	*Cerastoderma edule*	N
		Common periwinkle	*Littorina littorea*	N
		Mussels	*Mytilus* spp.	N
		Tellins	*Tellinidae* spp.	N
	Polychaeta	Sand mason worm	*Lanice conchilega*	N
	Crustacea	Australasian barnacle	*Austrominius modestus*	INNS
		European shore crab	*Carcinus maenas*	N
		Rockpool shrimp	*Palaemon elegans*	N
	Tunicata	Orange sheath tunicate	*Botrylloides violaceus**	INNS
	Porifera	Breadcrumb sponge	*Halichondria panicea*	N
Total	**5**		**11**	
Cuskinny (marsh nature reserve)	Mollusca	Common cockle	*Cerastoderma edule*	N
		Mussels	*Mytilus* spp.	N
		Dog whelk	*Nucella lapillus*	N
		Tellins	*Tellinidae* spp.	N
	Polychaeta	Sand mason worm	*Lanice conchilega*	N
	Crustacea	Australasian barnacle	*Austrominius modestus*	INNS
		Amphipods	*Amphipod* spp.	N
		Edible crab	*Cancer pagurus*	N
		European shore crab	*Carcinus maenas*	N
		Opossum shrimp	*Neomysis integer*	N
		Rockpool shrimp	*Palaemon elegans*	N
	Tunicata	Orange sheath tunicate	*Botrylloides violaceus*	INNS
Total	**4**		**12**	
Blackrock (riverine)	Crustacea	Australasian barnacle	*Austrominius modestus*	INNS
Total	**1**		**1**	

* *D. vexillum*, *B. violaceus* and *H. panicea* samples were individual colonies





DEVELOPMENT OF RIFAMPICIN AND NOVOBIOCIN RESISTANT *Aeromonas salmonicida* STRAINS AND THEIR POTENTIAL AS LIVE ATTENUATED VACCINE CANDIDATES

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Aeromonas salmonicida, the causative agent of furunculosis, poses a significant threat to a diverse range of hosts in both fresh and marine fish. Currently, there are no commercial vaccines available to treat furunculosis in non-salmonid fish. Limited vaccine options exist to prevent furunculosis in sablefish specifically. This problem is evident as sablefish aquaculture has intensified in the Pacific Northwest, furunculosis continues to cause high mortality rates during production. The primary objective of this study is to develop a live-attenuated vaccine suitable for immersion administration, aiming to effectively treat furunculosis in sablefish for commercial aquaculture production. Attenuated strains of typical and atypical *A. salmonicida* were generated using rifampicin and novobiocin through successive passages on TSA with escalating concentrations of the antibiotics, reaching up to 400 mg/ml for both rifampicin and novobiocin. Resulting isolates were cultured at temperatures up to 30 for additional attenuation. Partial and full attenuation of *A. salmonicida* strains was confirmed through in vivo virulence challenges in sablefish and rainbow trout. Subsequently, an in vivo vaccine trial was conducted with the attenuated typical *A. salmonicida* Isolate (A5) in rainbow trout. The vaccination trial using the A5 isolate showed significant *A. salmonicida* specific IgM titers for both immersion and injection vaccination methods. Overall, the proposed live-attenuated vaccine candidates to prevent furunculosis in sablefish and rainbow trout could become an asset to sustainable aquaculture management if proven effective with further vaccination trials.

USING CHEMICAL CUES RELEASED BY BLUE CRABS TO CREATE MORE PREDATOR-RESISTANT OYSTER SEED THROUGH CHEMICALLY INDUCED MORPHOLOGICAL CHANGES

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Chemical signaling is critical in predator-prey relationships in marine systems. Not only do chemical cues elicit behavioral responses that aid in prey detection and predator avoidance, but they can also induce morphological changes during early development. For instance, prior research has shown that the eastern oyster, *Crassostrea virginica*, responds to chemical cues from crab predators with stronger shell development. This project aimed to induce this morphological response in a hatchery setting to create more predator-resistant oyster seed stock.

Several thousand newly single-set juvenile oysters were reared in setting trays within closed recirculating systems and exposed to chemicals released by live blue crabs, *Callinectes Sapidus*, for 16 weeks. The crabs were isolated in separate mesh cages to avoid predation and fed shucked oysters daily to enhance chemical release. A control group of unexposed juvenile oysters was also reared in setting trays in separate closed recirculating systems. Samples of exposed and unexposed oysters were collected at 12, 14, and 16 weeks, respectively, and underwent scanning electron microscope imaging analyses to assess shell thickness. Results showed that at 12 weeks, the exposed oysters were thicker than the unexposed ($p=0.05$). At 14 and 16 weeks, all exposed oysters were shorter than unexposed oysters ($p<0.001$). These results suggest that the presence of crab chemical cues may lead to shifts in the energetic budgeting of oysters, with exposed oysters prioritizing thicker shell development and unexposed oysters prioritizing traditional shell growth (length). To determine if increased shell thickness results in greater predator resistance in a natural setting, the remaining oysters will be deployed to field sites with and without predator exclusion, and survivorship and growth will be compared between treatments. Field sites with known crab and or oyster drill presence will be purposely selected. The development of predator resistance in seed oysters through chemical-induced morphological change may have significant implications for both aquaculture and restoration efforts.

DETERMINING THE OPTIMAL MACRONUTRIENT REQUIREMENTS FOR JUVENILE WHITE STURGEON *Acipenser transmontanus* GROWOUT DIETS USING A MIXTURE DESIGN

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Currently there is a lack of information on the most efficient grow out diets for juvenile white sturgeon from 1 year to 3-5 years, when sexing can occur. Most studies have focused on sturgeon below 1 year and over 7 years of age. During growout, producers maintain excess fish inventories and feed at high rates to maintain rapid growth. A majority of the feed costs incurred by producers occur in this growout period before sexing. Identifying the optimal diet for growout could allow for a significant reduction in feed costs for producers in the Western U.S. The study objective was to determine which of the experimental diets provided a 90% optimum response in terms of growth parameters.

A 20-week feed trial was conducted using 1.5-year-old juvenile white sturgeon, with a mean weight of 1105 ± 11.57 g. 14 fish were randomly assigned to each of the 24 400L tanks used. The flow rates were 3.0 L min^{-1}, water temperature was 16 ^{O}C, and a natural photoperiod was maintained to mimic commercial culture conditions in the Hagerman Valley. The sturgeon were fed at 0.7% of their body weight per day. At the onset of the study and at weeks 8, 14, and 20 the sturgeon from each tank were bulk weighed. Utilizing a simplex mixture design model with a constrained region, 14 experimental diets were formulated. The constrained region was defined using the known requirements of macronutrients for white sturgeon. The macronutrients in the diets ranged from 45-65%, 10-30%, 13.67-37.84% for fishmeal, fish oil, and wheat starch, respectively on a dry matter basis.

The data gathered suggested that the ideal diet with an optimum response of 90% would have a blend ratio of 0.6559 FM, 0.1884 FO, and 0.1556 WS. With this ratio the predicted SGR, DGI, and TGC are 0.33 %/day, 1.25 g$^{1/3}$/day, and 0.07 g$^{1/3}$/degree day, respectively. The FCR is predicted to be approximately 1.62 while the mean weight gain is expected to be 37.73%. This predicted blend will have an approximate survival rate of 96.62%.

Figure 1. Analysis of the diet response surface at the end of the 20-week feed study. The predicted optimal diet surface area, in white, with the suboptimal regions shaded in. The point is the 90% optimal response with the ratio of 0.6559 fishmeal, 0.1884 fish oil, and 0 .1556 wheat starch.

INTEGRATED MULTI-TROPHIC AQUCULTURE – HOW GEOPOLITICS CAN IMPACT ITS VIABILITY AND SUSTAINABILITY AS MUCH AS ECOLOGY OR BIOLOGY

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Ecological aquaculture, or polyculture, has been practiced for centuries in Southeast Asia and Africa but is still relatively uncommon in Western countries (Beveridge and Little, 2021). In Australia, as in other regions, commercial aquaculture is increasingly facing criticism for environmental impacts and product quality (Mazur and Curtis, 2006). With growing interest in seaweed and the circular economy in Western economies, we examined whether seaweed farming within an Integrated Multi-Trophic Aquaculture (IMTA) model could improve environmental and economic outcomes and boost social acceptance of aquaculture.

Our findings showed that while seaweed can be grown successfully in Australia alongside higher trophic-level species (Visch et al., 2024), there were several non-biological challenges which could influence the success of IMTA:

- **Regulations**: Strict policies focused on biosecurity or conservation agendas often limit co-production of species due to licensing and proximity rules.
- **Public Perception**: Many people misunderstand the nutrient cycling and nature positive benefits in IMTA, leading to concerns about using waste streams to feed other species.
- **Business Models**: Building integrated business models or IMTA partnerships is complex.
- **Conflicting Priorities**: There's often a mismatch between economic growth goals and environmental sustainability.

These challenges may discourage farmers and companies from adopting IMTA or reduce its effectiveness limiting the benefits that IMTA could offer like improved waste management, better nutrition from a more diverse range of aquaculture products, and more efficient resource use. To achieve sustainable outcomes, we must promote the value of integration at ecosystem, operational, political, and societal levels. Aligning policies and governance with new aquaculture practices like IMTA could help communities and markets better understand and support these initiatives, avoiding outcomes that undermine sustainability.

APPLYING MARK-RECAPTURE TECHNIQUES IN OYSTER AQUACULTURE TO IMPROVE MANAGEMENT DECISIONS

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Shellfish farmers face complex challenges in optimizing production across multiple variables, and with so many factors affecting oyster performance at Hog Island Oyster Company (HIOC), traditional production tracking methods often lack the resolution needed for data-driven management decisions.

In this presentation, we discuss how HIOC has adapted mark-recapture techniques commonly used in fisheries management (like RFID tagging in salmon and floy tagging in cod) to track oysters through their growth cycle. Additionally, by implementing a tagging system at the point of gear closure, we can trace individual groups of oysters from initial deployment through harvest while accounting for variables such as genetics, ploidy, seed size, planting date, gear type, and location. This methodology provides granular data on mortality rates, growth performance, and size class distribution across different production scenarios, enabling more informed decision-making for farm management in a changing climate.

DEVELOPMENT OF A PCR-BASED DIAGNOSTIC ASSAY FOR SCREENING *Enterocytozoon hepatopanaei* (EHP) IN FORMULATED AQUAFEED

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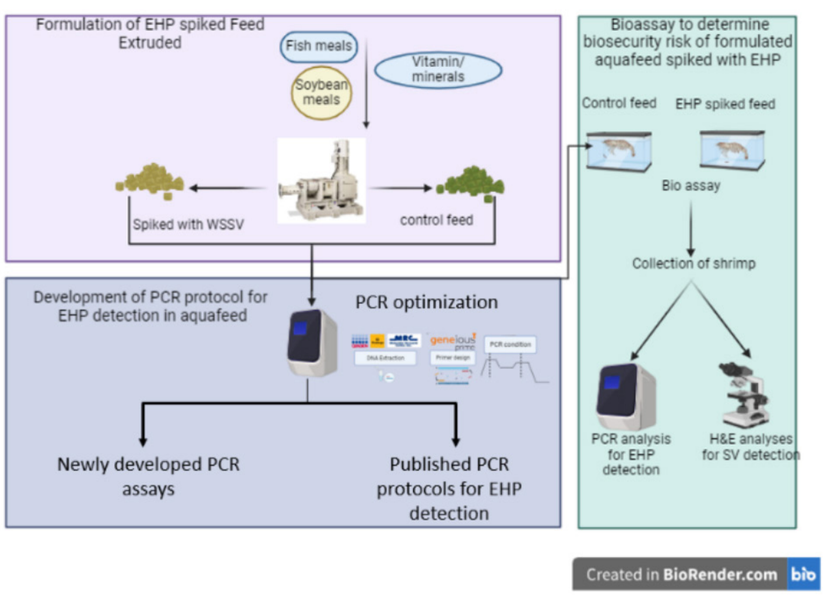
The use of formulated aquafeed shows potential as a safer option than fresh feed for large-scale farming of crustaceans and fin fish, offering numerous advantages. However, the current limitations of PCR-based detection methods for EHP and other crustacean pathogens hinder their widespread application for testing a diverse range of aquafeed and feed ingredients. In response to this challenge, we are developing a robust and validated PCR-based diagnostic assay for detecting EHP in aquafeed. This assay, once fully developed, will play a crucial role in enabling disease-free certifications of formulated aquafeeds, thereby ensuring the health and well-being of farmed shrimp.

Experimental shrimp diets were produced by incorporating EHP-infected tissue at 0.2% and 2% (w/w) in commercial-type feed formulation before extrusion. To find an optimal method of DNA isolation from aquafeed, total genomic DNA was isolated using four different methods. DNA polymerase gene was selected as a target gene to develop a PCR protocol for EHP detections since the polymerase gene plays a vital role in EHP replication. Four sets of primers/probes (corresponding to three conventional PCR protocols and one real-time PCR protocol) were developed. Three independent assays were conducted for each primer pair to determine the limit of detection (LOD). The LOD was 100 copies/reaction and 10 copies/reaction for conventional PCR and real-time PCR assays, respectively. The newly developed primers and probes detected only EHP, and no other pathogens known to infect crustaceans, in the specificity test.

The PCR assay using EHP polymerase as a target gene for PCR amplification demonstrates an unparalleled sensitivity and specificity in detecting EHP in formulated aquafeed. Since polymerase is a critical gene in EHP replication, a lack of amplification of polymerase would indicate that aquafeed does not contain infectious EHP. The EHP detection method described here provides an effective means of assessing biosecurity risk of formulated aquafeed and feed ingredients. By providing a rapid and robust means to evaluate the infectivity of aquafeed products, we empower industry stakeholders to make more informed choices, reduce waste, and optimize the utilization of resources, ultimately benefiting both the environment and economic sustainability of formulated feed used in shrimp farming.



AQUAGRIS: A NEW GLOBAL INFORMATION SYSTEM FOR CHARACTERISING AND RECORDING GENETIC RESOURCES FOR AQUACULTURE

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The Food and Agriculture Organization of the United Nations (FAO) was requested by its Members, through the Commission on Genetic Resources for Food and Agriculture, to assess the status of aquatic biodiversity used in aquaculture and to develop tools and policy responses to address the principal needs and challenges for its effective management. Following the publication of *The State of the World's Aquatic Genetic Resources for Food and Agriculture* in 2019, FAO developed, in active consultation with members, *The Global Plan of Action for the Conservation, Sustainable Use and Development of Aquatic Genetic Resources for Food and Agriculture* as a policy response. The Global Plan of Action is a voluntary, non-binding policy framework that identifies key strategic priorities and actions that countries and the international community should undertake for a more responsible management of aquaculture species, and their farmed types (the equivalent of livestock breeds and crops for the aquaculture sector) and wild stocks.

Information is power and availability of clear, consistent and harmonized information on the status of genetic resources, especially at the level below species ("farmed types" and wild stocks), promotes clearer understanding of the priority needs prevalent in countries and can inform the development of appropriate strategies, policies, management plans and human resource capacity. Very few countries possess information systems on their aquatic genetic resources and those that exist are not harmonized and utilize different characterization methods and different descriptors of genetic resources. FAO is addressing this need through the development and application of AquaGRIS, a global information system for aquatic genetic resources. A fully functional version of AquaGRIS was release in mid-2024 and FAO is now supporting countries in the application of AquaGRIS to develop national registries of their genetic resources for aquaculture.

Whilst the aquaculture sector in North America has developed and utilizes numerous developed farmed types (strains and varieties) of important aquaculture species the continent is also home to key reservoirs of genetic diversity for several important aquaculture species, including salmonids and ictalurid catfish. Due to the presence of a well-resourced and active research community, there is often a richness of information available on the genetic status of cultured species and their wild relatives, related to conservation, sustainable use and development these genetic resources. FAO is looking to engage with national focal points on aquatic genetic resources globally and to support them to create national registries of their aquatic genetic resources. Given the richness of data available on genetic resources in North America it would be interesting to explore the opportunity of utilizing AquaGRIS as a single depository of this information and to explore the benefits that could accrue to stakeholders in aquaculture and in cultured species more broadly from such a registry.

DISTRIBUTION, POPULATION STRUCTURE, AND FISHERY POTENTIAL OF THE GOLDEN DEEP-SEA CRAB *Chaceon somaliensis* IN THE KENYAN COAST IN EAST AFRICA

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This study focused on *Chaceon somaliensis*, a species in the geryonid family, which is commonly found in the Horn of Africa. The species has global commercial value, yet little is known about it.

The study adds to our understanding of the species by identifying its distribution, population structure, and fisheries potential in the Kenyan Coast.

Maxent modelling assessed the appropriate environmental variables and predicted potential species distribution and hotspot locations. Regression was used to explain *Chaceon Somaliensis* distribution and some aspects of the population structure.

Stratification by depth was observed with large male crabs (CW>150 mm, weight=1100 g) found in shallower depths (depth<501 m), while females, smaller in size (CW=92 mm, weight=316 g), seemed to prefer higher depths of >500 m. The males were dominant (0.94), and females and juveniles comprised only 0.06 of the population. The population was found to be skewed towards males of large size (CW> 140 mm, weight 1100 g). Bathymetry and environmental variables associated with feeding and nutrients, such as phytoplankton, iron, and silicate, were the best predictors of species presence. Potential sites occurred on a ridge at gentle slopes (0.98°– 4.31), with the hotspot areas being spatially about 3,230 km^2 of 61,694 km^2.

The fishery was considered productive and suitable for maintaining marine biodiversity (catch> 94% adults). Based on this study, it is recommended to prioritize the sustainable management of *C. somalien*sis fisheries, taking into account the depth stratification of the population, environmental variables, and identified hotspot locations to ensure long-term productivity and marine biodiversity conservation.

THE AUTOMATED FINGERLING CARRIER

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The transportation of fish seed (fingerlings) from hatcheries to farms or other aquatic environment is a critical aspect of aquaculture operations. Ensuring the health and safety of fingerlings during transportation is essential for the success of aquaculture enterprises. The automated fingerling carrier presents the design of a novel fingerling transportation system utilizing a portable and durable transparent bucket equipped with a 12 volts water pump, carbon dioxide sensor and batteries for power source to both the sensor and the Arduino board. The system aims to maintain optimal environmental conditions within the carrier, thereby enhancing the survival rate and overall fish health of transported fingerlings. The carrier involves, water circulation mechanisms for the purpose of dissolving oxygen in the water, to create an efficient and reliable transportation system for fingerlings.

The changing global environment is increasingly threatening life forms because countries are focused on development without caring about the environmental damage caused by pollution and degradation of agricultural lands. More factories are being built, using harmful chemicals, and people are using plastic bags, which harm the environment (Jalil et al., 2011). Fingerlings transportation plays a crucial role in the aquaculture industry, as it facilitates the transfer of juvenile fish from hatcheries to grow-out facilities or natural environments. During transportation, fingerlings are susceptible to stress, oxygen depletion, and accumulation of metabolic wastes, which can adversely affect their survival and growth. To address these challenges, innovative transportation systems equipped with monitoring and control mechanisms are being developed. This research focuses on the design and implementation of a fingerlings transportation system that utilizes advanced technology to maintain optimal environmental conditions within the transport container.

SALINITY DRIVES RELATIVE ABUNDANCE AND DISTRIBUTION BY SEX OF BLUE CRAB *Callinectes sapidus* THROUGHOUT THE PROVIDENCE RIVER ESTUARY

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Despite being well established in the Providence River Estuary (PRE), research on the blue crab (*Callinectes sapidus*) has historically focused on the mid-Atlantic and Gulf of Mexico, regions where the species has been commercially significant for decades. The species' population structure in New England, including the PRE, remains poorly understood by comparison. As water temperatures rise, blue crab populations may be driven northward in their range, prompting New England states to consider adopting commercial fishing regulations. In anticipation of potential management and regulatory decisions, understanding local population dynamics in the region is increasingly important.

An ongoing juvenile fish survey conducted by The Nature Conservancy in Rhode Island has collected data on the abundance of *C. sapidus* in the PRE, creating new insights into the species' population structure in the estuary. Seines were hauled along the shoreline at twelve sites in the Providence and Seekonk Rivers. Surveys were repeated once per month from May to October between 2021 and 2023. Crabs captured were then identified to genus or species, measured to the nearest millimeter at the widest point of the carapace, identified by sex, enumerated, and released. Additionally, temperature, salinity, and dissolved oxygen were recorded during sampling.

The sites were grouped into two distinct salinity regimes: five of the twelve study sites were classified as mesohaline (~5–18 ppt), while seven of the sites were classified as polyhaline (~18–30 ppt). *C. sapidus* were more abundant at the mesohaline sites and salinity is a factor influencing the ratio of males to females observed. In particular, a higher proportion of males were observed at the mesohaline sites. Furthermore, salinity is a factor influencing the length of individuals observed. Overall, these data demonstrate the continued importance of estuaries as nursery sites for juveniles. Within the estuary, they shed light on the role of salinity as a contributor to determining the abundance of crabs and the structure of subpopulations.

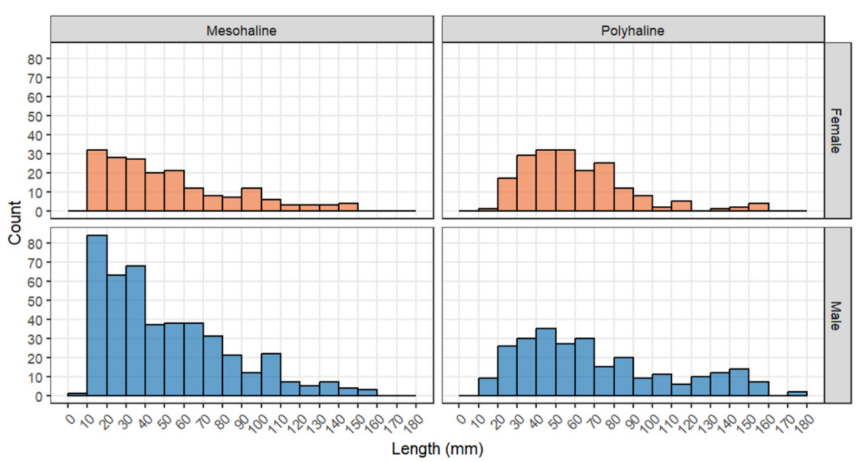

Figure 1: Length frequency distribution of blue crabs (n = 1081) in mm by sex at mesohaline and polyhaline sites

TRASH TO TREASURE: HARNESSING LOCAL RESILIENCE TO COMBAT GHOST NETS IN BANGLADESH

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The ocean and aquatic ecosystems are vital lifelines for countless species and human communities, yet they are increasingly endangered by plastic pollution, especially from "Ghost Nets," which are lost, abandoned, or discarded fishing gear that can persist in the environment for centuries. In Bangladesh, a country intricately tied to its water resources where fish is integral to both diet and economy and globally second in freshwater fish production ghost nets pose an especially critical threat.

With the rapid growth of aquaculture, more people are engaging in fisheries, contributing to an influx of synthetic, non-biodegradable fishing gear. As these nets accumulate, they introduce long-lasting environmental risks by entangling marine life, generating microplastics, and jeopardizing fish stocks that millions depend on for their livelihoods.

This presentation outlines a proposal for a year-long initiative to address ghost nets in Bangladesh's waterways through a phased approach.

We aim to remove ghost nets from high-risk areas, establish collection hubs, conduct research on material composition to support recycling, and develop economically viable products from recycled gear. Additionally, this project will promote awareness of ghost gear's detrimental impacts and engage coastal communities as active participants in sustainable solutions.

By fostering a circular economy in fishing gear and building environmental resilience, this initiative aims to protect Bangladesh's aquatic biodiversity while supporting the economic well-being of communities reliant on these precious water resources.

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TRIPLOID SABLEFISH *Anoplopoma fimbria* DISPLAY INHIBITION OF FEMALE REPRODUCTIVE DEVELOPMENT AND SKEWED SEX RATIOS THROUGH SEX REVERSAL OF GENETIC MALES

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Sablefish (or black cod, *Anoplopoma fimbria*) is an emerging aquaculture species typically reared in open water net pens. Escape of farmed fish is an ecological concern associated with net pen aquaculture of sablefish and other marine fishes. To mitigate this risk, we investigated the use of triploidization for the induction of reproductive sterility in sablefish for the first time. Triploidy was induced using a cold shock protocol (-1.5°C, applied 10 min post-fertilization for 120 min). Control diploids and cold shock-induced triploid sablefish were screened for external deformities, co-reared in duplicate tanks for growout, and periodically sampled over 15 months.

We found the control diploid sablefish grew significantly larger (1.48 ± 0.04 kg; mean ± SEM; $p < 0.001$) than triploids (1.17 ± 0.04 kg) and had greater condition factor (diploid: 1.22 ± 0.004, triploid: 1.16 ± 0.003; $p = 0.007$), however, the overall specific growth rate (SGR) did not significantly differ between controls (0.58 ± 0.01) and triploids (0.57 ± 0.02). Diploid control females (XX) had ovaries composed of well-developed primary oocytes, while triploid females (XXX) had ovaries that exhibited suppressed development, with mostly empty lamellae (i.e., lacking germ cells) and reduced numbers of smaller primary oocytes. Diploid males (XY) and 35% of the triploid males (XXY) had testes that appeared similar to each other, composed of type-A spermatogonia. Interestingly, we found that 65% of the triploid male (XXY) sablefish developed ovaries that were similar in structure to those of triploid females (XXX). The sex reversal observed in XXY genetic males skewed the overall sex ratio of triploid fish to female biased. The gonadosomatic index (GSI) of ovaries (regardless of genotypic sex, XXX or sex reversed XXY fish) was reduced by 7-fold in triploids relative to diploids, while there was no difference in GSI between testes of triploid (XXY) and diploid (XY) phenotypic males. Gonadal expression of sex related genes, such as *dmrt1* and *cyp19a1a*, retained characteristic sex-specific patterns, with XXY individuals exhibiting patterns that aligned with their phenotypic sex.

These results indicate that triploidy is disruptive to ovarian development in sablefish and likely renders females effectively sterile. Testicular development failed to occur in the majority of XXY fish, which instead developed ovaries, while the testes of XXY males appeared similar to those of XY diploids. It is likely that there are yet uncharacterized differences between testes of diploids and triploids, and that future XXY sperm would be aneuploid and effectively sterile. One possibility for the development of ovaries in 65% of triploid males (XXY) could be a gene dosage effect associated with the two X-chromosomes overriding the male sex-determining gene. More work is needed to fully characterize the XXY triploid-associated sex reversal and determine the utility of triploidy induction for mass sterilization of sablefish for aquaculture.

EGG PRODUCTION IN SIZE TRUNCATED OYSTER POPULATIONS AND ITS RELATIONSHIP TO RECRUITMENT

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Oysters, *Crassostrea virginica*, are considered relatively long-lived protandrous hermaphrodites exhibiting aggregated recruitment that, in turn, facilitates fertilization during broadcast spawning. While recovering from major epizootics in the 1990s through early 2000s, current oyster populations in the Virginia Chesapeake Bay remain size and age truncated. Oysters in excess of 5y and 140 mm shell length (SL) are rare, yet fossil records indicate that ages in excess of 20 y and 160mm SL were commonplace. We explore the question "how does age truncation influence egg production across the extant demographic among oyster populations in the sub-estuaries of the Virginia Chesapeake Bay?" Source demographic data is taken from quantitative long-term population surveys and focused examination of the size and age at transition from male to female as described by Harding et al (DOI: https://doi.org/10.1017/S002531541200032X). Initial exploration indicates that the abundance of 2y old oysters, at sub market size of <76mm SL, can produce approximately half of total egg production depending on year and site – that is egg production does not appear to be limited by the protandric transition from male to female.

The availability of both egg production and recruitment data (from population demographics) prompts a second question "what is the relationship between the two parameters?" Substantial efforts have been made by fisheries biologists to describe stock-recruit (S/R) relationships in fish stocks. There are, however, strong arguments that these have limited applicability in bivalves (Timbs et al https://doi.org/10.2983/035.037.0507), especially so for oysters where substrate availability is critical for recruitment. None the less, the egg production versus recruitment comparison represents a rare occasion (throughout marine invertebrate larval ecology, not just oysters) wherein mortality in this critical early life period is quantified over both time and space in several populations. We discuss these observations in the context of larval mortality versus loss and/or supplementation through advection in these sub-estuarine locations.

PROFILE OF MANGROVE PLANTERS THROUGH COASTAL COMMUNITY-BASED CONSERVATION IN PUJADA BAY, CITY OF MATI, PHILIPPINES

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Mangrove ecosystems play an integral role in coastal communities. They are crucial in mitigating climate change, supporting coastal ecosystems, and providing habitat for marine fauna, making them both ecologically and economically significant. The Province of Davao Oriental has the longest coastline in the country with a stretch of 513.2 km. In addition, the City of Mati, the Capital City of the province of Davao Oriental is home to diverse floral and faunal species within its coastal ecosystems, including 18 estimated rare and endangered mangrove species. Despite their importance, mangrove populations are deteriorating due to anthropogenic factors such as the booming tourism industry, increasing population, mining, overfishing, fishpond and beach resort conversion. This alarming decline has spurred increased efforts in mangrove planting and conservation.

In response, the Regional Integrated Coastal Resource Management Center XI (RIC XI) of the Davao Oriental State University, in collaboration with Mama Earth Foundation Inc., Department of Environment and Natural Resources (DENR), Bureau of Fisheries and Aquatic Resources (BFAR), and the local government unit, has spearheaded the Community-based Mangrove Rehabilitation and Enhancement Project in Pujada Bay. This initiative aims to restore mangroves through reforestation while also providing livelihoods for indigenous people and members of non-governmental organizations like the Women's Association of the City of Mati.

This report presents the community-based efforts focused on mangrove reforestation by the Women's Association on its economic and environmental opportunities, and challenges at the mangrove sites of the Malizia Mangrove Park within Pujada Bay. The Mangrove Park, initiated by the Mama Earth Foundation, is a reforestation project that encourages active community participation in planning and implementing mangrove rehabilitation and development. The project goal is to plant 1,084,000 of local species of mangroves present in their sites like *Rhizophora stylosa, R. mucronata, R. apiculata, Avicennia marina, A. officinalis, Ceriops tagal, Sonneratia alba,* and *Bruguiera gymnorrhiza* seedlings within coastal barangays of the City of Mati. Approximately 380-400 women participated from the Women's Association of nine coastal barangays. About 854,500 mangrove seedlings were planted as reported in 2023. Results indicated widespread recognition among the women's stakeholders and other local people of the social and economic benefits of the reforestation and enhancement of the mangrove park. Despite there being economic and organizational challenges, there is a strong desire to develop the area for mangrove ecotourism in the City of Mati. Empowerment and strengthening community involvement in planning and decision-making are recommended for sustainable socio-economic development for the coastal communities.

BIOLOGICAL PERFORMANCE OF RED ABALONE *Haliotis rufescens* ON AN EXPERIMENTAL MARINE BOTTOM CULTURE SYSTEM

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Since the mid-1970s, wild abalone populations in California (USA) and Baja California (MX) have significantly declined owing to overfishing, illegal catches, diseases, and warming seawater. This decline led to the development of abalone farming. In Mexico, the commercial aquaculture of red abalone (*Haliotis rufescens*) is performed in land-based facilities. However, abalone farming has faced several challenges recently, including slow growth rates and pathogens exacerbated by climate change. To address these issues, pilot projects for abalone mariculture have been developed to conserve the fishery and restock natural stocks. Offshore suspended sea cages have allowed abalone to reach commercial size faster and at lower costs than land-based farms. However, more information is needed on managing abalone sea farming in the context of climate change. Given the effectiveness of sea farming, we evaluated the biological performance of juvenile red abalone cultured in a bottom-sea cage system.

Red abalone farming was conducted over seven months at the small bay of Puerto Arbolitos, Ensenada, Baja California, MX. A bottom sea cage system containing 6 Australian baskets, each with 100 juvenile abalone (3 mm shell length), was placed at seven meters of depth. At sea, abalones were monitored every 15 days when fed, and water samples were taken to assess environmental parameters. A parallel land-based system was set up at the CICESE Marine Biotechnology Laboratory, consisting of a recirculating system with six 250-L semicircular tanks. Water temperature was maintained at 16°C, and ammonia, dissolved oxygen, and pH levels were measured daily. Biometric measurements were performed in both systems every two months to determine growth and mortality rates. In both systems, abalone were fed a fresh *Eisenia arborea* and *Pterygophora californica* diet, collected in the field. After 7 months, land-based abalone farming showed a higher growth increase in shell length (Figure 1) and wet weight than sea-based farming. Furthermore, the mortality rate was only 1% on land compared to 5% at sea. Environmental factors, including severe storm surges in December, predation risk, and system location, played a significant role in the sea-based system, adversely impacting marine culture and highlighting the challenges of sea farming.

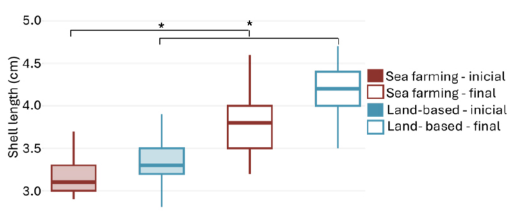

Figure 1. Average abalone growth (shell length in mm) of sea farming and land-based system.

IMPROVING WATER QUALITY IN MEDIA-BASED AQUAPONIC SYSTEMS WITH NANOBUBBLE AERATION TECHNOLOGY

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Aquaponics and recirculating aquaculture systems are often affected by poor water quality, which can lead to low plant yields and the potential collapse of fish stock. Key to these systems is dissolved oxygen (DO), which, if not present in sufficient concentrations, can result in the accumulation of toxic ammonia and nitrite. Conventional macrobubble (i.e., millimeter-sized bubbles) aeration, usually via porous ceramic diffusers, cannot maintain high DO, leading to poor water quality. Nanobubble (NB) aeration technology, a novel gas delivery method that produces bubbles with size less than 1000 nm, can effectively alleviate these bottlenecks, supersaturate DO concentrations (above 8 mg L^{-1}), and improve overall water quality. Mechanistically, their small size allows for a long retention time due to reduced buoyancy forces, and high interfacial surface area for enhanced oxygen mass transfer to the aqueous phase.

In this study, NB aeration (IDEC, AgriGALF 15, Japan) was applied at an airflow rate of 1.2 L min^{-1} in a media-filled aquaponic system to grow radish (*Raphanus sativus*), with effluent supplied by Nile tilapia (*Oreochromis niloticus*). This system operated in parallel with a control system (ceramic diffuser) under the same airflow rate. Both systems consisted of five components, arranged according to which water flowed: a fish tank, settleable solids removal sump, aeration sump (where NB/control aeration is applied), biofilter, and grow bed filled with clay aggregates.

Results showed that nitrate concentrations were higher in the NB-aerated system, with a final concentration of 74.2 mg-N L^{-1} and a nitrate accumulation rate of 12.9 mg-N L^{-1} $week^{-1}$, compared to 66.0 mg-N L^{-1} and 10.9 mg-N L^{-1} $week^{-1}$, respectively, in the control system (Figure 1A). Moreover, the total and volatile solids were lower across most components (i.e., fish tank, solids removal tank, biofilter, and grow bed) in the NB-aerated system (Figure 1B). In addition, the total fresh yield of radish was 32% higher. These findings suggest that NB aeration enhanced nitrification and sludge mineralization, likely because of improved DO availability (Figure 1C). Overall, the application of this technology holds promise for improving water quality in aquaponic and aquaculture systems.

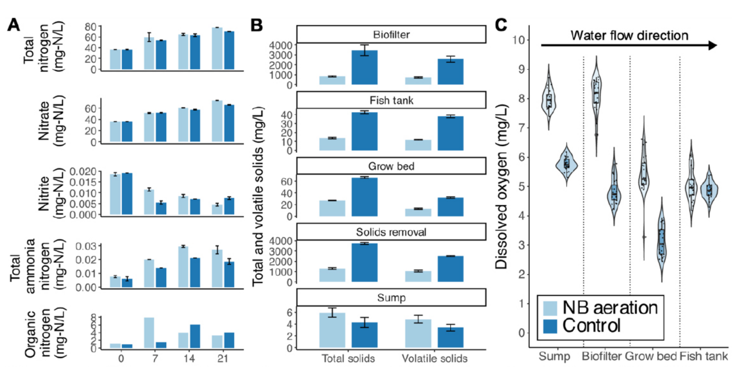

Figure 1. Nitrogen dynamics overtime (A), total and volatile solids (B), and DO (C) across the system components. Organic nitrogen was calculated.

MITIGATING AFLATOXIN B_1 (AFB_1) TOXICITY IN NILE TILAPIA *Oreochromis niloticus* FEED USING NATURAL CLAY CHISUMBANJE VERTISOL

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Contamination of feed and food with aflatoxin B_1 (AFB_1) is a prevalent issue in the tropical and subtropical regions. This contamination is exacerbated by environmental conditions that favour fungal proliferation and mycotoxin production, particularly affecting aquaculture because of the industry's vast use of plant-based feedstuffs. AFB_1 contamination in tilapia feed leads to diminished fish health, reduced productivity, economic losses, and potential health risks to human consumers. While mitigation strategies exist, more effective, safe, and affordable solutions are needed. This study investigated the efficacy of Chisumbanje Vertisol, natural clay found in Zimbabwe, as an AFB_1 adsorbent in Nile tilapia feed. Laboratory tests assessed the effects of clay inclusions (0%, 2.5%, and 5%) on pellet physical quality. For growth response, a seven-week feeding trial was conducted on Nile tilapia juveniles (20 ± 1g) using six treatment diets: Basal diet + no additive (Diet 1), Basal diet + 200ppb AFB_1 (Diet 2), Basal diet + 2.5% Clay (Diet 3), Basal diet + 5% Clay (Diet 4), Basal diet + 2.5% Clay+200ppb AFB_1 (Diet 5), Basal diet + 5% Clay+200ppb AFB_1 (Diet 6). The results showed significant ($P<0.05$) improvements in pellet physical quality with the inclusion of clay. Specifically, the 5% clay treatment exhibited the highest bulk density (0.39±0.01 g/mL), percentage floatability (73.10±0.41 after 35min), and durability (96.12±0.65%). Growth performance metrics (FBW, NWG, ADG, and SGR) were signicantly higher ($P<0.05$) in fish fed 5% clay diets (Table 1). Moreover, the combination of 5% clay and 200ppb AFB_1 showed significantly ($P<0.05$) better growth parameters than diets without clay inclusion, indicating the protective role of clay against aflatoxicity. FCR and survival rate were unaffected ($P>0.05$) across treatments (Table 1). Overall, Chisumbanje Vertisol at 5% inclusion improves pellet physical quality and promotes better growth performance in Nile tilapia, offering a natural solution for the mitigation of AFB_1 toxicity in aquaculture.

Table 1: Final body weight (FBW), net weight gain (NWG), average daily gain (ADG), specific growth rate (SGR), feed conversion efficiency (FCR), and survival (%) of Nile tilapia juveniles fed on six practical diets over a period of 7 weeks. Means sharing similar letters in a row were statistically non-significant ($P>0.05$).

	Diets					
	1	2	3	4	5	6
FBW (g)	58.3±1.5 ^{b}	48.7±3^{b}	58.3±1.2^{b}	64±1^{a}	51.7±1.5^{b}	59.3±0.6^{a}
NWG (g/fish)	38.3±2.5^{b}	28±4^{b}	38±0^{b}	42.7±0.6^{a}	31±1^{b}	38.3±0.6^{a}
ADG (g/fish/day)	00.78±0.05^{b}	0.57±0.08^{b}	0.78±0^{b}	0.9±0.01^{a}	0.63±0.02^{b}	0.78±0.01^{a}
SGR (%/day)	20.19±0.15^{b}	1.75±0.22^{b}	2.15±0.08^{b}	2.24±0.03^{a}	1.87±0.02^{b}	2.12±0.08^{a}
FCR	10.54±0.10^{a}	2.56±0.71^{a}	1.76±0.19^{a}	1.84±0.21^{a}	1.91±0.34^{a}	1.69±0.22^{a}
Survival (%)	93.3±5.77^{a}	83.3±5.77 ^{a}	86.7±5.77^{a}	86.7±5.77 ^{a}	90±10 ^{a}	86.67±5.77^{a}

SPECTRAL SENSITIVITY OF *Puntius titteya* PHOTORECEPTORS THROUGHOUT LARVAL DEVELOPMENT

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The cherry barb (*Puntius titteya*) is popular within the freshwater ornamental trade for its bright red coloration and schooling behavior within community tanks. The species is widely available from overseas producers but is also currently in aquaculture production in the United States. There has been little research on the life history or culture techniques for this species, and such research is needed to address bottlenecks in larval rearing to improve production efficiency in the United States. Larval fish encounter several 'critical' periods during development which can lead to mass mortality events that involve the loss of significant quantities of larvae. Larval mortality is often attributed to poor nutrition during development. Identifying appropriate nutritional protocols is vitally important for the survival and growth of larvae. Chemosensory and visual cues are important for successful prey capture by larvae. Larval feeding response can be impacted by additional factors, but low feeding incidence in the presence of abundant prey items may be indicative of poor prey visualization. Gaining a better understanding of visual system ontogeny in larval fish can help predict appropriate lighting and environmental protocols required to increase feed ingestion rates.

For this study, retinal ontogeny of *P. titteya* will be described using microspectrophotometry. Microspectrophotometry (MSP) can determine the spectral absorption properties of photoreceptor cells and then be used to characterize the development of the visual capabilities of fish larvae throughout early life history. *P. titteya* larvae will be dark adapted before undergoing dissection for retinal isolation. The retina will be macerated on a cover slip to target individual photoreceptors with the MSP. Measurements obtained will illustrate a maximum absorbance curve for a singular photoreceptor (Figure 1). The collected data can help inform culture conditions such as wavelength and light intensity that would be most conducive for effective prey identification, capture, and ingestion by larval fish. Manipulations in lighting conditions during the larval stage may help to promote larval feeding incidence, growth, and survival, and ultimately increase production efficiency of *P. titteya*.

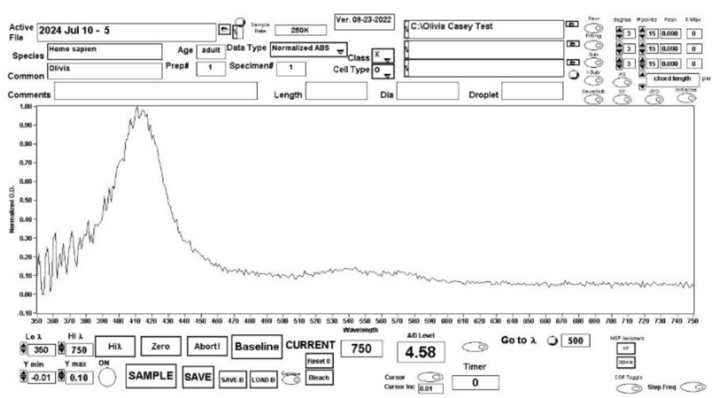

Figure 1. Example of a maximum absorbance curve obtained from the MSP.

CHARACTERIZING THE DIGESTIVE CAPABILITIES OF *Dascyllus auripinnis* TO INFORM LARVAL FEEDING

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The golden domino damselfish (*Dascyllus auripinnis*) is an ornamental fish native to the Indo-Pacific and highly sought after by aquarium hobbyists. Closely related to the three-spot damselfish (*Dascyllus trimaculatus*), the appearance of the golden domino damselfish differs with a distinct golden underbelly. Development of early culture protocols for this species contributed to successful culture of juveniles through metamorphosis at 42 days post hatch (DPH) at the University of Florida. With an extended larval period and heavy reliance on live feeds, such as rotifers (*Brachionus* spp.) and *Artemia* spp. nauplii, the transition to an inert diet would help reduce reliance on costly live feeds and simplify larval rearing to facilitate future commercial culture of this species. However, inert diets often contain more complex macromolecules that are difficult to digest compared to the free amino acids and highly unsaturated fatty acids (HUFAs) found in live feeds. To ensure proper weaning of larvae from live feeds to inert microdiets, the maturation of the larval digestive tract should be understood to select appropriate weaning timepoints, which can be species specific. This study sought to characterize the digestive physiology of *D. auripinnis* to inform timing for introduction of microdiets and reductions in live feed consumption.

Larval digestive physiology of *D. auripinnis* was examined from 3 – 30 DPH, where larvae were sampled at six time points throughout development. Digestive tract maturation will be indicated by the formation of a functional stomach, which will be determined through histology and digestive enzyme quantification. Histology will verify the onset of intestinal coiling and activation of gastric glands in the stomach via periodic acid-Schiff staining. Additionally, the activity of digestive enzymes such as pepsin, trypsin, and lipase will also be measured via standard microplate assays characterize the ontogeny of these enzymes. Results of these analyses will determine when *D. auripinnis* larvae can digest a more complex diet, ultimately reducing live feeds, and providing insights into the development of other closely related species.

IMPROVEMENTS IN SULFUR DENITRIFICATION REACTOR CONSTRUCTION TO FACILITATE NITRATE REMOVAL

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Biofiltration is arguably one of the most important factors in life support system design. The conversion of ammonia to nitrate allows nitrogen to accumulate in a system in a less toxic form. However, high concentrations of nitrate in aquarium systems could still have a harmful impact. Invertebrate species, such as corals and anemones, will not survive in high nitrate conditions and fish spawning could potentially be inhibited. Water changes will prevent the build-up of nitrate, but they can be expensive especially to facilities without access to seawater. Denitrification uses chemoautotrophic bacteria to convert nitrate to nitrogen gas, which can be released into the atmosphere. While sulfur denitrators are not new to LSS design, their designs can be labor intensive and unreliable. This study examined the efficiency of sulfur denitrification and how to maximize nitrate removal. An experimental reactor was constructed and installed on an independent system, where nitrate was dosed to simulate the production of nitrate by biofiltration. Once the bacterial population matured, multiple sets of experiments were performed to determine the relationships between oxidation-reduction potential (ORP), pH, flow rate, and nitrate removal rates. We concluded that higher pH significantly increases nitrate removal over time ($p = 0.04$, $\alpha = 0.05$) and that a design accommodating this would be preferable. There were also linear correlations between ORP, flow rate, and nitrate removal. Our findings allow LSS operators to use ORP as an indicator for optimal nitrate removal no matter how large or concentrated a tank is. An additional reactor was designed following these experiments to incorporate improvements, such as temperature control and to make the functionality user-friendly for easier maintenance.

OYSTER REEF RECOVERY: RESPONSES TO SHELL REPLENISHMENT ON PUBLIC FISHING GROUNDS

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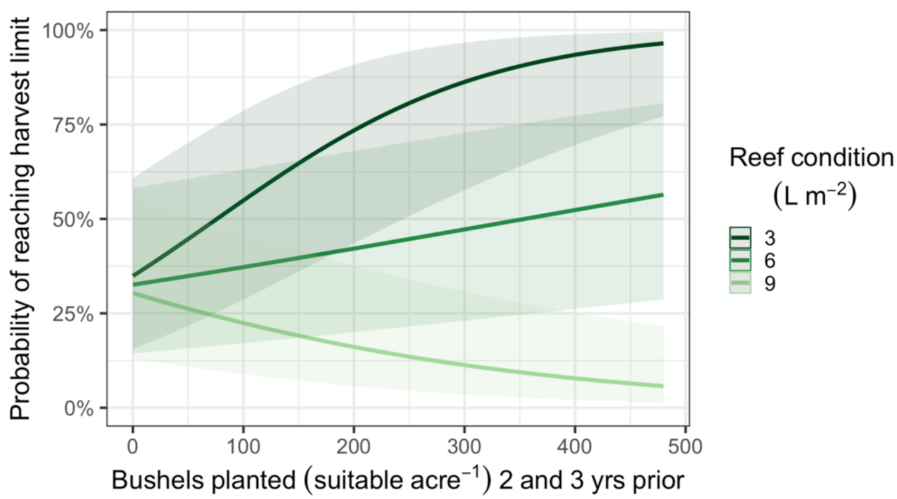
Oysters are a benthic dominant, critical ecosystem engineer, and important fishery species in temperate estuaries worldwide. Despite their importance, oyster populations have declined globally. The Rappahannock River in Virginia was essentially closed to harvest for several decades due to epizootics and low oyster abundance. In 2007, the Virginia Marine Resources Commission (VMRC) implemented rotational management in the Rappahannock River. VMRC created 6 management areas, where 2 areas are open per year and each area is open to harvest every 3 years. Oyster harvest rotation allowed for the reestablishment of an active fishery in the Rappahannock River. In addition, VMRC routinely performs shell replenishment, the provisioning of additional shell material or hard substrates, when the underlying reef falls below management thresholds. Oyster shells have high turnover rates and are a limited, expensive resource; thus, optimizing repletion efforts to maximize restoration success is critical to maintain ecosystem services and support local economies. This project integrates long term data sets including annual oyster population surveys, shell replenishment records, and commercial harvest reports to understand both biological and economic responses to shell replenishment. We used generalized linear mixed effects models to explore how harvester efficiency, market sized oyster abundance, recruitment, and the underlying reef structure respond to shell inputs. Research findings will be used to create a decision tool for managers to inform management decisions and replenishment practices.

Fig. 1. Marginal effect plot displaying the effect of bushels planted in a harvest area across reef conditions (weighted mean brown shell substrate) at the time of planting on the probability a harvester reaches the harvest limit. Shading represents 95% confidence intervals.



EVERYTHING'S BIGGER IN TEXAS – 60-ACRE OYSTER PARK

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This project updates the collaborative effort between Texas Sea Grant and a community of Hispanic, underrepresented minority fishermen displaced by regulatory changes in the Gulf of Mexico. The project aimed to empower these fishermen by providing training in oyster aquaculture, ultimately leading to the establishment of a cooperative and the permitting of a 60-acre oyster park in Keller Bay, Texas. All training sessions were conducted entirely in Spanish to ensure maximum accessibility and understanding for the target population. The project engaged aquaculture specialists from across the United States to provide comprehensive, high-quality instruction. The training program fostered community engagement and collaboration, leading to the formation of a cooperative to manage the oyster park and market the harvested product. The cooperative received the necessary permits to operate the 60-acre oyster park, ensuring sustainable and legal aquaculture practices. This highlights the potential of targeted outreach and training programs to support under-represented communities and promote sustainable economic development in coastal regions. By providing the necessary skills and resources, these individuals can transition to new livelihoods and contribute to the revitalization of local economies.

DEVELOPMENT OF AN INSECT-BASED FEED USING BLACK SOLDIER FLY *Hermetia illucens* FOR NILE TILAPIA *Oreochromis niloticus* IN A RECIRCULATING AQUAPONIC SYSTEM

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Aquaponics combines the technologies of hydroponics and aquaculture to produce fish and vegetable crops with significantly reduced external fertilizer and water use. The nutrients available to the fish and plants are directly related to the feed given to the fish, making high quality feed essential for effective crop production. The objective of this research is to analyze the effects of Black Soldier fly (*Hermetia illucens)* meal as a supplemental protein source integrated within existing commercial feed for Nile Tilapia (*Oreochromis niloticus*).

Purina Catfish 32 Floating Feed was ground down, and Black Soldier fly meal was added as 15% and 25% the total weight. The feed was re-pelletized, and six aquaponic systems were run for 12 weeks within a greenhouse, each with 40 Nile tilapia and 16 plants of Ethiopian Kale and 16 Georgia Southern Collard Greens within a deep-water growing bed. Two systems were each fed 15% BSFM feed, two fed 25% BSFM feed, and two fed 0% BSFM feed. They each were fed 1% daily of the average total system fish weight, taken weekly. Plants were harvested upon reaching maturity and analyzed for leaf length, leaf color, root length, root color, shoot weight, and root weight. Tank and biofilter ammonia, nitrite, and nitrate concentrations were taken weekly.

Results revealed the BSFM supplemented feed to be similar in effect to the pure commercial mix feed. There was no significant difference in tilapia, collard green, or kale yield due to feed type. Feed type similarly had no significant effect on ammonia, nitrite, and nitrate content within the tanks or biofilters.

AQUAPONICS, A METHOD TO PRODUCE HEALTHY FOOD

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The aquaponic system includes combined production of fish and plants. In the present study, the quality of tilapia and plant products produced in the aquaponic have been investigated. Results of sensory evaluation of the cooked fillet showed high score for sensory parameters. In all plant products, the amount of nitrite and nitrate were considerably lower than permissible limit of consumption. The amount of protein and iron in plant products were high, however calcium and potassium were a few lower than the desired amount.

The aquaponic system includes combined production of fish and plants. in which the wastes and metabolites produced by farmed fish are removed from the environment through nitration and absorbed by plants. The plants and fish produced in this system are healthy due to the absence of fertilizers and pesticides. In the present study, the quality of tilapia and plant products produced in the aquaponic system including mint, peppermint, pennyroyal, green basil, purple basil, fodder beet, Swiss chard, various cultivars of lettuce, watercress, celery, and tomato have been investigated. Results of sensory evaluation of the cooked fillet showed high score for sensory parameters. For the purpose of producing better quality farmed fish, it is recommended to use high quality feed especially in the last month of breeding. In all plant products, the amount of nitrite and nitrate were considerably lower than permissible limit of consumption. Peppermint, fodder beet, Swiss chard, watercress and celery had the lowest proline content, while peppermint, fodder beet, Swiss chard were also richer in chlorophyll. Higher levels of proline were obtained in lettuce and basil, indicating more severe environmental stress conditions for them. The amount of protein and iron in plant products were high, however calcium and potassium were a few lower than the desired amount. Considering the health of the products of the aquaponic system and its applicability in different scales, it is a suitable option for the production of fish and plants.

TABLE 1. Content of fatty acids in tilapia fillets and Nutritional Fact (N. F.) values

Fatty acid		Present study		N. F.
		%	(g/100g)	(g/100g)
Meristic acid	C14:0	2.89	0.22	0.05
Palmitoleic acid	C16:1	5.32	0.40	0.1
Palmitic acid	C16:0	28.8	2.16	0.42
Linoleic acid	C18:2	10.66	0.80	0.16
Oleic acid	C18:1	44.78	3.36	0.38
Stearic acid	C18:0	6.79	0.51	0.11
Arachidic acid	C20:0	0.78	0.06	<0.01

TABLE 2. Amount of nitrate and nitrite (mg/kg) in dry/wet weight in the plants

	(mg/kg)	Peppermint	Common mint	Oregano	Green basil	Purple basil	Fennel
Nitrate	Dry weight	1106	7190	1004	874	690	810
	Wet weight	90.7	805.3	131.5	78.7	56.6	89.1
Nitrite	Dry weight	34	22	8	10	24	8
	Wet weight	2.8	2.5	1.0	0.9	2.0	0.9

EVALUATING THE INFLUENCE OF STOCKING DENSITY ON GROWTH INDICES, CARCASS COMPOSITION AND MICROBIAL DYNAMICS OF *Oreochromis niloticus* UNDER BIOFLOC CULTURE

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Culturing of fish at high stocking densities is a hallmark of biofloc aquaculture. Current work aimed to explore the influence of medium stocking densities of Nile tilapia (*O. niloticus*) i.e. 30, 40 and 50 fish/m^3 on growth indices, carcass composition and microbial dynamics in biofloc system. Juvenile fish was reared for 180 days in tarpaulin sheet tanks having biofloc with feeding of 25% CP feed @ 3% of body weight per day. To maintain C/N ratio as 20:1, starch was used as carbon source. All the treatments showed significantly different response towards all parameters in question during the experiment. *O. niloticus* showed a gradual decrease in weight gain as stocking density increased. Best gain in weight (303.2g) was recorded at 30 fish/m^3 density, while minimum (264.2g) at 50 fish/m^3. Better FCR and survival of fish was also recorded for 30 fish/m^3 density. Carcass analysis revealed a decline in crude protein (CP: 52.65 to 49.43%) and ether extract (EE: 24.49-23.36%) with increasing stocking densities 30 to 50 fish/m^3, although dry matter (DM: 92.75 to 93.67%) and ash content (13.45 to 14.15%) increased. Water quality parameters viz. temperature, pH, nitrite-nitrogen (NO_2), nitrate nitrogen (NO_3), total ammonia-nitrogen (TAN) and total suspended solids (TSS) remained under optimal ranges. Maximum floc volume (20.76 ml/lit) was recorded in 50 fish/m^3 treatment, while minimum (18.22 ml/lit) was observed at 30 fish/m^3 treatment. Maximum concentrations (24.6×10^4 CFU/ml) of total heterotrophic bacteria in biofloc were observed at 50fish/m^3 density, while minimum (15.3×10^4) concentrations were observed at 30fish/m^3 density. Gut microbial community (CFU/g) were higher (35.5×10^7) at 50 fish/m^3 whereas, lower value (28.2×10^7) were observed at 30 fish/m^3. From the results of experiment, it is evident that varying stocking density of *O. niloticus* significantly affect the growth, carcass composition and microbial dynamics under biofloc culture.

APPLICATION OF LOW-COST GENOTYPING STRATEGIES FOR GENETIC IMPROVEMENT OF CULTURED *Crassostrea virginica* IN TEXAS

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Genotyping individuals to estimate genetic covariance can increase selection accuracy, reduce generation interval of selection candidates, and enable more efficient breeding program design, but genotyping methods can vary widely in cost and genome coverage. A possible low-cost, high information approach is to use low-density genotype coverage to impute high-density genotype coverage in selection candidates based on genetic information from broodstock. Using hatchery-bred oysters exposed to field and lab challenges to measure traits of commercial importance, we examined the relative effectiveness of several genotyping strategies to estimate genetic parameters in *Crassostrea virginica* in Texas.

RELIABLE MILK CONCH CAPTIVE BREEDING POPULATION HELPS TO PAVE THE WAY TO ESTABLISH A QUEEN CONCH CAPTIVE BREEDING POPULATION

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A recent breakthrough at FAU Harbor Branch offers promising advancements in conch aquaculture by potentially eliminating the need for wild-sourced egg masses to supply hatcheries. Sixteen milk conch (*Macrostrombus costatus*) broodstock, consisting of nine females and seven males, were provided with a sand substrate, flow-through seawater, and a diet predominantly consisting of diatom-rich algal turf scrubbers (Fig. 1). These scrubbers were cultured using nutrient-rich wastewater from an Integrated Multitrophic Aquaculture system. This diet has been one of the contributing factors that resulted in reliable and prolific spawning, compared to previous studies that used formulated feeds. Between April to September 2024 (24 weeks) this population produced an average of 9.2 egg masses per week, totaling 221 egg masses, of which 76% had viable offspring.

The listing of the queen conch, *Aliger gigas* as threatened on the Endangered Species Act has underscored the need for a recovery plan for this ecologically and economically important species. As a close relative of *A. gigas,* the milk conch (*M. costatus)* serves a similar ecological role and is harvested in certain Caribbean regions. This proximity allows milk conch to be used as a surrogate species to test the viability of establishing a captive queen conch breeding population, thereby reducing the dependence on wild egg mass collection for restoration hatcheries. This study has shown that a key to a reliable captive conch breeding program is the use of algal turf scrubbers as the primary nutritional component. Future studies with queen conch adults should use this food source to encourage egg laying in captivity for use in restoration aquaculture.

FIGURE 1. a-c (a) Captive milk conch using its proboscis to graze on the algal turf scrubbers. (b) Milk conch from the captive breeding colony engaged in copulation with the female (right) grazing on filamentous algae. (c) Milk conch egg mass produced by the captive breeding colony.

ASSESSING PROTEIN CONTENT AND ANTIOXIDANT ACTIVITY IN MICROALGAE *Chlorella* sp.

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Chlorella is a genus of green microalgae which produces high biomass, presenting higher photosynthetic efficiency when compared with other microalgae. The objective of this study was to analyze the protein content and antioxidant activity of *Chlorella* sp. *Chlorella* has important uses in bioindustry and biopharmaceuticals. Biosynthesis of bioactive metabolites is also important for industrial uses. To combat issues associated with its inherent low growth rate, chlorella can be genetically transformed to produce more biomass. For this reason, bioproducts from microalgae are in demand and high value. In our experiment, we used BG11 media to culture microalgae and assessed its protein content and antioxidant activity. Microalgae cultures were first obtained from colonies on plates with BG11 solid media. Clones were then cultivated and grown in BG11 liquid media. Once these cultures reached the stationary phase, biomass was extracted by aqueous abstract, methanolic abstract, and ethanolic extract. Bicinchoninic Acid (BCA) method was used to analyze protein content. The antioxidant activity was then calculated using (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. BCA method and ABTS method results indicate that *Chlorella* sp. is the ideal candidate for biomass production and monitoring antioxidant activity.

SEABIRD INTERACTIONS WITH FLOATING OYSTER GEAR AND *Campylobacter* PREVALENCE AT OYSTER FARM SITES

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Recent outbreaks of campylobacteriosis, a gastrointestinal illness caused by bacteria in the genus *Campylobacter*, linked to the consumption of raw oysters have raised questions on how oysters become contaminated with *Campylobacter*. The leading hypothesis implicates seabird interactions with floating oyster aquaculture gear. Due to seafood safety concerns, operational plans are required for oyster farms to deter birds or mitigate their effects. Unfortunately, research related to this issue is limited. This project focuses on three objectives: 1) to survey the species of seabirds interacting at oyster farm sites, 2) to test the efficiency of a simple, inexpensive, nonlethal bird deterrent, and 3) to screen oysters and seabirds found at oyster farm sites for *Campylobacter*.

Six experimental floating cages were deployed at an experimental oyster plot in the Mississippi Sound, three equipped with a zip tie bird deterrent and the other three serving as non-deterrent controls. Cameras monitored bird interactions at half hour increments. *Campylobacter* presence in seabird fecal matter was determined using a selective and differential culture medium. An enrichment procedure was used to detect *Campylobacter* in oysters.

A total of 15 bird species were observed, with Brown Pelicans and Double-crested Cormorants being the most abundant. Bird deterrents reduced bird interactions by approximately 85%. *Campylobacter* positivity rates for seabird feces and oyster homogenates were 5.9% and 13.4%, respectively. These results do not rule out the potential for birds to transfer *Campylobacter* to oysters in floating aquaculture gear; however, more information is needed to determine if the *Campylobacter* species detected present a significant human health risk.

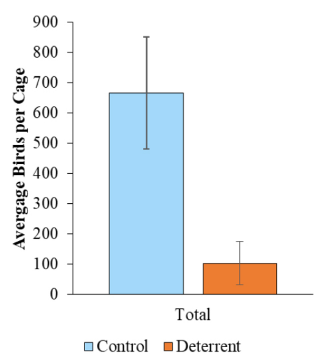

Figure 1. Bird interactions per cage type over entire study. $P < 0.05$

	Species/Cage	Positive	Negative	Positivity Rate
Seabirds	Brown Pelican	4	48	7.69%
	Royal Tern	5	99	4.81%
	Sandwich Tern	1	23	4.17%
	Common Tern	1	14	6.67%
	Ruddy Turnstone	1	0	100.00%
Oysters	Control	8	26	23.53%
	Deterrent	4	27	12.90%

Table 1. Incidence of *Campylobacter* in samples collected from seabirds and oysters. Seabirds are reported per species and oysters are reported per cage type.

BLACK SOLDIER FLY (*Hermetia illucens*) MEAL IMPROVES GROWTH, FEED UTILIZATION AND INTESTINAL HEALTH OF NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

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Insect meals are promising ingredients in aquafeeds, offering potential to meet the rising demand for protein driven by the rapid expansion of the aquaculture sector. Black soldier fly (BSF; *Hermetia illucens*) larvae meal is particularly promising due to its high nutritional value and environmentally sustainable production. This study investigated the effects of BSF meal on the growth performance, feed utilization, and intestinal health in Nile tilapia (*Oreochromis niloticus*) juveniles.

A 5-week feeding trial was conducted with Nile tilapia juveniles (initial body weight: 7.28 ± 0.09 g) in tanks (13 L each) in a RAS. Three isonitrogenous and isolipidic diets were formulated based on the known nutritional requirement of tilapia to contain 0 % (Control: BSF0), 20 % (BSF20), and 40 % (BSF40) of defatted BSF meal (Table 1). The fish were divided into 3 groups of 16 fish in triplicate tanks and were fed the diets at 5 % biomass per day. After the feeding trial, the final body weight (FBW), weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion ratio (FCR) were improved significantly with increasing BSF meal inclusion levels in the diet (Table 2). The survival rate (SR) of the fish were not affected by BSF meal compared with the control. Histology analysis revealed that intestinal mucosal fold length (MFL), muscularis thickness (MT), and goblet cell density (GCD) were significantly elevated by BSF meal compared with the control (Data not shown). In addition, intraepithelial lymphocytes (IELs) and the enterocyte microvilli length (MVL) were elevated significantly in BSF40 fed fish compared with the control.

Discussion/conclusion
Results of this study aligns with most previous research on Nile tilapia. Unlike earlier studies that primarily replaced fishmeal with BSF meal, this study maintained fishmeal at lower-than-typical levels and incorporated BSF meal at the expense primarily of soybean meal and corn gluten meal. Hence, the results show that BSF meal can be used as an alternative to these in Nile tilapia fed low fishmeal diets.

Table 1: Experimental diets composition (g/100g).

Ingredient (g/100g, DM basis)	Experimental diets		
	Control (BSF0)	BSF20	BSF40
Soybean meal	38.00	25.00	11.35
Black soldier fly meal (BSF)	0.00	20.00	40.00
Corn gluten meal	25.09	16.22	3.00
Sunflower meal	25.00	25.00	36.00
Sunflower oil	5.50	4.74	3.88
Corn starch	3.15	5.81	2.70
Fish meal	1.00	1.00	1.00
Carboxymethyl cellulose	0.50	0.50	0.50
Fish premix	0.50	0.50	0.50
Fish oil	0.50	0.50	0.50
Lysine HCL	0.46	0.43	0.27
Gelatin	0.30	0.30	0.30
Proximate composition (% as fed)			
Dry matter	91.48 ± 0.23	91.92 ± 0.54	92.03 ± 0.92
Crude protein	45.94 ± 1.26	45.69 ± 0.50	45.05 ± 0.35
Crude lipid	7.03 ± 1.38	7.14 ± 0.25	7.16 ± 1.42
Ash	4.82 ± 0.06	5.43 ± 0.33	6.29 ± 0.87

Table 2: Growth and feed utilization (Mean±SEM) of Nile tilapia juveniles fed with BSF meal.

	Control (BSF0)	BSF20	BSF40
Initial body weight (g)	7.25 ± 0.13	7.38 ± 0.00	7.21 ± 0.07
FBW (g)	14.02 ± 0.48[a]	16.71 ± 0.32[b]	17.96 ± 0.19[c]
WG (g)	6.77 ± 0.40[a]	9.34 ± 0.32[b]	10.75 ± 0.22[c]
SGR (%/day)	1.88 ± 0.07[a]	2.33 ± 0.05[b]	2.61 ± 0.04[c]
FCR	1.75 ± 0.07[c]	1.38 ± 0.04[b]	1.20 ± 0.02[a]
PER	1.24 ± 0.07[a]	1.58 ± 0.03[b]	1.85 ± 0.04[c]
SR (%)	97.91 ± 3.61	93.75 ± 6.25	100.00 ± 0.00
Condition Factor	1.65 ± 0.00[a]	1.66 ± 0.02[a]	1.71 ± 0.02[b]

MAINE SHELLFISH AND SEAWEED AQUACULTURE APPRENTICESHIP: ORIGINS, PROGRESS, AND THE PATH AHEAD

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Maine's aquaculture industry faces a significant workforce shortage, which threatens to limit its growth potential. In response, the Gulf of Maine Research Institute (GMRI), Maine Aquaculture Association (MAA), and Educate Maine developed The Maine Aquaculture Workforce Development Strategy. This strategic roadmap outlines a cohesive training pipeline to meet current workforce needs while anticipating future demands as the industry evolves. Since the Strategy's launch in 2020, substantial progress has been made. MAA, in partnership with GMRI and Educate Maine, created the first-generation Maine Aquaculture Occupational Standards to standardize workforce training. Building on these standards, and with funding from USDA NIFA, the Shellfish and Seaweed Aquaculture Apprenticeship program was registered with the Maine Department of Labor. The apprenticeship includes 2,000 hours of On-the-Job Training (OJT) at aquaculture businesses and 144 hours of Related Technical Instruction (RTI) provided by Southern Maine Community College (SMCC). All SMCC training is reviewed and approved by an industry steering committee to ensure alignment with sector needs. The program recently celebrated the graduation of its first cohort of apprentices, with the second cohort now in progress. Feedback from both apprentices and employers has been highly positive, signaling the program's success in meeting the industry's workforce demands. This session will explore the creation of the apprenticeship program, its outcomes, and our vision for expanding it to further support Maine's growing aquaculture sector.

BREEDING FOR THE FUTURE: ENHANCING RESILIENCE IN AQUACULTURE WITH GENOMIC OFFSETS

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As global temperatures rise and become more variable, the capacity of farmed species to adapt while maintaining production efficiency is becoming a pressing concern. Aquaculture species are particularly vulnerable due to their exposure to heightened variation in wild aquatic environments. In this context, Genotype-by-Environment (GxE) interactions pose a significant logistical challenge for selective breeding, as traits that perform well in one environment may not in another. These interactions complicate the design of breeding programs that aim to ensure long-term resilience while optimizing short-term productivity. Genomic offsets, a novel metric that quantifies the genetic changes required for populations to adapt to anticipated environmental shifts, may offer a promising solution. Here, we explore potential applications of genomic offsets in aquaculture, including their use as tools for risk assessment, selective breeding, and cryopreservation. We will also discuss how genomic offsets can overcome the hurdles posed by GxE interactions, addressing practical considerations such as data requirements and methodological frameworks, and needed validation efforts. By predicting maladaptation risks and guiding the selection of individuals best suited for changing environmental conditions, genomic offsets may help breeders proactively enhance resilience of aquaculture populations.

SALMON RANCHING MITIGATES THE NORTH PACIFIC ECOSYSTEM FOR LOST SPAWNING AND REARING HABITAT

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Pacific Salmon (*Oncorhynchus* spp.) ocean ranching maintains valuable fisheries and compensates the North Pacific Ecosystem for freshwater habitat loss. These anadromous fish spawn, incubate eggs and early rear in streams, rivers and lakes around the North Pacific Rim. Following early rearing they smolt and migrate seaward to productive ocean waters where they spend the majority of their life and put on over 95% of their body weight. In the southern portion of their range freshwater habitat is heavily impacted by dams, logging, agriculture, urbanization and pollution resulting in low natural production requiring hatchery smolt production to maintain fisheries. In the Northern portion of their range hatchery production is utilized to enhance fisheries. Since 1990 combined mitigation and enhancement production has leveled out around 5 billion fish (Figure 1). This production level is about 17-20% of the overall North Pacific salmon population with combined hatchery and wild production being less than historic levels (Figure 2). During their first spring and summer at sea Pacific Salmon reside in the productive nearshore epipelagic zone feeding on zooplankton as well as crustaceans (crabs and shrimp), cephalopods (squid) and fish larvae. In turn they are a crucial food source for breeding marine birds, nursing seals, porpoise, dolphins and baleen whales. Following their initial spring-summer entry they progress to larger prey and in turn are preyed upon by additional predators including lamprey, sharks, sealions beluga and killer whales. Many of these avian and marine mammal predators dependent on salmon productivity are legally protected and/or charismatic fauna with high economic value to the tourist, wildlife viewing and film industries. The negative impact current salmon production has on crab, shrimp and squid fisheries is likely less than historic levels. Resource managers establishing hatchery smolt release numbers should consider both their ecosystem and fishery value with the goal of maintaining overall smolt production at historic levels.

FIGURE 1. North Pacific Rim hatchery releases by species in thousands from 1952 to 2023 based on NPAFC statistics accessed on June 2024 (using lines to emphasize species trends).

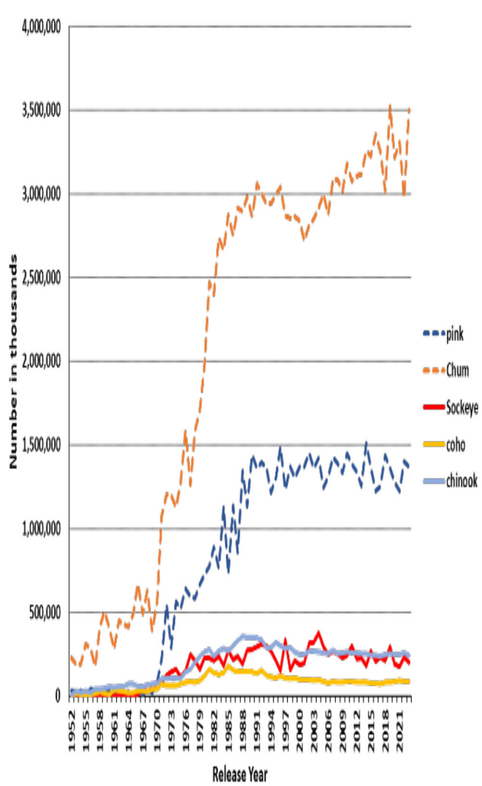
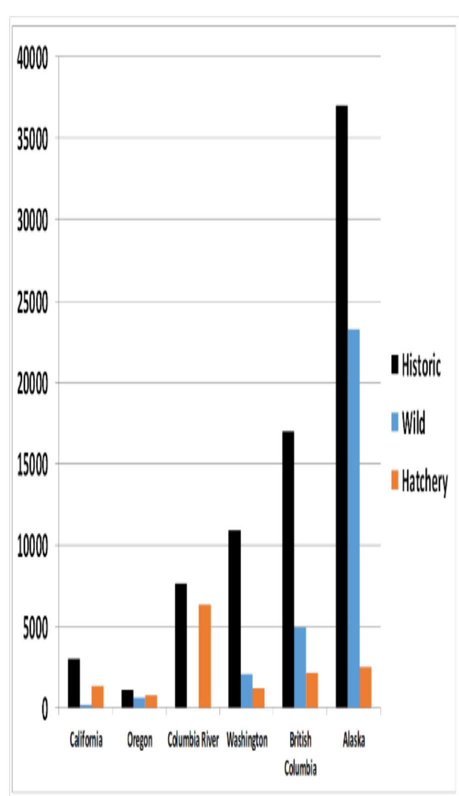


FIGURE 2. Biomass estimates in kg (x 1,000) for historic (1880-1920), wild (1988-1997) and hatchery smolt production (1993-1997) for five species of Pacific Salmon (Schoonmaker et al 2003).



HORMONAL SEX REVERSAL IN SACRAMENTO PIKEMINNOW *Ptychocheilus grandis* USING 17β-ESTRADIOL

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Sacramento Pikeminnow (*Ptychocheilus grandis)* are a large (up to 1.4m) piscivorous cyprinid native to the Sacramento-San Joaquin basin in California. They were introduced in the upper mainstem of the Eel River, CA in 1979. Over time, it has become one of the most prevalent fish species within the Eel River ecosystem, raising concerns due to its predation and competitive interactions with juvenile salmonids and other native fishes. Because of this, there have been many suppression efforts to eradicate the Sacramento Pikeminnow populations in the Eel River, but none of them have been successful in reducing their populations in a meaningful way. Despite the Sacramento Pikeminnow being as abundant as they are, there is very little information on their life history and especially on their reproduction. The overall goal of this study is to create Trojan YY supermales, male fish with YY chromosomes that only produce male offspring, as a new method to reduce the populations of Sacramento Pikeminnow. By skewing the population's sex ratio, this approach aims to eventually decrease the overall population size and even eradicate the species entirely in the river. The specific objective of this study is to test the duration and dose needed to create XY female Sacramento Pikeminnow using 17β-Estradiol.

Histology revealed that Sacramento Pikeminnow gonads begin developing at 14 cm. In this preliminary study, 30 juvenile Sacramento Pikeminnow (8-10 cm) were used to test the duration and dose of 17β-estradiol needed for sex reversal. The fish were housed in a 130 L circular tank and maintained at 20°C. The 17β-estradiol was initially mixed into their feed at a dose of 50 mg/kg using ethanol, but the fish refused to eat. Switching from ethanol to fish oil as the carrier improved feed acceptance. Three treatment durations were tested: 60, 90, and 120 days. After each period, 10 fish were transferred to their own 130 L tank.

Because gonads are difficult to locate in Sacramento Pikeminnow under 15 cm, the fish will be elastomer-tagged to differentiate between trials and moved to 2120 L tanks for further growth before dissection.

SELECTIVE CLEARANCE OF MACROALGAL-DERIVED ORGANIC MATTER BY GREEN-LIPPED MUSSELS (*Perna canaliculus*) MAY BE LINKED WITH PHLOROTANNIN CONCENTRATIONS AND NUTRITIONAL PROFILES

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Two, no-choice feeding experiments were used to test how initial concentration and age affected clearance rates by the green-lipped mussel (*Perna canaliculus*) of macroalgal-derived particulate material (MDPM) originating from common large brown algae with relatively high (fucoids: *Cystophora retroflexa, Cystophora torulosa*) and low (laminarians: *Macrocystis pyrifera, Undaria pinnatifida*) phlorotannin concentrations from southern New Zealand. Mussels removed fresh MDPM originating from each species but removal rates of MDPM were higher for the low phlorotannin species than for the higher phlorotannin species. Clearance rates of MDPM ranging from fresh, 14 and 28-day old material decreased with age in *C. retroflexa* and *M. pyrifera*, but not for MDPM from *C. torulosa* and *U. pinnatifida*. Initial MDPM concentrations present to mussels ranged from 0.017 to 0.126 g L^{-1} and removal rates were positively correlated with increasing concentrations from fresh MDPM from *C. torulosa, M. pyrifera,* and *U. pinnatifida.* We did not observe a relationship with increasing concentration and removal rates of MDPM from *C. retroflexa.* In addition, we report carbon to nitrogen mass ratios (C:N) and nutritional profiles of mature blade material collected from *C. retroflexa, C. torulosa, M. pyrifera,* and *U. pinnatifida* to consider the implications of selective clearance of MDPM on nutritional profiles from macroalgal detritus. We found that the two laminarian species had lower median C:N ratios than the fucoids, which was largely reflective of significant differences in total carbohydrate and crude protein content. Interspecific differences in phlorotannin concentrations and nutritional quality of organic matter produced by large brown algae have important consequences for the availability of organic matter originating from macroalgal communities and ecosystem function of coastal food webs.

GENETIC PARAMETERS FOR SALINITY STRESS TOLERANCE IN NORTHERN QUAHOGS *Mercenaria mercenaria*

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The northern quahog dominates shellfish aquaculture in the state of Florida and is ranked 3rd in the nation for total production. The clam industry in Florida faces two environmental challenges, extended low salinity caused by hurricanes and heavy storms. In this study, the feasibility of improving low salinity tolerance in northern quahogs was evaluated using a challenge experiment on ~1-year-old clams from 4 distinct groups. These groups consist of two commercially produced stocks (Com and BF) and two research-produced stocks (HS and Ctl), one of which is the F1 offspring of heat-tolerant broodstock. Tolerance to acute low salinity stress was conducted at 5 ppt for 18 days by decreasing salinity from 22 ppt to 5 ppt over 6 days. Any mortalities observed during the challenge period were recorded and tissue was taken for genotyping and parentage analysis. The clams were brought back to 22 ppt over 4 days. All survivors at LD 50 were remeasured and weighed to compare the amount of stress applied throughout the challenge. Siphon clips and hemolymph samples were taken of survivors for later genotyping and the biopsied survivors were deployed in a fluctuating-salinity lease site for continued culture.

During the low-salinity challenge, peak mortality occurred on day 18 at 5 ppt, which coincided with LD 50 for the challenge. Of the 4 stocks of clams used for this challenge, the HS stock, which was the F1 heat shock-resistant clams, showed the most susceptible with a significantly lower survival rate. After 8-month of cultivating in the field, the survival and biometrics of these clams were recorded. Both research stocks had a high survival rate (HS-FC = 71.43% and Ctl-FC = 65.95%) compared to the commercially produced stocks (Com-FC = 39.47% and BF-FC = 47.42%).

These final survivors will be used as broodstock for F1 offspring production and will be genotyped for heritability of tolerance and genetic correlations analysis. This study is an initiation of low-salinity clam breeding. It is expected that the results will lay the foundation for further clam breeding for resistance to low salinity and summer heat stress to support the sustainability of the Florida clam industry.

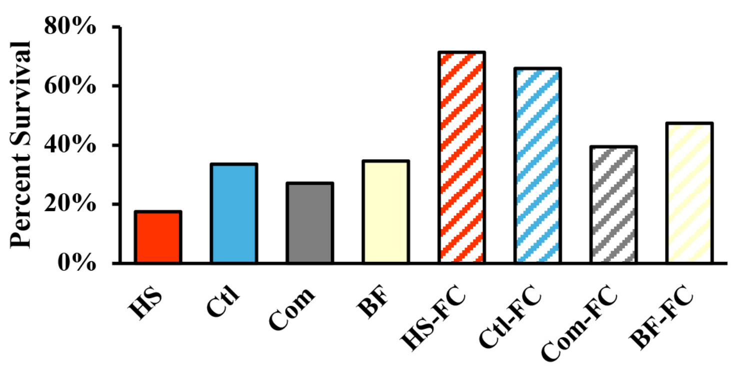

Figure1. Percent survival from acute low salinity challenge and percent survival from field culture 8 months post challenge (-FC).

DNA METHYLATION IN RESPONSE TO HYPOXIC STRESS IN EASTERN OYSTERS

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Oysters are experiencing numerous environmental shifts as the threat of climate change intensifies. Rapid adaptive response is required for these organisms to survive and manage these environmental stressors. It is becoming increasingly critical to understand not only the immediate response to stress, but how the previous environment may influence this response. This concept is known as within-generation carryover effects. However, the mechanisms behind carryover effects remain largely unexplored. Here, we look at DNA methylation as a response to environmental stress. This epigenetic mark can be more sensitive to environmental change than broader phenotypic measures. Additionally, DNA methylation is believed to facilitate adaptive responses in invertebrates.

In a fully factorial experiment, we exposed juvenile oysters to two phases of water treatments (two treatment options; no stress and diel-cycling hypoxic water, <2 mg/L) to simulate climate change predictions. After the second exposure to environmental stress, whole body tissue samples were collected and processed for MethylRAD-sequencing. Our findings reveal context-dependent methylation patterns (Fig. 1), meaning methylation is not a uniform response, but specific to the environment experienced. Additionally, we found that the timing of environmental stress influences the oyster methylome. These results suggest that the methylated response is influenced by previous exposure and emphasizes the importance of studying multiple stress events. Enhancing our understanding of these processes can provide insights to efficient and effective management practices to ensure the persistence of oyster populations in a changing climate.

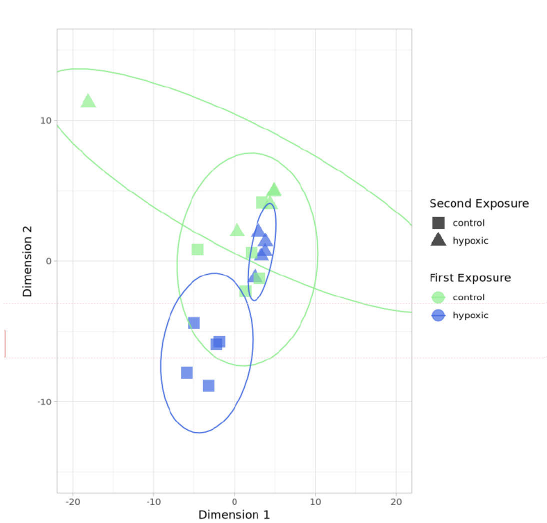

Figure 1. NMDS plot of methylation patterns of oysters subjected to an initial exposure to hypoxia (blue) or control (green) conditions, followed by a second exposure to either hypoxia (triangle) or control (square).

ECOLOGICAL INTERACTIONS OF SHELLFISH AQUACULTURE RELEVANT TO PUGET SOUND NEARSHORE HABITATS AND A REVIEW OF RELEVANT TOOLS, MODELS, AND CALCULATORS

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In Washington State there are over 250 shellfish farms across more than 700 sites, the majority of which are in Puget Sound. A growing interest in maintaining and expanding shellfish aquaculture in Puget Sound, encompassing oysters, clams, and geoducks, could increase interactions with the surrounding ecosystem. These interactions can have positive, negative or neutral effects, on different aspects of the ecosystem. And currently, our understanding of aquaculture-environment interactions is limited, especially interactions with protected species like salmon. In Puget Sound, WA, juvenile Chinook salmon (*Oncorhynchus tshawytscha*), which is listed as threatened under the Endangered Species Act, depend on the nearshore habitat in Puget Sound and likely interact with shellfish farms, but to what extent and effect is unknown. Potential positive impacts of shellfish farms may include an increase in habitat structure and nutrient removal associated with harvest in eutrophic areas. Negative impacts may include reduced primary production related to nutrient removal at times when forage is limited for juvenile salmonids, reduced light affecting productivity, and disturbance of eelgrass beds and eelgrass-associated invertebrates and fish. Considerations of these interactions at local and seascape scales are also important. In addition, climate change may shift the type, intensity, and outcome of aquaculture-environment interactions. To address knowledge gaps regarding interactions between shellfish aquaculture and the environment, we reviewed literature on shellfish aquaculture and environment interactions. Managers are keenly interested in understanding these interactions and the available approaches to assess them. While there are a variety of tools, models, and calculators developed to examine these interactions, many are region- or species-specific and it can be difficult to evaluate if any given tool can be adapted to a different region. We conducted a literature review to synthesize shellfish aquaculture-environment interactions and tools, models, and calculators used to assess these interactions. This synthesis will inform our plans to incorporate information about shellfish aquaculture-environment interactions into a calculator currently used for assessing the effects of anthropogenic change in nearshore habitats on Chinook salmon.

GLUTAMATE IS AN EXCITATORY NEUROTRANSMITTER IN CEREBRAL GANGLIA OF THE BIVALVE *Crassostrea virginica*

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In the oyster *Crassostrea virginica* and other bivalves, gills are innervated by serotonin and dopamine nerves. The innervation originates in the cerebral ganglia (CG), which connect to the visceral ganglia (VG) and then to the gills. In gill lateral cells (GLC), serotonin is cilio-excitatory and dopamine is cilio-inhibitory. In mammals glutamate (Glut) neurons are major excitatory neurons. In humans, disfunction of Glut neurons are associated with several disorders including Parkinson's disease, Alzheimer's disease, Huntington's disease, autism, depression and schizophrenia. Glut receptors are generally classified as ionotropic NMDA, AMPA and kainate types or metabotropic group I, II, III and GluR7 types. Not much has been reported concerning Glut neurons in bivalves. However, ionotropic Glut receptors were found involved in regulating bivalve larvae metamorphosis in *C. gigas*, *Mercenaria mercenaria* and *Mya arenaria*; and genetic studies showed genes for ionotropic Glut receptors present in Pacific oysters. Glut neurons had not previously been reported in or have a physiological function in adult *C. virginica*. Recently our lab used immunohistofluorescence and Western Blotting to detect Glut neurons and the ionotropic Glut receptor GluR1 in VG of *C. virginica*. We also showed the Glut neurons in VG excited serotonin neurons to increase GLC cilia beating rates. Based on these findings, we sought to determine if Glut had a neurophysiological role in CG of *C. virginica*. We hypothesize Glut would be an excitatory neurotransmitter in CG, resulting in an increase in GLC cilia beating rates. To test this Glut was applied directly to the CG of CG preparations in which gill innervation from CG and VG were kept intact. Shells were removed and preparations placed in chambers with a barrier so drugs could be discretely applied to CG without coming in contact with VG or gill. Beating rates of GLC cilia were measured by stroboscopic microscopy. Our results showed applying Glut (10^{-5} - 10^{-3}M) to CG, of CG preparation, caused a dose dependent increase in GLC cilia beating rates from a basal of 13 to 19 beats/sec. This response was mimicked by applying the ionotropic agonist, homocysteic acid (10^{-5} – 10^{-3}M) to the CG, which also caused a similar dose dependent increase in cilia beating from 13 to 19 beats/sec. This cilio-excitatory effect of Glut on the CG was prevented by the presence of the Glut ionotropic antagonist, DL-2 amino-5-phosphonopentanoic. The study confirms a physiological role for Glut as an excitatory neurotransmitter in CG, most likely exciting CG serotonin neurons to increase GLC cilia beating rates. Our pharmacological results also show the Glut receptors in CG are ionotropic. The bivalve mollusc gill is a useful model to study regulatory mechanisms of cilia activity as well as the pharmacology of drugs affecting biogenic amines in nervous systems.

This work was supported in part by grants 0537231071 of the CSTEP Program of NYSED, P120A210054 of the MSEIP Program of the DoEd, 2R25GM06003 of the Bridge Program of NIGMS, and NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University.

EVALUATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DETECTING *Vibrio vulnificus* AND TOTAL AND PATHOGENIC *Vibrio parahaemolyticus*

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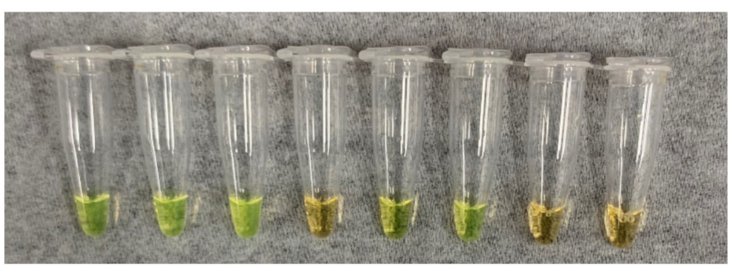
Vibrio parahaemolyticus (Vp) and *V. vulnificus* (Vv) are the leading causes of shellfish consumption-associated infections and mortality in the United States. The current National Shellfish Sanitation Program (NSSP)-approved DNA probe/colony hybridization method for isolate confirmation cannot be conducted as the manufacturer of the alkaline phosphatase (AP) probe has discontinued production. The AP probe is used in the NSSP for post-harvest process (PHP) validation/verification sample testing. It is important to find a cost-effective replacement for the AP method so PHP testing can continue to provide confidence that these processes are reducing the *Vibrio* pathogens to "non-detectable" levels in shellfish. Loop-mediated isothermal amplification (LAMP) was identified as a possible replacement. LAMP occurs at a constant temperature and amplification can be seen by the naked eye so expensive thermal cyclers are not needed, and it can be highly specific with up to six sets of primers per target species. Published LAMP assays targeting *tox*R for Vp and *vvh*A for Vv were evaluated and optimized for enzyme system, amplification temperature, and DNA preparation method against a panel of 185 isolates. Optimal conditions were tested for specificity and sensitivity. When non-specific amplification was seen in LAMP, chromogenic and selective agars were evaluated as a prescreening step to prevent non-target species from being tested in LAMP assays. Prescreening with chromogenic and selective agars increased specificity. Upon further testing, the *tox*R assay detected 49/50 (98%) Vp isolates and the *vvh*A assay detected 48/50 (96%) Vv isolates. Isolates that were not detected did not look typical on chromogenic or selective agars and were not picked to LAMP for confirmation. The completed research demonstrates that the published LAMP assays tested would need to be redesigned for increased specificity so prescreening would not be necessary before moving forward.

Figure 1. Image of LAMP results. Green, turbid results are positive. Orange results are negative.



ENVIRONMENTAL IMPACTS AND RISK ON JUVENILE OYSTER GROWTH AND SURVIVAL IN LONG ISLAND SOUND

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Eastern oysters *(Crassostrea virginica)* are ecologically and economically important throughout the Atlantic and Gulf of Mexico coasts. Oysters improve water quality by filter feeding and increase biodiversity by providing substrate and habitat for other species. Oyster aquaculture is a major industry that is worth over $14,000,000 in Connecticut alone. As a result, restoration projects and a push to bolster the aquaculture industry are prevalent in the Northeast, USA, however, climate change, disease, and sedimentation threaten restored and aquacultured populations. To discern environmental and biological effects, we measured growth and survival of juvenile oysters at two sites during summer 2024. Differential mortality between sites was attributed to heavy predation by oyster drills at one site but not the other. Oysters at the site with less predation also had a marginally higher growth rate. In anticipation of increased interest to establish new aquaculture leases, we incorporated these data into a risk assessment framework. Risk assessment and habitat suitability models have been successful in other parts of the world to identify optimal habitat for restoration, so we suggest future work centered around combining existing methods in a way that is relevant to the specific needs of Connecticut aquaculture. These recommendations are intended to serve as building blocks for scientists, industry, and local government to create a standardized framework for assessing the suitability of potential farm or restoration sites in Connecticut.

AQUACULTURE TECHNICAL ASSISTANCE AND OUTREACH AT DELAWARE STATE UNIVERSITY: PAST, PRESENT AND FUTURE

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Despite ongoing research and extension efforts, aquaculture in DE is currently limited in terms of the number of commercial producers, with the industry historically being dominated by finfish production (e.g. tilapia, striped bass, hybrid striped bass). In 2013, then-Governor Jack Markell signed legislation to allow leasing of subaqueous lands in the DE Inland Bays for shellfish aquaculture. This represented a significant opportunity to expand DE's aquaculture industry, and also poses a challenge as there is a new/expanded demand for technical assistance, education, and outreach.

The Delaware State University (DSU) Aquaculture Technical Assistance and Outreach Program was developed with a mission of fostering the creation of an aquaculture industry in DE. The Program's goals are to identify suitable aquaculture species for use in the mid-Atlantic region and identify low-input methods to raise them profitably so that as industry grows it is economically and environmentally sustainable. Implementation has followed three avenues:

1. Conduct applied research investigating the suitability of aquaculture species and culture techniques for use in the region,
2. Disseminate information to all interested parties about aquaculture species and technologies through workshops, one-on-one farm visits and fact sheets, and
3. Demonstrate various aquaculture production methods and techniques at the DSU Aquaculture Research and Demonstration Facility (ARDF).

Since its inception in 2004, DSU's Aquaculture Technical Assistance and Outreach program has become a preeminent source of aquaculture information in DE, which, until recently, was focused primarily on freshwater finfish production. Our existing infrastructure - namely the DSU ARDF - serves as a laboratory, classroom, and demonstration farm site where we conduct research, teaching and extension programming. With recent grant funding, we are expanding the reach and scope of our program to include a shellfish aquaculture demonstration farm in the DE Inland Bays. Once established, this will allow a space to conduct shellfish-focused extension, teaching and research projects. Currently, no such experimental or demonstration farm exists within DE waters, inhibiting the confident growth of our shellfish aquaculture industry by farmers that lack the experiential knowledge to grow oysters. Providing such a facility, and unique learning opportunities, will help potential shellfish farmers transition to proficient aquaculturist and lessen their economic uncertainty.

EXPLORING THE ART OF MORE-THAN-HUMAN INTELLIGENCE IN *Micranthemum Tweediei*

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The objective of this research is to experiment with organically composing the growth structures of aquatic plant life in a freshwater aquarium with the intent of exploring more-than-human intelligence. The outcome of my research will be to reveal the connections between composing an aquascape, and the *umwelt* of emersed plants. The German phrase umwelt, coined by Estonian Biologist Jakob von Uexküll, is defined as 'the world as it is experienced by a particular organism.' The physical predisposition of any living organism directly affects the way they perceive and move about the world; and in return, the way humans perceive them. As plants grow very slowly, their intelligence seems foreign to us, but in reality, they quiver, stretch, and yearn to live in real-time.

Currently, the research is focusing on *Micranthemum Tweediei,* also known as Monte Carlo pearl grass; this species is easy to cultivate, and quickly spreads across surfaces. The tank holds approximately 10 gallons, is heated, and dosed weekly with CO_2. I have created 3D fabricated 'snoots', a mechanism used in photography to centralize light. These snoots are utilized to direct plants to form a dense web of interconnected life. The Monte Carlo was originally only planted on the outer edges of the aquarium and is slowly coaxed to the center by the lowering of the snoot, which creates a shrinking pinnacle of light. The work, which will be documented as a timelapse, is titled *SOL (solar solace).* As their light source shrinks, viewers may witness new life, as well as decay as the plants respond to their umwelt.

Through timelapse video documentation, humans can observe the qualities of plants we may not notice through our perception of time, potentially allowing us to become more empathetic toward their form.

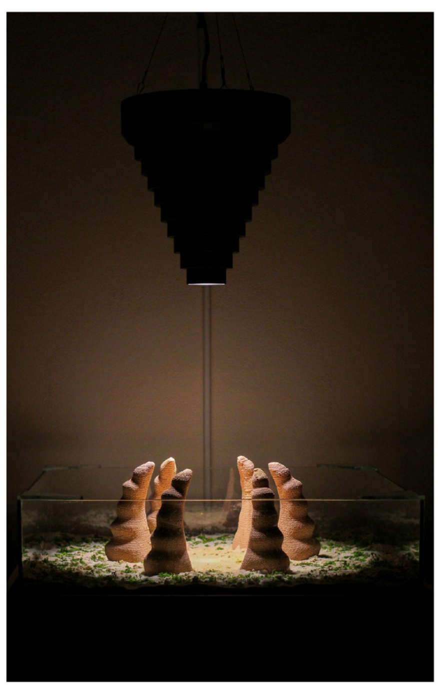

Figure 1:
SOL (solar solace) WIP
glass, substrate, grow light, 3D printed hand

OPTIMIZING ALGAL TURF TECHNOLOGY FOR ENHANCED NUTRIENT REMEDIATION AND BIOMASS PRODUCTION IN AGRICULTURAL SYSTEMS

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Algal Turf Technology represents an innovative biosystem that effectively removes excess carbon, nitrogen, and phosphorus from wastewater, thereby enhancing water quality and facilitating the recycling of water for agricultural irrigation and industrial use. This project focuses on the implementation of Algal Turf Technology in aquaculture settings to evaluate its efficacy in biomass production and nutrient remediation, with particular emphasis on the comparative analysis of algal growth over different study periods. Preliminary investigations suggest that systems operating at higher flow rates yield greater algal biomass and achieve significant nutrient removal, notably in phosphorus. This research will further delve into algae species composition, chlorophyll content, and the biochemical characterization of the algal biomass, particularly examining lipid and fatty acid profiles for potential applications in fish feed. We aim to provide a comprehensive assessment of biomass quality and its implications for biofertilizers, bioenergy, and aquaculture. Our goal is to equip Delaware farmers with the knowledge and tools necessary to adopt Algal Turf Technology, ultimately improving wastewater management practices and fostering sustainable agricultural systems.

CHALLENGES RELATED TO CULTIVATING BULL KELP (*Nereocystis luetkeana*) IN AN EXPOSED LOCATION IN THE PRINCE WILLIAM SOUND, ALASKA, USA

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The conditions required for the successful cultivation of bull kelp (*Nereocystis luetkeana*) in Prince William Sound, Alaska, USA (PWS) are not well understood. PWS represents the northern most region of the species' range. Growth, buoyancy, nutrient availability, salinity, and current exposure to better understand the requirements for cultivating this valuable and unique species.

Bull kelp was grown on modified 1 m x 1 m crab pots (kelp pots) threaded with 20 mm nylon strapping cord at the Native Village of Eyak's Sheep Bay Research Farm. Five kelp pots were deployed in November 2023 at a water depth of 20-35 m. The pots were suspended by polyform buoys in the water column at a depth of 6 m. Bull kelp seed string was planted on four of the kelp pots in December 2023, while one control pot was left unseeded to compare buoyancy measurements due to biofouling. Another pot was designated for measuring kelp morphology by sacrificial means. Semi-monthly measurements of bull kelp morphology followed the Bull Kelp Research Squad working group's standard operating procedures, which included measuring bulb + stipe length (BSL), stipe length, sub-bulb diameter, supra-holdfast diameter, mid-stipe diameter, and bulb diameter. Buoyancy measurements were conducted using a crane scale by weighing pots and kelp completely submerged in the water and also fully removed from the water. Additionally, an acoustic Doppler current profiler (ADCP), and HOBO water temperature loggers were moored at the site. Each field visit included CTD casts (RBR Concerto) and water quality sampling from 3 m depth to assess nutrient availability.

Presented here are data from the first year of a two-year study. Bull kelp morphology was not measured until April 2023 due to a lack of growth during the winter months. Water temperature and salinity did not significantly vary from past years' data. Nutrient and current data analyses are pending. Anecdotally, bull kelp appeared nutrient starved based on the pale-yellow coloration of thalli. Sugar kelp grown nearby also displayed poor nutrient exposure with pale-yellow blades and transparent blade tips. From December 2023 to August 2024, bull kelp exhibited minimal growth. The peak average BSL was 51.7 ± 17.2 mm in June, with a density of 40.2 individuals m^{-1}. The small size of the kelp prevented accurate buoyancy measurements due to the lack of sensitivity of the crane scale. By August, fewer than two ind. m^{-1} remained on the kelp pots.

A new crop of bull kelp will be seeded on the kelp pots in January 2025, and growth and buoyancy will be evaluated using the same methodologies to assess temporal variability.

INTEGRATED MULTI-TROPHIC AQUCULTURE – HOW GEOPOLITICS CAN IMPACT ITS VIABILITY AND SUSTAINABILITY AS MUCH AS ECOLOGY OR BIOLOGY

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Ecological aquaculture, or polyculture, has been practiced for centuries in Southeast Asia and Africa but is still relatively uncommon in Western countries (Beveridge and Little, 2021). In Australia, as in other regions, commercial aquaculture is increasingly facing criticism for environmental impacts and product quality (Mazur and Curtis, 2006). With growing interest in seaweed and the circular economy in Western economies, we examined whether seaweed farming within an Integrated Multi-Trophic Aquaculture (IMTA) model could improve environmental and economic outcomes and boost social acceptance of aquaculture.

Our findings showed that while seaweed can be grown successfully in Australia alongside higher trophic-level species (Visch et al., 2024), there were several non-biological challenges which could influence the success of IMTA:

- **Regulations**: Strict policies focused on biosecurity or conservation agendas often limit co-production of species due to licensing and proximity rules.
- **Public Perception**: Many people misunderstand the nutrient cycling and nature positive benefits in IMTA, leading to concerns about using waste streams to feed other species which are produced for human consumption.
- **Business Models**: Building integrated business models or IMTA partnerships is complex and often implemented retrospectively.
- **Conflicting Priorities**: There’s often a mismatch between economic growth goals and environmental sustainability.

These challenges may discourage farmers and companies from adopting IMTA or reduce its effectiveness limiting the benefits that IMTA could offer like improved waste management, better nutrition from a more diverse range of aquaculture products, and more efficient resource use. To achieve sustainable outcomes, we must promote the value of integration at ecosystem, operational, political, and societal levels. Aligning policies and governance with new aquaculture practices like IMTA could help communities and markets better understand and support these initiatives, avoiding outcomes that undermine sustainability.

EFFECTS OF FISHMEAL SUBSTITUTION BY DEFATTED BLACK SOLDIER FLY LARVAE AND SOY PROTEIN MEALS ON THE GROWTH AND HEALTH OF ATLANTIC SALMON *Salmo salar*

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Atlantic salmon (*Salmo salar*) aquaculture continues to grow, and research into alternative feed ingredients is crucial for its sustainable production. This study aimed to evaluate the substitution of fishmeal (FM) with defatted black soldier fly larvae (DBSFL) and soy protein meals (SOY) in the diet of Atlantic salmon using growth performance, health parameters, and nutrigenomics approaches.

A 12-week feeding trial was conducted in a recirculating aquaculture system at UMaine - ARI. Thirty Atlantic salmon parr (36.4 ± 0.1g, initial weight) were randomly stocked into triplicate tanks (300 L each). A control diet (100%FM) and six test diets (factorial design) containing three levels of DBSFL (10%, 15% and 20% of the diet) and two levels of SOY (12% and 19% of the diet) were evaluated.

All dietary treatments had high survival (greater or equal to 99%). Dietary DBSFL significantly affected fish growth, with a high final weight (141.84 ± 2.67g) and weight gain (291.25 ± 6.45%) in fish fed 10%DBSFL+12%SOY diet compared to those fed 20%DBSFL+12%SOY (116.61 ± 7.43g and 221.53 ± 21.35%, respectively), but similar with the other treatments. Two-way interactive effects were found on hepatosomatic index, plasma alanine aminotransferase, immunoglobulin M, total iron-binding capacity (TIBC), cortisol and head-kidney insulin-like growth factor-I (*P*= 0.005, 0.043, 0.009, 0.006, 0.004 and 0.022, respectively). The highest plasma IgM level was measured in the group fed 15%DBSFL+12%SOY diet. The 20%DBSFL+12%SOY and 10%DBSFL+19%SOY diets significantly improved plasma TIBC in contrast to 20%DBSFL+19%SOY. Plasma cortisol and alkaline phosphatase levels were significantly elevated in fish fed the 20%DBSFL+19%SOY diet compared to those fed the control and 15%DBSFL+19%SOY diets. DBSFL meal significantly altered the relative expressions of immune-related genes (IgD and IFN-γ), while SOY influenced the growth (IGF-I) and immune-related (IgM, IgD, IgT, IFN-γ and IL-1β) genes in the head-kidney of Atlantic salmon. IGF-I and IgD genes expression were upregulated in fish fed the 15%DBSFL+12%SOY diet compared to those fed the 20%DBSFL+19%SOY. IgM and IgT expressions were significantly increased in fish fed the 15%DBSFL+12%SOY diet in contrast to those fed high-SOY diets, except 10%DBSFL+19%SOY. The 20%DBSFL+19%SOY diet significantly elevated head-kidney pro-inflammatory cytokine gene (IFN-γ) expression compared to the low-SOY diets. The overall results suggest that DBSFL meal (up to 15% of the diet) could replace fishmeal without compromising Atlantic salmon growth and health; and alleviate inflammation caused by anti-nutritional factors derived from soy protein.

BEHAVIORAL OBSERVATIONS, RELATIVE CONDITION, AND ESTIMATED PRODUCTION OF BLACK SEA BASS *Centropristis striata* USING OYSTER AQUACULTURE CAGES AND BOULDERS AS HABITAT

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Black sea bass are a temperate reef finfish found in natural and artificial structured habitats. This study uses fish behavior, condition indices and enhanced production estimates to evaluate how oyster aquaculture cages serve as artificial reefs for black sea bass, relative to natural rock reefs at study sites in central Long Island Sound. We recorded underwater video on shellfish farms and a rock reef in Milford, CT to identify and quantify black sea bass behavior associated with oyster cages and boulders. We sampled juvenile black sea bass from farms and reefs in Clinton and Milford, CT to assess relative condition factor and energy density as measures of habitat quality. We used young-of-the-year abundance data to estimate fish production enhancement provided by shellfish farms. Black sea bass demonstrated more shelter and grouping behavior on cages as compared to boulders. Instances of courtship/reproduction, escape, foraging, and territorial behavior were similar across cage and boulder habitats. Measurements of relative condition factor and energy density in juvenile black sea bass showed no difference in physiological condition of fish on farms and reefs. Enhanced production of black sea bass was estimated based on the greater abundance of young-of-the-year fish associated with oyster aquaculture cage farms relative to the natural rock reef. Our results suggest that aquaculture gear provides valuable habitat and ecosystem services for black sea bass throughout their life history, similar to other man-made structures that are considered Essential Fish Habitat.

AN OVERVIEW OF SIX YEARS OF RESEARCH ON HABITAT PROVISIONING BY EASTERN OYSTER AQUACULTURE GEAR

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NOAA's GoPro Aquaculture Habitat Project uses video from underwater cameras to study fish abundance, behavior, and community composition on oyster aquaculture cages at shellfish farms and natural rock reef habitat in the Connecticut waters of Long Island Sound. We attached two action cameras to each study cage, one camera positioned to record across the cage top and the other with a view across two cage sides and the interface between the cage and the seafloor. We used timers to record video at 8-minute intervals hourly over a full tidal cycle. In 2018, we placed 4 study cages on both a high-density (40+) commercial oyster farm, and on featureless seafloor, respectively, to compare how cage abundance affects fish activity. To compare cages to natural structured habitat, we also constructed T-platform stands to mount cameras among boulders that provided a similar perspective to cage-mounted cameras and minimized added structure. In 2019, we compared two styles of oyster cage, shelf & bag versus stacked tray, at three oyster farms in central and western Long Island Sound. From 2017 to 2023, we monitored fish abundance on cages adjacent to the Milford commercial oyster farm to assess interannual changes in fish activity on cages. During 2022, we used GoPro cameras in stereo configuration to collect continuous video on farms and reefs in Clinton, Milford and Noank, CT that will be used to measure the body length of fish in these habitats. In 2023, we collected juvenile black sea bass from Clinton and Milford farms and reefs to assess fish condition using energy density and relative condition factor as indicators of habitat quality. To increase efficiency of processing fish for abundance estimates, we are collaborating with researchers at National Research Council-Canada to develop a machine-learning model to automate identification of the four most common temperate reef species associated with oyster cages in Connecticut.

To date, our video analysis has documented fish of all sizes and life stages using cages as habitat, including commercially and recreationally important black sea bass, scup and tautog. These temperate reef species occurred in similar or higher abundance on cages as compared to boulders. Overall, fish abundance did not differ on cages at large and small farms and or between styles of oyster cage. We have observed a variety of fish behavior associated with habitat provisioning on oyster cages including courtship/reproduction, escape from predators, feeding, grouping/schooling, sheltering and territoriality. Using 2018 data, we are estimating enhanced production of black sea bass based on the greater abundance of young-of-the-year fish we observed on oyster cages relative to the natural rock reef. We found no difference in physiological condition of fish on farms and reefs based on energy density and relative condition factor metrics. These results suggest that oyster aquaculture cages provide habitat services to fish in much the same way as natural boulder habitat and that oyster farms may act like artificial reefs providing structured habitat for fish on otherwise featureless seafloor.

SOLIDS CONTROL FUNDAMENTALS IN INTENSIVE FLOW-THROUGH AND RECIRCULATING AQUACULTURE SYSTEMS

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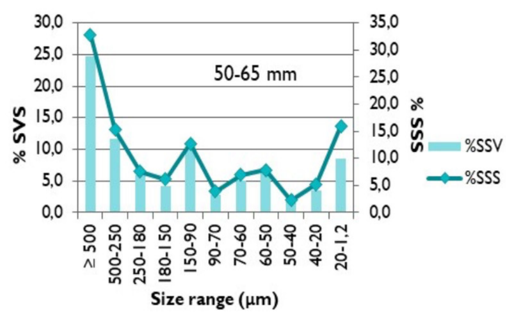
The main particulate waste found in aquacultural water recirculation systems includes feces, uneaten food, and mucus. Fish have been particularly studied, and it is indicated that for salmon, for every 1 kg of food, between 0.3 and 0.4 kg of solids are produced as feces, and in the case of catfish between 0.2 and 0.7 kg of solids as feces.

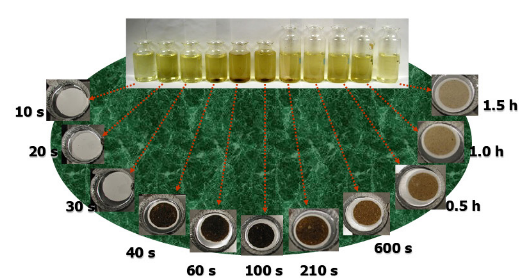
In aquaculture it has been reported that the densities of the solids that are produced particularly in salmon farming have an average density between 1050 and 1190 kg/m^3 (with a specific gravity of 1.19). These suspended particles vary greatly in size (from cm to µm) and shape.

The mean sedimentation rate for SS found in rainbow trout farming facilities was 1.7 cm/s and for feces extracted manually by massage a mean value of 0.7 cm/s was determined. In the case of salmon faeces, it has been described that the sedimentation rate is between 4 and 6 cm/s. Salmon feed has a higher sedimentation rate than faeces with values between 15 and 33 cm/s. The sedimentation rate of extruded diets for sea bream and sea bass is between 3.9 and 10.6 cm/s.



In relation to the size of the solids, it has been described for fish farm effluents for salmon ranging from 8.4 to 155 µm. In recirculating systems, sizes between 0.4 and 12 µm have been reported, with most particles in the range between 0.4 and 2 µm. It is generally known that 95% of suspended solids in recirculating aquaculture systems are less than 20 µm and that they represent between 40 and 70% of the total mass of SS.

The distribution of sedimentation rates and particle sizes are bioengineering data required for the design of gravity solids removal devices and for the selection of solids removal devices by size. This presentation will address the fundamentals of solids control by sedimentation and screening technological devices.



NUTRIENTS REMOVAL AND MICROBIAL SUCCESSION IN MARINE PERIPHYTON BIOFILTER: ONE STEP CLOSER TO UNDERSTANDING THE ECOLOGICAL ASPECT OF A COST-EFFECTIVE BIOFILTER IN SUSTAINABLE AQUACULTURE SYSTEMS

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Sustainable and inexpensive technologies for treating mariculture effluents are needed in both integrated multi-trophic aquaculture (IMTA), and recirculating aquaculture systems (RAS). Marine periphyton can be used to remove excess nutrients from fishponds effluent while converting waste into edible biomass but the technology has not been developed commercially so far. Toward this goal, we developed and examined a marine periphyton-based biofilter in both an IMTA system and intensive RAS. Growth performances, nutrient removal, and biochemical content were studied along with the cultivation of periphyton in the biofilter. In addition, we examined the assembly processes of the microbial community in the periphyton to expand our understanding of the forces that govern community composition, functionality, and networking.

In the IMTA, the periphyton-based biofilter removed up to 76% of the total N and P in the effluents of the fishponds (*Mugil cephalus)*. In RAS, low levels of toxic ammonia, nitrite, and nitrate were maintained even at a high stocking density of fish (*Sparus aurata)* of 50 kg m^{-3} and minimal addition of fresh water (10% day^{-1}) to compensate for evaporation.

The periphyton prokaryotic and eukaryotic communities revealed significant differences in their succession patterns. The bacterial community consisted of 23 phyla, but *Proteobacteria*, *Bacteroidetes*, *Planctomycetes,* and *Cyanobacteria* accounted for >95% of the total ASVs. The bacterial successional process became more stochastic over time while the eukaryotes were more deterministic. The bacterial community assembly was governed by both nutrient regimes and temporal dynamics. However, the force of time produced a stronger effect on the differentiation in prokaryotic community composition compared to nutrient input. Interestingly, the lowest diversity was detected in the mid-time of the periphyton development course, implying a competition process before the community reaches a stable stage. This event coupled with a drastic reduction of *Vibrio* prevalence and an increase in bacterial genes related to resilience and stress resistance. Further research about pathogenic occurrence in this system will complete a full picture of community dynamics, which in turn will support a more holistic mariculture practice.

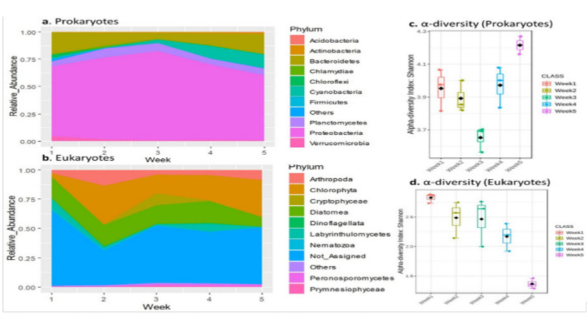

Figure 1. Changes in the bacterial and eukaryotic community in marine periphyton during five weeks of succession are shown by changes in the relative abundance (a, b) and Shannon diversity (c, d). (n = 15).

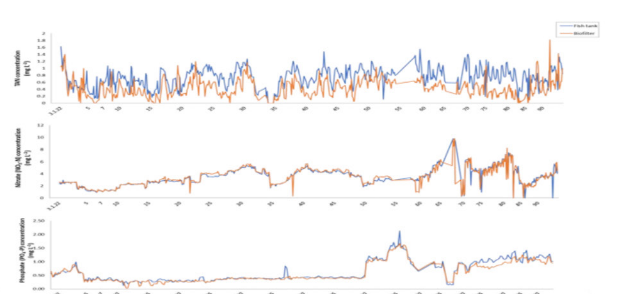

Figure 2. Fluctuations in levels of TAN (up), NO_2-N (middle), and NO_3-N, in the culture water (blue lines) and the periphyton biofilter (orange lines) of marine RAS, over a period of four months.

SEX CLASSIFICATION OF DELTA SMELT BASED ON PHENOTYPIC APPEARANCE USING DEEP LEARNING METHODS

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Delta Smelt, an endangered fish species, plays a critical role in maintaining biodiversity, genetic diversity, and ecological balance in California. Accurate sex identification of Delta Smelt is crucial for optimizing breeding and operational strategies and enhancing the conservation of this species. Currently, sex identification of Delta Smelt primarily relies on egg stripping during the sexually mature stage. Determining their sex is almost impossible without the presence of eggs or milt in the field.

This study presents a novel approach to recognize sex of Delta Smelt by utilizing deep neural networks to recognize differences on phenotypic appearance. We collected data from Delta Smelt across three life stages—before the spawning season (Section 1), during the spawning season (Section 2), and at the end of the spawning season (Section 3). The framework consists of two key steps: first, segmenting the fish from the background using a fundamental visual model, followed by applying a classification model to distinguish the sex of the Delta Smelt. Segment Anything Model, as a visual fundamental model, is employed to segment fish from the background with the visual prompt, reducing the cost of label annotation and model training. Transfer learning-based fine-tuning techniques are used to train the parameters of final convolutional and fully connected layers for the classification task.

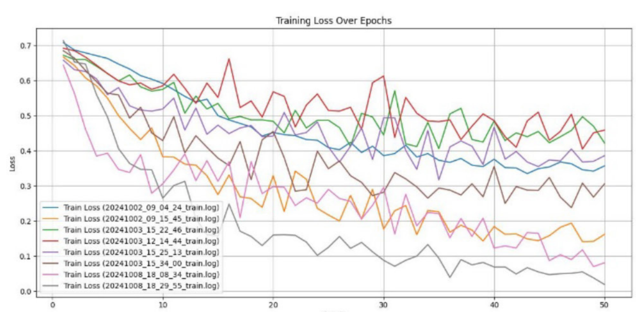

Fig1. Training loss curves of different methods in Delta Smelt (Section 3).

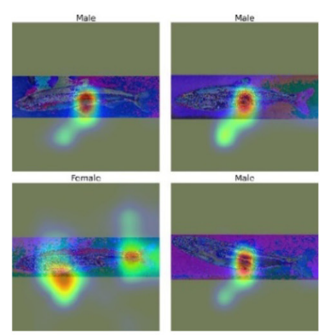

Fig2. Class activation mapping of the finetuned model.

THE SOCIAL DIMENSIONS OF AQUACULTURE: COMMUNITY OF PRACTICE OPEN DISCUSSION

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At Aquaculture America 2024, the Social Dimensions of Aquaculture session centered on the development of a linked community of practice (CoP). We discussed desired actions and efforts related to the CoP, including the creation of a group space on the Aquaculture Information Exchange. The final block of this session will be used to provide an update on the CoP as well as the opportunity to discuss next steps and goals.

THE SOCIAL DIMENSIONS OF AQUACULTURE: ENABLING COMMUNICATION AND COLLABORATION THROUGH A COMMUNITY OF PRACTICE

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The “Social Dimensions” of aquaculture are a regular part of discussions surrounding industry development, specifically related to challenges, opportunities, and accessibility. Additionally, presentations centered on the social dimensions are not uncommon at meetings like Aquaculture 2025, but typically are represented by a single “socially leaning” talk in a broader session or through sessions focused on specific topics within the social dimensions (e.g., perceptions of aquaculture, diversity and inclusion, women in aquaculture). Through a special session at Aquaculture America 2024, we sought to bring together individuals and organizations conducting work in the social dimensions of aquaculture to 1) build awareness of the range of different efforts occurring in this space (e.g., research, extension, communication, policy, and others), 2) provide a setting for communication and collaboration amongst social dimensions practitioners, and 3) discuss the formation of a related community of practice. Last year’s session represents the first of what will hopefully continue to be a regular session at Aquaculture America, the National Shellfisheries Association annual meeting, and likewise the triennial Aquaculture meetings in addition to providing an at least annual in-person meeting opportunity for the community of practice. This presentation will give an overview of the Social Dimensions of Aquaculture Community of Practice, including its goals and activities thus far.

COMMUNITY CONTEXT FOR OFFSHORE AQUACULTURE DEVELOPMENT: VULNERABILITY, RISK, AND OPPORTUNITY PROFILES FOR COASTAL COMMUNITIES IN THE GULF OF MEXICO

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The promise of the blue economy and a growing interest in sustainable, reliable, and accessible sources of protein has enhanced interest in the potential of offshore aquaculture. To maximize the possible benefits of offshore aquaculture, it is essential to consider how its development might impact shoreside communities - positively, negatively, or otherwise. This study focused on coastal areas in the Gulf of Mexico in order to better characterize the impact that offshore aquaculture development could have on communities in the region.

Forty-five coastal communities in the central and western Gulf of Mexico were evaluated to consider vulnerability, risk, and opportunity related to the potential development of offshore aquaculture. Sub-regional community profiles were developed using three publicly available datasets: 1) NOAA Fisheries Community Social Vulnerability Indicators (CSVIs), 2) FEMA's National Risk Index (NRI), and 3) NOAA Gulf of Mexico Integrated Ecosystem Assessment (IEA) – Aquaculture Indicators; findings were further ground-truthed through in-person interviews and community visits as part of a complementary project.

Resulting profiles illustrate a range of community characteristics that should be considered as part of any future planning or permitting process, in addition to informing thoughtful community engagement for any prospective offshore operation (e.g., considerations of existing infrastructure limitations given increasing storm events). In many cases, offshore aquaculture has the potential to both exacerbate existing vulnerabilities or provide potential solutions, depending on operation-specific decisions. The results of this study underscore the need to consider best practices for community engagement and context for offshore development to have the most positive impact on local communities.

DETECTION AND QUANTIFICATION OF *Escherichia coli* IN SHELLFISH BY COMBINED MPN-qPCR METHOD

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There is substantial need for the rapid and efficient detection and quantification of *Escherichia coli* and other fecal coliforms in shellfish intended for human consumption. Outbreaks of diarrhea, hemorrhagic colitis, and Hemolytic Uremic Syndrome (HUS) in humans have been associated with *E. coli*, most commonly the O157:H7 strain due to its production of pathogenic Shiga-toxins. The current FDA-approved most probable number (MPN) method used for quantifying pathogenic *E. coli* in shellfish is outdated and can take up to four days to complete. In this project, two triplex quantitative Polymerase Chain Reaction (qPCR) assays were developed using previously published primers and probes. These assays were intended to be combined with the overnight enrichment from the standard FDA MPN method to streamline the detection and quantification of the pathogenic O157:H7 *E. coli* strain.

This qPCR-MPN method was first tested with shellfish collected from both contaminated and uncontaminated sites on Cape Cod, MA; however, no O157:H7 was identified by qPCR or by plating on O157:H7-specific MacConkey Agar Medium with Sorbitol. To troubleshoot and validate the assays, a spiking experiment using known amounts of O157:H7 *E. coli* cultures and MPN-qPCR analysis was conducted as follows (Fig. 1). Sets of 10 oysters were sterilely shucked and homogenized. A 1:10 dilution series was created, then used to spike oyster homogenates before inoculation of the 10-mL lauryl sulfate tryptose broth (LST) tubes. Following an overnight enrichment, gas-positive LST tubes were presumed positive for coliforms and used to inoculate 8-mL *E. coli* media (EC) tubes to confirm the presence of *E. coli*. Following another overnight enrichment, gas-positive EC tubes were presumed positive for fecal coliforms. DNA was extracted from both LST and EC positive tubes and run in the newly designed qPCR assays. Positive results from qPCR were compared with MPN positive results with the goal of eliminating the EC media step, saving up to two days.

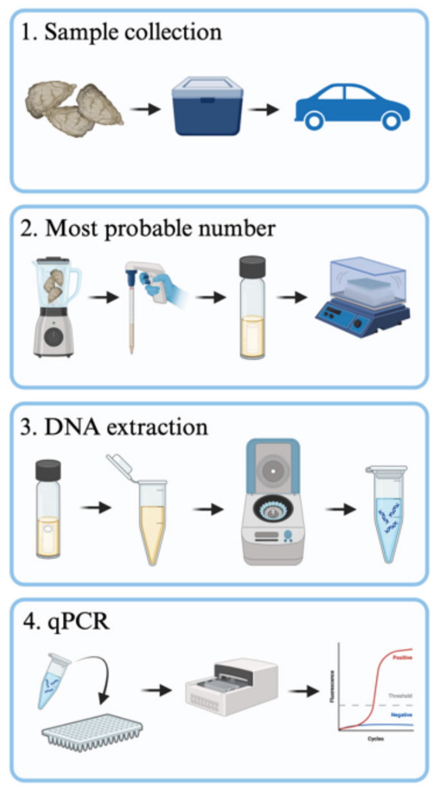

Figure 1. General workflow of MPN-qPCR analysis. Graphic made using BioRender.com.

ANATOMICAL COMPLEXITY ALLOWS FOR HEAT-STRESSED GIANT CLAMS TO UNDERGO SYMBIONT SHUFFLING AT BOTH ORGANISM AND ORGAN LEVELS

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Giant clams are photosymbiotic mollusks, hosting Symbiodiniaceae dinoflagellates. Serving as an alternative model organism for ecophysiological studies within reef environments, giant clams differ from corals due to their anatomical complexity, with extracellular symbionts present in multiple organs. We aimed to determine if clams, under thermal stress, exhibit symbiont shuffling both at the organism level and across individual organs. Therefore, we exposed the fluted giant clam, Tridacna squamosa, to control and heat-stress temperatures of 26 and 30 ºC, respectively, for 45 days. Subsequently, we assessed the degree of bleaching through quantification of symbiont cells and chlorophyll-a loss via fluorometric detection and photometric analysis. The relative composition of Symbiodiniaceae ITS2 rDNA profiles across ten different organs was determined using metabarcoding by next-generation sequencing. Findings show that the outer mantle of heat-stressed clams lost approximately 30% of its symbionts and 45% of the chlorophyll-a content. Extensive shuffling took place at the organism level, with the downregulation of thermally-sensitive Durusdinium phylotype D4/D5, and upregulation of thermally-tolerant, homologous and generalist phylotypes belonging to Symbiodinium and Cladocopium genera. At the organ level, shuffling took place only in the outer mantle, the only organ directly exposed to light. The other organs did not undergo compositional changes in symbiont phylotypes and may potentially serve as symbiont reservoirs. Our results illuminate the complexities of symbiont shuffling within an anatomically intricate organism, offering perspectives for other photosymbiotic reef organisms. Additionally, our study advances the knowledge regarding bleaching in giant clams, a relevant resource that has experienced substantial population declines.

REPRODUCTIVE BIOMARKER DISCOVERY IN THE SKIN-MUCUS TRANSCRIPTOME OF THE NORTH AMERICAN ATLANTIC SALMON *Salmo salar*

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The simultaneous analysis of numerous genes and endocrine factors in fish skin-mucus is a new approach to facilitate a deeper understanding of the physiological status of fish. In the broodstock management of North American Atlantic salmon (*Salmo salar*), non-invasive skin-mucus sampling combined with transcriptomics and bioinformatics, has the potential to identify novel predictive biomarkers, which may reflect the reproductive status and quality of gametes.

We collected and processed skin-mucus from broodstock of the St. John's strain of Atlantic salmon for transcriptome analyses at different reproductive stages, i.e. mid-vitellogenesis (September), late vitellogenesis (November), and ovulation (December), and documented spawning success, fecundity, and egg quality. Preliminary results from the Agilent 2100 Bioanalyzer produced RNA integrity numbers (RIN) as high as 10, indicating very high quality and quantity of RNA in fish mucus.

Transcriptomic results using Illumina sequencing technology were mapped to an annotated reference genome and then combined with bioinformatic tools including DEseq2 for statistics which revealed seasonal differential gene expression in the skin-mucus when comparing September vs. November, November vs. December, and September vs. December. Protein coding genes Cathepsin-L and Gap junction Cx32.2 exhibited the highest log2fold changes of 14.1 and 13.9, respectively, between September and December fish. Gene ontology (GO) analysis revealed that the most represented biological processes between September and December for upregulated genes was G protein-coupled chemoattractant receptor activity, lysosome activity, and c-type lectin receptor signaling pathways. For downregulated genes between September and December, GO analysis revealed a high level of ribosomal and translational activity.

Indeed, the female salmon skin-mucus transcriptome may be viable matrix for predicting the timing and quality of female sexual maturation and ovulation. The presence of highly upregulated genes including cathepsin-L and gap junction, which are both well known for their roles in ovarian follicle growth and maturation, is encouraging. In principle, we aim to develop a non-invasive, farmer-friendly detection and egg quality prediction technology to support North American Atlantic salmon aquaculture broodstock management and improve spawning success.

CULTURE-INDEPENDENT META-PANGENOMICS ENABLED BY LONG READ METAGENOMICS

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The gut microbiome is important for nutrition with potential positive and negative on host linear growth. However, current microbiome approaches based on 16S amplicon or short-read (SR) metagenomics from a single ‘cross-sectional’ time point are limited in that they only have the resolution to evaluate a snapshot of changes in taxonomic or functional abundances across participants. We hypothesized that complete metagenome-assembled-genomes (cMAGs), generated from a longitudinal, long-read (LR) metagenomics cohort, were critical for pangenome and microbial GWAS (mGWAS) analyses for identifying microbial genetic associations with pediatric linear growth trajectories. Here, we showed that single molecule sequencing (PacBio ‘PB’ and ONT) approaches generate 51-72x more cMAGs per Gbp than legacy SR approaches and that PB generated the most accurate, complete cMAGs at the lowest cost making the approach feasible and accessible for microbiome research. When applied to a pediatric undernutrition cohort in Malawi Africa, we generated 985 cMAGs (831 circular) from 47 samples, performed independent functional pangenome and mGWAS analyses across multiple clades, and identified microbial genetic associations with various environmental and biological phenotypes related to undernutrition. Our longitudinal pangenome and mGWAS analysis of these cMAGs revealed genetic differences within the same bacterial species found in different participant phenotypes highlighting the importance of this approach for new diagnostic methods or therapeutic development. We revealed new insights to strain abundances and genome evolution at both the collective population along with the individual participant as it related to linear growth trajectories laying the foundation of a new framework and resource to understand undernutrition. The metagenome resource we present here demonstrates that longitudinally sampled LR-derived cMAGs establish a new standard for microbiome association studies as well as providing a rich dataset for others to develop analysis tools. Finally, we describe how this approach can be utilized to improve food production of fish and shellfish by identifying the genetic associations of gut microbes with growth phenotypes.

SEASONAL VARIATION DRIVES GILL MICROBIOME DIVERSITY AND REVEALS A NOVEL SYMBIONT, *Shewanella spp.,* IN A FORAGE FISH, *Scomber japonicus,* IMPLICATIONS FOR HOST EVOLUTION AND PHYSIOLOGY

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Scomber japonicus is an economically important fishery, and is an emerging aquaculture species in Asia. Mackerel, crucial to marine ecosystems as forage fish and prey, are highly nutritious due to their rich omega-3 content and can be caught year-round in San Diego (wide temperature range 10-29 oC); thus, it is an ideal fish to study the impacts of seasonal variation on the microbiome. Here, we evaluated the extent to which the microbiome varied seasonally by sampling at least four fish per month for three years (2017- 2019) from a fixed location (Scripps Institution of Oceanography Pier, La Jolla, CA). We hypothesized that the gut microbiome would be the most impacted as prey items are known to differ seasonally and that temperature would be the strongest environmental driver with winter and summer months varying the most. Microbiome samples from the gill, skin, and hindgut were processed from a total of 230 fish individuals using the EMP pipeline. Seasonal effects were greatest in the gill microbiome followed by skin, but absent in the gut. The gill and skin microbiomes varied most between spring (high diversity) and fall (low diversity) which may be driven by upwelling and general nutrient availability rather than the hypothesized summer-winter extremes driven by temperature. A single, highly abundant, *Shewanella* ASV was found in every gill sample, and was negatively associated with microbial diversity and *Vibrio* abundance. In a comparison to 101 other coastal marine fish species, *Shewanella* enrichment in the gill is unique to mackerel. We isolated and sequenced two *Shewanella* isolates with Oxford Nanopore, and performed a pangenome analysis with all 153 complete *Shewanella* genomes in NCBI which suggested our isolates are a new genus with the metabolic capacity for utilzing urea waste from fish gills and a type III secretion system. We next generated a chromosomal-level reference genome of mackerel (788 Mbp, scaffold N50 33.19 Mbp, BUSCO 96.9%) and resequenced 22 mackerel genomes spanning the three years to verify there was no population structure of the fish confounding seasonal variation in microbiome. We assessed whether gill microbiome biofouling negatively impacted fish respiration. Older fish had higher microbial biomass, which was linked to declining respiration efficiency, suggesting a role for the gill microbiome in aging. Our study shows how seasonal effects primarily impact the external mucosal surfaces of the gill and skin of a coastal marine forage fish while identifying a previously undescribed gill symbiont, *Shewanella*.

MONITORING HARMFUL ALGAL BLOOMS: A HIGH-RESOLUTION STUDY OF *Akashiwo sanguinea* IN COASTAL GEORGIA

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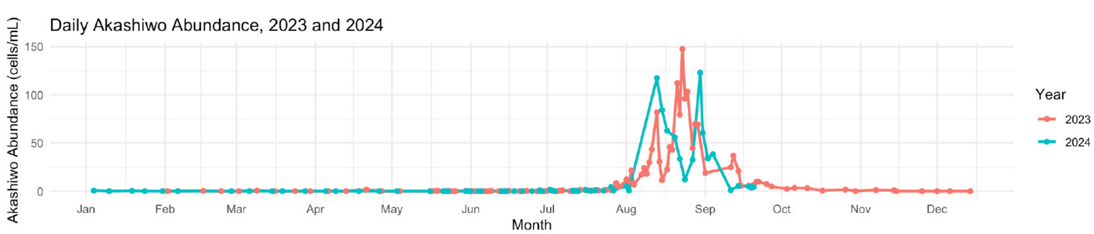
Harmful algal blooms (HABs) are a growing concern in coastal Georgia, posing threats to oyster aquaculture industries and larger ecosystem health. Until recently, Georgia has lacked a monitoring framework to address the timing, duration, and environmental drivers of these events. We began the state's first high-resolution HAB monitoring campaign in the Skidaway River Estuary to target *Akashiwo sanguinea*, a HAB-forming dinoflagellate known for its damaging effects on marine life in temperate waters globally. The dataset spans two years and contains information on phytoplankton community composition via fluid imaging, physicochemical parameters, and nutrient concentrations. Findings from 2023 and 2024 reveal correlations between *Akashiwo* abundance and warmer waters with low ammonium and nitrate/nitrite levels, suggesting the species may have a competitive advantage when nitrogen concentrations are low.

To put this seasonal dataset in a broader context, these findings are compared to qualitative assessments performed by citizen scientists as part of NOAA's Plankton Monitoring Network (PMN) on Skidaway Island over a 15-year period. PMN data correlated well with our results, as *Akashiwo* was observed during high temperature and mid-salinity conditions. Elevated *Akashiwo* levels in the estuary, as seen in PMN records and our dataset, align with records of oyster population crashes in the nearby UGA Marine Extension Shellfish Research Lab.

A regional survey was conducted via boat sampling, from the ocean break to the upper estuary. This spatial analysis revealed that *Akashiwo* blooms are most prominent in the brackish mid-estuary waters. The spatial distribution indicated that *Akashiwo* favors moderate salinity zones, with lower abundance observed closer to the ocean and in more riverine settings. While broad trends were evident, small differences in temperature and salinity between sites often corresponded with significant variations in abundance, suggesting that multiple environmental factors—such as temperature, turbulence, and nutrient availability—interact in complex ways to drive the spatial distribution of *Akashiwo*.

Hourly sampling with depth during periods of high abundance showed a mix of rapid fluctuations in total *Akashiwo* abundance through the water column and relatively stable concentrations throughout the diel cycle. A strong daily vertical migration signal was not apparent. Instead, rapid changes in abundance suggest that blooms are highly patchy, even in the well-mixed, turbulent environment of the Skidaway River Estuary. These insights highlight the complexity of *Akashiwo sanguinea* growth dynamics and will be valuable for informing bloom prediction models and coastal management strategies.



ENHANCEMENT OF STRESS RESILIENCE AND IMMUNE FUNCTION IN NILE TILAPIA *(Oreochromis niloticus)* FED TRYPTOPHAN-SUPPLEMENTED DIET

Israt Mishu*, Abdullahi Idowu, Lindee Mason, and Ahmed Mustafa

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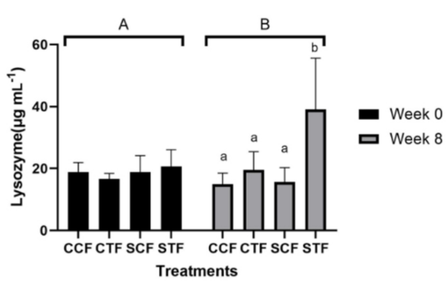
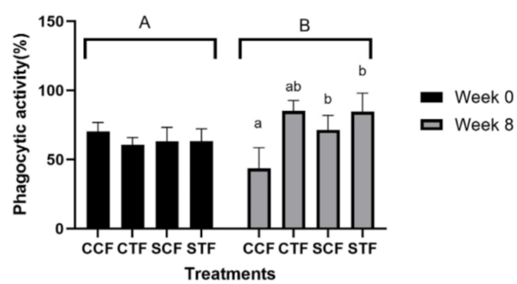
Nile tilapia (*Oreochromis niloticus*) is a major species in aquaculture due to its rapid growth and adaptability. However, intensive farming conditions expose these fish to stressors such as overcrowding and poor water quality, which can weaken their immune system and increase susceptibility to diseases. Ensuring strong immunity is crucial for maintaining the health and productivity of tilapia in aquaculture settings. Tryptophan, an essential amino acid, has been found to reduce stress and boost immune function by acting as a precursor to serotonin, a neurotransmitter involved in stress regulation. Supplementing fish diets with tryptophan can help alleviate stress-induced immunosuppression, thereby improving overall health and disease resistance. In this study, Nile tilapia was divided into four dietary groups:1) control feed (CCF), 2) tryptophan-supplemented feed (CTF), 3) cortisol-induced stress with control feed (SCF), and 4) cortisol-induced stress with tryptophan-supplemented feed (STF). The fish were monitored over an eight (8) -week period to evaluate the impact of dietary tryptophan on immune and physiological parameters. The results showed that tryptophan supplementation significantly enhanced lysozyme activity, indicating improved innate immune response, especially under stress conditions. Macrophage phagocytic activity, essential for pathogen defense, was also significantly higher in the tryptophan-supplemented groups. Additionally, the hepatosomatic index (HSI), which indicates liver health, improved in tryptophan-treated fish, suggesting enhanced stress tolerance and overall physiological condition. These findings demonstrate that tryptophan supplementation can effectively strengthen the immune system and reduce the negative effects of stress in Nile tilapia, making it a beneficial dietary addition in aquaculture practices.





Figures: Lysozyme and macrophage activities. Different letters are significantly different (P<0.05).

DESIGN, FABRICATION AND PERFORMANCE EVALUATION OF A BRIQUETTE POWERED CONVECTION DRIVEN FISH DRYER

Mkaanem, David Tertsegha.* Solomon, S.G., Olufeagba S., and Ataguba G.A.

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This study targeted to design, fabricate and use a modified smoking kiln to reduce the drudgery in use of traditional smoking kiln with the aim of obtaining quality products that keep for longer periods. The dryer consisted of four major functional units; combustion chamber, heat exchanger, the blower, and drying chamber. Briquettes were made from *Gmelina arborea* and *Swietenia macrophylla* saw dust residues. The effects of using briquettes as source of fuel on the quality of *Oreochromis niloticus* samples was investigated. The drying rate for sample smoked was 0.276kghr-1. The average marginal effect of a 1°C increase in temperature on the moisture was a 0.1176% decrease in the moisture content. The proximate composition of smoke-dried sample showed moisture content of 10.81±1.23 and Crude Protein of 62.50±3.21. It also revealed optimum crude protein, fat and ash contents at decreased moisture level. Sensory evaluation of the smoked product using the 8 point Hedonic scale showed a score of 8.6±1.55 for smoked samples. Phenolic content of the smoky taste of fish species tested 75mgkg-1 for smoked samples. The smoked fish conformed to acceptable European standards for Phenol content. There is less risk of lung infection as common with the use of wood. In conclusion, the kiln proved effective in the smoking of fish to safe moisture level at a record time. The proximate composition of smoked fish showed a positive impact on the nutritive component of the fish. The test on the total phenolic content showed the products to be within the acceptable limits for safe consumption and sensory components presented fish to be of top quality.

OZONE IMPREGNATED NANO BUBBLE TECHNOLOGY – MITIGATION AND CONTROL OF HABS, TOXINS, CONTAMINANTS, NUTRIENTS AND THEIR IMPACTS: A NOVEL GREEN APPROACH TO WATER SAFETY AND SECURITY

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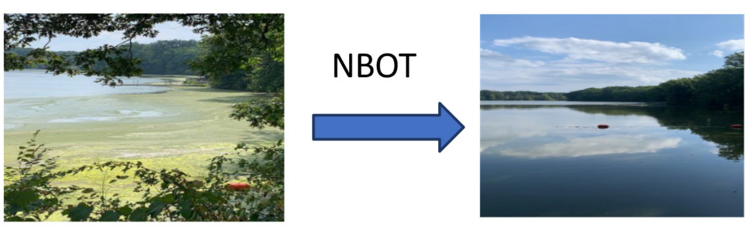
Nano Bubble Ozone Technology (NBOT) represents an emerging process that safely harnesses the power of ozone through impregnation of the gas into nano bubbles. NBOT provides a powerful tool for use as a HAB and HAB toxin mitigation and control technology. NBOT is capable of addressing several facets of HAB control including microbial destruction, toxin and contaminant elimination as well as nutrient reduction in open water. This technology capitalizes on the use of nano bubbles as an effective and safe ozone carrier eliminating the danger of raw ozone use in open water treatments. NBOT is scalable and configurable adapting to many different applications focused on clean water. This technology is green, ultimately leaving only oxygen in the water when operations are complete. It leaves no legacy components behind making it ideal for use in aquaculture applications.

A recently patented process also highlights NBOT's use as a truly destructive (as opposed to collective/isolative) technology for PFAS analogs affecting both shellfish and finfish aquaculture will also be discussed.

We will report on past and current incorporation of NBOT into HAB mitigation/control processes focused on aquaculture programs in both fresh and salt water.

Active commercial NBOT applications include ballast water treatment, industrial waste effluent, nutrient reduction and HAB control on large scale and mariculture operations (Oysters, Flounder, Yellow Perch, Redfish).

HAB Control/Nutrient Reduction



NBOT Mariculture

EPIGENETIC MODIFICATIONS ASSOCIATED WITH OOCYTE AGEING IN COMMON CARP *Cyprinus carpio*

Azin M. Samarin*, Swapnil G. Waghmare, Fabian Schumacher, Burkhard Kleuser, Trine K. Dalsgaard, Marianne Danielsen, Hanne S. Møller, Tomas Policar, and Azadeh M. Samarin

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Aberrant epigenetic modifications are a hallmark of ageing process. To investigate the involvement of epigenetic modifications in the progress of fish oocyte ageing the present study focused primarily on the epigenetic mechanisms of DNA methylation and histone acetylation. Investigating and understanding the molecular mechanisms underlying oocyte ageing is a research gap in fish and other vertebrates.

Common carp *Cyprinus carpio* oocytes were aged *in vitro* at 20 °C and their capacity to fertilize and progress to the larval stage were evaluated. The two important cytosine epigenetic marks, 5-methyl-2´-deoxycytidine (5-mdC) and 5-hydroxymethyl-2´-deoxycytidine (5-hmdC), were analyzed in the wholegenome in fresh and different aged oocytes and in the emerging embryos using liquid chromatography tandem-mass spectrometry (LC-MS/MS) method. Additionally, we examined global histone modifications and the probable changes in the acetylation patterns of selected lysine residues on histonesH3 and H4 during oocyte ageing by immunoblotting.

No significant difference was detected in the global DNA methylation between the fresh and aged oocytes. However, with the progress of oocyte ageing the genome-wide DNA hydroxymethylation decreased significantly in 30-h embryos originating from aged oocytes, as compared to those produced from freshly ovulated oocytes. Since studies have indicated the diagnostic and prognostic value of the mechanism of decreased 5-hmC in the whole genome, there might be a potential use of 5-hmC in the study of phenotypic failures associated with post-ovulatory oocyte ageing. *In vitro* oocyte ageing for 28 hours led to significantly increased levels of H4K12 acetylation. Furthermore, the activity of histone acetyltransferases showed an upward trend during oocyte ageing. Since 5-hmdC plays the role as a DNA demethylation intermediate, further studies on TET activity are needed. Investigation of the probable involvement of other epigenetic mechanisms such as histone methylation, the involvement of miRNAs, and the regulation of epigenetic-associated genes in the progress of oocyte ageing is of our interest for future studies.

Acknowledgments: This study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic, project: LRI CENAKVA LM2018099, and by the Czech Science Foundation (GACR No. 20-01251S).

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PROMOTING CAREERS IN AQUACULTURE: EDUCATING HIGH SCHOOL STUDENTS ABOUT WORKING IN THE AQUACULTURE INDUSTRY

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This innovative program to educate and promote aquaculture careers requires high school students to complete custom-developed online training modules about aquaculture as a prerequisite. Students who have successfully completed the online training then have a week-long in-person bootcamp to gain hands-on experience, after which they are placed with employers. The program serves to: 1) increase high school juniors and seniors' understanding of what aquaculture is and what jobs are available in the industry; 2) expands educational opportunities that meet employers' needs; and 3) places interns with industry partners, enabling them to gain real-world experience with specific employers.

The program has completed its second year of operation, based in the Middle Peninsula and Northern Neck regions of Virginia and will expand to the Hampton Roads area next year. This program is funded by the Virginia Initiative for Growth and Opportunity in Each Region (GO Virginia), an economic development program. The Promoting Careers in Aquaculture program, led by Virginia Tech, has unique aspects, including a wide variety of partners from diverse institutions including high schools, community colleges, and industry. It has received positive feedback from industry partners, as illustrated by the comment from Sarah Matheson-Harris, Owner and Chief Marketing Officer of Matheson Oyster Company: "When we started working with the interns Virginia Tech placed with us, we had such a good working relationship with them, they ended up bringing on their friends and family members. That led to us developing our own internship program. And it all started with the Virginia Tech program".

This session will provide background information about the program's development, implementation, and results, with the goal of spurring development of similar programs in other areas and exchanging information about best practices in aquaculture workforce development.

CHARACTERIZATION OF THE (GREEN) MICROBIAL AND PHYSIOLOGICAL DIVERSITY ON RETENTION PONDS IN THE LAKE ONTARIO WATERSHED

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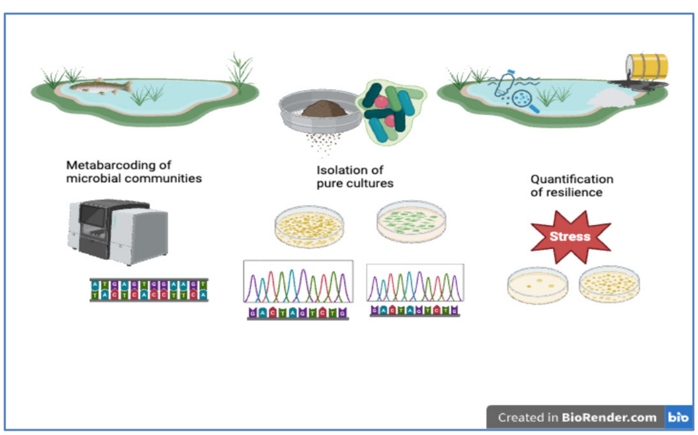
Extreme rainfall events across the US are projected to get 20% more severe and 200% more frequent in the next decades due to warming. This is concerning for the health of aquatic systems as runoff is a prevalent source of aquatic pollution. In farmlands, but also in urban areas, heavy rains wash from soil the excess nutrients from fertilizers. These nutrient-rich waters often find their way into water bodies fueling Harmful Algal Blooms (HAB) in rivers, freshwater lakes, lagoons and coastal areas. One common solution to mitigate the excess runoff alongside reducing flooding has been the construction of retention ponds/wetlands to catch stormwater. The role of these artificial reservoirs in protecting water quality and infrastructure is undoubtable, but also creates runoff-fed pond/wetland ecosystems that expose organisms to high levels of pollutants selecting for organisms with an increased tolerance to chemicals. Here we capitalize on these man-made environments to explore the ecological consequences of urban and suburban runoff in the diversity of water and sediments' microbial communities, with special emphasis on those often linked to HABs, such as cyanobacteria and eukaryotic algae.

We used a combination of microbial community characterization using high-throughput sequencing of the 16S ribosomal gene, strain isolation methods and lab-based testing under common garden conditions to investigate the changes in community membership and diversity of the microbial communities in retention ponds heavily impacted by urban run-off in the Lake Ontario watershed (Rochester, NY). We contrasted the response of the organisms isolated from these environments with those collected in retention ponds, relatively unimpacted by anthropogenic actions. Common garden experiments indicated that members of the green algal community are extremely susceptible to the presence of NaCl road salt, $MgCl_2$ road salt (marketed as environmentally friendly), or Miracle Gro Lawn Food. The common herbicide Roundup Weed & Grass Killer was able, even at extremely low concentrations, to inhibit algal growth completely. This has important consequences for blooms in freshwater environments as cyanobacteria, the major cause of HABs in lakes, are often resistant to herbicides. Combined stressors such as warmer temperatures, presence of nutrients and toxics and a decrease of other members of the phytoplankton can create ideal conditions for cyanobacteria HABs.



COMPARING THE AQUACULTURE POTENTIAL OF TWO TROPICAL ROCK-OYSTER SPECIES

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Developing a hatchery-based aquaculture of tropical rock oysters provides opportunities for likelihood diversification in developing countries. In the Indo-Pacific region, several species stand out as promising candidates for modern hatchery production programs, particularly *Saccostrea cucullata* and *S. echinata*. However, no formal studies have compared the productivity of these species, despite the importance of this information for effective resource management and planning. Our study aimed to produce both species in the hatchery, and compare their growth performance during key phases of the production process (larval, nursery, and grow-out).

Broodstock oysters collected from two sites were identified using multiplex PCR tests based on COI mitochondrial primers. Batch spawning was triggered by a mix of desiccation and thermal stress. During the larval phase we monitored growth and development. Competent larvae settled on oyster-shell cultch, and were subsequently transferred to nursery raceways for a period of one year. Growth during the nursery phase was followed for 98 days under two conditions: (1) raceways provided with microalgae produced in the laboratory, and (2) upwelling cylinder sieves provided with seawater from an earth pond maintained with 'semi-natural' microalgae blooms. To compare performance during the grow-out phase, animals were placed in baskets and deployed across three sites capturing a gradient in food availability: (1) Vairao Lagoon (nutrient-poor), (2) Phaeton Bay (natural, nutrient-rich), and (3) earth pond (managed, nutrient-rich). After two months, we recorded growth and survival as a function of species and grow-out site.

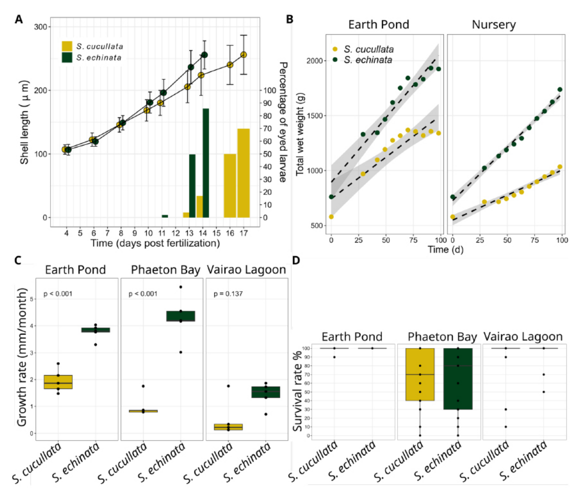

FIGURE 1. *Saccostrea cucullata* and *S. echinata* growth performance during (A) larval, (B) nursery, and (C) grow-out production phases. (D) Survival during the grow-out phase.

We completed the reproduction of *S. cucullata* and *S. echinata*, with fertilization rates higher than 90%. Results showed that *S. echinata* consistently outcompetes *S. cucullata*. *S. echinata* larvae grew faster and developed earlier than *S. cucullata* (Fig. 1A). *S. echinata* was more productive in the nursery, though independently of the growth system used (Fig. 1B). Grow-out trials also revealed higher growth rates for *S. echinata*, but only in nutrient-rich sites (Fig. 1C). Survival was lower at the natural, nutrient rich site, likely due to fouling (Fig. 1D). Finally, *S. cucullata* invests more energy into reproduction, suggesting a possible mechanism to explain the differences in growth.

THE EFFECTS OF HYPOXIA AND WARMING ON THE GROWTH AND NUTRIENT STORAGE OF THE EASTERN OYSTER *Crassostrea virginica*

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Eastern oysters *(Crassostrea virginica)* are a valuable commercial and recreational fishery, as well as an important ecosystem engineer in coastal systems, providing habitat for an array of marine organisms and improving water quality by removing excess nutrients such as nitrogen. Oysters filter nitrogen-rich plankton as they feed and assimilate it into their tissue and shell (bioaccumulation), thereby removing nitrogen from the system. Their ability to remove and store nitrogen can be affected by the environment, as oysters eat and grow differently depending on the conditions they are exposed to. Climate change is affecting environmental conditions in shallow water systems where oysters live, potentially influencing their ability to deliver this ecosystem service. Specifically, dissolved oxygen is declining in shallow water estuaries like the Chesapeake Bay, driven in part by rising water temperatures associated with climate change. The interactive effects of warming and low dissolved oxygen on oyster growth and nitrogen storage are therefore critical to understanding how climate change will affect coastal systems.

We conducted a manipulative laboratory experiment that quantified how low dissolved oxygen (hypoxia) and warming affects the growth and nitrogen storage of juvenile eastern oysters. Early life stages are often particularly sensitive to environmental stress and changes that occur early in life may have persistent effects later in life. We hypothesized that when exposed to warming and hypoxia alone, oysters would grow less and store less nitrogen, but that exposure to warming and hypoxia together would have no effect on growth or bioassimilation of nitrogen, in accordance with previous studies. This talk will explore these effects and discuss how changes in oyster performance driven by climate change would impact their ability to deliver ecosystem services.

DEVELOPMENTAL BIOMARKERS IN ATLANTIC SALMON (*Salmo salar*) FOR PREDICTION OF FAVORABLE TRAITS IN DOMESTICATED LINEAGES: GROWTH, SURVIVAL, AND TEMPERATURE TOLERANCE

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Development and refinement of Atlantic salmon (*Salmo salar*) family strains that favor high survival and optimal growth are essential to the continued rise of the salmonid aquaculture industry in North America. Identifying relationships between traits of early life stages (egg and alevin) and market size fish will assist in refining broodstock lineages that favor desirable and limit unfavorable traits. In this study, we compiled an extensive suite of metrics on multiple families of Atlantic salmon to better understand the relationships between early life stages and endpoints of interest to the industry. The endpoints of focus include survival, growth, and temperature tolerance. Survival of eggs post-fertilization to hatch has seen a major decline in the previous decade and has become a serious bottleneck for the industry; salmon growth and growth rate are essential metrics for profitable salmon stock; and temperature tolerance is an important, but often overlooked, aspect of the future of the salmon aquaculture industry. In cooperation with the USDA's National Marine Cold Water Aquaculture Center, whose Atlantic salmon selective breeding program has been in operation since 2003, this longitudinal study anticipates multiple comparative timepoints of familial siblings for correlative analysis beyond the current findings.

EXPLORING ALTERNATIVES TO GLACIAL ACETIC ACID FOR THE PREPARATION OF DAVIDSON'S AFA SOLUTION USED FOR FIXATION OF SHRIMP TISSUES

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The growth and sustainability of shrimp farming worldwide relies on the detection of infectious diseases. With continued growth of the industry, disease outbreaks become more common and new diseases continue to emerge. To fight the spread of disease, farmers rely on regular health assessments. Histopathology and PCR-based diagnostics are the main means of conducting these health assessments within a laboratory setting but rely on adequate fixation.

Davidson's Alcohol Formalin Acetic acid fixative (DAFA) has been the gold standard for the preservation of shrimp tissues for histopathological analysis for half a century. However, in many regions around the world, chemical-grade glacial acetic acid (GAA) is very difficult to acquire, expensive, and is identified as a hazardous substance that also impacts sample shipping. In an effort to make shrimp sample preparation and shipping more accessible and environmentally friendly, we evaluated 30% industrial-strength vinegar (ISV) as an alternative to GAA in DAFA. While chemically similar to GAA, ISV is inexpensive, and is readily available worldwide due to its use as a household cleaner.

For initial testing, healthy *Penaeus vannamei* shrimp were fixed with either DAFA or a modified fixative with ISV in place of GAA. The shrimp were then processed according to conventional techniques for paraffin embedding and sectioning. Sections were stained with Mayer-Bennett's hematoxylin/eosin- phloxine (H&E) and examined via light microscopy. Preliminary analysis revealed that the modified fixative provides the same quality of tissue preservation as traditional DAFA, indicating that it may be a viable replacement that would give greater accessibility to this crucial technique. Results indicate that fixative solution formulated with ISV in place of GAA yields equivalent ability to prevent autolysis, preserve tissue morphology, decalcify the cuticle of shrimp, and functionally identical staining properties as DAFA formulated with GAA.

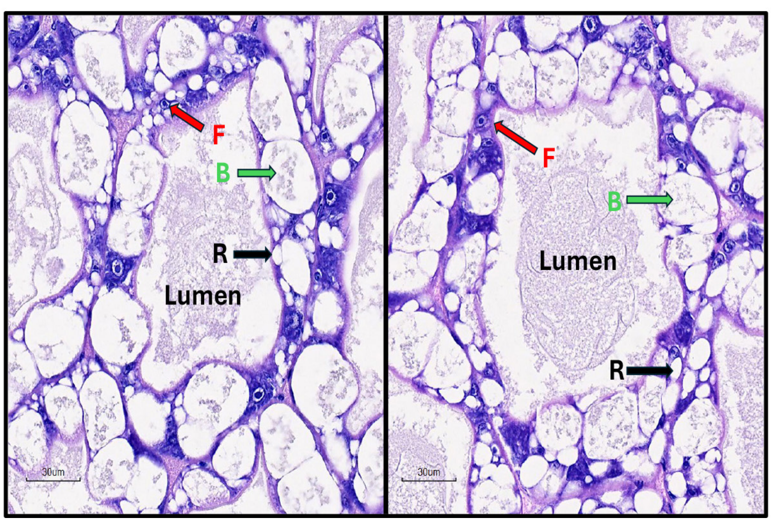

Figure 1. Photomicrographs of H&E-stained section of hepatopancreas tissue. Major cell types are labeled. (A) Original DAFA fixative, and (B) modified DAFA with ISV.

A COMPARISON OF METHODS TO ESTIMATE AGE AND SIZE-AT-MATURITY FOR CHANNELED WHELKS *Busycotypus canaliculatus* IN MASSACHUSETTS

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The channeled whelk (*Busycotypus canaliculatus*) is a benthic marine gastropod that supports a niche fishery in Massachusetts (USA). Typically, due to cost, sexual maturity for channeled whelks is determined macroscopically; and age is determined via the operculum. Yet, histological analysis of gonads and aging of statoliths are alternative techniques to determine reproductive stage and age, respectively. Hence, this study compared two techniques to estimate age and size-at-maturity through the analysis of 2157 (n=1406 males, 748 females) whelks. Macroscopic results showed males reached 50% sexual maturity at a smaller shell width (59.8 - 72.9 mm) than females (86.3 - 103.1 mm). Histology was used to verify size-at-maturity for sexes. Due to its location the operculum is generally used to age whelks; however, the internal calcium carbonate statolith is thought to provide a more precise age. A subset (n=40) of whelks were used to compare aging between the statolith and the operculum. The percent agreement (PA = 2.5%) between the anonymous readers was low for the statoliths, and none of the readers agreed on the age with the opercula. Since, whelk life history traits differ at small spatial scales; an accurate and time and cost-efficient method to quantify these life history traits is needed to inform best fishery management practices.

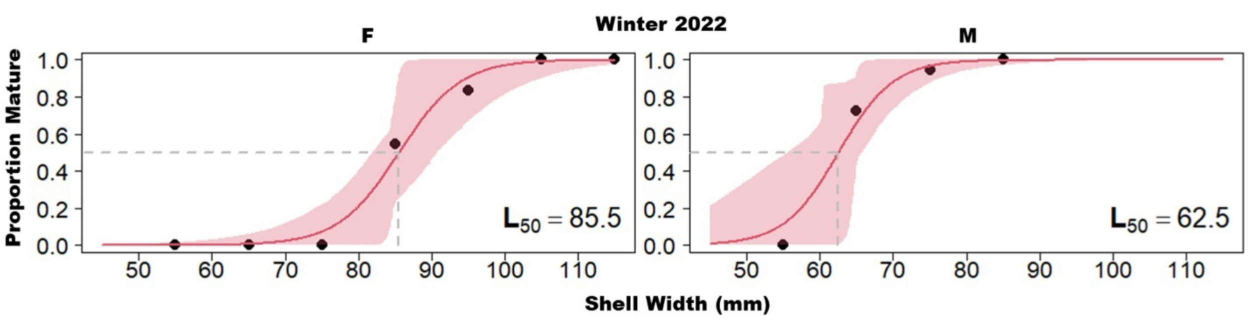

Figure 1. Proportion of size (shell width; mm) at maturity for male (M) and female (F) channeled whelk from Buzzards Bay (based on macroscopic assessment of gonads. Whelks were grouped into 10 mm size classes. Observations (black circles) presented with mean model estimates (color line) + 95% confidence intervals (color shading). The L_{50} for maturity is given grey dashed lines depict L_{50} estimates on the curve.

SPECIES, MARKETS, AND REGULATIONS, OH MY!: SCOPING MACROALGAE MARICULTURE IN COASTAL ALABAMA

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Seaweed mariculture is a quickly growing sector of the aquaculture industry, especially in the United States. Macroalgae are being cultivated as food products, feedstocks, and sources of nutraceuticals, biopolymer compounds, and water quality bioremediation. While most growth in the North American seaweed sector has taken place in colder climates (Maine/Alaska/Pacific Northwest), warm water macroalgal culture has lagged behind. Coastal Alabama may provide an ideal location for piloting some of the first commercial warm water macroalgal farms in the northern Gulf of Mexico.

Potential locations within Alabama state waters were screened for environmental suitability factors (salinity, turbidity, depth, benthic substrate, critical habitat), maritime usages (shipping, military, existing aquaculture), and infrastructure considerations (proximity to ports). Nearshore farms offer benefits of lower operational costs due to simplified regulatory requirements, proximity to processors, and less costly gear arrays. Additionally, nearshore macroalgal culture can provide nutrient remediation of coastal river plumes and enhance availability of complex 3-dimensional habitat for fish and invertebrates. Past studies conducted by the Auburn University Shellfish Lab have shown that macroalgal culture is not optimal in tandem with oyster aquaculture, as these areas are shallow with high turbidity, temperatures, and salinity flux. However, sites south of the coastal barrier island complex may yield more ideal growing conditions.

Based on the physical environmental regimes and the native algal species assemblages in the northern Gulf of Mexico, target genera for cultivation include: rhodophytes such as *Gracilaria* and *Eucheuma*, chlorophytes such as *Caulerpa* and *Ulva*; and phaeophytes like *Sargassum*. Some of these genera are clonal, and can be cultivated via fragmentation, whereas others need to be raised in a land-based nursery to seed ropes for grow-out.

Few of the proposed algal species are ideal for human consumption, so this region would primarily serve agriculture (both fertilizer and livestock feed), biopolymer, bioplastic, and biofuel markets. Approaching algal mariculture as industrial scale commodity production - as opposed to boutique and artisanal food production- presents unique challenges, namely in scale, labor, regulation, and environmental impact. If this project is carried out responsibly and thoughtfully, these same challenges become opportunities - not just for economic impact, but for large-scale environmental benefit in the excess-nutrient encumbered Gulf of Mexico.

CULINARY EXCHANGE IN SUPPORT OF THE SCALLOP FARMIG INDUSTRY IN MAINE AND THE NORTHEAST US

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The state of Maine is pioneering the development of aquaculture production of the sea scallop (*Placopecten magellanicus*) and is the only state in the nation where whole and live scallops can be routinely sourced. Markets focus on the adductor muscle of the scallop – the 'meat' – and between domestic landings and imports, the US market approaches $1B USD.

Much less well-known in the US are products and dishes made with other parts of the scallop: the gonad, the mantle, and the entire animal. Culinary uses in other countries regularly use whole scallops or other parts besides the adductor: 'Coquilles St. Jacques' is a popular dish in France, and Japan is well known for its spectrum of products made from scallops and different preparations. The US has traditionally had a much narrower range of products and uses made from scallops, but there is significant opportunity in broadening those offerings.

Given that approximately 80% of US seafood is consumed in restaurants, it is vital to engage chefs and restaurateurs about preparations with whole scallops; they serve as the ambassadors to consumers, and interpreters of unusual dishes. Culinary professionals are critical to achieving demand from consumers.

With funding from NOAA Sea Grant in their FY 2023 *Aquaculture Technologies and Education Travel Grants*, a group of chefs, seafood professionals, educators, economic development specialists and writers have embarked on an effort to create an avenue for culinary technology transfer, in support of broadening interest in both farmed scallops from Maine, as well as the dayboat-quality that the state's wild fishery is recognized for. The group visited with chefs, fishermen and others in France, and have implemented programming to engage and educate chefs here in the US.

We will review the activities of our travel, lessons learned, and the follow-on activities already in progress and yet to come, in support of raising the profile of Maine's farmed and wild sea scallops.

DEVELOPING TOOLS TO QUANTIFY ECOSYSTEM SERVICES PROVIDED BY AQUACULTURE - INTEGRATING HABITAT AND FISH PRODUCTION

Ryan Morse*, Julie M. Rose, Renee Mercaldo-Allen, Alexandria Ambrose, Brendan Campbell, Paul Clark, Emma Cross, Zachary Gordon, Jonathan Grabowski, Edward Hale, Peter Kiffney, Stephen Kirk, Julia McDowell, Daphne Munroe, Gillian Phillips, Dylan Redman, Beth Sanderson, Kelsey Schultz, Jenny Shinn, and Christopher Schillaci

IBSS Corporation in support NOAA Fisheries
NEFSC, Milford Laboratory

Shellfish aquaculture gear that creates complex structure can increase the abundance and diversity of wild fishes relative to reference habitats devoid of vertical heterogeneity. Ongoing research programs using underwater action cameras in Connecticut, Delaware, Massachusetts, New Jersey, and Washington state seek to quantify fish interactions with aquaculture gear. Using a synthesis of the existing literature, and regionally-relevant data from our partners, our program seeks to provide shellfish resource managers with an assessment of habitat provisioning by shellfish farms in the Northeast and Pacific Northwest regions. Observations of fish behavior in Connecticut suggest that cages provide food, shelter, refuge and other ecological services, much like natural structured habitat. Association of young-of-the-year fish with oyster cages across multiple research locations suggest that aquaculture gear may be utilized as nursery habitat by fish during the early life history stages. An assessment of data availability for finfish and invertebrates was conducted to identify opportunities to develop tools to aid consideration of habitat benefits into aquaculture permitting. We integrated existing methodologies for estimating growth and mortality parameters for fish species in order to estimate total production as an ecosystem service factor. We created a comparison of commonly observed species by region/state based on available data from our Northeast and Northwest Pacific research programs. When possible, we used bottom trawl survey data to determine age-at-length and length-weight relationships in order to estimate mortality parameters. Habitat maps were created from existing base maps of sediment type, bathymetry, and seabed characteristics. These maps serve as a baselayer for determining the effectiveness of aquaculture gear as habitat modifiers for productivity enhancement. Results from the synthesis and the regional research programs will be discussed, and data gaps identified. Information on region-scale variation in ecosystem service provisioning associated with shellfish aquaculture can aid resource managers in developing a permitting framework that includes consideration of environmental benefits in addition to potential impacts. A better understanding of how shellfish farming influences fish communities will increase social license for aquaculture among coastal communities.

AQUACULTURE EXTENSION PROGRAMMING AT MAINE SEA GRANT

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The Marine Extension Team (MET) at the University of Maine is a formal collaboration between the Maine Sea Grant College Program and University of Maine Cooperative Extension. The MET works closely with industry, science, management and education relative to aquaculture. Given the scale and scope of aquaculture in Maine, the extension program requires nimbleness, a grasp of issues both fine-scale and broad, and strong relationships along the coast, nation-wide and internationally.

Principal activity areas include:
<u>Training of new and established producers, networking, and engagement</u>: These programs support skill-building and professional development for producers, and support connections between producers, science, management and related sectors.

<u>Applied Research</u>:
These activities advance our knowledge of better production practices, environmental interactions, and a more diversified and resilient working waterfront economy.

<u>Outreach and extension services</u>:
This work allows investigators to work in Maine more efficiently and effectively, informs investigators with valuable local information, gets the resulting information into circulation more thoroughly, and assists with delivering programs efficiently in times of need.

<u>Education</u>:
The MET participates in a broad spectrum of educational efforts: supporting K-12 programs and other programs with direct, hands-on learning opportunities; the *Coastal Conversations* radio show that examines issues of importance to Maines' coastal communities; mentoring and advising undergraduate and graduate students; participating in school of local organizational meetings to present information about aquaculture practices, science, *et cetera*. This work develops and maintains connections with all generations of learners.

The MET works also with a growing number of partners, as aquaculture gains importance and size; universities and colleges, private not-for-profit entities, scientific institutions, community groups, and the like. In an increasingly-active field, the MET looks to its core competencies and values to guide its work portfolio: connections to- and reliance upon science-based information on the issues of the day, an honest-broker approach toward treating all citizens and collaborators with fairness and transparency, and a recognition of the deep interconnections between our natural resources and the people who depend on them.

PARTICIPATORY SCIENCE APPROACH TO MITIGATE THE EFFECTS OF HARMFUL ALGAL BLOOMS IN COSTA RICA

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Marine biotoxins are poisons that are produced by certain kinds of microscopic algae that are naturally present in marine waters, normally in amounts too small to be harmful. Biotoxins accumulate in shellfish to levels that can cause illness or death in humans and other mammals that ingest them. Of critical concern is the neurotoxin, saxitoxin and related derivates. Ingestion of saxitoxin contaminated shellfish and finfish is responsible for the illness known as Paralytic shellfish poisoning (PSP). Saxitoxin is produced by the dinoflagellates *Alexandrium*, *Pyrodinium*, and *Gymnodinium*. Along the coast of Costa Rica, blooms of *Pyrodinium* and *Gymnodinium* have been reported since 1981. During 1999, blooms of these species caused 70 cases of PSP with 6 fatalities, leading to a shellfish closer for over 2 years because of consecutive blooms and a lack of resources to analysis these toxins in Costa Rica. Commercially important shellfish such as thorny oyster (*Spondylus calcifer)*, Panama pearl oyster (*Pinctada mazatlantic)*, mangrove cockle (*Andara turculosa)*, and Guiana swamp mussel (*Mytella guyanensis*) are all known vectors for human intoxication.

The NOAA National Phytoplankton Monitoring Network is a community-based volunteer science approach to monitor harmful algal blooms and their associated toxins. Founded in 2001, the network has trained over 600 volunteers across 36 U.S. states and 4 countries including Costa Rica. Since 2022, the network has partnered with Innoceana, a global marine conservation organization that works to preserve the ocean for future generations by empowering coastal communities through access to education and innovative tools, to monitor potential harmful algae along Isla Violin. This project will expand the initial sampling conducted by increasing the number of sites coupled with toxin detection and involve local schools and community groups to monitor the entire coast of Costa Rica.

DEVELOPMENT OF AN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM TO RESTORE HAWAII'S VULNERABLE LIMU (SEAWEED) POPULATIONS

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The goal of this project was to develop an Integrated Multitrophic Aquaculture (IMTA) system for limu culture using nutrients supplied by fish and shrimp pond effluent. A 3 x 3 experimental design was used to assess the effects of effluent quality on growth and nutrient uptake by three limu species from Hawaii. The experiment consisted of two independent variables (limu species and effluent type), each with three levels for a total of nine treatments, and there were three replicates per treatment. Experimental units consisted of 55-gallon, plastic tanks containing limu with flow-through effluent. Carnivorous fish effluent came from a tank containing giant trevally (*Caranx ignobilis*), herbivorous fish effluent came from a pond with striped mullet (*Mugil cephalus*), and shrimp effluent came from a pond with Pacific white shrimp (*Litopenaeus vannamei*).

Two dependent variables (limu production and nutrient uptake) were monitored in each of the 27 tanks. Limu biomass was measured weekly and nutrient concentrations (TAN, NO_3^-, PO_4^{3-}) were measured entering and exiting the tanks twice per week over the 6-week trial. Results indicate that shrimp effluent promoted the highest production for all three limu species, followed by carnivorous fish effluent, then herbivorous fish effluent. *Ulva* exhibited the highest production in all three effluent types, followed by *Gracilaria*, then *Gratelupia*. *Gracilaria* production decreased after week 4, in carnivorous and herbivorous fish effluent, and exhibited significant fragmentation. All three limu species were effective at removing TAN from all three effluent types, and all three limu species were effective at removing NO_3^- and PO_4^{3-} from shrimp and carnivorous fish effluent. NO_3^- and PO_4^{3-} concentrations were not reduced from herbivorous fish effluent under the conditions of this experiment.

Results from this study indicate that limu from Hawaii can be grown in fish and shrimp pond effluent as a potential secondary cash crop while acting as a biofilter to remove nutrients from the effluent stream.

Treatment	Limu Species	Effluent Type
1	*Gracilaria parvispora.*	Carnivorous fish
2	*Gracilaria parvispora.*	Herbivorous fish
3	*Gracilaria parvispora.*	Shrimp
4	*Grateloupia phuquocensis*	Carnivorous fish
5	*Grateloupia phuquocensis*	Herbivorous fish
6	*Grateloupia phuquocensis*	Shrimp
7	*Ulva fasciata*	Carnivorous fish
8	*Ulva fasciata*	Herbivorous fish
9	*Ulva fasciata*	Shrimp

THE ROLE OF DISEASE DIAGNOSTICS, CHALLENGE ASSAYS, AND BOISECURITY IN THE SELECTIVE BREEDING OF *Litopenaeus vannamei*

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Over the last 45 years, infectious diseases have negatively impacted shrimp aquaculture production worldwide, resulting in the loss of billions of dollars in revenue. Despite this challenge, annual shrimp aquaculture production increased from ~0.15 million metric tons (MMT) in the early 1980s to >7 MMT currently. Arguably, the largest driver of this increase was the availability of Specific Pathogen Free (SPF), genetically improved Pacific white shrimp, *Litopenaeus vannamei* and their subsequent introduction to Asia. In fact, global shrimp production was <1 MMT in the early 2000s when *L. vannamei* farming began in Asia, but quickly increased to ~3 MMT by 2006.

In 1984, the U.S. Marine Shrimp Farming Program (USMSFP) was formed to solve problems constraining the U.S. shrimp farming industry. At that time, *L. vannamei* had become the dominant farmed shrimp in the Western Hemisphere and the USMSFP committed resources to develop culture technologies for this species, including domestication. Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) was not lethal to *L. vannamei*, but it caused an economically significant disease where infected populations exhibited reduced growth and cuticular deformities. Thus, efforts to eradicate this pathogen from captive shrimp populations were needed to improve commercial production. In 1989, USMSFP members Oceanic Institute (OI) and the University of Arizona (UAZ) began developing the world's first SPF population of *L. vannamei* which was free of IHHNV and other known pathogens at that time. In 1994, OI started the first family-based breeding program to improve growout performance and pathogen resistance of *L. vannamei*. During these early years, OI generated basic information about shrimp genetics and distributed SPF, selectively bred shrimp to the U.S. industry. By 2010, OI distributed >2.5 million shrimp, with U.S. broodstock suppliers playing a critical role in catalyzing *L. vannamei* farming in Asia. Selective breeding has continued to gain importance within the industry and *L. vannamei* farming now relies exclusively on selectively bred lines.

Developments in disease diagnostics and pathogen-challenge assays, as well as the implementation of biosecurity measures, have greatly aided selective breeding efforts and the shrimp farming industry as a whole. Histopathology has played a significant role in developing and maintaining SPF populations, as pathogen-specific diagnostic tools are typically developed after the disease etiology is known. In the late 1990s-2000s, molecular techniques for disease diagnostics were developed and the widespread use of these techniques, particularly PCR, has played a critical role in the implementation of biosecurity measures. Routine pathogen testing at commercial facilities has greatly reduced risks associated with the transfer of diseased shrimp. An effective disease-challenge assay for Taura syndrome virus was developed in the mid-1990s and was used to develop lines of *L. vannamei* that are highly tolerant to this pathogen. These lines were the first to be introduced to Asia and they continue to be the foundation for many selectively bred populations. Challenge assays continue to be used to develop lines of shrimp which are tolerant/resistant to a number of important shrimp pathogens.

DIRECT EVALUATION OF THE INFLUENCE OF SIZE DISTRIBUTIONS ON THE ATLANTIC SURFCLAM *Spisula solidissima* FISHERY IN THE U.S. MID-ATLANTIC

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Warming bottom water temperatures are increasingly impacting sensitive sedentary bivalves within the Mid-Atlantic, including the economically important Atlantic surfclam, *Spisula solidissima.* A primary characteristic of the surfclam is the regional variability in average maximum size as measured by the von Bertalanffy parameter L-infinity (Linf) varying from around 100 mm to above 170 mm. Variation in maximum length in this species is likely due to its wide geographic distribution coupled with its genetic capacity to reach considerable size under appropriate conditions. The influence of bottom water temperatures on surfclam maximum size is a good example of the temperature-size rule, which states that animals of larger size will be found in cooler climates. The physiological basis for this rule is found in the surface area to volume relationship relating the surface area of the gill (ingestion) to the volumetric demand of tissue maintenance (respiration). These two temperature-dependent physiological attributes cap maximum size and shape the gradient with temperature.

The challenge, as global warming persists, is to evaluate the influence of temperature-determined variations in surfclam size frequency relative to fishery performance under the present regulatory framework that includes a landings size-limit of 4.75″ (~120 mm). As warming continues and Linf declines, smaller clams are more often caught and the probability of the landings size-limit impacting the fishery increases. An agent-based fisheries model, SEFES, is applied to directly evaluate the impact of size-limited growth on fishery performance and its regulatory framework. SEFES utilizes a newly developed algorithm capable of predicting trends in the von Bertalanffy parameters k and Linf under present-day bottom water temperatures and in the coming years as warming of the northwestern Atlantic continues. This model provides a unique opportunity to holistically simulate the implications of size limits, and the entire impact of the size-temperature rule, as global warming continues.

DEVELOPMENT, IMPLEMENTATION AND INITIAL RESULTS OF A STRATEGY FOR THE SUSTAINABLE DEVELOPMENT OF AQUACULTURE IN THE COUNTRY OF GEORGIA

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Georgia possesses substantial resources and potential for the development of aquaculture production, alongside a rich tradition and history in this sector. However, state-level regulation has only emerged in recent years. Growing awareness of the country's diverse and abundant natural hydrobiological resources, coupled with private sector interest and contemporary international challenges, has laid the groundwork for a unified vision and strategy for aquaculture development.

For the first time, Georgia has developed a strategy and action plan for the sustainable development of aquaculture. This process began with a comprehensive assessment of the aquaculture sector, involving state and international organizations. Stakeholder meetings, in-depth interviews, expert surveys, and economic studies were conducted to gather relevant data. Through this analysis, we identified the sector's strengths and weaknesses, prioritized needs, and addressed key issues. Priorities were informed by both domestic conditions and international strategies.

The primary goal of the strategy is to enhance Georgian aquaculture by ensuring the sustainable use of aquatic biological resources, implementing advanced fish breeding technologies, producing competitive products, and meeting market demands as well as comply with environmental goals. A target indicator is to increase current aquaculture production in Georgia by fivefold by 2028. The action plan outlines thematic tasks and activities, including ecosystem inventory, ecological approaches in aquaculture, knowledge dissemination, quality improvement of fish products, and support for small farms.

The challenge of developing this strategy has been met, and it is set to take effect in 2024. Implementation of the action plan activities has already begun. We believe that the experience gained from creating and executing this strategy will significantly shape the future of aquaculture policy in Georgia, positively influencing government actions, policy makers, and private sector stakeholders as key actors and beneficiaries.

EVALUATION of ONE CURRENT™ AS A DIETARY STRATEGY FOR INCREASING RESILIENCE IN RAINBOW TROUT

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Ralco, a US-based functional ingredient supplier currently partnering with Rangen/Wilbur-Ellis Nutrition, has developed a feed supplement package, ONE CURRENT™, for use in aquaculture feeds. Containing a proprietary blend of MICROFUSED® essential oils, ACTIFIBE® prebiotic, and *Yucca schidigera*, ONE CURRENT purports to improve gut health and growth performance of intensively cultured finfish and may also have beneficial effects on water quality in such rearing systems. Studies have been conducted to evaluate ONE CURRENT in feeds for various finfish, but the product has not been rigorously evaluated in salmonids. Accordingly, the present work is proposed to evaluate the response of Rainbow Trout to ONE CURRENT-supplemented feed in optimal vs. sub-optimal rearing conditions.

Juvenile Rainbow Trout were fed a commercially manufactured diet with or without supplementation of the functional feed ingredient ONE CURRENT and reared for 8 weeks under optimal environmental conditions (1st use water) followed by an additional 8 weeks of rearing under suboptimal conditions (3rd use water). Dietary supplementation of ONE CURRENT had no adverse effects on growth performance in juvenile Rainbow Trout under both optimal and suboptimal rearing conditions. Both feeds were readily accepted and feeding behavior was aggressive during rearing under optimal conditions. Feeding was expectedly less active during rearing under suboptimal conditions, but fish fed the ONE CURRENT-supplemented feed exhibited a significantly more robust feeding response during this phase. Survival in 1st use water was nearly 100% in both groups, but mortality attributed to reduced environmental quality and gill hyperplasia steadily increased throughout the time fish were in 3rd use water. However, the onset of mortality was delayed, and cumulative mortality was significantly lower among fish fed the ONE CURRENT supplemented feed. Greater feeding response and survival following exposure to 3rd use water suggests that dietary supplementation with ONE CURRENT may increase trout resilience and help to maintain performance under challenging rearing conditions.

PLASTIC POLLUTION AND HEALTH METRICS IN THE UNIQUE GALÁPAGOS BLACK STRIPED SALEMA (*Xenocys jessiae*)

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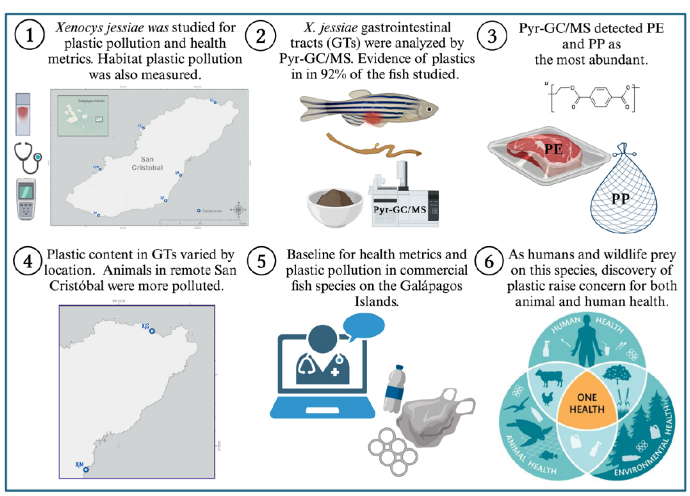
Marine vertebrates that feed on plankton, such as small fish and filter feeders, are particularly vulnerable to plastic pollution. This is due to their feeding behaviour, which primarily involves the ingestion of detritus in the water column or filtration of large volumes of water. As a result, these organisms are at an increased risk of ingesting microplastics. However, the extent to which plastics affect wildlife remains unclear. In this study, 61 black striped salemas (*Xenocys jessiae*) sourced from six different bays on one Galápagos National Park (GNP) Island (San Cristóbal) were subjected to a thorough examination of the prevalence of plastic in their gastrointestinal tracts (GT) and compared it with a suite of health metrics (vital signs, hematology, and blood chemistry). Plastic pollution in the surrounding habitats was also assessed, and it was found in all sampled *X. jessiae* habitats. Pressurized liquid extraction with double-shot pyrolysis-mass spectrometry gas chromatography (Pyr-GC/MS) revealed plastic pollution in the GT of the fish. Polyethylene (PE) $\bar{x}$ = 535±743 (range:0-2327) µg/g was the polymer with the highest mass concentration in GT, and polypropylene (PP) $\bar{x}$ = 138±143 (range:0-751) µg/g was also abundant. Furthermore, these levels differed significantly according to the sampling site. The highest concentrations of plastics were found in fish collected from off-limits GNP and fishing areas. Consequently, the fishing and tourism industries are likely the primary contributors to this problem. This study is the first to use Pyr-GC/MS to quantify the plastic pollution in commercial fish in Galápagos. This technique detected and quantified plastic pollution in the GT of 56 *X. jessiae* (92%) of the sampled population. Our collected data on plastic pollution, vital signs, morphometry, and blood values served as a baseline for future comparisons.



TRANSCRIPTOMIC CHARACTERIZATION OF TRANSMISSIBLE DISSEMINATED HEMIC NEOPLASIA IN *Mercenaria mercenaria*

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Hemocytic Neoplasia (HN) is a transmissible cancerous disease-causing significant mortality in *Mercenaria mercenaria* (quahog) aquaculture in Massachusetts and has been identified in quahog stocks in other states including Rhode Island, New Jersey, and Florida. Previous studies have identified candidate genetic markers for detecting HN in quahogs; however, they were unspecific to HN and were only effective at identifying moderate to severe cases of the infection. In this study we aim to differentiate gene expression profiles between HN and non-HN cells to pinpoint cancer-specific genes as well as characterize key pathways and molecular functions associated with HN. Naive HN-negative quahogs were exposed in cohabitation with HN-positive clams for up to 90 days, and samples of hemocytes were collected at different times after exposure to represent early and late-stage infection. Pools of hemocytes from infected neoplastic and non-neoplastic control clams (as determined using hemocyte smears) were processed for single cell sequencing analysis using 10X Genomics Single Cell Multiome ATAC + GEX Gel Beads followed by 10X Genomics Chromium Single Cell Sequencing. Data analysis revealed 23 clusters of hemocytes based on their individual gene expression profile (Fig. 1). Differential gene expression analysis identified significantly upregulated genes that are only present in neoplastic clams and are signatures of cancer in humans (*e.g.* transforming growth factor-beta-induced protein ig-h3-like, glutathione S-transferase-like, cytochrome P450 2B19-like, probable G-protein coupled receptor CG31760, secreted frizzled-related protein 3-like, and synaptotagmin-4-like). Additionally, KEGG pathway enrichment analysis revealed that several pathways are significantly enriched only in HN-positive cells. These pathways include RNA degradation, ribosome biogenesis, and nucleotide excision repair, all of which are involved in genetic information processing. Further work is currently underway characterizing enriched pathways and regulated genes in quahogs at early stages of infection. This information will be used to develop accurate and fast molecular methods of HN diagnosis of the disease using an RT-qPCR method.

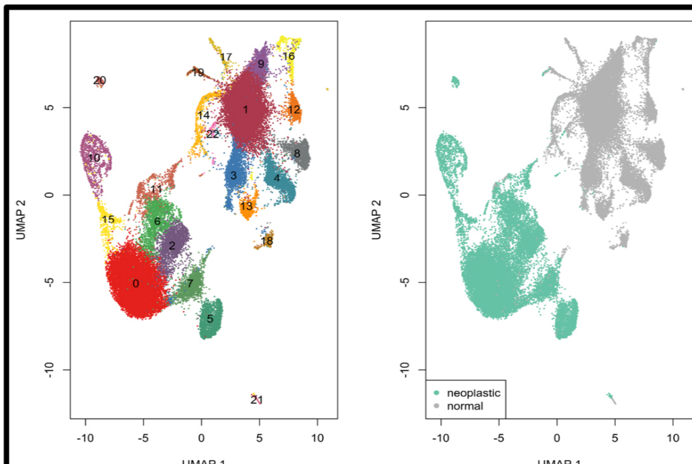

Figure 1. Uniform Manifold Approximation and Projection (UMAP) of the clusters identified (left) from neoplastic and normal hemocytes.

RAS FUNDAMENTALS, HEATING AND CHILLING

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Thermal control in RAS systems is a key function for optimal growth, metabolic activity, and survivability during maturation, spawning, grow out and brood stock maintenance.

To achieve thermal control, heating (heat pumps & hydronic boilers) and chilling (DX chillers & hydronic chillers) systems are an integral part of RAS architecture. This equipment can be deployed in multiple configurations to control temperature for induvial tanks or across entire systems.

Heating and chilling equipment must be sized to properly offset the heat flow due to the ambient environment (evaporation, convection, conduction, and solar irradiance) as well as mechanical, biomass and filtration thermal loads that appreciably affect the temperature of an aquaculture system. By understanding how heat flows through a RAS application, a properly sized and selected heating and chilling system will enable the grower to achieve the desired system temperature year-round in an energy efficient manner. Stable and controlled temperatures directly contribute to regular and increased production.

INVESTIGATING THE EFFICACY OF CALCEIN MARKING TECHNIQUES FOR HOGFISH *Lachnolaimus maximus*

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Hogfish (*Lachnolaimus maximus*), popular sport and food fish, have historically experienced overfishing throughout portions of their range prompting desire for intervention from management agencies to support impacted populations. Aquaculture and subsequent stock enhancement are potential opportunities that can support impacted populations, but evaluation of stocking success first requires effective assessment tools (e.g., mark-recapture experiments). Various marking methods exist but utility may be limited by cost, labor intensity, and species-specific sensitivity. Calcein is an efficient, non-lethal, batch fluorescent chemical marker that has potential to avoid some of the problems associated with more traditional marking methods. Standard calcein marking practices use osmotic induction, however, induction protocols, marking efficacy, and retention may be specific to species and environment. Four trials were conducted to evaluate the efficacy of calcein marking for hogfish. First, a trial was completed to determine the most efficient salinity pre-treatment for osmotic induction of calcein. A second trial determined the calcein concentration that resulted in the brightest mark. Two subsequent trials examined the effects of time and lighting scenarios on mark-retention of calcein. Osmotic induction experiments showed positive correlations between mark brightness, calcein concentration, and salinity pretreatment (Fig. 1). Retention experiments suggested vulnerability of marks, with rapid degradation of mark brightness over three to eight weeks. These results, alongside its current regulatory status, cast doubt on the viability of calcein as an external mark for stock assessment studies in hogfish, although more research is needed to assess its viability in natural settings. Results from this research will help guide future aquaculture and stock enhancement efforts for hogfish and add to the growing body of literature for this valuable marine species.

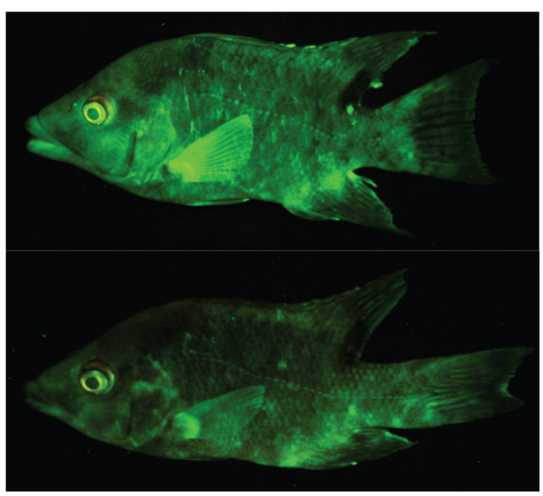

Figure 1. Hogfish exhibiting calcein staining at two brightness levels.

INVESTIGATING LIQUID DIETS AS LIVE FEED REPLACEMENTS IN FRESHWATER ANGELFISH *Pterophyllum scalare* LARVICULTURE

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Freshwater angelfish (*Pterophyllum scalare*) are common aquarium fish belonging to the family Cichlidae. This species is popular in the aquarium trade due to their aesthetic quality, with several different color morphs and fin lengths available. They grown via commercial aquaculture production; however, few empirical studies have been conducted, especially when considering larviculture. Larviculture of *P. scalare* often depends on *Artemia* spp. nauplii as a first feed, increasing production costs due to fluctuating cyst prices, limited availability, and labor-intensive culture maintenance. Reducing the use of live feeds can therefore streamline larviculture and increase cost efficiency, given that larval survival, growth, and quality are maintained. Liquid *Artemia* replacement diets are inert liquid diets that claim to mimic the smell, taste, and size of *Artemia* spp. nauplii while maintaining appropriate nutritional profiles. These diets have been used in shrimp aquaculture but have yet to be investigated for fish larviculture. Three trials were conducted to investigate the efficacy of replacing *Artemia* spp. nauplii with inert liquid diets. First, the performance of two brands of liquid diets, EZ Artemia and Liqualife, were compared in a 14-day trial. Next, the inclusion level of the liquid diet that best promoted larval growth, survival, and quality after 14 days from first feeding was investigated. Lastly, three timepoints (3, 6, and 9 days post-hatch) were selected to determine the effects of gradually weaning *P. scalare* from live feeds to a liquid diet. In general, larval fish did not perform well when fed liquid diets; larval survival was reflective of the inclusion rate of the liquid diet, regardless of the brand (Fig. 1). Overall, tanks fed diets the highest inclusion of live *Artemia* spp. had the highest larval survival. These results suggest that inert liquid diets may not be a suitable replacement for live feeds for *P. scalare.* Further research into *Artemia* spp. density requirements for this species is warranted.

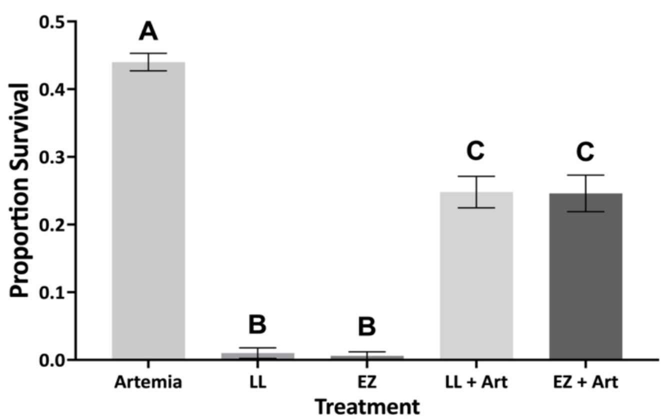

Figure 1. Mean proportion survival (±SEp) of larval *P. scalare* fed one of four experimental replacement diets after 14 days of feeding (n=5): 100% Liqualife (LL), 100% EZ Artemia (EZ), 50% *Artemia* spp. & 50% LiquaLife (LL+Art), or 50% *Artemia* spp. & 50% EZ Artemia (EZ+Art) compared against those fed 100% *Artemia* spp. nauplii diet. Different letters above bars indicate statistical significance.

EVALUATING THE POTENTIAL OF FEED ATTRACTANTS AND LIQUID DIETS TO REDUCE THE USE OF LIVE FEEDS FOR LARVAL *Trichopodus leerii*

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Freshwater ornamental larviculture typically relies on *Artemia* spp. nauplii as a first feed due to their ability to elicit larval feeding responses, high digestibility, and appropriate nutritional profile. However, *Artemia* spp. can be costly to produce and maintain, adding complexity and costs to the larviculture stage. Reducing the use of live feeds often involves transitioning larvae to inert feeds, but these inert diets often do not elicit a strong feeding response and may be more difficult to digest. This study aimed to reduce the use of *Artemia* spp. nauplii for the pearl gourami (*Trichopodus leerii*), a high-value ornamental fish known for its challenging larviculture requirements. Two types of inert feeds were investigated in this study: microparticulate diets (MD) top-dressed with feed attractants and inert liquid diets designed to replicate the smell, taste, and size of *Artemia* spp. nauplii. Feed attractants (FAs) are powdered amino acids that are used to top-dress pelleted diets to increase ingestion rates via chemosensory activation. Different inclusion rates of three feed attractants (FA), L-alanine, betaine, and L-tryptophan were tested at four inclusion rates (0%, 0.25%, 0.5%, and 1%) to determine the lowest effective dose needed to improve larval feeding performance in three 7-day trials. Next, two liquid diets, Zeigler EZ Artemia and Cargill LiquaLife, were tested at 100% and 50% replacement levels of *Artemia* spp. nauplii in a 14-day larviculture trial. L-alanine diets resulted in an approximately 5% increase in larval survival with both the 0.25% and 1.0% inclusion rates (Fig. 1A). The betaine and L-tryptophan experiments yielded results with similar survival rates as the control diet treatments (Fig. 1B,C). Additionally, replacing up to 50% of *Artemia* spp. nauplii with either brand of liquid diet did not significantly affect larval *T. leerii* survival. Together, these data provide baseline protocols for *T. leerii* larviculture that reduce live feed usage. Further research into appropriate liquid diet density and weaning to inert diets is necessary to refine these protocols.

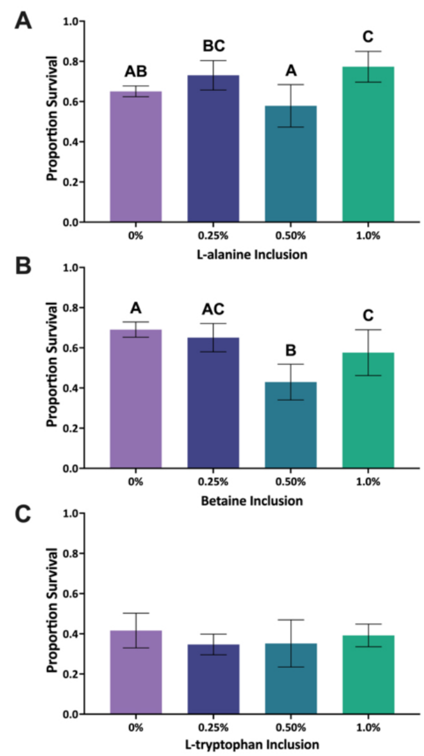

Figure 1. Mean proportion survival (±SEp) of larval *T. leerii* fed MDs top-dressed with 0%, 0.25%, 0.50%, or 1.0% L-alanine (A), betaine (B), or L-tryptophan (C) after 7 days of feeding. Different letters above bars indicate statistical significance.

ASSESSING THE EFFECTS OF DIFFERENT WATER HARDNESSES ON COPPER TOXICITY TO THE MARSH RAMSHORN SNAIL (*Planorbella trivolvis*)

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The catfish trematode (*Bolbophorus damnificus*) is a problem in the catfish industry in the southeastern USA, resulting in significant economic losses. The life cycle of *B. damnificus* consists of a planorbid snail as the first intermediate host, catfish as the second intermediate host, and the white pelican (*Pelecanus erythrorhynchos*) as the final host. The marsh rams-horn snail (*Planorbella trivolvis*) is recognized as the common first intermediate host of *B. damnificus.* Controlling populations of this snail species in aquaculture ponds can significantly reduce the infection impacts of *B. damnificus*. Among molluscicides, copper (Cu) is an effective treatment agent for pond snails. Previous studies found that marsh ramshorn and related species were sensitive to Cu. However, the effect of Cu on snails is dependent on water quality, such as temperature, pH, hardness, alkalinity, total suspended solids, and dissolved organic matter. While these water quality parameters in aquaculture ponds vary geographically, research on the influence of water quality on Cu toxicity to these snail species has not been conducted. This study examined the influence of water quality on Cu bioavailability and toxicity to the marsh rams-horn snail. The standard 96-h toxicity test was used to determine the toxicity of Cu on the snail. The initial results found as hardness increased, Cu toxicity decreased during the first 24 to 48 hours of exposure, likely due to more competition of hardness ions with Cu at the biotic ligand of the snail. The 24-h LC_{50} increased from 265 µg/L Cu in soft water to 652 µg/L Cu in hard water or 510 µg/L Cu in very hard water. Interestingly, the LC_{50} values remained similar for all hardness levels when the exposure time was increased to 96 hours. These results suggest that the snails may have reached their Cu threshold by this timeframe regardless of hardness. More research needs to be conducted to understand this interesting observation and determine the influence of other water quality characteristics on Cu toxicity to the marsh rams-horn snail. The effect of hardness water quality parameters on Cu toxicity to the snail will be presented.

A REVIEW OF BIODIVERSITY AND CHEMICAL CONTAMINANTS IN FISH FROM LAKE VICTORIA, UGANDA

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Lake Victoria connects the East African nations of Uganda, Kenya, and Tanzania. We present here a review of the scientific literature on biodiversity and contaminants (heavy metals, pesticides) in fish from Lake Victoria on Jinja, Uganda. The review was performed using PubMed and PMC databases at the National Library of Medicine (https://www.ncbi.nlm.nih.gov/). Preliminary results will be presented.

The most common fish found in Lake Victoria, Uganda include among others: Nile perch (*Lates nilotocus*), Nile tilapia (*Oreochromis niloticus*), dagaa (*Rastrineobola argentea*), catfish (*Xenoclarias eupogon*), elephant-snout fish (*Mormyrus kannume*), Nila killfish (*Micropanchax loati*), marbled lungfish (*Protopterus aethiopicus*) and cichlid fish (*Haplochromis thereuterion*).

Very limited information was found about chemical contaminants in fish of Lake Victoria in Uganda. Most references found were about metals in Nile perch. Results showed that Nile perch generally contains low levels of heavy metals (lead, mercury, cadmium, copper, zinc). Some studies have found that fish in the lake can bioaccumulate these metals to levels that exceed WHO, EU, and USEPA limits. However, other studies have found that some heavy metals are present at levels that are safe for human consumption (Ogwok et al. 2009). Mercury and lead were detected in most samples while arsenic and cadmium were below detection limits, suggesting that Nile perch may accumulate significant amounts of chemical contaminants.

Considering the new CAFW's initiative of Rotary International (RI) with the United Nations Environment Programme (UNEP) to protect, restore, and sustain freshwater ecosystems (www.rotary.org/en/rotary-unep-partnership), we plan to build collaborations with Rotaracts and Rotary Clubs from countries connecting Lake Victoria to obtain preliminary results and prepare a global grant to perform research on conservation of biodiversity and pollution to address food safety and food security issues.

FUNDAMENTALS OF BIOREACTORS IN RAS

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Bioreactors are a vital component of recirculating aquaculture systems (RAS). Often called the heart of the RAS, bioreactors carry out the important function of converting the toxic ammonia produced by the fish to the less toxic nitrate. This conversion process, called nitrification, is carried out by nitrifying bacteria or archaea. Typically, these microorganisms reside in biofilms, which are a complex conglomerate of microorganisms attached to surfaces in the bioreactors. Bioreactors are often treated as a black box and as simply another technical equipment at a fish farm. In fact, bioreactors are thriving with a multitude of microorganisms many orders of magnitude greater than the fish. These microorganisms can influence the physicochemical and microbiological water quality, thus directly or indirectly affecting the fish. Therefore, care must be taken to select for the right microbes. The bioreactor must be treated as a "living organism", taking into consideration the needs and optimal conditions for the desirable microorganisms residing within.

We will look into different types of bioreactors and their design. The types of microorganisms living in bioreactors and the factors influencing the nitrification performance will be presented. Startup, operation, maintenance, troubleshooting and disinfection of bioreactors will be covered.

It is important to remember that both microorganisms and fish are being grown in a RAS. The two must co-exist in harmony to ensure good water quality and healthy fish.

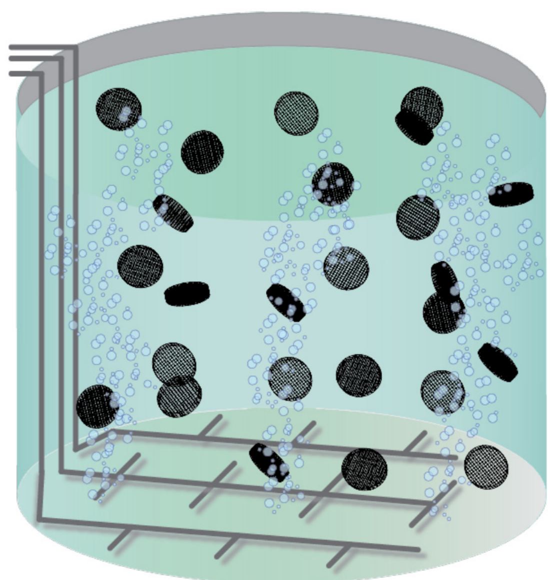

Figure 1: Schematic of a moving bed biofilm reactor (MBBR) commonly used in RAS

ENHANCE YOUR AQUACULTURE EDUCATION, EXTENSION BUSINESS AND MORE THROUGH BACKWARDS DESIGN

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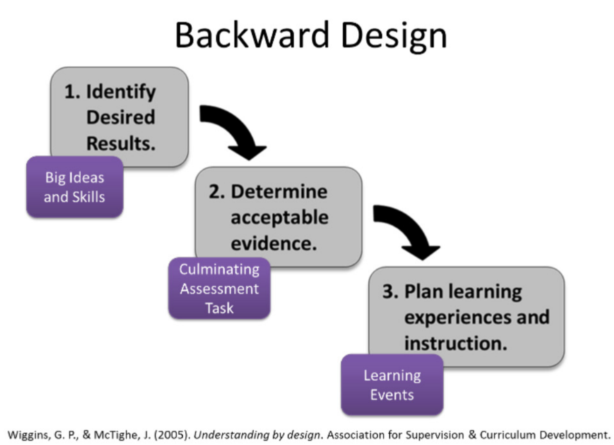
Aquaculture is the fastest growing sectors of food production worldwide and is vitally important to obtaining sustainable food security in the future. However, in the United States aquaculture continues to grow at a sluggish pace and is often misunderstood or completely unknown to many U.S. consumers. A large amount of dollars from the federal government are now going towards research, education and outreach, with the goal of enhancing and growing the aquaculture industry. But with the overwhelming number of barriers and challenges to growth in the aquaculture industry, extension and education programing can often lack a focus towards clear outcomes. A core research-backed practice in education planning is known as backwards design. This theory was recently shared with international partners and works towards enhancing all aquaculture education, programing and business.

In an effort to increase effectiveness of growth focused initiatives in aquaculture, Michigan Sea Grant recently embarked on an international exchange with Indonesian aquaculture extension and education partners. The goal of the exchange was to share best practices in education and outreach efforts and to exchange knowledge on how to effectively implement aquaculture extension activities. Backwards design techniques were shared, and implemented by both partners. The results of this international exchange, along with backwards design, logic model and other planning techniques are now being shared to help enhance the aquaculture industry across all aspects.



CHALLENGES AND SOLUTIONS TO MUSSEL AND OYSTER AQUACULTURE BIOLOGY, ENGINEERING AND BUSINESS: FOSTERING A CULTURE OF INNOVATION

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The development of profitable mussel and oyster aquaculture in Maine over the past 45 years has required the development of a culture of innovation which finds solutions to challenges using a variety of approaches, ranging from research discoveries and technology transfer to home-grown ingenuity. An understanding of the factors affection growth and yield on a farm must be translated into a profitable business plan and operations which not only solve culture bottlenecks but also provide resiliency in the face of new challenges.

A series of case studies spanning 4 decades demonstrates a tried and true method which involves:
1. A clear definition of the problem and how it affects operations.
2. See if somebody else has solved the problem and importing the solution to your operations with adaptations as necessary.
3. Development of a custom solution yourself with a team of the right people.

I will discuss solutions to a number of challenges which have been solved, including:
site selection, gear development, predation mortality, storm and drift ice damage, biofouling, mooring and anchoring systems, processing efficiency, mortality from disease, fecal coliform pollution, seed collection, upweller design, operation scale and labor costs.

Fostering a culture of innovation where problem solving, improvements in efficiency and cost savings are valued is a key element in shellfish aquaculture success, and indeed a big part of why its rewarding.

GROWTH PERFORMANCE, BLOOD CHEMISTRY AND GUT MICROBIOTA OF FLORIDA POMPANO *Trachinotus carolinus* FED DIFFERENT LEVELS OF CORN FERMENTED PROTEIN AND YEAST DIETS

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If species-specific diets for the Florida pompano (*Trachinotus carolinus*) are to be developed, we need information not only on nutritional requirements but also on how the species responds to various ingredients in practical diet formulations. Considerable work has been completed that has reduced the levels of animal protein to ~15% of the diet using solvent-extracted soybean meal (SBM) as a primary protein source. Further cost reductions can be obtained if other low-cost proteins are then substituted for SBM. We evaluated a corn-fermented protein (CFP) as a substitute for soybean meal by conducting a 12-week growth trial with juvenile Florida pompano (6.08 ± 0.55g) using a fishmeal-free basal diet. The basal diet contained poultry meal (15% diet) and SBM (52% diet) as primary protein sources. The SBM was then incrementally replaced with CFP (5, 10, 20% diet) on an equal protein basis. Although final weights increased from 45g to 56g, there were no differences due to data variability. There were also no differences in FCR, protein, or energy retention. Furthermore, analysis of serum metabolits and gut microbiota revealed no indications of impaired health confirming that CFP could be used as a substitute for SBM in practical diets for Florida pompano.

Table 1. Growth performance of Florida pompano (*Trachinotus carolinus*) with an initial weight of 6.08 ± 0.55g (mean ± SD) offered corn-fermented protein (CFP) diets for twelve weeks.

Diets	Final weight (g)	Weight gain (%)	TGC	Feed Intake (g fish^{-1})	FCR	Survival (%)	Protein Retention (%)	Energy Retention (%)
Basal	45.00	565	0.55	63.86	1.70	83.33	32.22	28.27
CFP-5	48.99	622	0.61	68.31	1.64	81.67	33.22	26.95
CFP-10	50.17	636	0.63	69.79	1.64	81.67	30.48	28.48
CFP-20	56.98	738	0.72	77.55	1.56	91.67	31.70	29.24
FSC-2	44.61	557	0.55	66.03	1.75	88.33	30.30	25.41
PSE	3.53	47.75	0.05	4.29	6.25	6.25	1.40	1.21
P-value	0.123	0.085	0.110	0.238	0.312	0.728	0.56	0.22

ETHANOL CO-PRODUCT AS SUSTAINABLE PROTEIN SOURCES IN WHITE SHRIMP *Litopenaeus vannamei* DIETS

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Utilizing corn-fermented protein (CFP) as a substitute for soybean meal (SBM) in aquafeeds presents a promising option due to its dependable supply and cost-effectiveness. In this study, two growth trials were performed to evaluate the effectiveness of CFP products such as CFPA (48% crude protein - CP), CFPB1 (50% CP), and CFPB2 (60% CP) from two different sources (A, B) in practical diets for the juvenile white shrimp *L. vannamei*. The test diets in both trials were formulated to be isonitrogenous and isolipidic (36% crude protein, 6% crude lipid). These diets were produced by supplementing the basal diet with 5, 10, 15, and 20% CFPA or 4, 8, 12, and 16% of CFPB1 and CFPB2 to replace SBM on an isonitrogenous basis. In the first trial, shrimp (1.02 ± 0.02g mean weight, 15 shrimp per tank, n=6) were offered diets for six weeks. Growth parameters and protein retention showed no significant differences among the diets. However, a significant feed conversion ratio (FCR) increase was observed when shrimp were fed a diet containing 20% CFPA compared to the basal and 5% CFPA diets. In the second seven-week trial, shrimp (0.55 ± 0.01g, 15 shrimp per tank, n=5) received experimental diets, including CFPB with two protein sources, CFPB1 and CFPB2. No differences were observed in growth performance. This research highlights the potential of CFP as a protein source in shrimp diets and improves aquaculture's economic and environmental sustainability by expanding the range of feed components and identifying optimal inclusion levels.

Table 1. Growth of *L.vannamei* fed different levels of CFPA, B1/2 in 6 and 7 weeks experimental period.

	Diets	FW (g)	WG (g)	PWG (%)	TGC	FCR	SR (%)
Trial 1	Basal	10.49	9.48	932.74	0.27	1.46[b]	92.22
	CFPA 5%	10.78	9.77	961.69	0.28	1.46[b]	87.78
	CFPA 10%	10.49	9.47	933.47	0.27	1.55[ab]	85.56
	CFPA 15%	10.03	9.02	887.76	0.26	1.50[ab]	93.33
	CFPA 20%	10.47	9.45	931.99	0.27	1.61[a]	82.22
	CFPA 25%	10.19	9.17	903.21	0.26	1.54[ab]	90.00
	PSE	0.18	0.18	18.9	0.01	0.03	3.11
	P-value	0.09	0.09	0.12	0.05	0.01	0.14
Trial 2	Basal	12.34	11.78	2119.98	0.29	1.38	89.33
	CFPB1 4%	12.25	11.69	2096.32	0.28	1.47	88.00
	CFPB1 8%	12.67	12.12	2174.17	0.29	1.4	86.67
	CFPB1 12%	12.48	11.92	2135.69	0.29	1.44	86.67
	CFPB1 16%	11.99	11.43	2055.27	0.28	1.52	84.00
	PSE	0.2	0.21	43.78	0.01	0.07	4.28
	P-value	0.22	0.23	0.42	0.38	0.59	0.93
	CFPB2 8%	12.55	11.99	2151.47	0.29	1.42	88.00
	CFPB2 12%	12.6	12.05	2165.35	0.29	1.45	84.00
	CFPB2 16%	12.26	11.7	2104.99	0.28	1.42	90.67
	PSE	0.25	0.25	51.57	0.01	0.04	3.53
	P-value	0.76	0.72	0.83	0.7	0.77	0.59
	Ingredient	0.72	0.75	0.78	0.77	0.54	0.78
	Level	0.15	0.14	0.31	0.17	0.73	0.91
	Ingredient * Level	0.66	0.67	0.74	0.82	0.56	0.52

EVALUATION OF THE EFFECTS OF DIETARY TREATMENTS ON THE INHIBITION OF *Enterocytozoon hepatopenaei* (EHP) ON PACIFIC WHITE SHRIMP *Litopenaeus vannamei* UNDER LABORATORY CONDITION

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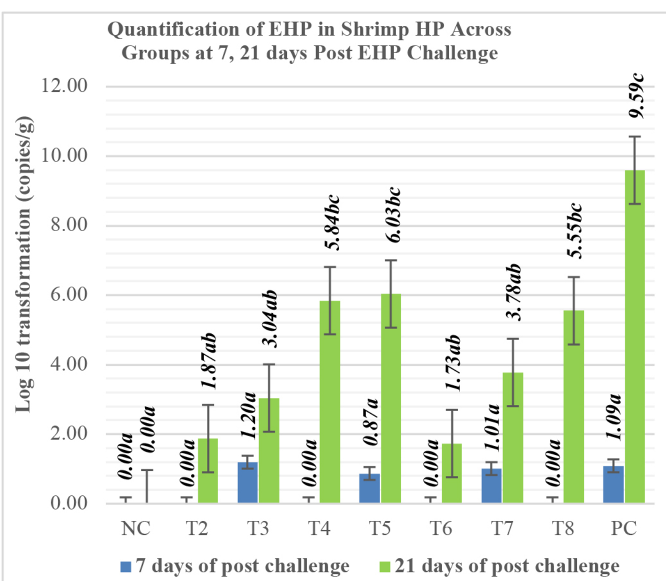
The microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) is currently one of the most severe diseases in many of major shrimp producing countries, causing devastating effect on shrimp production with significant reduction in success rate and profit. A nine-week trial was conducted to evaluate the efficacy of preventative diets in suppressing EHP in white leg shrimp *Penaeus vannamei* under controlled laboratory conditions. A diet with 43% protein was used as a basal diet for negative control (NC) and positive control (PC) treatments. 7 preventative diets were then supplemented with functionally preventative packages to deliver different modes of action for controlling EHP in the hepatopancreas of shrimp. Each of the 9 treatments was replicated 5 times. Specific pathogen free (SPF) shrimp were stocked in 250L tank of seawater with 50 shrimp each (~200 shrimp/m2). Following a 14-day pre-challenge period, shrimp were exposed to EHP via oral challenge (*Per os* method) for 7 days. Post-challenge monitoring continued for 42 days. According to the results of our study, there were no significant difference in the EHP load among groups of treatment after 7 days of challenge. EHP load in the hepatopancreas of shrimp, however, increased dramatically in positive control treatment after 21 days of challenge. In contrast, shrimp in the groups fed preventative diets T2, T3, T6, T7 showed pretty good EHP control with significantly lower EHP loads compared to the positive control treatment. Other growth performance including average daily gain, feed conversion ratio and survival rate were also measured after the trial which indicated the best performance of shrimp in T2. In conclusion, the trial results showed the potential of preventative diets as a solution for EHP management in shrimp aquaculture.



Growth performance	Difference of T2 and PC
Average daily gain (g/d)	+21%
Survival rate (%)	+8%
Feed conversion ratio	-12%
EHP density	- 20% for 2nd qPCR

ECONOMIC EVALUATION OF HIGH AND LOW PROTEIN FEED APPLICATION IN HIGH STOCKING DENSITY OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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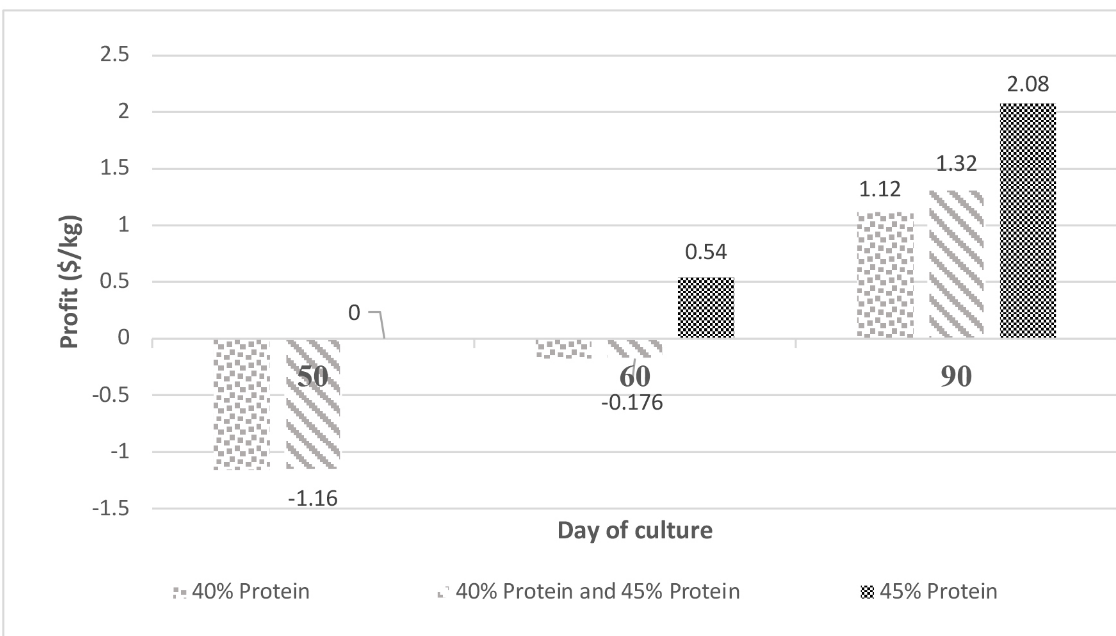
Shrimp farmers have been facing complex challenges. Internally, there are the challenges from disease outbreak such as TPD, EHP, AHPND, …; influence of weather condition and climate change; and inconsistent post larvae quality and availability, ... These difficulties combining with low farm gate prices, complicated global market dynamics are creating the biggest difficulties ever for shrimp farmers. With all challenges, success rates and profits of shrimp farmers had been reduced significantly during the past year. As feed costs representing about 50-55% of total production cost in shrimp faming with high stocking densities, many aquafeed companies and shrimp farmers switched from premium feeding programs with high protein feed (45% protein) to medium (40%, 45% protein) and standard feed (40% protein) with lower protein contents with the assumption to reduce feed cost and total production cost thereby enhancing profit in the end. The economic model was applied to evaluate the profits of farmers with different feeding programs in which 200 proofs of performance were collected from the market. These proofs of performance were converted to 50, 60 and 90 days of culture to better visualize the performance of shrimps and estimated profits. According to the result, farmers fed high protein feed showed the best profit after 90 days of culture at about $2.08/kg, whereas lower profits at $1.32/kg and $1.12/kg were observed in farmers fed lower protein feeds. With the utilization of 45% protein feed, farmers could also shorten days of culture to reach the break-even point.



IMPLEMENTING AUTOMATION AND REAL-TIME FEED MANAGEMENT IN SHRIMP FARMING: RESEARCH AND PRODUCTION RETROSPECT

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Automated feeding systems, whether timer based or using passive acoustic feedback, have garnered attention as advances in shrimp feed management. Despite being available for less than a decade, passive acoustic monitoring (PAM) is widely utilized in shrimp production. However, the temporal effects of hand feeding, timer feeding, and PAM on shrimp performance and the general benefits of automated feeding have yet to be fully examined. This research aimed to fill these gaps by assessing the effects of three principal feeding strategies on the development trajectory of shrimp and overall production results. The study utilized production data from research facilities over 18 years and from commercial production facilities over 4 years. We also highlight benefits and obstacles faced in the implementation of automated feeding technology, based on the experiences of field personnel. The findings indicated that total biomass, yield, average weight, and weight gain were markedly superior in production farms using timer and sonic feeding methods compared to hand feeding over three consecutive years ($p<0.05$). In research settings where genetic background and stocking density were not considered, the 18-year dataset exhibited a consistent trend: shrimp fed with the sonic and timer systems achieved significantly higher survival rates, total biomass, average weight, and total feed input ($p<0.001$) compared to those reared using hand feeding. Figure 1A presents a logistic growth model showing that while all three feeding techniques reached an inflection point around week 7, the average weights at this point were 10.14 g, 13.98 g, and 17.82 g for hand, timer, and sonic feeding practices, respectively. Figure 1B illustrates a smoothing spline model of weekly weight gain from week three onward, highlighting the highest average weekly gains of 4.1 g/week, 3.2 g/week, and 2.0 g/week at eight weeks post-stocking for sonic, timer, and hand feeding techniques, respectively. Interestingly, while weight gain began to decline simultaneously across all feeding methods, automated feeding systems consistently produced superior cumulative growth rates compared to the labor-intensive hand feeding approach, with the rate of decline being similar across all feeding regimes. Increased growth rates with automated systems also required nearly a 50 % increase in daily feed input compared to other feeding systems which required stakeholders to alter water quality management strategies. Implementation of this technology also required greater capital expenditures and specific training for operators. Although these limitations exist, temporal data from research and on-farm production contexts demonstrate the benefits of improved technologies, allowing higher production yields with improved economic returns reported by farmers.

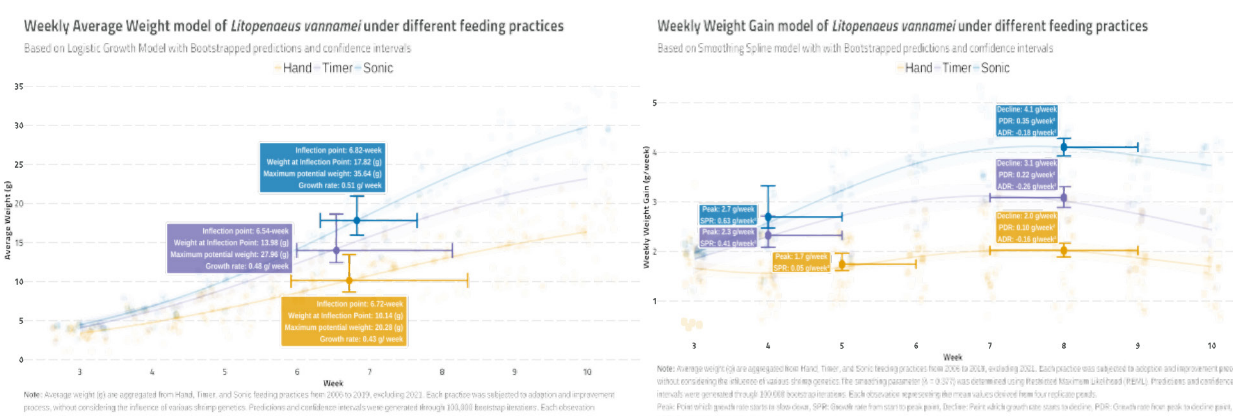

Figure 1. Logistic and smoothing spline models of weekly average weight and weekly weight gain of *Litopenaeus vannamei* under Hand, Timer, and Sonic feeding practices.

ASSEMENT OF MORPHOLOGICAL DIFFERENCES AND GENETIC DIVERSITY IN FLORIDA POMPANO (*Trachinotus carolinus*)

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Genomics research is applied in selective breeding to improve the genetic quality of broodstock and seedstock for the U.S. warmwater marine finfish aquaculture industry. This pilot study originates from a long-term multidisciplinary project (USDA Agreement #59-6034-9-007) supporting the expansion of the U.S. warmwater marine finfish aquaculture industry. Florida pompano (*Trachinotus carolinus*) is our model species due to its consumer popularity, market value, and known capacity to spawn in captivity. We focus on developing techniques that will contribute to a DNA marker-assisted selection (MAS) breeding program and provide information on growth performance for *T. carolinus* in aquaculture. This study incorporates restriction-associated DNA (RAD) sequencing to analyze single nucleotide polymorphisms (SNPs) on genes linked to phenotypic traits such as growth and understand how these genetic markers relate to measurable morphological differences. The experiments were conducted in partnership with Aquaco Farms, in Fort Pierce, Florida. In response to the observation of atypical growth patterns among a particular cohort (progeny of known brood), we collected twenty fish 68 DPH (days post hatch) for comparative assessment and DNA extraction. Individual juveniles were collected and separated into two groups, ten with "normal" head sizes and ten with "abnormal" head sizes. Twelve types of morphological measurements were recorded for each fish "before" and "after" freezing the samples. DNA was extracted from fish fin clips for RAD sequencing on the Illumina HiSeq platform. Sequencing data will be analyzed for SNPs associated with growth differences using a variety of publicly available bioinformatics packages for RAD-Seq analysis.

DEVELOPMENT OF SUSTAINABLE MUD CRAB RAS UNIT

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Background

The mud crab aquaculture industry, despite being highly lucrative, especially in regions like Southeast Asia and India, faces persistent challenges that threaten its sustainability and long-term growth. **Disease outbreaks**, particularly the **Mud Crab Reovirus (MCRV)**, have emerged as a critical issue. MCRV, responsible for 100 percent chronic mass mortalities in mud crab farms, has devastated the industry, and its presence has been reported across India's farming systems as well as in wild populations (Weng et al., 2007; Sravani et al., 2022; Ganesan et al., 2023; Sravani et al., 2024; John et al., 2024). This pathogen poses a severe threat, making disease management the foremost concern in mud crab culture.

Beyond disease, **s**ustainability issues related to **feed** further complicate mud crab farming. In developing countries like India, Vietnam, and Indonesia, the high cost of commercial feeds designed specifically for mud crabs makes their consistent use impractical for many farmers. While formulated feeds offer balanced nutrition, research shows that there is no significant difference in the growth performance of mud crabs fed low-value fish compared to those fed formulated feeds. Thus, farmers continue to rely heavily on low-value fish, an unsustainable practice that over-exploits marine resources and contributes to environmental degradation.

Additionally, **cannibalism**, though often considered a secondary issue, worsens both disease transmission and mortality rates. It has been found that diseases like Mud Crab Reovirus (MCRV) lead to high mortality rates in open culture systems, where individual crabs are not isolated. However, in compartmentalized systems, survival rates can improve by up to 60%, demonstrating that cannibalism plays a significant role in worsening disease transmission. When a healthy crab consumes an infected dead crab, it further aggravates the spread of the virus, making the situation even more critical in case of MCRV (Nivas et al., 2023).

These **three interconnected issues**—disease outbreaks, unsustainable feeding practices, and cannibalism—are undermining the potential of mud crab aquaculture, a sector vital to the livelihoods of many coastal communities. Addressing these challenges demands immediate, innovative solutions that can enhance the sustainability, efficiency, and economic viability of the industry. Without such interventions, the mud crab aquaculture industry risks stagnation and decline, impacting both local economies and global seafood markets.

How we address the issues?

Our proposed Recirculating Aquaculture System (RAS) for mud crab culture directly tackles the three major challenges identified in the industry: disease outbreaks, unsustainable feeding practices, and cannibalism. The design integrates advanced filtration, water quality control, and feeding strategies, ensuring a more sustainable, efficient, and biosecure mud crab farming system.

The **filtration system** in the proposed Recirculating Aquaculture System (RAS), comprising biofilters, UV treatment, and plasma ozonation, coupled with a nano bubble generator, offers enhanced biosecurity by ensuring efficient pathogen removal. Plasma ozonation and UV filtration, in particular, are critical for neutralizing harmful pathogens, while the nano bubble generator improves oxygenation and water quality. This combination creates an environment that significantly reduces the risk of pathogen proliferation, providing a more controlled and secure aquaculture setting.

Additionally, the **customized crab culture unit**, designed with PVC piping, minimizes housing space, reducing pathogen accumulation. It is well-established that increased surface area in aquaculture systems promotes biofilm formation, which can harbor communities of microorganisms, including harmful pathogens. The presence of these biofilms elevates the risk of disease outbreaks, as maintaining cleanliness and biosecurity becomes more challenging in systems with extensive surface areas. By minimizing available surface area, our system limits the potential for biofilm development, thereby reducing pathogen load and addressing one of the primary sources of disease in aquaculture systems.

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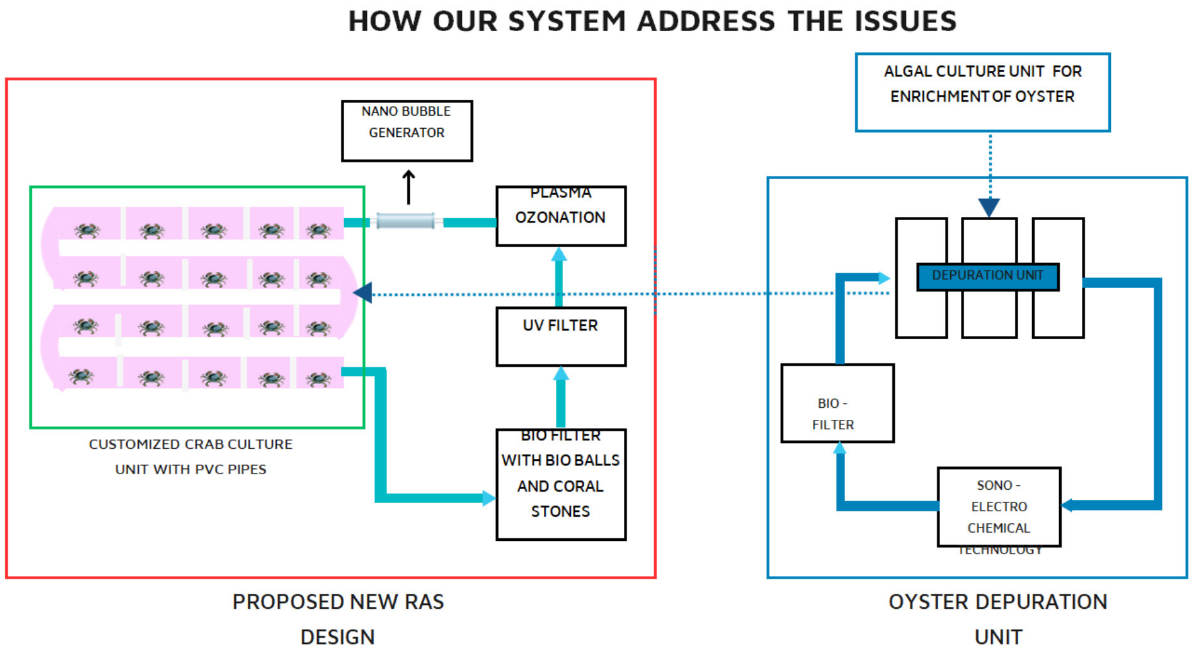

Feed selection also plays a pivotal role in pathogen management. The use of oysters as a primary feed source for mud crabs is a sustainable alternative to traditional wet feeds such as trash fish, shrimp head waste, and poultry by-products. Oysters have been shown to promote better growth in mud crabs due to their high lipid and cholesterol content, which supports moulting. Moulting is a critical phase for growth, during which crabs can gain 25–30% of their body weight. However, mollusc feed can harbor significant microbial loads, posing a biosecurity risk. To mitigate this, a 24-hour depuration process is employed, effectively reducing microbial contamination. During this depuration, oysters are enriched with algae species known to enhance the nutritional profile of the feed. Specifically, brown algae, which contains fucoidan, is incorporated to improve the innate immunity of mud crabs. Fucoidan supplementation has been shown to reduce mortality in viral infections (Jin et al., 2021), adding an additional layer of protection against disease.

The depuration process occurs within a dedicated RAS, which incorporates **biofilters** and **advanced sono-electrochemical technology**. This technology synergistically combines ultrasound (sonication) and electrochemical processes to enhance pathogen disinfection and the degradation of contaminants in the water. Sonication disrupts microbial cell walls, making them more susceptible to the reactive species generated during electrochemical oxidation. This combination effectively neutralizes pathogens by damaging cellular components such as DNA and proteins, leading to more comprehensive pathogen removal.

Lastly, the **source of the crab seed** itself is a significant consideration. As hatchery technology for mud crabs is not yet widely adopted, the industry continues to rely on wild-caught seed for cultivation. Wild seed presents an inherent biosecurity risk, as it may introduce pathogens into the culture system. To address this, all crabs undergo a 5-day quarantine period before being introduced to the RAS, during which they are treated with antibiotics to reduce the potential for infection. This quarantine procedure is crucial for minimizing the risk of introducing diseases into the system from external sources.

Objective

1. To compare the performance of mud crabs with respect to Mud crab reo virus (MCRV) infection in cage and open pen system.
2. To design and standardize a Recirculating Aquaculture System (RAS) unit for the culture of mud crab (Scylla serrata), addressing the issues present in existing mud crab culture systems
3. To compare the efficiency of the proposed system and to evaluate the effectiveness of different microalgae-enriched oysters as wet feed in enhancing the growth performance of mud crabs (Scylla serrata) within the developed system.

AQUACULTURE, A PROMISING SOLUTION FOR FOOD INSECURITY POVERTY AND MALNUTRITION IN KENYA

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Food insecurity remains one of the most visible dimensions of poverty. The increasing population amid competition for land and water resources means that the global demand for food will continue to increase. In Kenya, the food insecurity trend is worrying as the population is expected to hit 55 million by 2020 against an annually declining arable land per capita and consequent increase in food prices. The Kenyan agricultural sector has failed to either eliminate or reduce malnourishment for poor populations as the annual national production for both staple food and livestock products fall short of national consumption levels. With increasing food production challenges like dwindling capture fisheries and impacts of climate change becoming more eminent, solutions to food insecurity and malnutrition in Kenya must bring quick results in food availability by stimulating more own-food production. Aquaculture has so far been recognized as an important opportunity to enhance household food security in developing countries. Aquaculture, the controlled land-based or open-ocean farming of aquatic organisms such as tilapia, catfish, finfish, shellfish and plants, is the fastest growing food sector globally alongside terrestrial crop and livestock production. Fish provides protective effects on a wide range on health including obesity, stroke, high blood pressure, and coronary heart disease. Fish has a nutrient profile superior to all terrestrial meats, an excellent source of high quality animal protein, omega-3 polyunsaturated fatty acids (PUFAs) and vitamins. Unfortunately, in Kenya, fish has been only marginally included in the national debate on reduction of micronutrient deficiency, precisely where it could potentially have the largest impact.

EVALUATION OF SELECTED HORMONAL AGENTS AND ILUMINATION IN HATCHERY PROPAGATION OF *Heterotis niloticus* (Cuvier, 1829)

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The study examined four hormonal preparations from *Heterotis niloticus* pituitary extract, ovaprim, *Clarias gariepinus* pituitary extract and carp pituitary extract, under three different light conditions in the hatchery propagation of *Heterotis niloticus*. The study was carried out at Abak, Akwa Ibom State, Nigeria. One thousand *Heterotis niloticus* juveniles with the size range of 8-10cm and weight, 5-10grams each, were collected from Omambala River in Otuocha, Anambra State and stocked in an earthen pond. After being raised to brood stage for two years, thirty-six males and females were selected and used for the study and divided into four groups of nine fishes each and exposed to three light conditions. The females in each group were induced to spawn with same hormone under each light condition and replicated thrice within groups. Sperm from one male was used to fertilize eggs stripped from a particular female. The effect of the hormones on latency period, fecundity, fertilization, hatchability and larval rearing was investigated. Latency period was significant ($p<0.05$) among the treatments. The period lasted for 10hours, 9hours, 12hours, and11hours for *Heterotis niloticus* pituitary extract, ovaprim, *Clarias gariepinus* pituitary extract and CPE, respectively at 28^{o}C. Percentage egg fertilization among the treatments under different light conditions was significant ($p<0.05$). Fertilization rates within *Heterotis niloticus* pituitary extract under no illumination (89.66%) and ovaprim under no illumination (89.33%) were significantly higher than all others with the lowest recorded in *Clarias gariepinus* pituitary extract under full Illumination (67.33%). Percentage egg hatchability was significant among treatments under different light conditions ($p<0.05$). Eggs produced with ovaprim under no illumination (84.16%) was higher, followed with eggs produced with *Heterotis niloticus* pituitary extract under no illumination (80.66%); the lowest hatchability rate was recorded with eggs produced with *Clarias gariepinus* under full illumination (57.33%) and CPE under full illumination (59.50%). Percentage larval survival among treatments under different light conditions was significant ($p<0.05$), the highest survival was recorded with eggs hatched with ovaprim under no illumination (68.66%) followed with CPE under no illumination (66.66%) and *Heterotis niloticus* pituitary extract under no illumination (63.16%) with the lowest larval survival in *Clarias gariepinus* pituitary extract under full illumination (47.33%) and *Heterotis niloticus* pituitary extract under full illumination (49.50%). At the end of this research, there were disparities in the efficiency of the four hormonal preparations and it was evident that the results of ovaprim were outstandingly impressive, while *Heterotis niloticus* pituitary extract and CPE subjected under No Light condition gave average performance relative to *Clarias gariepinus* pituitary extract. Ovaprim hormone gave the highest reproductive indices in fecundity, fertilization, hatchability and larval survival, as it can be easily accessed but expensive.

WHICH CAME FIRST: THE SCALLOP OR THE SPAT? A BIOECONOMIC COMPARISON OF HATCHERY AND WILD SPAT COLLECTION FOR THE ATLANTIC SEA SCALLOP *Placopecten magellanicus*

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Shellfish aquaculture is dependent on a reliable and affordable supply of juveniles in the form of newly settled seed or older spat. Aquaculture growers may elect to collect spat from the wild or purchase seed from a hatchery. When both options are available, the grower's decision is a trade-off between the increased cost of hatchery seed and wild spat collection efficiency/ uncertainty. However, what market pressures lead to the development of a hatchery system if there is no industry already present? In a 'chicken and the egg' scenario, without a grower demand for seed, what market pressures would facilitate hatchery development?

Our research evaluated the incipient Atlantic sea scallop aquaculture industry. Wild spat collection currently supports a cottage (<1 mt annual sales) industry in Maine, however collection efficiency is low. This has spurred interest in developing a hatchery process to supply scallop seed. Our scenario analysis compared hatchery and wild spat collection economic metrics using results from a three-year hatchery research project and published wild spat collection sub-model. Our results determined that, while hatcheries could reliably compete with wild spat at annual production scales exceeding one million seed, the current annual grower demand is less than 100,000 seed, making commercial hatchery operations unprofitable. Our scale-efficiency framework can be applied to all shellfish species, particularly as climate change drives uncertainty in wild settlement higher (Fig. 1).

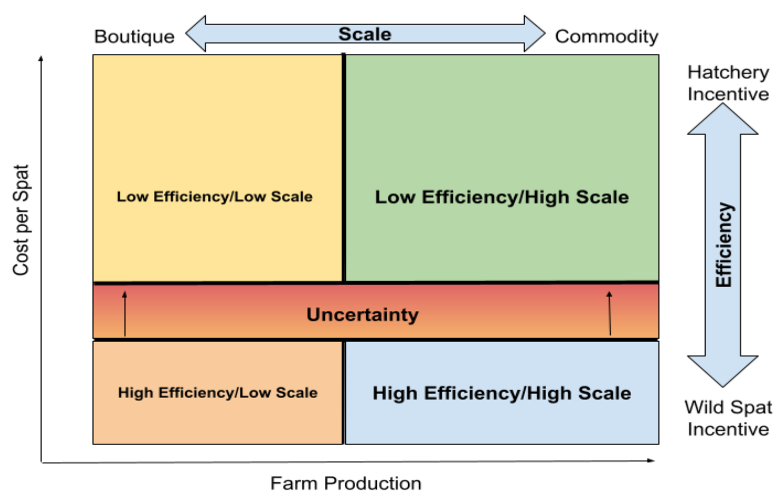

Figure 1: Scale-efficiency-uncertainty framework influencing grower economic preference to collect spat from the wild or purchase seed from a hatchery.

DIFFERENTIAL PERFORMANCE OF DIPLOID, MATED TRIPLOID, AND INDUCED TRIPLOID PACIFIC OYSTERS UNDER VARIED ENVIRONMENTAL CONDITIONS: INSIGHTS INTO IMPACTS OF TEMPERATURE, DISSOLVED OXYGEN, AND $p\mathrm{CO_2}$.

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Pacific oysters (*Crassostrea gigas*) are an important aquaculture species due to their fast growth, high market demand, and adaptability to various environmental conditions. Triploid oysters, have an additional set of chromosomes relative to diploids, grow faster and are sterile, making them more marketable. Thus, triploids comprise a large proportion of this species grown worldwide. Triploid oysters, however, are reported to experience higher mortality rates than diploids. Growers must, therefore, make farm management decisions that balance the risks and rewards of growing triploids. Understanding how these stressors affect these oysters is essential, not only to understanding the drivers of triploid mortality but also to prepare for the impacts of climate change stressors on these individuals in aquaculture. In this study, we examined the impact of temperature, dissolved oxygen (DO), and $p\mathrm{CO_2}$ on diploid, chemically induced triploid, and mated triploid Pacific oysters. Diploid and induced triploid groups were full siblings, while mated triploids were half-siblings. We measured whole organism physiological responses—growth, mortality and dissolved oxygen—after a 4-week exposure to one of these stressors and an additional acute heat stress. Survival was high in all groups across a broad range of temperature and DO and temperature levels. Survival of mated triploids, however, was negatively impacted at lower (but still higher than ambient) $p\mathrm{CO_2}$ levels compared to the other two groups. Diploids and induced triploids had similar oxygen consumption patterns across temperature and $p\mathrm{CO_2}$ experiments, but diploids consumed more oxygen across all dissolved oxygen treatments. The differing performance of mated triploids suggests that their genetic background may contribute to their resilience or susceptibility to climate change stress. Thus, the development of locally adapted tetraploid broodstock production of mated triploid oysters is essential to the future of triploids in the aquaculture industry in a changing climate.

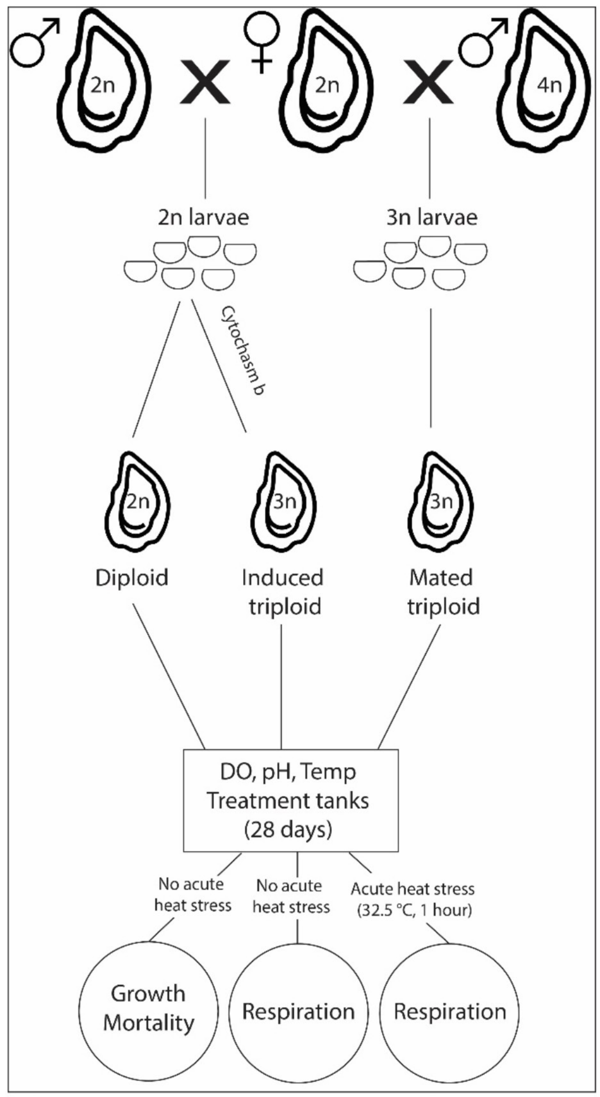

Figure 1. Outline of experimental design showing spawning, treatment and responses measured.

INVESTIGATING WATER QUALITY IMPROVEMENT DUE TO RESTORATION OF SUBTIDAL EASTERN OYSTERS *Crassostrea virginica* IN PAMLICO SOUND

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Oysters are a filter feeding species of bivalve that have been known to have significant positive contributions to water quality. Over many years, populations of Eastern oysters have been decimated due to factors such as habitat loss, pollution, over-harvesting, changing environmental conditions, and other human impacts. Oyster restoration projects such as the Oyster Sanctuary Program in North Carolina aim to rebuild oyster populations through artificial reefs. According to the NC Division of Marine Fisheries, the Oyster Sanctuary Program encompassed an estimated 788 acres as of September 2023. Previous investigations of the impacts of oyster restoration have found that large-scale restoration efforts have positively contributed to water quality and that these changes are detectable by remote sensing. However, these studies have primarily focused on oyster reefs in the Chesapeake Bay, and water quality impacts in North Carolina have not been investigated at this scale. This project aims to investigate the water quality impacts of subtidal oyster restoration in the Pamlico Sound in Eastern North Carolina, including changes to water quality improvement speed following natural disasters such as hurricanes.

Using imagery from the NASA Aqua satellite and the onboard Moderate Resolution Imaging Spectroradiometer (MODIS), we intend to analyze changes in Total Suspended Matter (TSM) at n = 6 sites in the Oyster Sanctuary Program as well as control sites which have not been restored. The chosen restored sites are fully encompassed by the archival imagery and data available, with the earliest TSM measurements available from 2012, the earliest restoration beginning in 2013, and the latest restoration ending in 2022. This remotely sensed information will be utilized alongside survey data from NC Division of Marine Fisheries' annual survey of all sites in the Oyster Sanctuary Program network. This will allow an analysis of restoration impact by acreage, density, and by materials used for artificial reef construction. By evaluating these factors, we will investigate differences in water quality impact based on restoration methods, siting, and sanctuary size.

Figure 1: Map of Oyster Sanctuary Program sites in Pamlico Sound. Sites included in this study are indicated in red.

NUTRIENTS PROVIDED BY SEABIRDS AT FLOATING FARMS APPEAR TO GENERATE BIOGEOCHEMICAL HOTSPOTS

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Seabirds can aggregate in large numbers on the structure provided by floating shellfish operations (hereafter farms), particularly during the fall/spring migrations. We have previously estimated bird-related nitrogen (N) fluxes on the scale of 0.2-16.9 g of nitrogen m^{-2} y^{-1} at several North Carolina farms, putting bird nutrient inputs on a similar scale as other major estuarine N fluxes. However, the biogeochemical effects of concentrating avian biomass—and consequently associated nutrient inputs—over these farms is unknown.

Given the relative insolubility of uric acid, the primary N component of guano, and its likely degradation in ammonium (NH_4^+) at the benthic-water interface, we sought to determine if water column [NH_4^+] at floating farms tracked with observed changes in bird biomass. Surface water samples were collected from the edge of five floating farms and paired, fixed-point reference locations biweekly during a daytime low tide from July to December (n=60; only first 40 shown). Samples were analyzed fluorometrically using the OPA method. The degree to which farm [NH_4^+] exceeded expected (i.e. reference) concentrations was positively correlated to observed bird biomass (Figure 1), suggesting that farm-scale biogeochemical hotspots may form from bird nutrient inputs. In environments where N is often limiting, the introduction of these nutrients may support local water column and benthic primary productivity and enrich food webs.

This work is a step towards understanding how the bird habitat created by floating farms can direct bird nutrients into farms and farm-adjacent ecosystems. It may be possible to place farms or farm-associated gear strategically to maximize benefits to birds and harness their nutrients in ways that (re-)create nutrient subsidies lost in many developed settings. Going forward, scientists and managers must work to determine if the potential ecological benefits of bird aggregations at floating farms can be balanced with the potential for shellfish contamination, as a best-case scenario may be one in which the ecological functions of these farms are maintained while human health risks are minimized.

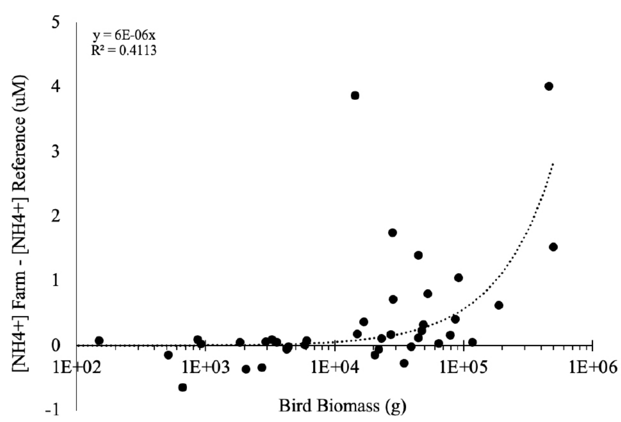

Figure 1. The relationship between seabird aggregation size and surface [NH_4^+].

GLUTAMATE POSTSYNAPTIC RECEPTORS IN VISCERAL GANGLIA OF THE BIVALVE *Crassostrea virginica* ARE IONOTROPIC TYPE RECEPTORS

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Glutamate (Glu) neurons are major excitatory neurons in mammalian brains and various invertebrate ganglia. In humans, disfunction of Glu neurons are associated with a number of disorders including Parkinson's disease, Alzheimer's disease, Huntington's disease, autism, depression and schizophrenia. In higher animals Glu receptors are classified as ionotropic NMDA, AMPA and kainate types; or metabotropic group I, II, III and GluR7 types. NMDA type Glu receptors recently have been reported to be involved in regulating bivalve metamorphosis in *C. gigas*, *Mercenaria mercenaria* and *Mya arenaria*. Other studies also are showing that genes for ionotropic Glu NMDA (iGluRs) type are present in *C. gigas*. In *C. virginica* and other studied bivalves, gills are innervated by serotonin and dopamine nerves from their visceral ganglia (VG). Serotonin is cilio-excitatory, while dopamine is cilio-inhibitory to gill lateral cell (GLC). Recently our lab detected Glu neurons in ganglia of *C. virginica*, and showed they excite serotonin neurons to increase GLC cilia beating rates. Using immunohistochemistry and PAGE and Western Blotting we also showed the presence of the ionotropic GluR1 receptor in the VG. Previously, the presence of Glu neurons had not been reported in the adult *C. virginica* or to have a physiological function. In this project we sought to further study the physiology of ionotropic Glu receptors in VG of *C. virginica*. We hypothesize that applying a GluR1 agonist to the VG of *C. virginica* would stimulate the receptor, whereas a GluR1 antagonist would block the receptor, preventing the cilio-excitatory actions of Glu on the GLC. To test this, we used VG preparations in which the VG innervation to the gill is kept intact. We examined effects of applying a GluR1 ionotropic agonists and antagonists to the VG while we observed GLC cilia beating rates. Shells were removed and preparations placed into chambers with a barrier so drugs could be discretely applied to the VG without coming in contact with the gill. Beating rates of GLC cilia were measured by stroboscopic microscopy. Our results show that applying the ionotropic agonist homocysteic acid (10^{-5} – 10^{-3}M) to the VG caused a dose dependent increase in cilia beating from 12 to 18 beats/second. Applying 10^{-5}M of the ionotropic antagonist DL-2 amino-5-phosphonopentanoic acid (DL2) to the VG prevented Glu (10^{-5} – 10^{-3}M) from increasing cilia beating rates. The study thus far confirms a physiological role for Glu as an excitatory neurotransmitter in the VG, most likely exciting serotonin neurons to cause an increase in GLC cilia beating rates. The results of the ionotropic agonist homocysteic and antagonist DL2 indicate that the Glu receptor type is ionotropic. The bivalve mollusc gill is a useful model to study regulatory mechanisms of ciliary activity as well as the pharmacology of drugs affecting biogenic amines and other neurotransmitters.

This work was supported in part by grants 2R25GM06003 of the Bridge Program of NIGMS, 0537231071 of the CSTEP Program of NYSED, P120A210054 of the MSEIP Program of the DoEd, and NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University.

INVESTIGATION OF THE PRESENCE AND PERSISTENCE OF BACTERIA IN SEAWATER AND OYSTERS *Crassostrea virginica* FROM SALLY COVE IN REHOBOTH BAY, DELAWARE

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The Eastern oyster (*Crassostrea virginica*) is consumed raw by nearly twenty million Americans and can filter approximately five to twenty-five liters of water per hour while accumulating various disease-causing contaminants within its tissues. These diseases are more likely to be caused by bacteria, as they contribute to most seafood-borne infections. Sally Cove in Rehoboth Bay (Longitude: Latitude; 075˚ 07.631' W: 38˚ 38.932' N) is one of the oyster aquaculture farms along the Atlantic coast of Delaware that produces oysters through off-bottom and bottom cultures for consumption in Delaware. However, the risk of bacterial contamination from consuming raw oysters from this farm is unknown. The objective of this study was to evaluate the presence and persistence of diverse bacteria in seawater and oysters from off-bottom and bottom cultures at Sally Cove. A control site within Sally Cove that is without oyster cultures was included in this study.

Six liters of seawater and twelve oysters each from the off-bottom and bottom cultures at Sally Cove and six liters each of off-bottom and bottom seawater from the control site were collected once per month from July to October 2023. The seawater and oysters were processed, pre-enriched, and cultured on selective media to obtain colonies of targeted bacteria.

Molecular confirmation **(Figure 1)** with PCR and rt-PCR showed that *Vibrio parahaemolyticus,* Shiga-toxin-producing *Escherichia coli*, *Salmonella enterica, Shigella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were present and persisted in seawater and oysters from both cultures at the farm throughout the study. These findings indicate that consuming raw oysters from Sally Cove poses contamination risks from several bacteria, predominantly in summer months.

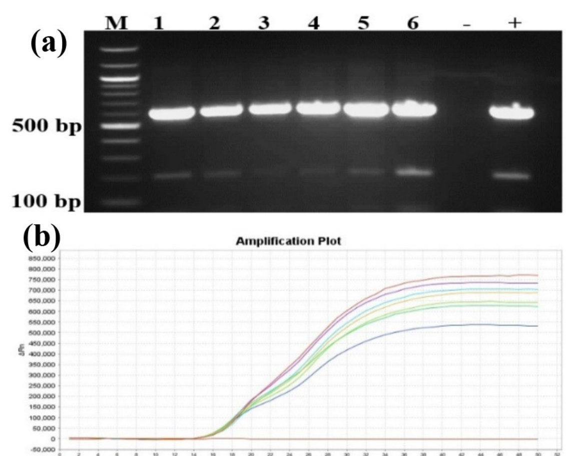

Figure 1(a-b). Presence of *Shigella* spp. in seawater and oysters sampled in July from Sally Cove and control site.

Note: M- 100 bp ladder, 1- Bottom seawater from Sally Cove, 2- Off-bottom seawater from Sally Cove, 3- Bottom seawater from control site, 4- Off-bottom seawater from the control site, 5- Bottom oysters from Sally Cove, 6- Off-bottom oysters from Sally Cove, 7- Negative control, 8- Positive control.

EFFECTS OF TEMPERATURE VARIATION ACROSS GEAR TYPES AND LOCATIONS ON BAY SCALLOP GROWTH AND SURVIVAL

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This study investigates how water temperature variation impact growth and survival in bay scallops (*Argopecten irradians*) across different environments and gear types. The research evaluates scallop performance in both nursery and grow-out settings, focusing on seasonal temperature effects, especially in the fall and winter. The study's objectives are to (1) assess temperature variation across gear types (bottom-based vs. floating), (2) evaluate scallop growth and survival in different locations, and (3) identify optimal gear configurations for productivity and sustainability in different seasons.

The study is conducted at Roger Williams University's outdoor flow-through nursery system and a 2-acre aquaculture farm in Mount Hope Bay, Rhode Island (RI Coastal Resources Management Council File No. B2022-04-046). Gear types include bottom cages, floating plastic baskets, and boxed bags, each stocked with 150 scallops of uniform size. Onset HOBO® pendant temperature loggers record data hourly, enabling precise monitoring of temperature variation across gear types. Additional environmental parameters, including pH, dissolved oxygen, total ammonia nitrogen (TAN), and nutrient levels, are monitored to assess the broader environmental impact on scallop performance. Statistical methods, including linear mixed models (LMMs) and analysis of variance (ANOVA), will be used to assess temperature variability and performance across gear and location settings.

We hypothesize that floating gear will show greater temperature variation and thus more variable growth rates, while bottom-based systems may provide stable temperatures that enhance survival rates. Analyzing seasonal temperature fluctuations, especially during colder months, provides data-driven insights on gear suitability for varying environmental conditions. This study will guide best practices for bay scallop farming, optimizing growth and supporting sustainable aquaculture in changing conditions. Findings aim to improve economic viability and sustainability, guiding aquaculture producers toward strategies that enhance productivity and environmental resilience.

PROSOCIAL MANAGEMENT OF A PARASITE IN A FOOD PRODUCTION NETWORK: EVIDENCE FROM SALMON LICE IN AQUACULTURE

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We model and evaluate prosocial management of a parasite in a food production system. With contagion effects a food production system can be modelled as a production network with parasites as a bad intermediate input. We highlight the important difference between the physical and economic production network when regulating the system. Well defined property rights aligns the two networks, generating prosocial behavior eliminating the external diseconomy due to cross-site parasite contagion, and moves the system closer to the social optimum.

Empirically we test and verify the relevance of prosocial behavior by comparing parasite treatment and outcomes for independently owned and common owned production sites in Norwegian salmon aquaculture.

We show that common owned production sites demonstrate consistently lower spatial clustering of parasites, and significantly higher spatial coordination of treatment. Furthermore, we document a positive relationship between the geographic density of independently owned sites and the extent of parasites at site level. This density relationship is not present for common owned sites. We use seaport access as an instrument variable to identify excess clustering of independently owned sites and quantify the resulting causal effect of site density on parasites in the system.

Our results provide evidence for pro-social management of salmon lice among common owned sites suggesting that siting policies should distinguish between common owned and independently owned sites.

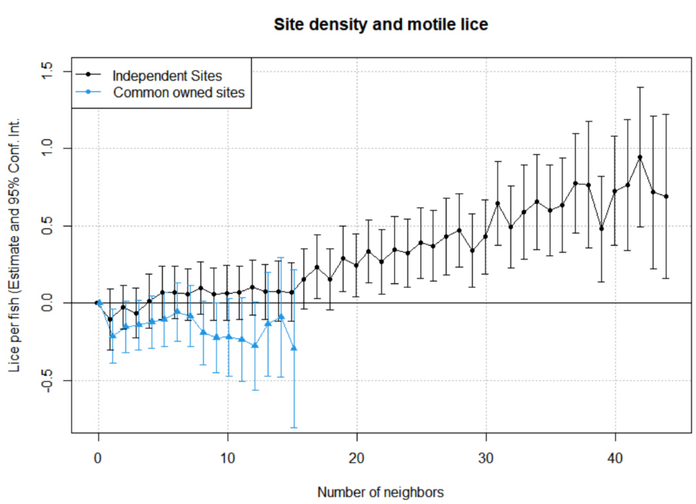

FIGURE 1. *Site density and motile lice.*

DOWN ON THE FARM: EXPLORING THE EVOLUTION AND POTENTIAL OF ALTERNATIVE OYSTER CULTURE IN LOUISIANA

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Louisiana's oyster industry has long relied on oystermen who harvest by traditional dredge methods from the 1.7 million acres of public reefs or from their managed 400,403 acres of private leases from the state. From 2003 through 2022, Louisiana accounted for 32.3% of the nation's oyster landings and produced 7,220,000 pounds of oyster meat in 2022 alone. This traditional harvest practice persists today, but the emergence of Alternative Oyster Culture (AOC) has introduced a complementary approach: off-bottom oyster farming using floating cage systems. This "Down on the Farm" session explores the historical context and future potential of this innovative sector within Louisiana's oyster industry.

Since about 2007, the influx of freshwater from natural and human-induced processes has diminished the principal public oyster grounds on the east side of the Mississippi River resulting in a precipitous transition from an approximate 50:50 public-private production split to the private leases now accounting for over 95% of the state's oyster landings. This shift has increased harvest pressure on private oyster leases and focuses on water bottoms which may not be suitable for traditional harvest methods but could be suitable for off-bottom AOC cage culture.

In the past two decades, off-bottom oyster culture has spread across Louisiana and other northern Gulf and South Atlantic states. Louisiana boasts a deep-rooted fisheries tradition, and many of the AOC farmers, even if they are not traditional commercial fishers themselves, often have a family history in the oyster industry spanning generations. At present, most of the AOC farmers operate within four aquaculture maritime zones i.e., aquaculture parks, where water bottoms are subdivided into smaller leased plots, facilitating easier entry into AOC operations.

Louisiana's AOC parks include Grand Isle (53 acres), Cameron (47 acres), and Dos Gris, a private park near Grand Isle (48 acres). Besides the three aquatic parks, totaling 148 acres, there are presently another 117 acres of AOC permitted water bottoms located on privately owned or state-leased land. Initially, AOC grew slowly but is now gaining momentum, with all three Louisiana AOC parks fully leased. This maritime zone model can potentially continue to expand within Louisiana's private and traditionally leased water bottoms.

A notable initiative in this area is a branding and marketing campaign led by the Jefferson Economic Development Commission (JEDCO), in collaboration with the Grand Isle Port Commission and Louisiana Sea Grant. This campaign promotes Grand Isle's off-bottom oysters, cultivated within the Grand Isle Park, through marketing videos, brand graphics, and a dedicated website to enhance the visibility and appeal of Grand Isle oysters. Although initially met with little interest within the state, AOC aims to diversify the oyster industry, and its growth continues.

PROVIDING BETTER ACCESS TO MEDICATED FEED FOR KENTUCKY AQUACULTURISTS AND A SUBSEQUENT SURVEY OF THEIR OPINIONS AND PERCEPTIONS OF THIS SERVICE

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Fish farmers in Kentucky and surrounding states are often faced with challenges when trying to obtain medicated feed to treat internal bacterial infections of their fish. The lack of infrastructure in the region (feed mills, supply sources, etc.) causes fish farmers to choose treatment options other than medicated feed due to difficulties in obtaining it and ordering medicated feed can be expensive due to ordering small quantities with high shipping costs. Most fish farmers in this part of the country report that it takes approximately 11-15 days for the medicated feed to arrive, which is after the peak of the disease mortalities. Objectives of the project were:

- To evaluate the perception of farmers towards the effectiveness of medicated feed treatment options,
- To evaluate the perception of farmers to the accessibility of medicated feed (containing Aquaflor®, Romet® or Terramycin®) in Kentucky and surrounding states influenced by establishment of the Kentucky State University Aquaculture Research Center (KSU ARC) as a VFD distributor, and
- To evaluate the perception of farmers towards the effectiveness of fish health services of KSU ARC.

The outcome of this research project may assist in reducing economic losses currently experienced by fish farmers in Kentucky and surrounding states and foster the growth and sustainability of fish farming in the region.

SEXUAL GROWTH DIMORPHISM IN JUVENILE SOUTHERN FLOUNDER *Paralicthys lethostigma*

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The southern flounder (*Paralicthys lethostigma*) is a large commercially and recreationally important flatfish common in coastal waters of the southeastern US. Fisheries stocks show signs of decline across the Gulf of Mexico. Because of its fast growth rate, excellent flesh quality, euryhaline habits, and the limited and unpredictable availability from fisheries, this species has drawn interest from the aquaculture industry. However, southern flounder females reach much larger sizes than males, which reduces productivity in farms if mixed-sex stocks are cultured. The sexual growth dimorphism must be understood and managed to optimize aquaculture potential, but to date little data on sex-specific growth patterns in controlled environment are available. The objective of this work is to describe sex-specific growth rates in southern flounder from the juvenile stage to market size by tracking individual growth of males and females.

On 8/29/2024, one hundred and twenty 200-day post-hatch (DPH) southern flounder juveniles (mean total length 12.79 ± 2.03 cm, mean weight 24.87 ± 1.15 g) were randomly selected, individually tagged with a full duplex nano 8mm x 1.25mm magnetic PIT tag, and stocked at 20 fish/tank in 6 blue fiberglass tanks (1572 L each) connected to two recirculating systems maintained at a salinity of 25 ppt and a temperature of 26°C. Fish are being fed a sequence of extruded pellets (Skretting, Nutreco; 55% crude protein and 18% lipid) at a rate of 5.24%/body weight/day. Feed ration is adjusted every 7 days to account for growth and biomass change. All fish are measured (total length and body width) and weighed every six weeks. Growth monitoring will continue until fish reach an average Total Length of 12 inch.

Sex will be determined at the end of the experiment by observation of gonad morphology. Growth rates of individual males and females between sampling points and through the experimental period will be estimated using the Thermal Growth Coefficient (TGC) and Specific Growth Rate (SGR). Alternative models will be considered if growth trajectories depart from the simple models used in these two coefficients. ANOVA will be used to test the significance of differences between sexes accounting for replicate recirculating system and replicate tank within system. At the end of the experiment, morphometric and carcass quality traits including gut, visceral fat, gonad, filet and fileting waste yields will be recorded and compared between sexes after correction for allometric growth.

USE OF FLUORESCENT MICROSPHERES TO DEFINE LIVE FOOD ORGANISM PREFERENCES OF LARVAE OF YELLOW DOMINO DAMSELFISH (*Dascyllus auripinnis*), SPINECHEEK CLOWNFISH (*Amphiprion biaculeatus*) AND PIGFISH (*Orthopristis chrysoptera)*

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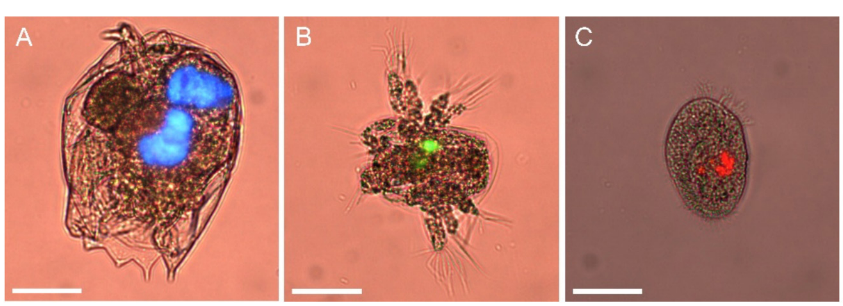
Successful culture of marine fish is reliant on feeding live feed organisms to small larvae to maximize survival. Currently most larvae are cultured by feeding live rotifers and *Artemia* nauplii and occasionally copepod nauplii. Larvae consume various zooplankton in the wild, including copepod nauplii, ciliates, and less commonly rotifers. Research was conducted to define live food preferences during the early larval stages of three marine fish larvae.

This study used different colored fluorescent microspheres (1-2 µm diameter) to mark different prey organisms and then fed them to larvae at specific densities. Larvae were yellow domino damselfish at 1 and 3 days post hatch (dph), spinecheek clownfish at 0 dph, and pigfish larvae at 3 dph. Rotifers (*Brachionus rotundiformis*), copepod (*Parvocalanus crassirostris*) nauplii, and the ciliate (*Euplotes* sp.) were each marked with different colored fluorescent microspheres (Figure 1) and fed in different combinations to larval fish. Larvae were preserved and placed under a fluorescent microscope which clearly identified the prey organisms consumed.

Results indicated that rotifers were the least preferred by all species, while copepod nauplii were favored by most larvae, confirming their importance as a primary feed organism. Ciliates, a novel live food organism not commonly used in aquaculture, were consumed by all larvae, highlighting their potential as a valuable prey organism especially for small larvae that cannot consume larger prey due to their small mouth gapes. Variations in prey preferences by species at different developmental stages justifies further research to investigate feeding novel prey types at different developmental stages and to define shifts in prey preference as larvae grow.

Figure 1. Rotifer (*Brachionus rotundiformis*) marked with blue microspheres (A). Copepod nauplii (*Parvocalanus crassirostris*) marked with yellow-green microspheres, Ciliate (*Euplotes* sp.) marked with red microspheres.



DEVELOPMENT OF RELATIVE TEMPERATURE GROWTH INDEX (RTGI) FOR LARGEMOUTH BASS *Micropterus nigricans*

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Growth of Largemouth Bass (LMB) as a food fish in Kentucky is constrained by a modest growing season. Understanding the relationship between temperature and growth is instrumental in developing a practical feeding protocol for LMB to take full advantage of growth potential throughout the year.

Two separate studies were conducted at different times over a 9-week period at the Aquaculture Research Center of Kentucky State University. In each study, seven recirculating systems with each system comprised of four replicate aquarium tanks were randomly assigned temperature treatments. Study 1 evaluated 15, 18, 21, 24, 27, 30, and 33 °C and Study 2 evaluated 9, 12, 15, 18, 21, 24, and 27 °C. Both studies utilized feed-trained juvenile LMB of the same size. The first study featured six fish per (tank size) tank (57.5±1 g), while the second study used 10 fish per tank (56.2±1.8 g). Fish were hand-fed once daily to apparent satiation using a slow-sinking 6.5 mm commercial trout feed (45% protein, 20% fat). Water quality was monitored weekly to ensure optimal conditions for growth. The batch weight of each treatment was recorded at stocking and harvest.

Regression analysis of the combined data (figure 1) revealed the following equation:

SGR = 0.0187 - 0.00367 C + 0.000243 C^2 - 0.00000434 C^3 where C is temperature (°C) and SGR is specific growth rate (%BW/day). Maximum SGR was 1.113 at 26.8 °C and R^2 was 0.91. RTGI was created by assigning this maximum SGR a value of 100% and generating a data set across the temperature range for SGR values divided by 1.113. The resulting relationship creates an equation describing relative growth and temperature based on data from 60 g LMB (Figure 2): RTGI = 1.494 - (0.301 * T) + (0.0204 * T^2) - (0.000367 * T^3), where T is water temperature in degrees Celsius.

Figure 2: Relationship between Specific Growth Rate and Temperature

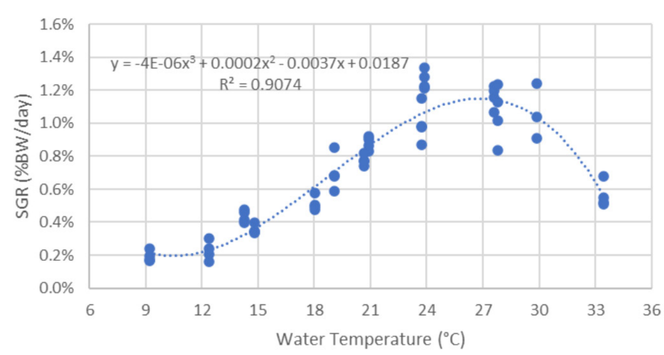
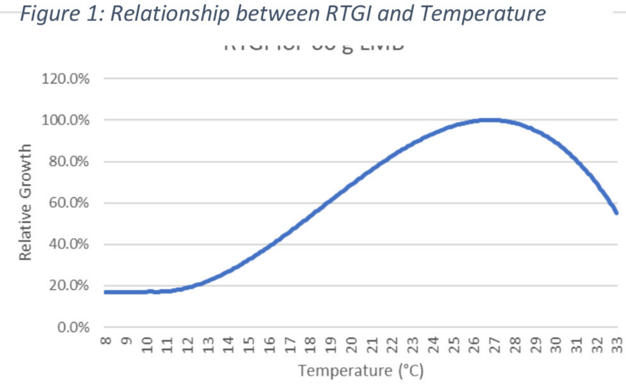


Figure 1: Relationship between RTGI and Temperature



INVESTMENT ANALYSIS OF S^{3}AM ADOPTION IN OYSTER FARMS: EVALUATING THE FEASIBILITY OF PURCHASE Vs. SUBSCRIPTION OPTIONS

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The Smart Sustainable Shellfish Management (S^{3}AM) framework combines aquatic drone and monitoring technology to enhance the efficiency and sustainability of oyster production. It provides advanced tools for assessing sub-tidal lands for profitable lease development, precision seed planting on the seafloor, monitoring inventory growth, and optimizing harvests. These features aim to boost productivity by improving seed survival rates, reducing fuel usage, and lowering labor hours through guided harvesting.

S^{3}AM technology offers oyster growers two main adoption options: purchasing the technology outright or subscribing to a service model. In the purchase model, growers face a high upfront cost, which could slow adoption, especially for smaller farms. This initial investment might also lead to a digital divide, as only larger, financially secure farms may be able to afford the technology suite (Rotz et al., 2019). However, a subscription-based model, where growers pay a fixed fee for services like mapping, monitoring, and inventory management without purchasing the entire technology, provides a more accessible alternative. This subscription model allows smaller farms to access smart production technology by purchasing only the data needed for their operations.

Given that most oyster growers in Maryland are small-scale, our study seeks to determine which of these two options would optimize farm profitability in the long run. We will conduct an investment analysis for both purchasing and renting options, comparing their viability for Maryland's growers. The study will employ investment appraisal techniques—including Return on Investment (ROI), Net Present Value (NPV), Modified Internal Rate of Return (MIRR), and Payback Period—to evaluate the financial outcomes of both models and provide growers with information for their decision-making. Our investment analysis will be unique, as S^{3}AM is a monitoring technology designed to enhance production efficiency by reducing errors and time spent on tasks. Unlike full automation technologies that replace labor, S^{3}AM complements labor, boosting productivity per labor hour, which can then be traded for additional paid work or leisure and can be added in the model as additional gain.

For this study, we will use the 2018 enterprise budget as a baseline and simulate results from over 10,000 hypothetical oyster farms. The purchase model will consider a quasi-fixed cost, while the subscription model will use a fixed cost structure. We will compare results across three production scenarios: farms with outputs of 200 bushels, 2,000 bushels, and 6,000 bushels, focusing on Maryland, where traditional bottom culture is the primary oyster production method. The results from this study will guide Maryland's oyster growers in making informed technology adoption decisions based on production scale and financial outcomes.

BIO-ECONOMIC ASSESMENT OF NON-CONVENTIONAL PROTEIN SOURCES IN NILE TILAPIA (*Oreochromis niloticus*), USING NOVEL FUTA AQUA-FEED FORMULATON SOFTWARE

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This study evaluated the bio-economic assessment, growth performance, and nutrient utilization of non-conventional protein sources as substitutes for traditional feed ingredients in Nile tilapia (*Oreochromis niloticus*) diets. Four test diets were formulateded with various combinations of non-conventional protein sources, including agricultural by-products, rendered animal meals, and insect components, detailed in Table 4. These diets were formulated using the novel FUTA aquafeed software and administered to tilapia over a period of 56 days. Biological parameters such as weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival rate were compared with the control diet containing the conventional fishmeal. Economic analysis, based on feed formulation costs and production yields, was also conducted. The results presented indicated that significantly higher (P>0.05) mean weight gain, FER, PER and better FCR was recorded in fish fed with a diet comprising 30% poultry viscera meal and 10% Mopane worm meal (Diet C), in comparison to the control and diets containing Palm kernel cake (Diet A) and blood meal (P<0.05). However, the control diet recorded the best profitability per unit biomass produced. These findings highlighted the potential for the widespread adoption of specific non-conventional feed ingredients, including blood meal, poultry intestine, mopane worms, water fern, palm kernel meal, and wheat meal, to decrease feed costs without compromising tilapia growth.

TABLE 01: Gross Composition of the Experimental Diet (g/100g) for Culturing *Oreochromis Niloticus*

FEED STUFFS	CONTROL	DIET A	DIET B	DIET C
Blood meal	0.00	32.50	0.00	0.00
Palm kernel cake	0.00	13.00	0.00	0.00
Water fern	0.00	9.00	0.00	0.00
Wheat meal	0.00	0.00	19.00	0.00
Mopane worm	0.00	0.00	20.00	22.0
Poultry Intestine	0.00	0.00	0.00	18.00
Ground nut cake	8.00	0.00	0.00	0.00
Fish meal	44.10	0.00	0.00	0.00
Soya bean meal	7.90	0.00	23.44	16.00
Maize	30.0	26.50	25.00	25.00
Soya bean oil	6.00	5.00	5.00	5.00
Alginate	2.00	2.00	2.00	2.00
Vitamin	2.00	2.00	2.00	2.00
Methionine	0.00	5.00	5.00	5.00
Lysin	0.00	5.00	5.00	5.00

INCORPORATING AI/MACHINE LEARNING INTO A MOBILE RESPONSIVE CLINICAL FISH HEALTH DATABASE

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Aquaculture plays a crucial role in food security but faces challenges like infectious diseases. Pathogens, including viruses, fungi, bacteria, and parasites, can cause significant losses, especially in closed systems like Recirculating Aquaculture Systems (RAS). Early disease detection is crucial to prevent mortality that can even be up to 100%.

This project aims to integrate machine learning/AI into the current Mobile Responsive Clinical Fish Health Database created at Kentucky State University (KSU). This integration will allow the database to analyze images and videos of fish to identify disease progression in different species, including clinical signs such as scale loss, abnormal skin growth, and the early stages of lesions. In addition, abnormal fish behavior will be documented and compared to healthy fish.

The project initially focuses on the early detection of columnaris disease in largemouth bass, caused by *Flavobacterium columnare*, using machine learning. This approach would help detect diseases several days earlier than usual, giving fish farmers a head start on treatment and reducing fish mortalities. The project will also expand the database's capabilities to include other pathogens such as Saprolegnia fungus, *Ichthyophthirius multifiliis*, and Aeromonas bacteria. Data from U.S. diagnostic labs will enhance a database for proactive fish health management, benefiting aquaculturists worldwide by improving disease management and reducing losses.

WOMEN AND YOUTH PARTICIPATION IN COASTAL AQUACULTURE ACTIVITIES IN SOUTHWEST NIGERIA

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The contribution of women and youths to the economies of developing nations differs from sector to sector. Aquaculture being the fastest growing food production sector in the world holds high potential for engaging vulnerable societal groups in its value chain.

In this study, therefore, the perspective of coastal aquaculture stakeholders from the southwestern region of Nigeria was investigated on the role of women and youth in the aquaculture value chain. The study's data was gathered using field observations and key informant interview of 44 stakeholders from Ondo, Ogun, and Lagos states in Nigeria.

The responses obtained were quantitatively analysed, and the results obtained are presented herein. The study shows that cultured fish are predominantly freshwater finfishes of indigenous (Catfishes and Tilapias) and exotic (e.g. Carp, *Pangasius*) origins despite the close proximity to the ocean in the study area.

The responses received suggest that men, women, and youth are pretty involved in all aspects of the aquaculture sector activities understudied in the southwest coastal regions of Nigeria. However, the level of involvement appears to be biased, differing from one activity to another. The activities of men were more into farming, harvesting, and transportation compared to those of women, who dominated the post harvest activities which include packaging, marketing, and sales.

Also, adults seem to be more engaged in the aquaculture value chain than youths in all the activities evaluated. Nevertheless, the level of involvement of all groups seems to be influenced by traditional role prescription in the society.

Diversifying the aquaculture species that can futher engage youth and women in mariculture practices is recommended.

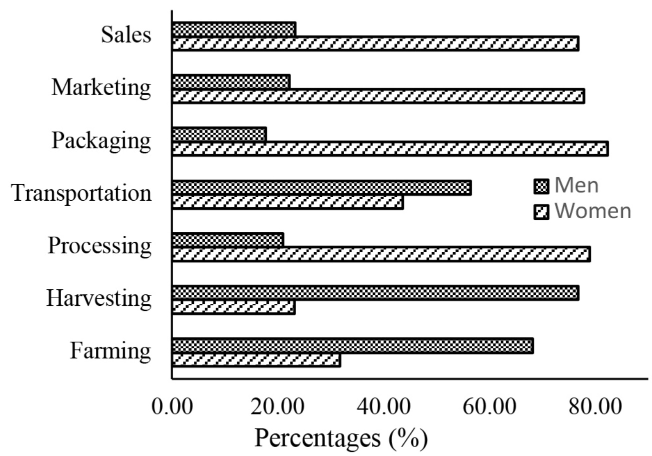

Figure 1: Gender involvement in the aquaculture activities of Southwest Nigeria

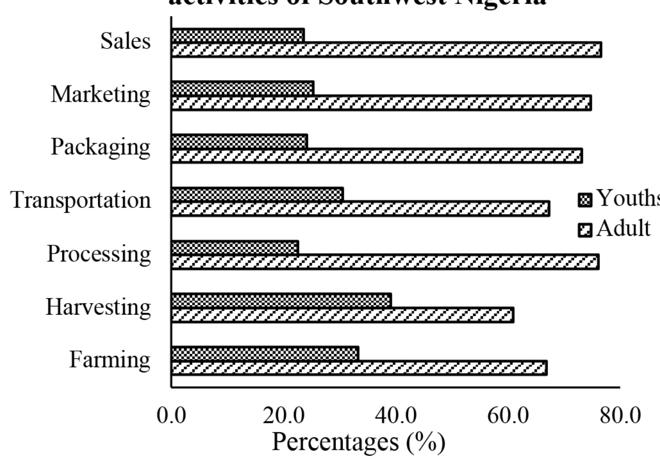

Figure 2: Youth and adult involvement in the aquaculture activities of Southwest Nigeria

HUMIC SUBSTANCES COMBINED WITH BUTYRIC ACID AND/OR YEAST CELL WALLS PROMOTES GROWTH PERFORMANCE AND RESISTANCE TO *Flavobacterium covae* AND *Edwardsiella ictaluri* CO-INFECTION IN CHANNEL CATFISH *Iclaturus punctatus*

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Bacterial infections pose a persistent threat to U.S. catfish aquaculture. Pathogen interactions have also been shown to drive disease and increase mortality in catfish ponds, thus complicating treatment interventions. Previously, adding humic substances to diets has shown antibacterial and immune influences in fish, including improving channel catfish resistance when experimentally challenged with *Flavobacterium covae*. However, their prospect against polymicrobial infection is unknown. This study investigated the potential of dietary humic substances with butyric acid (HS & BA) as catfish immunomodulators for growth performance and resistance to co-infection with two virulent catfish pathogens. Yeast cell wall (YCW) components, an immunostimulatory polysaccharides, were also added as a treatment factor.

A feeding trial was conducted in a recirculating aquaculture system with channel catfish *Ictalurus punctatus* fingerlings (19.7±0.2g). Fish were offered either a practical basal diet (Basal; 32%), a diet supplemented with IFC4 (HS & BA blend; 4 lb/US ton; 32%), a diet supplemented with YCW (1 lb/ US ton; 28%) or a diet supplemented with both IFC4 (2 lb/US ton) and YCW (0.5 lb/ US ton) (IFC4+YCW). After 45 d, catfish were pooled by treatment and transferred into a biosecure wet lab for an *in vivo* pathogen challenge. All treatment groups were contested in an immersion challenge using triplicate tanks with either a single dose of *Edwardsiella ictaluri* (S97-07; 7.4 x 10^6 CFU mL^{-1}), *F. covae* (ALG-00-530; 1.2 x 10^6 CFU mL^{-1}), or co-infection with simultaneous half-doses of *E. ictaluri* and *F. covae*. Fulton's condition factor post-feeding differed across groups (*P*=0.014), with an increase in IFC4 catfish compared to the Basal group (*P*=0.009). Following the 14-day bacterial challenge, cumulative percent mortality (CPM) comparisons showed differences in infection type (*P*=0.011) and across dietary treatments (*P*=0.004) with no significant interaction effects (*P*=0.598). CPM increased in the co-infected group compared to the single *E. ictaluri*-infected catfish group. No mortality was observed in the single *F. covae* groups due to the low immersion dose. Single infection CPM with *E. ictaluri* across treatments were IFC4 (22%), YCW (30%), IFC4+YCW (38%) and Basal (48%). Overall CPM increased in the co-infected groups (IFC4 (28%), YCW (55%), IFC4+YCW (70%), and Basal (70%)), with lower mortality in IFC4-fed catfish compared to the Basal (*P*=0.010) or YCW (*P*=0.010) groups.

These research findings have potential practical implications for fish health, providing an alternative tool for managing co-infections in the U.S. catfish industry. This information provides the industry with actionable knowledge to make informed decisions and develop effective strategies for disease management.

BACTERIAL COMMUNITIES ASSOCIATED WITH ZOOPLANKTON IN A HYBRID (*Ictalurus punctatus* × *I. furcatus*) CATFISH POND

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The early diet of catfish fry consists primarily of zooplankton. Therefore, the zooplankton-associated microbiome represents some of the first microbes catfish come into contact with. These microbes may be an important contributor to establishing the catfish gut microbiome and subsequent health and production outcomes. In the present study, the bacterial communities associated with four zooplankton taxa commonly observed in catfish ponds (*Ceriodaphnia* spp., ostracods, calanoid copepods, and cyclopoid copepods) were characterized. Additionally, different quantities of organisms were evaluated to determine appropriate methods for future studies.

Zooplankton samples were obtained from a single hybrid (*Ictalurus punctatus* × *I. furcatus*) catfish pond in Stoneville, MS, via water sampling with a 80 µm mesh Wisconsin-style net. The sample was filtered through a glass-microfibre disc filter. Then individual zooplankton were sorted taxonomically into culture plates containing deionized water. Zooplankton from each taxonomic plate were filtered again and rinsed with nuclease-free water. Individual zooplankton were then aliquoted into microtubes containing a RNA stabilization buffer in quantities of 5, 10, and 20 organisms per tube, for a total of 12 samples (3 quantities each of 4 taxa). DNA was extracted from the samples, subjected to amplification of the 16S rRNA gene using primers 27F and 1492R, and sequenced on an Oxford Nanopore Technologies GridION. Reads were quality-filtered, mapped to the Greengenes2 database with minimap2, and taxonomic classifications made with a lowest common ancestor approach. Diversity metrics, statistical analyses, and plotting were completed using R.

Ten organisms was the lowest quantity tested which provided sufficient DNA yield for all taxa. Although composition of communities varied among the different quantities within each zooplankton taxa, composition was more different among zooplankton taxa. Communities were primarily composed of species from the phyla Proteobacteria and Bacteroidota. Proteobacteria was the only phyla with at least 30% relative abundance in almost all *Ceriodaphnia* spp., calanoid copepod, and cyclopoid copepod samples. Ostracods had high relative abundances of Proteobacteria as well (34-44%), but with substantial proportions of Bacteroidota (29-40%). Ostracods had the richest communities, containing an average of 1523 bacterial species, compared to averages between 532 and 974 for the other taxa. These results provide valuable information for guiding additional studies of the microbiome associated with zooplankton in catfish aquaculture ponds. Additionally, the presented data represent the first characterization of the bacterial communities associated with this critical food source for catfish fry.

BIOGEOCHEMICAL WATER QUALITY MODELING OF PROPOSED PACIFIC OCEAN AQUAFARMS SITES - A PRECEDENT APPROACH FOR PERMITTING ANALYSES

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Pacific Ocean Aquafarms propose to construct and operate cage finfish (*Seriola dorsalis*) aquaculture facilities offshore of Long Beach and San Diego. An analysis of the impacts to water quality from the release of residual solids (i.e., residual feed and fecal matter) is required to inform environmental review and issuance of the EPA National Pollutant Discharge Elimination System (NPDES) permit. DHI Water & Environment Inc. was commissioned to carry out appropriately designed biogeochemical models to examine water quality impacts. The presented study applied an internationally recognized - offshore aquaculture tailored nutrient, phytoplankton, zooplankton and detritus (NPZD) modeling approach that relied on the integrated modeling capabilities of MIKE Powered by DHI, i.e. MIKE 3 FM (hydrodynamics), MIKE PT / MIKE MT (deposition of solids), and MIKE ECO Lab (Water Quality).

As apparent, hydrodynamic modeling forms the basis of the applied model complex as it provides the necessary underlying oceanic conditions that drive subsequent sediment dispersal and ECO Lab modeling. This is evident (see diagram) for the operational releases of residual solids via the solid orange line from the 'Hydrodynamics' box to the 'MIKE MT Module box', ending with, the 'Solids Deposition and Dispersion' results box. The full integration of all model components is apparent for the ECO Lab Model modeling of nutrients by the orange line from the 'Hydrodynamics' and 'Solids Disposition / Dispersion' result boxes to the 'ECO Lab' Template box. These model outputs, along with various baseline data, ultimately allow for the biological algorithms of ECO Lab to generate the required water quality results.

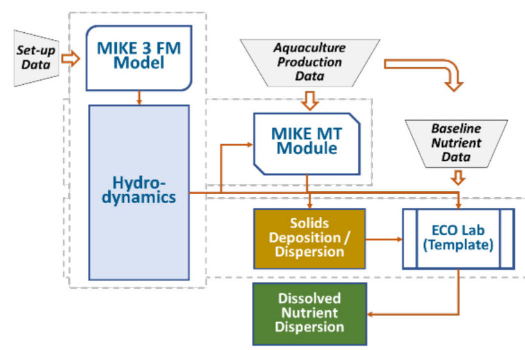

Figure 1 Diagram of the Applied Model Complex

The modeling study, which consisted of an initial phase involving 1 year of hindcast modeling and a second phase involving 5 years of hindcast modeling, produced net difference output for both predetermined compliance water quality parameters (i.e., Ammonia, Dissolved Oxygen, pH, and TSS), and a number of additional general water quality indicators (i.e., Dissolved Inorganic Nitrogen, Chlorophyll-a, Particulate Organic Carbon, Total Ammoniacal Nitrogen, and Total Phosphorous.

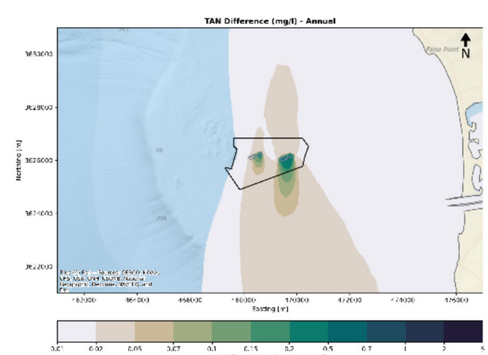

Figure 2 Example of Model output for Total Ammoniacal Nitrogen

VARIANCE COMPONENTS AND GENOMIC PREDICTION FOR HARVEST WEIGHT IN A CHANNEL CATFISH (*Ictarulus punctatus*) BREEDING PROGRAM

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The Delta Select channel catfish breeding program at the Warmwater Aquaculture Research Unit (WARU – USDA-ARS), in Stoneville, MS, started selecting animals to increase the harvest weight and carcass yield in 2006. Variance components and breeding value estimation were based on pedigree and phenotypes initially and Genomic selection was initiated in 2015. Thus, our objectives in this study were to estimate the variance components and breeding values for harvest weight and verify the accuracy, dispersion, and bias of breeding values from pedigree and genomic models.

Data were provided by WARU – USDA-ARS, with harvest weight records of 46,450 animals from 2008 to 2021, of which 12,279 were genotyped using 53,976 single nucleotide polymorphisms (SNPs). Variance components were estimated using pedigree REML and single-step genomic REML (ssGREML) algorithms as implemented in the BLUPF90+ program. To validate the estimated breeding values (EBV) and genomic estimated breeding values (GEBV) we estimated the dispersion, bias, and accuracy according to the Linear Regression (LR) method, where the focal group was composed of 1208 animals born in 2021 with genotypes, phenotypes, and complete parent information. In the LR method, (G)EBV of animals from the focal group was estimated based on a full and reduced dataset (i.e., no phenotypes for focal animals). Thus, the reduced dataset had phenotypes up to 2020 and the complete one had phenotypes up to 2021. The EBV and GEBV were predicted using REML-BLUP, REML-ssGBLUP, and ssGREML-ssGBLUP, for reduced and complete datasets using the same relationship matrices according to BLUP and ssGBLUP methodologies. Random effects included the additive direct genetic effect, the family environment effect and residual, and the contemporary group effects (year-pond-sex), and age nested within sex were fixed.

The heritability was lower in REML (0.118) than in ssGREML (0.298), also the additive genetic variance presented the same pattern (6,626 and 17,548 for REML and ssGREML). The residual and common family variances were smaller in ssGREML. REML estimates in ssGBLUP provided the most accurate and least biased GEBV (0.22 and 0.68). The accuracy using REML estimates in ssGBLUP increased by 89% and 24% compared with REML estimates in BLUP (0.36) and ssGREML estimates in ssGBLUP (0.55). The most biased predictions (0.72) and the biggest dispersion (0.80) were when using REML estimates in BLUP.

Models considering the genomic information provided the smallest dispersion and bias and the most accurate breeding values.

MIXED METHODS AND MURKY MICROBIOMES: A LITERATURE REVIEW OF CURRENT MICROBIAL PROFILES AND ASSOCIATED CHALLENGES IN SEA URCHIN STUDIES

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With the rise in demand for commercial fisheries and marine invertebrate cultivation, understanding the biological needs of aquatic organisms for optimal health and growth has become critical. Sea urchins, valued for their biomedical research as model animals and cultured seafood, are increasingly studied for commercial and scientific purposes. The microbiomes - the living communities in the gut ecosystem of urchins, play a crucial role in echinoid health, influencing nutrition and metabolism.

While recent technological advancements have improved the accessibility of these microbiomes and made studying their effect on hosts logistically feasible, a lack of standardization has led to inconsistent methodologies in sample collection, dissection, experimental controls, genomic library preparation, and data analysis through informatic pipelines. Addressing these inconsistencies is essential to unlocking the full potential of microbiome research for enhancing urchin health and productivity.

At present, meaningful comparisons across urchin microbiome studies are challenging. This review aims to consolidate the current findings of the field, highlight differences in methodologies and reporting that contribute to variation across 16 studies involving 18 unique urchin species, and provide suggestions for standardizing methodologies and reporting for future studies. By focusing specifically on the gastrointestinal ecosystem and its associated microbiota, the standardization practices will help identify keystone and core taxa and their ecological roles in metabolic function, establishing a strong foundation for future ecological, biomedical, and nutritional sciences.

BURBOT *Lota lota* AQUACULTURE: INVESTIGATION OF IMMUNE DEVELOPMENT AND ASSESSMENT OF RECIRCULATING AQUACULTURE FOR EMBRYONIC AND LARVAL CULTURE

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As the aquaculture industry expands in the United States, investigation of the commercial aquaculture potential of novel species may allow producers to provide unique products for market. Burbot *Lota lota*, demonstrates potential for commercial production in northwestern states, all life stages of this cool to cold-water species can be cultured in captivity and grow-out culture conditions are shared with rainbow trout *Oncorhynchus mykiss*. Burbot offers a thick flake white fillet, delicacies such as roe and liver, and its skin can be used as a leather product. Finally, *L. lota* is refractory to many salmonid pathogens, offering an option to diversify existing trout production operations. The specific objectives for this USDA Agriculture and Food Research Initiative - Education and Workforce Development fellowship project are to: 1) characterize egg survival and incubator microbiomes within recirculating (RAS) and flow through (FT) systems, 2) characterize larval survival, development, and culture tank microbiomes within RAS and FT systems, and 3) investigate the development of immunological organs and proteins associated with adaptive immunity in larval diploid and triploid burbot.

Conventional burbot egg incubation and larval culture practices utilize single pass aquaculture methodology; however, recirculation technologies may be a viable method to improve sustainability and increase viable farming locations. Thus, a set of experiments were conducted to characterize embryonic and larval survival within RAS and FT systems. Six treatments were utilized: 1) flow-through (FT-NILL), 2) flow-through with hydrogen peroxide treatment (FT-H), 3) flow-through with UV treatment (FT-UV), 4) recirculating (RAS-NILL), 5) recirculating with hydrogen peroxide treatment (RAS-H), and 6) recirculating with UV treatment (RAS-UV). Initial experiments served to refine methods and yielded preliminary survival data. Highest embryonic survival was observed in treatments FT-H, FT-UV, and RAS-H, which were all statistically similar ($p = 0.05$). The highest larval survival was observed in FT-H, FT-UV, and RAS-NILL, which were all statistically similar ($p = 0.05$). Together, the embryonic and larval survival indicate that RAS may be a viable approach for burbot early life history culture.

Evaluation of adaptive immunity development in diploid and triploid larval burbot is pending, and refined experiments for the FT vs RAS will be conducted in spring of 2025, also including examination of culture system microbiomes. The results from this project will be of interest to commercial cold-water aquaculture producers and agencies utilizing burbot for management applications, as this may serve to improve the water resource use for burbot early life history culture.

ANALYSIS OF GROWTH, MORPHOLOGY, AND SURVIVAL OF WILD BURBOT *Lota lota maculosa* EGGS AND 1318 LARVAE FROM PARENTS EXPOSED TO HIGH ENVIRONMENTAL SELENIUM

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Extensive coal mining operations within the Elk River Basin from the late 1800s to present has contributed high concentrations of selenium, nitrate, sulphate, and cadmium to the Kootenai River watershed than would be expected under unimpacted conditions. Selenium is considered the pollutant of primary concern due to its toxicity and propensity to bioaccumulate in the food web. While selenium is an essential micronutrient in small concentrations, fish exhibit a narrow window between essentiality and toxicity. The selenium contamination of the Kootenai River ecosystem has become an international concern and is suspected to be contributing to the system's observed burbot population decline. Since current burbot restoration efforts within the Kootenai River ecosystem are focused on improving natural recruitment, and because of the known negative impacts of excess selenium on larval fish development, knowledge of the impacts of selenium on burbot embryo and larval performance is paramount. Thus, the survival and growth performance of embryos and larvae from a captive burbot population receiving only dietary levels of selenium were compared to the survival and growth of wild Kootenai River origin embryos and larvae. The average selenium concentration in the Kootenai River origin eggs, 11.33 mg/kg of dry weight (dw), was 2.5 times higher relative to a reference population in Northwestern Ontario, 4.55 mg/kg dw, and 7.8 times higher than the captive burbot population, 1.45 mg/kg dw. Despite the relatively high selenium concentration of the Kootenai River origin eggs, there was no statistical difference in embryo or larval survival relative to the captive population. However, eye deformities and edema, both classic symptoms associated with selenium toxicity, were observed only in Kootenai River larvae. While these deformities do not directly lead to death, these morphometric irregularities probably contribute to overall lower fitness. Thus, the observed selenium associated deformities in the Kootenai River larval burbot may contribute to low recruitment in the wild population of Kootenai River burbot. Future efforts should focus on identifying the concentration of selenium in burbot eggs that lead to embryo/larval mortality.

This research was funded through BPA2002-002-00 (The Kootenai River Habitat Restoration Project).

SHOULD TRACEABILITY SYSTEMS IN THE AQUACULTURE INDUSTRY BE BASED ON BLOCKCHAIN TECHNOLOGY?

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Transparency and traceability are key issues for food products in general, and for seafood products in particular. There are various reasons why the importance of traceability is increasing; partly it relates to the internal need for internal documentation and industrial statistics, and partly it is to meet customer and consumer requirements and preferences both in relation to product characteristics (species, feed type and ingredients, production parameters, ingredients, origin, processes undergone, etc.) and in relation to so-called secondary characteristics (certification, sustainability, emissions, eco-label status, ethics, fair trade, etc.). Traditionally these characteristics are recorded, stored in relational databases, and transmitted in the chain using some form of Electronic Data Interchange (EDI). In recent years building a traceability system on blockchain technology has become a viable alternative, and this presentation attempts to highlight the strengths and weaknesses associated with each option, and in particular to evaluate to what degree and under what circumstances a blockchain based traceability system is suitable for the aquaculture industry.

This presentation outlines applications, limitations, costs, and benefits related to the use of blockchain technology in the aquaculture industry, and in particular evaluates the pros and cons of having a blockchain-based traceability system compared to a traditional electronic traceability system. The core principles of blockchain technology are outlined, as well as the fundamental requirements and drivers relating to an electronic traceability system. The presentation compares traditional vs. blockchain-based food traceability systems in terms of database structure, data quality and veracity, immutability, integrity, transparency, confidentiality, trust, robustness, speed, efficiency, and interoperability.

DETERMINING THE CONSTRAINTS FACED BY CONSUMERS IN CONSUMING SAFER FISH IN BANGLADESH

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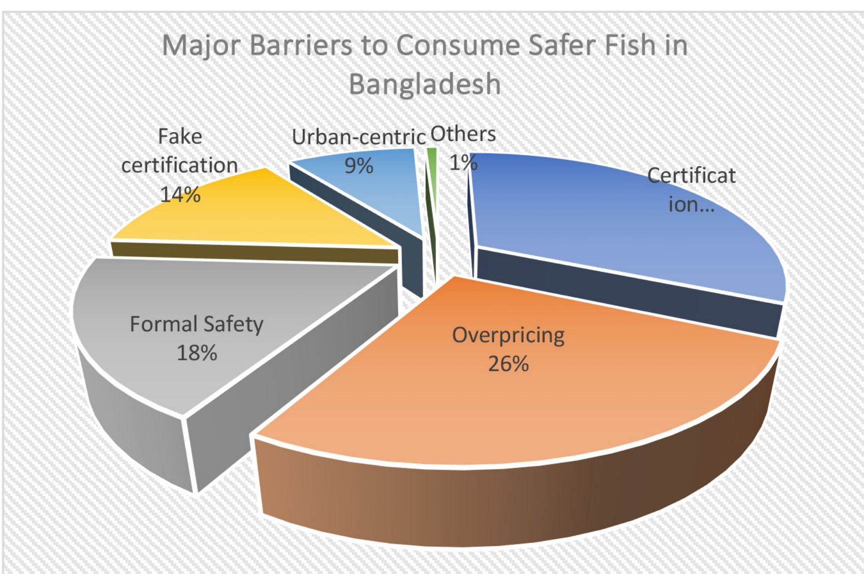
Bangladesh is the 5th largest aquaculture-producing country in the world. However, the aquaculture industry is more focused on quantity than quality, which can result in several health issues and deter customers from eating fish. The goal of this study is to identify and examine the key challenges that consumers encounter while trying to obtain and consume safer seafood in Bangladesh. Understanding these limitations is vital for formulating effective strategies to enhance food safety and public health.

The data was gathered through in-depth interviews and surveys with 600 participants from various regions within Dhaka, Mymensingh, Rajshahi, Bogura, Jashore, and Chattogram districts. The interviews provided qualitative insights, while the surveys measured the frequency and significance of each obstacle or limitation. The participants consisted of a diverse group of consumers from different socioeconomic classes to ensure a comprehensive representation of the public.

The findings reveal several significant challenges to the consumption of safer fish in Bangladesh. The lack of certification or labeling is identified as the most significant barrier, highlighting the urgent need for a uniform certification system that can provide consumers with confidence regarding the safety of the fish they purchase. Other major barriers include excessive pricing, mistrust towards certifying authorities, fake certifications, and urban-centric availability.

To overcome these challenges, a comprehensive strategy is needed. This strategy should involve the development of reliable certification systems, ensuring affordability, fostering consumer confidence through transparency, eradicating fraudulent certifications, and enhancing distribution networks to make safe fish readily available in rural regions.



DETERMINING THE TECHNICAL AND ECONOMIC EFFICIENCY OF TRADITIONAL SHRIMP AQUACULTURE IN INDIA

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India's aquaculture sector is experiencing rapid growth, establishing the country as a leading player in global shrimp production. While intensive and semi-intensive methods dominate, traditional shrimp farming remains significant, especially among small and medium producers. This study focuses on assessing the technical and economic efficiencies of traditional shrimp farming, which is essential for streamlining operations and enhancing productivity. Primary data were collected from 73 shrimp farmers in Kerala's (one of the leading sea foods producing area in India) major traditional shrimp-producing regions, Ernakulam and Alappuzha, through a survey detailing socio-economic characteristics, farm structures, input use, and revenue sources.

The analysis employs a Stochastic Production Frontier model—a widely recognized approach in economic research for efficiency measurement. The study reveals that 91.78% of the surveyed farmers are aged between 40 and 70, with varied educational backgrounds; 53.42% have not completed matriculation, 30.14% have completed matriculation and 6.85% have completed the college degree. Experience levels also differ, with 43.1% of farmers having 5 to 15 years in shrimp farming and 17.24% possessing over 30 years. Additionally, 73.97% are self-employed, with minimal representation from government or non-government job holders. Farm sizes vary, with 51.61% being less than 5 hectares and only 6.45% between 20-40 hectares. Shrimp accounts for 66.67% of production, averaging a yield of 850.65 kgs per acre annually, while other fish represent 33.33%, yielding 552.08 kgs per acre.

These socio-economic characteristics suggest potential factors influencing efficiency. The final analysis of technical and economic efficiency outcomes, which is currently underway, will be discussed at the conference. This study aims to contribute insights into optimizing traditional shrimp farming practices in India, thus supporting sustainable aquaculture growth.

UNDERSTANDING AQUACULTURE PRODUCERS' PERCEPTION AND ADOPTION CRITERIA FOR NOVEL AQUAFEED

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As the aquaculture industry expands to meet the growing global demand for food, the reliance on fishmeal has increased significantly. However, fishmeal production has declined in recent years due to overfishing and sustainability challenges. This shortage has led to rising prices and driven the industry to explore alternative feed sources. Therefore, there is a pressing need for the development of additional, cost-effective ingredients to fulfill the increasing demand for aquafeed. As an alternative source of aquafeed, cell-cultured fishmeal presents a promising alternative for several reasons: it alleviates pressure on marine ecosystems, helps preserve biodiversity by eliminating bycatch, stabilizes supply chains by reducing dependence on fluctuating wild fish stocks, and addresses ethical concerns related to traditional fishing practices. Figure 1 shows the overview of cell-cultured fishmeal production.

Undertesting aquaculture community's perception, willingness and barriers to adoption of novel cell-cultured fishmeal is instrumental for success of the emerging cellular aquaculture industry.

Therefore, a nationwide survey targeting aquaculture producers is being conducted to understand aquaculture and aquafeed producers' perceptions, acceptability, and criteria for adoption of novel cell-culture protein. We designed the survey based on the Decomposed Theory of Planned Behavior (DTPB) and Diffusion of Innovations (DOI) Theory.

The survey gathers descriptive data on respondents' operations, including fish types, system configurations, and current aquafeed usage. Additionally, respondents' knowledge, attitudes, and preferences toward cell-based aquafeed will be assessed Our presentation will outline the survey design and its components, as well as illustrating how the collected information can be used to develop aquafeed that meets the needs of the aquaculture industry.

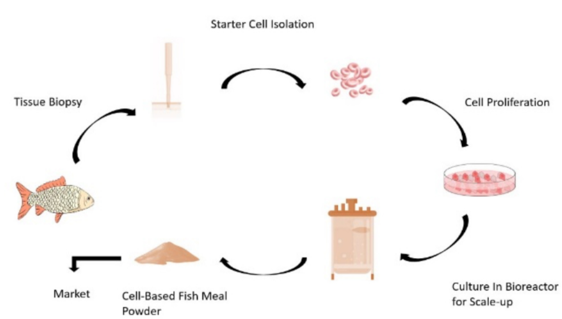

Figure 1: Overview of cell-cultured fishmeal production. (Credit: Rose Omidvar and Razieh Farzad, UF/IFAS Extension)

SEA CUCUMBER, *Neostichopus grammatus* DENSITY AND TANK CLEANING FREQUENCY AFFECT ABALONE, *Haliotis midae* GROWTH IN INTEGRATED MULTITROPHIC AQUACULTURE

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The integrated multitrophic aquaculture (IMTA) of sea cucumber and abalone has been proposed as a potential bioremediation tool that stimulates increased abalone growth. This study assessed the role of sea cucumber stocking density and frequency of tank cleaning in IMTA on growth, water, and sludge bioremediation. The study was conducted for 16 weeks and was made of four treatments and four replicates; abalone cocultured with sea cucumber (low density—27:1g[abalone(ab): sea cucumber (sc)]) with tanks cleaned once a week (L1); abalone cocultured with sea cucumber (low density) with tanks cleaned twice a week (L2); abalone cocultured with sea cucumber (high density— 15:1g [ab:sc]) with tanks cleaned once a week (H1) and abalone cocultured with sea cucumber (high density)with tanks cleaned twice a week(H2). Rearing water nitrite was significantly lower ($p=0.001$) at high stocking density of sea cucumbers, but sludge was unaffected. Tanks cleaned once weekly had higher sludge organic matter ($p = 0.015$) and sludge sulfur content ($p = 0.020$) and lower sludge carbon ($p = 0.003$) and nitrogen content ($p = 0.049$). At the end of the experiment, the stocking density of sea cucumber and frequency of tank cleaning affected abalone mean weight [$p = 0.047$; $p = 0.011$, respectively] without a significant interaction ($p = 0.517$). Abalone in H1 had a higher mean weight and shell length than abalone in L2 and H2 but was similar to those in L1. The stocking density and frequency of cleaning used in this study had no effect on the growth of sea cucumbers [$p = 0.150$; $p = 0.470$, respectively]. This study has shown that in an abalone–sea cucumber IMTA system, the stocking density of sea cucumber and the frequency at which tanks are cleaned influence abalone growth and bioremediation of the rearing water. Our result suggests H1 as the best density (ab:sc) and cleaning frequency. The tank cleaning frequency alone affects the tank sludge quality; tanks need not be washed too frequently as, in addition to causing animal stress, cleaning markedly increased carbon and nitrogen level of tank sludge. Both these effects are likely to negatively impact abalone growth.

CONTRIBUTION OF ROTARACTORS TO THE MANGROVES RESTORATION PROJECT IN THE KENYAN COASTAL AREAS

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The Mangrove Restoration Project by Rotaractors in Rotary District 9212 (Kenya, Ethiopia, Eritrea, and South Sudan) is a massive environmental project to restore the mangrove ecosystem in the Kenyan coastline, that is Mombasa, Kwale, Kilifi, and Lamu. This project aims at underlining the necessity of the ecological systems and benefits that mangroves provide such as climate change mitigation, shore protection, and the welfare of human society.

In annual regeneration exercises, Rotaractors and the existing communities, RCCs, and other players engage in planting of mangrove seedlings, while considering success factors of the site. It also incorporates leadership and training development for youths, women, fishers and local communities on knowledge and appreciation of mangrove values and sustainable management. The public plays a critical role in the implementation of the project since it would introduce commitment from everyone. It has also implemented the planting of over 180,000 mangrove seedlings, the restoration of vast areas of coastal forests; and the coordination and partnership with more than 25 Community Based Organizations, reaching out to more than 600 fishermen and women and 2500 pupils from various schools.

Some of the constraints for this project include weather barriers, inadequate funds, and sometimes people's resistance to the project. Despite these odds, the project has been branching through partnerships, funding, and cooperation. Further development includes: the consideration of additional coastal areas as the focal point of the project, and the elaboration of previously unutilized activities for furthering conservation and improving the standard of living of affected communities such as the eco-tourism business. The Mangrove Restoration Project provides a clear example of how such initiatives can be useful and bring a great advance in the restoration process. This should mean that the project retains its focus, cultivates partnerships and then looks for new solutions so that sustained positive change to the status of coastal ecosystems and to the people who depend on them can be realized.

EVALUATION OF ANESTHESIA PROTOCOLS FOR HANDLING TWO MARINE BAITFISH SPECIES: PIGFISH (*Orthopristis chrysoptera*) AND PINFISH (*Lagodon rhomboides*)

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Pigfish, *Orthopristis chrysoptera*, and Pinfish, *Lagodon rhomboides*, are marine baitfish species commonly used by recreational anglers in the southeastern United States. Previous aquaculture research conducted on these baitfish species demonstrated their potential for commercial production. Commercial scale production of pigfish and pinfish as baitfish will require safe anesthesia protocols for any type of handling performed such as transport, harvesting, sampling, and hormonal injection. The objective of this study was to determine the safety and effectiveness of two anesthetic agents, and to define recommended anesthesia dosages for pigfish and pinfish broodstock. Anesthesia effectiveness and safety was evaluated with adult pigfish (17.5-26 cm, 78.9-296.4 g) and pinfish (17-26 cm, 114.1-270.4 g) using TRICAINE-S® at 50, 100, and 150 mg/L, and AQUI-S 20E® (10% eugenol) at 100, 250, and 500 mg/L.

Optimal and preferred doses should induce light and surgical anesthesia in less than 5 minutes, with recovery times under 10 minutes, and result in zero mortality and absence of excitation behavior. Results for pigfish and pinfish broodstock, showed that both TRICAINE-S® (100-150 mg/L) and AQUI-S 20E® (250-500 mg/L) doses were safe and effective at inducing light and surgical anesthesia. TRICAINE-S® (50 mg/L) and AQUI-S 20E® (100 mg/L) doses were ineffective and should not be used for minor or invasive procedures. Both anesthetic agents at varying dosage levels proved to be safe and effective, nevertheless, the anesthetic agent AQUI-S 20E® is recommended over TRICAINE-S® due to its 0-day withdrawal period. Overall, pigfish and pinfish in this study responded similarly to other species tested with the same anesthetic agents and similar doses. In addition, ongoing trials with pigfish and pinfish juveniles are being conducted to determine safe and effective anesthesia dosages to induce light and deep sedation for handling and hauling of these two marine baitfish species. Results obtained from this trials will be included in the study and presented at the conference.

ASSESSMENT OF BROODSTOCK HUSBANDRY AND INDUCED VOLITIONAL SPAWNING OF HOGFISH (*Lachnolaimus maximus*)

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Food demands of the growing human population requires the expansion of sustainable and efficient food production systems. Aquaculture is crucial to ensure a consistent seafood supply while alleviating harvest pressure on wild fish stocks. Aquaculture of hogfish (*Lachnolaimus maximus*), a highly valued sportfish and foodfish species, could potentially alleviate pressure on wild hogfish stocks, while also meeting the increasing demand for this popular foodfish species in the seafood supply. Recent studies have defined initial culture methods for aquaculture of hogfish. While showing great promise, continued research is necessary to replicate results and improve culture efficiency. The objectives of this study were to define effective protocols for broodstock capture, transport, quarantine, harem formation, husbandry, conditioning, and induced spawning.

Broodstock were acquired by multiple methods and transported to the UF-IRREC. A total of six hogfish harems were formed. Broodstock conditioning and induced spawning were explored through photothermal manipulation and hormonal injections. Two hogfish harems were induced to spawn though photothermal manipulation and hormonal injections, yet no successful fertilization occurred. One harem was induced to spawn through photothermal manipulations while replicating natural hogfish spawning season, while a second harem was induced to spawn with injections of the product Ovaprim® in a single dose at 0.5 mL/kg for females. Unsuccessful spawning observed in this study could be caused by improper harem formation, developmental dysfunction, no synchronization between males and females, or reproductive dysfunction. The results from this study will help refine and improve aquaculture protocols for hogfish and inform producers best methods for commercial aquaculture of hogfish.

AQUEOUS ALMOND (*Terminalia catappa*) LEAF EXTRACT ENHANCED GROWTH PERFORMANCE, IMMUNOCOMPETENCE AND RESISTANCE OF *Heterobranchus longifilis* TO *Pseudomonas aeruginosa*

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Introduction

The growing demand for sustainable aquaculture has led to increased interest in natural feed additives. *Terminalia catappa* is a promising plant with bioactive compounds like flavonoids and tannins that can enhance fish health and growth (Abdel-Tawwab *et al*, 2018; 2019; Ajani *et al*., 2020). However, extraction methods significantly influence the quality of the extract (Bae *et al.,* 2012). This study aims to evaluate the impact of different *T. catappa* extracts on the growth, health, and disease resistance of *Heterobranchus longifilis*. Five experimental diets were formulated with varying concentrations of ethanol and aqueous extracts of *Terminalia catappa*. Diets were fed to triplicate groups of *Heterobranchus longifilis* for 70 days. Growth parameters, feed utilization, and hematological indices were measured. Liver samples were analyzed for antioxidant enzyme activity (SOD, GST, GPx) and lipid peroxidation (MDA). Fish were challenged with *Pseudomonas aeruginosa* to assess their disease resistance. Data were analyzed using one-way ANOVA and Duncan's Multiple Range Test to determine significant differences among treatments.

The study compared the efficacy of ethanol and aqueous extracts of *Terminalia catappa* on *Heterobranchus longifilis*. Ethanol extracts were found to have higher concentrations of bioactive compounds, but negatively impacted feed intake and growth performance. In contrast, aqueous extracts improved growth, feed utilization, and survival rates. This can be attributed to the varying polarity in the solvents. According to Archundia *et al,* (2019), the polarity in ethanol be described as medium while it is high in water. This suggests that aqueous extracts of *T. catappa* may be a more suitable dietary supplement for enhancing fish growth and health. Antioxidant indicators like GST and SOD were higher in aqueous extract diets, showing oxidative stress protection, which is required in fish during the normal feeding cycle. Fish fed *T. catappa* extracts showed increased resistance to *P. aeruginosa*, improving survival rates.

Table 1: Result of the quantitative screening of ethanoic and aqueous extracts of *T. catappa*

Phytochemical	Ethanoic extract concentration	Aqueous extract concentration
Total Phenolics (mg/g GAE)	89.139±0.01	42.462±0.00
Tannins (m/g GAE)	5.862±0.01	2.100±0.00
Alkaloids (mg/g)	13.66±0.03	10.30±0.03
Flavonoids (mg/g RE)	97.259±0.26	2.741±0.02
Saponins (%)	1.34±0.01	2.04±0.04

Table 2: Growth performance and survival of *H. longifilis* fed experimental diet for 70 days.

Parameter	AL1	AL2	AL3	AL4	AL5
Initial weight (g)	3.70±0.12	3.87±0.07	3.90±0.06	3.80±0.05	3.83±0.03
Final weight (g)	25.76±1.33^a	18.00±0.58^b	19.33±0.88^b	24.00±3.46^a	25.67±1.76^a
Feed intake (g)	33.96±0.64^a	28.20±0.69^b	29.41±3.76^b	31.43±2.41^a	33.61±1.45^a
Weight gain (g)	22.06±1.42^a	14.13±0.52^b	15.46±0.85^b	20.20±3.46^a	21.87±1.51^a
SGR (%g/day)	2.77±0.04^a	2.19±0.04^b	2.28±0.04^b	2.63±0.08^a	2.72±0.03^a
FCR	1.53±0.05^c	1.99±0.43^a	1.90±0.48^b	1.55±0.22^c	1.51±0.04^c
PER (%)	1.62±0.00^a	1.25±0.01^b	1.32±0.01^b	1.60±0.02^a	1.64±0.00^a
Survival rate (%)	90.00±7.64^a	85.00±2.89^{ab}	83.67±21.28^b	90.33±4.41^a	95.00±2.89^a

EXPANDING SEAWEED CULTIVATION IN SOUTHERN CALIFORNIA FOR SUSTAINABLE CULINARY USES

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Population growth, climate change, and resource constraints are driving increased demand for sustainable food sources in California, which highlights the importance of developing local seaweed aquaculture. Seaweed aquaculture presents significant opportunities to support California's blue economy and environmental goals, particularly in the Southern California region. This project establishes a framework for cultivating seaweed species with culinary applications, beginning with *Develaria mollis* (Pacific dulse) and *Ulva spp*. (sea lettuce), and aims to expand to six species that will include each of the major algal phyla.

Optimized growth trials and post-harvest treatments demonstrate the effectiveness of aerated storage in maintaining quality. For example, aerated *D. mollis* and *Ulva spp*. samples stored at 36°F retained quality for up to 11 days, preserving their high nutritional value and culinary appeal. Nutritional analysis (Table 1) highlights *Ulva spp*. as rich in potassium, magnesium, protein, and fiber, surpassing levels found in US established standards of leafy greens, thus enhancing its market viability. Heavy metal testing confirms that both species meet global safety standards with values well within limits set by the EU and Asian regulatory bodies. Color stability is a crucial quality parameter for consumer appeal. The CIE Lab* color model was applied to assess color changes in *D. mollis* over time, under various treatments and temperatures (Figure 1).

Results indicate significant differences in lightness (L*), red-green (a*), and blue-yellow (b*) axes across storage conditions, demonstrating the impact of temperature and treatment on color retention. Notably, samples stored at higher temperatures (6.1°C) showed lighter colors, while the Cleanworks treatment altered color balance along the a* and b* axes. These results emphasize the need for temperature-controlled storage to maintain visual quality and consumer appeal. Ultimately, this work aims to reduce reliance on imported food products and provides a scalable model for expanding sustainable seaweed aquaculture throughout California.

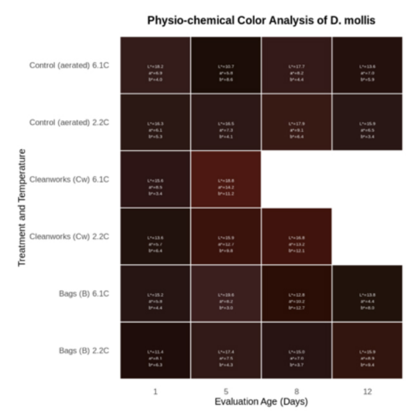

***Fig 1**. Tukey HSD Post-Hoc Test Results for Spool Quality among Twine Types.*

Table 2. Comparative Nutritional Content of Ulva spp., US produced 50 Baby Spinach 50 Spring Mix and US produced Spinach per 100g Portion

Nutritional Item	*Ulva spp.* (Sea lettuce)	%Daily Value	50 Baby Spinach 50 Spring Mix [1] (USDA)	Spinach, Mature[2] (USDA)
Calories	**50 kcal**	-	**24 kcal**	**28 kcal**
Total Fat	0 g	0	0 g	0.06g
Saturated Fat	0 g	0	0 g	-
Trans Fat	0 g	0	0 g	-
Sodium	**746 mg**	**32**	**94 mg**	**107 mg**
Total Carbohydrates (US)	8 g	3	3.53 g	2.64g
Dietary Fiber	**5 g**	**18**	**2.4 g**	**1.6 g**
Total Sugars	0 g	0	0 g	-
Protein	**4 g**	-	**2.35 g**	**2.91g**
Vitamin D	0 mcg	0	-	-
Calcium (Ca)	70 mg	5	71 mg	67 mg
Iron (Fe)	1 mg	6	1.69 mg	1.05 mg
Potassium	697 mg	15	506 mg	460 mg
Magnesium	**425 mg**	**101**	-	**93 mg**
Copper	-	-	-	0.079mg
Zinc	<25.0 mg/kg	-	-	0.42 mg

[1]U.S. Department of Agriculture, FoodData Central. (n.d.). *Food details: Baby spinach nutrients.* FoodData Central. Retrieved [access date], from https://fdc.nal.usda.gov/fdc-app.html#/food-details/956311/nutrients
[2]U.S. Department of Agriculture, FoodData Central. (n.d.). *Food details: Spinach nutrients.* FoodData Central. Retrieved [access date], from https://fdc.nal.usda.gov/fdc-app.html#/food-details/168462/nutrients

***Table 1.** Nutritional comparison of Ulva spp. (sea lettuce) with commonly consumed leafy greens, showing per 100g portions*

GROWTH PERFORMANCE OF *Clarias gariepinus* Burchell 1822 FED WITH COCOA BEANS SHELL WASTE AS DIETARY ADDITIVE

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This study was conducted to determine the growth performance of *Clarias gariepinus* fingerlings fed with Cocoa bean shell meal (CBS). One hundred and twenty *Clarias gariepinus* fingerlings were procured from a Negro farm in Ido-Ekiti, Ekiti State for the experiment. The fish were divided into ten plastic bowls (20 litres capacity) randomly at 12 fingerlings per bowl with 0%, 10%, 20%, 30%, and 40% inclusion of cocoa bean shell meal. The results for the growth parameters showed the highest mean weight gain (MWG) of 5.70g in the fish fed 40% Cocoa bean shell meal and the lowest mean weight gain of 4.44g was recorded in the fish fed 30% inclusion of cocoa bean shell meal. There was no significant difference ($p>0.05$) between weight recorded in all the diets. The highest standard length increase of 2.77cm was recorded in 40% inclusion of cocoa bean shell meal fed fish and the lowest value of 2.45cm was recorded in the fish fed 20% inclusion of cocoa bean shell meal with no significant difference ($p>0.05$) in length in all the diets. Specific Growth Rate (SGR) showed that Fish fed with 10% and 40% had the highest value of 1.0 and the lowest value of 0.8 was recorded in fish fed with 20% Cocoa bean shell meal. This study established that cocoa bean shell waste at 40% inclusion level can be incorporated into the diet of African catfish (*Clarias gariepinus*) without any adverse effect on growth and nutritional standard of the fish. However, further studies should still be done on the pre-conditioning and fermentation of cocoa bean shell waste for better growth, nutritional quality and also its effect on the water quality parameters of *Clarias gariepinus*.

POSTPRANDIAL AMMONIA EXCRETION, OXYGEN CONSUMPTION, AND CARBON DIOXIDE PRODUCTION OF CHANNEL AND BLUE CATFISH

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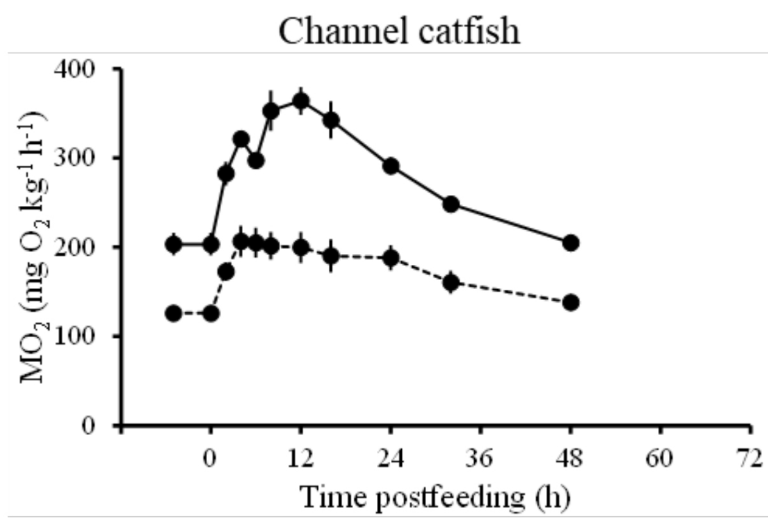
Fishes undergo physiological changes upon consumption of a meal, including an increase in oxygen consumption to support the metabolic cost of digestion (specific dynamic action; SDA) and an increase in the excretion of ammonia. Channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) are two species commonly used for commercial aquaculture production in the United States. Postprandial ammonia excretion and oxygen consumption of both channel and blue catfish were measured at 25 °C and 32 °C. Rates of both ammonia excretion and oxygen consumption increased quickly after feeding and were significantly higher within 2-hours postfeeding. Compared to fasting rates, ammonia excretion of channel catfish peaked at 6-hours postfeeding at both 25 and 32 °C; with peak ammonia excretion rates increasing 8.3 and 4.7-fold at 25 °C and 32 °C, respectively. Ammonia excretion of blue catfish at 25 °C peaked 12 hours postfeeding 6.6-fold higher than fasting rates and 6 hours postfeeding at 32 °C and 5.5-fold higher than fasting levels at 25 and, respectively. Relative to fasting levels, postprandial oxygen consumption of channel catfish peaked 1.8- and 2.0-fold higher at 25 °C and 32 °C, respectively. Blue catfish oxygen consumption peaked 1.9- and 1.8-fold higher at 25 °C and 32 °C, respectively. Both channel and blue catfish rapidly increase ammonia excretion and oxygen consumption in response to feeding, with temperature mostly affecting changes in peak and minimum rates.



INNOVATING U.S. AQUACULTURE WITH AI AND ROBOTICS: THE JOURNEY FROM HAUCS TO IREPA

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Despite having extensive coastal zones and ranking third globally for renewable water resources, the US produces only 1% of its aquaculture products. Consequently, over 90% of seafood consumed in the US is imported, leading to a $14 billion annual seafood trade deficit. US fish farming operations are often perceived as reactionary, labor-intensive, and inefficient. While advancements in AI, robotics, and Cyber-Physical Systems (CPS) have been widely adopted in other agricultural sectors, their integration into US aquaculture remains limited. This highlights the urgent need for a coordinated effort to innovate and secure the future of aquaculture in the US.

This presentation will outline our vision to create the fish farms of the future through Collaborative Intelligence (CI)—seamless collaborations between AI-driven systems and human operators. We see this as essential to overcome key barriers to the adoption of AI technologies in aquaculture: fish farmers' general risk aversion to new technology due to high initial costs and the typically low-profit margins of aquaculture operations.

We will discuss two projects that exemplify our initial endeavors to realize this vision: the Hybrid Aerial/Underwater Robotic System (HAUCS) and Intelligent Resource Efficient Pond Aquaculture (IREPA). These projects are supported by the NSF National Robotic Initiative (NRI) and CPS programs, with funding from USDA-NIFA.

CONTINUED ANALYSIS OF METAL IONS IN CLOSED LOOP AQUAPONICS

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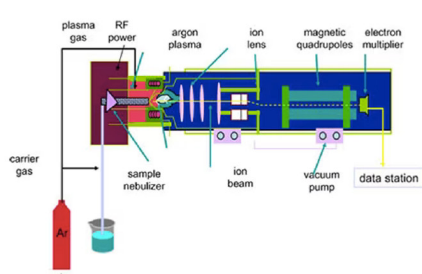
The LSSU Aquaculture club has been operating three aquaponics systems for approximately four years, one with yellow perch, another with Atlantic salmon, and our largest system with koi fish. Our water is sourced from Lake Superior, so the source water has very few ions, and is aged before entering the systems. After several sudden mortality events, we have continuously monitored the water quality of our system, using API freshwater test kits and real time Wi-Fi enabled digital water meters that allows us to monitor the water remotely. In general, water parameters appear to be optimal with the exception of slight variation of pH and ammonia levels.

Our analytical chemistry professors offered to run water samples using their Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipment, and discovered high levels of metal ions at least an order of magnitude higher that the source water. Water samples were collected regularly to see if the metals were accumulating from the food or other sources of contaminants such as condensation from overhead pipes. We suspect copper levels may be elevated due to copper pipes, but we later analyzed samples of our fish food, which showed high levels of metals.

We encourage the use of closed loop systems to conserve water, but realize the potential for increased concentrations of metal ions, and so are interested in finding indicators of high metal levels before the fish show signs of distress. Currently we are monitoring the pH and note that over time, the pH drops if no water is replaced. If the pH drops significantly, we are doing @20% water changes which brings the pH back to optimal levels. We are currently monitoring the pH and conductivity, while taking water samples for ICP-MS analysis to see which parameters can be used as an indicator of potential increases in metal ion concentrations.

We are still not sure of the effects of high metal ion concentrations on the quality of life for the fish or the plants. We plan to examine the tissues of frozen fish mortalities and plants growing in the system for traces of heavy metals using ICP-MS and Micro XRF spectrometer.



LAKE SUPERIOR STATE UNIVERSITY AQUACULTURE CLUB

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The aquaculture club at Lake Superior State University is currently 11 members strong and a proud student subunit of the U.S Aquaculture Society. Our club was formed in 2020 with 15 members. We have three deep water culture aquaponic systems, a recirculating aquaculture system, a vertical aeroponics system and zebra fish research tanks. Our aquaponic systems are growing Atlantic salmon, yellow perch, koi, and goldfish. We grow a variety of plants, anything from herbs and house plants to fruiting plants and trees.

The club puts on plant sales every month to have a continuous fundraiser. This schedule allows us time between sales to empty out the grow beds, clean and sanitize, before moving the next set of sprouts into the systems. We hold a yearly tie dye event where community and club members get to tie dye their very own fishman shirt or tote bag. The club has also hosted cooking demonstrations and soap making events.

We hold numerous tours of our facility throughout the year for K-12 groups, campus events, and community outreach. Our goals as a club are to educate the club members and community about aquaculture and aquaponic systems and practices. We also love to tour other aquaculture or aquaponic facilities. These activities allow the club members to gain knowledge about how others run their systems.

The club sends members to different conferences each year. Five members attended the Wisconsin/Minnesota Aquaculture conference in Bayfield, Wisconsin in February 2024. Two of those members had oral presentations and made posters, and all attendees made connections with local industry workers. The members also got the opportunity to visit the Red Cliff Fish Hatchery and Northern Aquacutlure Demonstration Facility before heading back to campus. Our advisor, two student sub-unit members, and one former sub-unit member gave talks at the San Antonio 2024 World Aquaculture conference. The opportunity to go to the conference was an eye opening and educational experience. The club looks forward to new members and new experiences each year.

CONTRIBUTION OF ROTARACTORS TO THE MANGROVES RESTORATION PROJECT IN THE KENYAN COASTAL AREAS

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The Mangrove Restoration Project by Rotaractors in Rotary District 9212 (Kenya, Ethiopia, Eritrea, and South Sudan) is a massive environmental project to restore the mangrove ecosystem in the Kenyan coastline, that is Mombasa, Kwale, Kilifi, and Lamu. This project aims at underlining the necessity of the ecological systems and benefits that mangroves provide such as climate change mitigation, shore protection, and the welfare of human society.

In annual regeneration exercises, Rotaractors and the existing communities, RCCs, and other players engage in planting of mangrove seedlings, while considering success factors of the site. It also incorporates leadership and training development for youths, women, fishers and local communities on knowledge and appreciation of mangrove values and sustainable management. The public plays a critical role in the implementation of the project since it would introduce commitment from everyone. It has also implemented the planting of over 180,000 mangrove seedlings, the restoration of vast areas of coastal forests; and the coordination and partnership with more than 25 Community Based Organizations, reaching out to more than 600 fishermen and women and 2500 pupils from various schools.

Some of the constraints for this project include weather barriers, inadequate funds, and sometimes people's resistance to the project. Despite these odds, the project has been branching through partnerships, funding, and cooperation. Further development includes: the consideration of additional coastal areas as the focal point of the project, and the elaboration of previously unutilized activities for furthering conservation and improving the standard of living of affected communities such as the eco-tourism business. The Mangrove Restoration Project provides a clear example of how such initiatives can be useful and bring a great advance in the restoration process. This should mean that the project retains its focus, cultivates partnerships and then looks for new solutions so that sustained positive change to the status of coastal ecosystems and to the people who depend on them can be realized.

In the future, we plan to follow the "Best Practice Guidelines for Mangrove Restoration" recently published by the Mangrove Alliance[1] and educate local communities using the 4-part animation video series on mangrove restoration prepared by Wetlands International.[2]

References

[1] Best Practice Guidelines for Mangrove Restoration. 2024. The Blue Carbon Initiative. Global Mangrove Alliance. https://www.mangrovealliance.org/best-practice-guidelines-for-mangrove-restoration/.

[2] Mangrove Restoration, a 4-part animation video prepared by Wetlands International, a member of the Global Mangrove Alliance.

INVESTIGATING ENVIRONMENTAL PARAMETERS AND *Vibrio* spp. LEVELS IN OYSTERS AND SEAWATER IN SLAUGTHER BEACH, DELAWARE USA

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Crassostrea virginica play a significant role in the ecosystem and food chain. The filtration of water by the oysters results in the removal of sediments, bacteria, and nutrients which leads to the bioaccumulation of different types of microorganisms in oysters such as *Vibrio* spp. Of various *Vibrio* species found in the aquatic environment common human pathogens, including *Vibrio vulnificus*, and *Vibrio parahaemolyticus,* cause wound infections, and gastrointestinal diseases. Other *Vibrio* spp., such as *Vibrio coralliilyticus* and *Vibrio tubiashii* are known as shellfish pathogens. Slaughter Beach, Delaware, USA has been considered as a potential area to establish an oyster hatchery. However, *Vibrio spp.* and water quality parameters remain unknown in this region. The aims of this study are to investigate and evaluate the *Vibrio* spp., in seawater and oysters and determine the relationship between water quality and total *Vibrio spp*., levels in oysters and seawater. Water samples were collected from three different sites (DuPont Nature Center, Cedar Creek, and Boat ramp) from June to November 2021 at high and low tides in Slaughter Beach, Delaware. The samples were collected bimonthly. The YSI 556 Multiprobe (Yellow Spring, Ohio, USA) was used to monitor (temperature (°C), salinity (g/L), dissolved oxygen in mg/L, and while a YSI 9500 Photometer was used to monitor chemical water quality (nitrate, nitrite, ammonia, phosphate). *Vibrio* spp. was monitored from oysters and water samples collected from these three study sites by using Thiosulfate Citrate Bile Salts Sucrose (TCBS. Our preliminary results revealed that the highest presumptive larval pathogenic *Vibrio* in the seawater was observed in July, August, and October in sites 2 and 3 with a peak at 95 ± 7.2 CFU/100 mL for site 2, and the highest presumptive human pathogenic *Vibrio* in the seawater was recorded during early July at site 2 (148 ± 40 CFU/100 mL). The highest human pathogenic vibrio in oysters was observed at site 2 during September (3.39 ± 0.01 Log cfu/100ml) and October (2.27 ± 0.03 Log cfu/100ml). Monitoring the pH of the seawater during the high tide and low tide at different sites from July to October demonstrated that the pH remained almost stable in a range from 7.22 to 8.89. Analyzing the seawater temperature from July to October revealed the highest sea temperature during high tide and low tide at site 2 during July with a peak of 28.96 ± 0.56 °C.

Acknowledgments: We acknowledge Delaware EPSCoR NSF Award# 1301765 “Meeting Delaware’s 21st Century Water and Energy Challenges through Research, Education, and Innovation (WICCED)” for funding this project.

EFFECT OF ELEVATED WATER TEMPERATURE ON PERFORMANCE AND IMMUNOLOGICAL PARAMETERS OF CHANNEL CATFISH *Ictalurus punctatus* AND HYBRID CATFISH *I. punctatus* × *I. furcatus* FED DIFFERENT ANIMAL PROTEIN SOURCES

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The U.S. catfish industry is predominantly based in the southern states, with Alabama alone accounting for ~30% of total food catfish production in 2023. The industry has been actively searching for alternative feed solutions due to the high costs of ingredients in particular protein sources. In addition to cost challenges, during the summer months, high water temperatures lead to increased health issues. Interestingly, when these catfish are examined, they consistently show no signs of bacterial or parasitic infections but display bloated intestines filled with undigested feed. This may indicate an interaction of feed or protein sources within the feed and temperature.

Two trials were conducted with channel catfish and hybrid catfish to evaluate this temperature effect. For each trial, one recirculation system was maintained at 30±1°C (High temp; HT) and a second at 26±1°C (Regular temp; RT) each stocked with 15 subadults per tank. Four dietary treatments were tested with soybean meal as the primary protein source: 1) a control diet with 6% fishmeal, 2) 6% porcine meal, 3) 6% poultry meal, or 4) 6% beef bone and meat meal. At the end of the trial, fish were weighed to assess survival and growth, while blood and liver samples were collected for further analysis, including plasma lysozyme assays and physiological gene expression.

Upon analysis of growth parameters in the channel catfish trial, no differences were found between treatments or different temperatures, but survival (P=0.031) and FCR (P=0.032) were increased in RT groups. Plasma lysozyme activity was also higher in the same group (P=0.009; Fig. 1), indicating a temperature-related innate immune change. From the gene expression analysis of four genes (*ghr*, *hsp70*, *igf1*, and *igf2*), no differences were found among dietary treatments or between temperatures. However, *hsp70* expression in the liver was greater in RT group (P=0.009). With respect to the hybrid catfish trial, mean weight (P=0.008), weight gain (P=0.007) and weight gain percent (P=0.010) were increased in RT group. No differences in plasma lysozyme activity were found in the hybrid catfish trial. Gene expression of the same 4 genes analyzed from liver tissue showed that *igf2* were upregulated in RT groups (P=0.002).

The findings from the two trials highlight the impact of temperature on catfish feed efficiency, growth and immune responses providing insight into optimal feed management in commercial catfish farming.

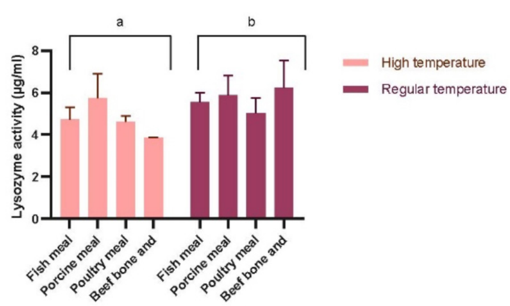

Fig 1: Plasma lysozyme activity of Channel catfish fed various protein ingredients at different water temperatures

DETECTION OF PATHOGENS IN AQUACULTURE USING NOVEL ACOUSTIC SENSORS

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Existing sensitive technologies for pathogen detection in aquaculture are slow and costly for large-scale deployment, presenting challenges to on-site decision making for pathogen control for preventing disease outbreaks. In this presentation, we discuss the development and performance testing of a novel acoustic biosensor for detecting pathogens in biological samples. The biosensor operation is based on immunoassay principles where capture bioprobes are designed to target pathogens via complementary binding, which is measured as an acoustic signal. Detection can be performed without requiring complex instrument or skills training for the performing personnel. The biosensor was tested for the detection of *Vibrio parahaemolyticus* in aqueous samples. Validation using plasmid-spiked samples showed high sensitivity, with detection ranges of 100-5000 gene copies/mL. These preliminary results demonstrate the potential of this biosensor as a rapid, efficient tool for pathogen monitoring in aquaculture. The biosensor's modular design has the flexibility for detection of other pathogens by replacement of target-specific capture bioprobes.

Table 1. Acoustic Detection of *V. parahaemolyticus* in Water

V. parahaemolyticus **(gene copies/ mL)**	**Frequency Shift (-Hz)**
100	265±11.2
500	435±5.2
1000	943±5.7
2500	1058±6.4
5000	1199±6.2

HARNESSING THE NUTRITIONAL POWER OF SEAWEEDS AND BIVALVES: TRANSFORMING DATA INTO DELICIOUS STRATEGIES FOR BOOSTING CONSUMPTION

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Despite their sustainability and health benefits, seaweed and bivalve consumption remains low due to gaps in accessible nutrition and food safety data. Over the past two years, Food + Planet and Food for Climate League have convened two roundtables with geographically diverse U.S. farmers to identify barriers and opportunities to increase demand for these products. Key needs identified include consumer-friendly talking points on nutritional compounds such as protein, omega-3 fatty acids, and calcium, as well as addressing food safety concerns, including iodine, PFAS, and heavy metal contamination. This presentation will synthesize the opportunities and challenges faced by U.S. farmers, showcase novel nutrition and food safety analysis data for 13 seaweed and 7 bivalve species produced in the USA, and highlight actionable strategies to enhance consumer confidence and drive consumption. These strategies aim to position seaweeds and bivalves as vital components of a nutritious, sustainable food system. By translating novel data into actionable insights, they empower farmers to confidently promote their products, fostering increased consumption and contributing to farmer livelihoods, planetary health, and nutrition security.

Species to be featured include: *Pectinidae, Mytilus edulis, Mercenaria mercenaria, Crassostrea virginica, Mytilus galloprovincialis, Venerupis philippinarum, Crassostrea gigas, Gracilaria mammillaris, Sargassum fluitans, Solieria filiformis, Palmaria palmata, Porphyra umbilicalis, Ulva lactuca, Laminaria digitata, Chondrus crispus, Alaria esculenta, Saccharina latissima, Saccharina groenlandica, Laminaria pallida, Nereocystis luetkeana, Saccharina latissima*

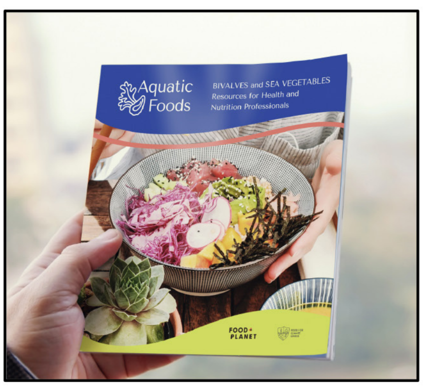

Figure 1: Bivalves and Seaweeds Toolkit for Nutrition Professionals

DE-NOVO GENOME ASSEMBLIES OF FOUR RAINBOW TROUT GENETIC LINES TOWARDS A PAN-GENOME REFERENCE

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Currently, we are working towards generating a rainbow trout pan-genome reference that will better represent the genetic diversity in this species using de-novo chromosome level assemblies. To that end we have previously generated the reference genome from the Arlee line, an improved assembly for the Swanson line, and a genome assembly from the Whale Rock Male line. The Swanson YY male line was originated from a semi-domesticated resident fish from the Kenai Peninsula in Alaska with a haploid number of 29 chromosomes. The Arlee doubled haploid YY male line has a different genetic background from the Swanson line. It has a haploid number of 32 chromosomes and was originated from a well-known domesticated hatchery strain that was originally collected from the northern California coast. The karyotype difference between the Swanson and Arlee lines is due to three fission or fusion events. The three Swanson metacentric chromosomes that are being divided into two acrocentric chromosomes in the Arlee karyotype are Omy04, 14 and 25. The Whale Rock line is also a YY male. Like the Arlee line, it has 2N=64 chromosomes and it was originated from the Central California Coast. The one known major difference from the Arlee line is that it was originated from a wild fish from a landlocked steelhead population. The fourth de-novo genome assembly is from a Keithley Creek (KC) male rainbow trout. The KC is from the inland lineage of rainbow trout (O. m. gairdneri) in contrast to the three previous assemblies that were done using fish from the coastal lineage (O. m. irideus). The wild resident KC population is from the Snake River Drainage of the Interior Columbia Basin. The exact Karyotype of the KC population is unknown. However, our de-novo chromosome level genome assembly provides strong support for 2N=60 number of chromosomes.

Table 1. Genetic lines used for the De-novo genome Assemblies.

Line Name	2N*	Sex	Geographic Origin	Life History Type	Wild or Domesticated Origin
Whale Rock Male	64	YY Male	Central California Coast	Landlocked Steelhead	Wild
Arlee	64	YY Male	Northern California	Resident	Domesticated
Swanson	58	YY male	Kenai Peninsula, Alaska	Resident	Semi-Domesticated**
Keithley Creek	60	XY male	Columbia Basin, Snake River	Resident	Wild

BREEDING SHELLFISH FOR IMPROVED YIELDS ACROSS THE PACIFIC COAST OF NORTH AMERICA

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Farming of bivalve shellfish produces significant economic values in the U.S. and has been recently recognized for its benefits to the environment. Farmed shellfish, however, can have inconsistent yield performances, with high mortality rates before harvest, placing a large financial burden on growers. Most shellfish farms on the U.S. Pacific Coast rely on hatchery-produced seed that are amenable to genetic improvement of desired production traits. Pacific Hybreed Inc. is a private research entity with a primary focus on shellfish breeding and genetics. By combining selection, crossbreeding, and on-farm trials, family-specific yield performances were evaluated across the Pacific Coast, from Alaska to Baja California.

Optimal yields may differ between different families at specific farm sites, suggesting the need to evaluate family-specific yield performances across sites to develop region-specific crosses. Results from different years further indicate the need for long-term breeding programs that work closely with commercial shellfish farms to optimize crop performances from seed to market-size products. Environmental monitoring at farm sites shows that while temperature has a significant effect on crop performance, it does not fully explain the difference in growth rate between sites. Besides contributing to the development of improved seed and broodstock for production, availability of multiple families with genetically based variation in response to the environment and the possibility to repeatedly produce such families will provide valuable resources to the research community with an interest in the relationship between genotype, phenotype, and the environment.

Figure 1. Test sites where bivalve families produced from experimental crosses are tested on the West Coast of North America.

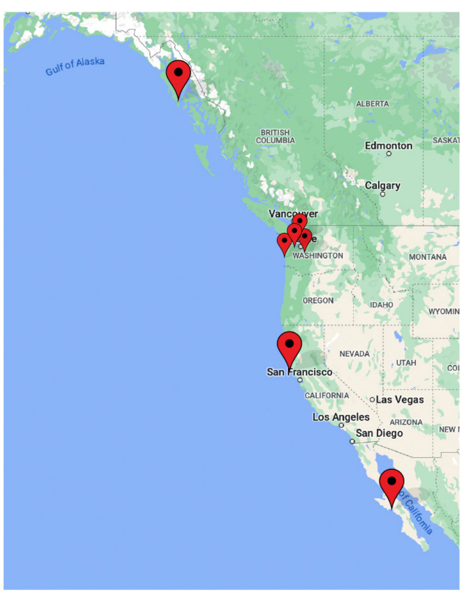

Table 1. Correlation of seed yields of oyster families deployed at different test sites.

	Site B	Site C	Site D	Site E
Site A	$r = 0.84$ $p < 0.01$	$r = 0.52$ $p = 0.03$	$r = 0.15$ $p = 0.57$	$r = 0.56$ $p = 0.02$
Site B		$r = 0.57$ $p = 0.02$	$r = 0.22$ $p = 0.39$	$r = 0.02$ $p = 0.59$
Site C			$r = 0.66$ $p < 0.01$	$r = 0.24$ $p = 0.34$
Site D				$r = 0.14$ $p = 0.58$

REVOLUTIONIZING FISH EGG QUALITY ASSESSMENT: RAPID FATTY ACID DETECTION WITH RAMAN SPECTROSCOPY AND AI

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Omega-3 fatty acids (FAs) are essential for fish health and growth, especially during early development, making them critical indicators of egg spawn quality. The current gold standard for measuring FAs is gas chromatography-mass spectrometry (GC-MS), which offers high sensitivity but requires complex sample preparation, skilled analysts, and slow turnaround times. In contrast, Raman spectroscopy allows for the acquisition of a chemical fingerprint directly from an egg sample within seconds. While Raman technology has been used in quality control for fish fillets, its application in fish eggs remains unexplored.

In this study, we applied Raman spectroscopy, coupled with machine learning algorithms that leverage existing GC-MS data, to develop a rapid, in-situ method for obtaining FA data in fish eggs. California Yellowtail (*Seriola dorsalis*) eggs were used in this study with samples comprised of floating, neutral, and sinking eggs across twenty spawns and three spawning seasons. These samples were homogenized and analyzed for FAs via GC-MS and their corresponding Raman spectra were collected. Our preliminary model successfully predicted DHA concentration with an R^2 of 0.896 and a mean square error (MSE) of 4.31%, demonstrating the ability to reliably predict egg FAs (Figure 1).

Importantly, this model can be shared to build larger databases, further enhancing predictive accuracy and reducing the need for in-house GC-MS analysis in aquaculture. This novel application introduces a cost-effective, time-efficient tool for egg quality assessment and can provide producers with immediate insights into spawn viability and quality—an application that was previously unattainable. Additionally, our research can potentially be expanded to include different fish species and enable rapid detection of other nutrients of interest such as vitamins, amino acids, and lipid classes.

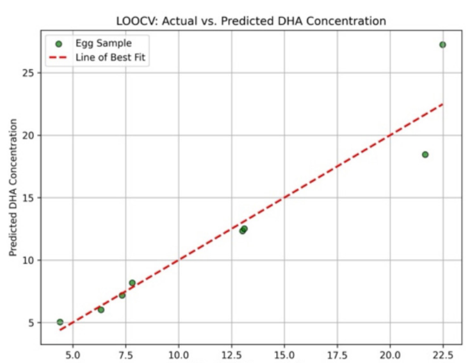

Figure 1. DHA concentration of eggs plotted against model-predicted concentration of eggs

A REOLIKE VIRUS (RLV) INFECTING THE HEPATOPANCREAS OF SPECIFIC PATHOGEN FREE *Penaeus vannamei*

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The international shrimp farming industry depends in part on shrimp supplied by breeding centers where the Specific Pathogen Free (SPF) status of their stocks is continuously monitored. According to guidelines by the World Organization for Animal Health (WOAH), the recommended methods for surveillance are based on PCR, which targets a limited number of listed pathogens. On occasion, a smaller number of samples is also included for histological analysis. Here, we report the re-discovery of a Reolike virus (RLV) facilitated by the inclusion of samples for histological analysis as part of a surveillance program.

Lesions highly suggestive of RLV infection were discovered during a health assessment of SPF *Penaeus vannamei* by using conventional H&E histology (Fig. 1A). Follow-up work by transmission electron microscopy (TEM) demonstrated the presence of viral particles with morphology consistent with RLV (Fig. 1B). Samples from the same population of shrimp were also analyzed by PCR for diseases listed by the WOAH and none were detected. The shrimp (3.5 g avg. wt.) had exhibited normal behavior and were otherwise healthy.

RLV infections have been reported in crustaceans, including penaeid shrimp. However, the relevance of RLV infection has been historically difficult to determine due to the presence of other ailments in the same shrimp. According to our observations, the virus appears to be very contagious, but we have not yet observed evidence of high virulence in the short term. We do not know what the long-term effect of the infection might be for example, on growth, survival, fertility, or resilience against other diseases or to stress due to sub-optimal grow-out or environmental conditions.

Current work on virus sequencing will provide insight into the taxonomic position and will allow us to design molecular tests based on PCR. These tests will be helpful in preventing the spread of a virus whose effect on the shrimp farming industry is yet unknown. This discovery exemplifies the usefulness of histological analysis and serves as a cautionary note to avoid relying solely on molecular methods for disease surveillance programs.

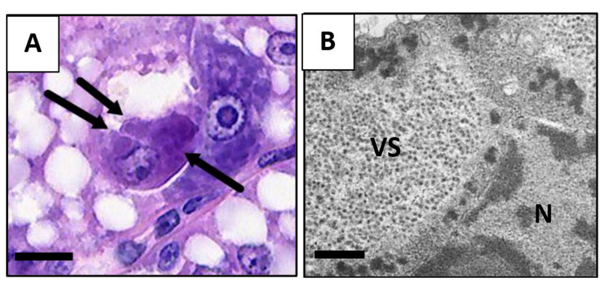

Figure 1. A) RLV basophilic cytoplasmic inclusions within a hepatopancreas "B-cell" (arrows). Bar=10µm. B) RLV particles within a virogenic stroma (VS) next to the host cell nucleus (N). Bar=500nm.

PREDATOR AND PREY DENSITY IMPACTS LARVAL PERFORMANCE OF LARGEMOUTH BASS IN AN INDOOR RECIRCULATING AQUACULTURE SYSTEM

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Largemouth bass (LMB), *Micropterus salmoides*, is a high-value freshwater aquaculture species with a rapidly expanding food fish market in North America. Proper transitioning from endogenous to exogenous feeding is pivotal for early rearing success. While some species can successfully be raised on artificial (i.e., commercial) diets from first feeding, LMB require a co-feeding period of both live prey and an artificial diet. Information regarding early feeding strategies for LMB are limited. Our objective was to determine how initial yolk-sac fry stocking density, prey density, and the stocking density × prey density interaction impacts LMB growth and survival.

LMB embryos were obtained from Red Hills Fishery (Boston, GA) and transported to Auburn University. At peak hatch, fry were stocked (~27^{o}C) in 25 L blue tanks equipped with recirculation technology at densities of 25, 50, and 100 yolk-sac fry/L. Starting at 3 days post-hatch (DPH), fry from each stocking density were factorially fed *Artemia* at 2, 4, and 8 *Artemia*/mL every 2-3 h from 07:00 to 23:00. All treatments received rotifers from 3-8 DPH and artificial diet (~0.5 to 1.0 g/tank) starting at 120 degree-days until 28 DPH. Mortalities and excess feed were removed daily. Rearing of offspring occurred under a 12-h light/12-h dark photoperiod at ~500 lux. Fish were randomly sampled weekly from 3-27 DPH for industry-relevant offspring performance traits, including notochord length, total length, eye diameter, myotome height, jaw length, yolk-sac area, body area, and fin-fold area. Daily survival and final tank biomass were recorded. To further understand phenotypic sensitivity to predator (fish) and prey density, we are currently following expression of genes associated with stress tolerance, growth, and development.

Preliminary results indicate that initial yolk-sac fry stocking density, prey density, and the stocking density × prey density interaction impacted offspring traits during this critical early life period. Highest survival (± SEM) and SGR were 90.3 ± 4.1% and 2.98 ± 0.1, respectively, when fry were initially stocked at 25 yolk-sac fry/L and fed 8 *Artemia*/mL. Highest biomass was 199.5 ± 5.6 g for the 100 yolk-sac fry/L and 8 *Artemia*/mL feeding treatment. Together, our approach will provide the global aquaculture sector with greater knowledge and technological innovation to enhance LMB hatchery production efficiency.

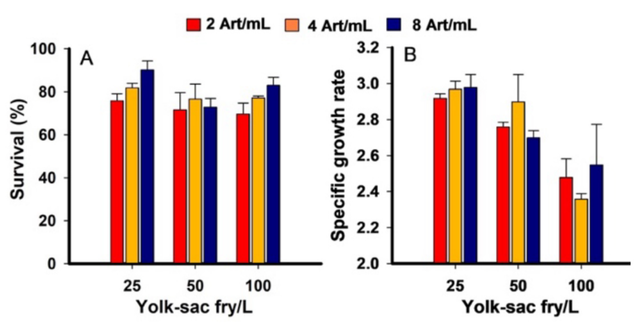

Fig 1. Impact of yolk-sac fry stocking density and prey density on survival and specific growth rate.

EFFECTS OF DIETARY CARBOHYDRATE SOURCES IN FLORIDA POMPANO (*Trachinotus carolinus*) GROWTH AND GLUCOSE METABOLISM GENE EXPRESSION

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Utilization of carbohydrates has reported beneficial effects when supplemented to aquafeed formulas. Carbohydrates are a well-known source of body energy, high-quality binding ingredient, and economical component for aquafeed. When inadequate or insufficient carbohydrates are presented in the diet, other energy-yielding nutrients, such as proteins and lipids are catabolized to produce energy. Thus, inadequate dietary carbohydrate sources could result in reduced growth performance, high feed conversion ratio, fatty liver deposition, and mortality. On the other hand, an appropriate carbohydrate source could lead to cost reduction of formulated diets, lessen ammonia excretion, improve protein consumption, and increase farm profitability. This protein-sparing effect of carbohydrates is becoming of great interest in aquaculture. Currently, little is known about carbohydrate metabolism in Florida pompano, *Trachinotus carolinus*. Thus, the objective of this research was to determine the effects of different carbohydrate sources on Florida pompano growth and glucose metabolism gene expression. To this end, five isonitrogenous, isolipidic, and isocaloric diets were formulated using different carbohydrate sources namely whole wheat grain flour, wheat starch, whole corn grain flour, corn starch, and dextrinized corn starch. At the end of the 10-week trial, fish were assessed for growth performance with fish fed whole wheat grain flour presenting higher weight gain and specific growth rate than fish fed corn-base diets. Liver transcriptomic analysis revealed differentially expressed genes related to glycolysis, gluconeogenesis, and lipogenesis.

Overall, the results indicated that from the tested carbohydrate sources whole wheat grain flour is the more adequate dietary carbohydrate based on the growth performance, physio-biochemical, and molecular approaches. These data are critical when formulating a complete commercial feed for sustainable and profitable culturing of Florida pompano.

A TALE OF TWO LEASES: COMPARING POTENTIAL SHELLFISH LEASES FOR FINAL SITE SELECTION

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This project uses an oyster bioenergetics model (Figure 1) to determine optimum growing conditions for Maryland oysters based on water quality at potential lease sites. Users will be able to get information to enter into the tool from publicly available data sources such as Maryland's Eyes on the Bay. Along with abiotic variables such as temperature and salinity ranges, users will be able to enter values to estimate food abundance for the proposed leases. The tool will include other variables such as bottom type and distance from land-based facilities to evaluate sites. Based on user inputs, potential leases will receive a numerical score that can be compared between sites to estimate which is better to grow oysters.

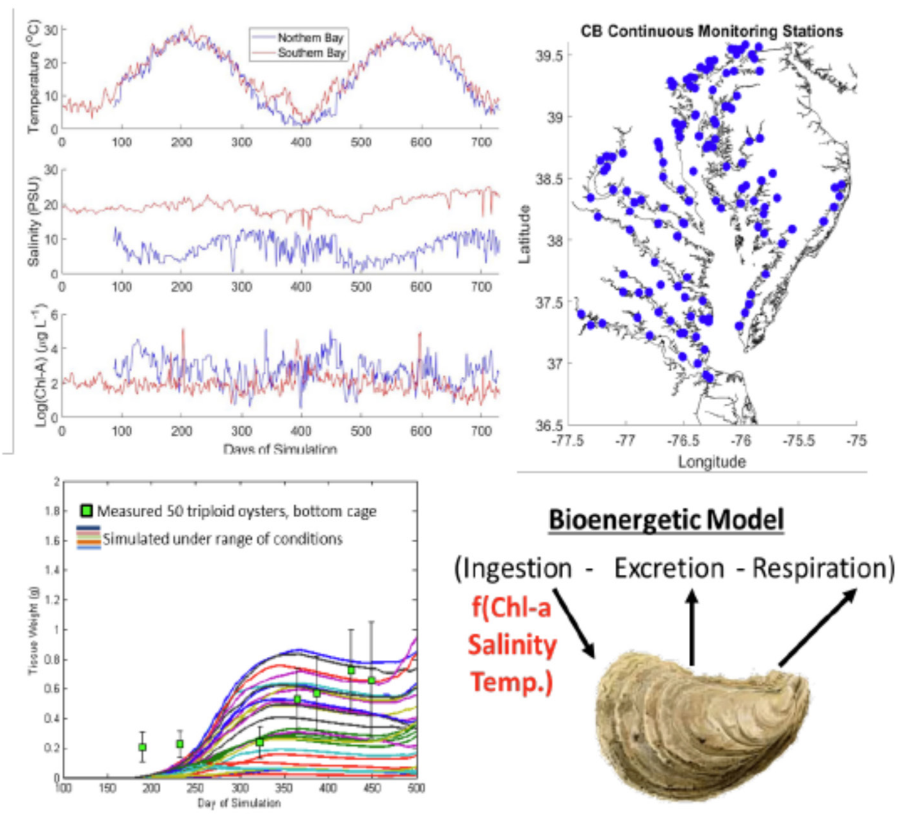

Figure 1. Montage of oyster growth model features including (clockwise from top left) example environmental data from two locations in Chesapeake Bay; available locations of multi-year environmental data already available in the Bay; simple schematic diagram of the bioenergetics model terms; and comparison of measured oyster soft tissue growth over a~2-year period with simulated growth under a range of representative Bay conditions.

INVESTIGATING FACTORS AFFECTING AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION IN THE UNICELLULAR GREEN ALGA *Chlorella* sp.

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This study establishes an Agrobacterium-mediated transformation platform for the unicellular microalga *Chlorella* sp. (UTEX B 3198). The microalga was cultivated in BG11 and Modified Bold 3N media, with total biomass and relative growth rate analyzed as key metrics to optimize downstream processes. The *Agrobacterium tumefaciens* strain LBA4404, carrying the pCAMBIA1305.1 plasmid (Add Gene plasmid number: 64401), expressing green fluorescent protein (GFP) and β-glucuronidase (GUS) reporter genes under a 35S promoter, was utilized for transformation. Several critical factors were systematically explored to maximize transformation efficiency. These included IPTG concentration, selection antibiotics, the ratio of microalga to *Agrobacterium*, and medium ratios, all of which were carefully optimized to ensure successful integration of foreign genes. The approach provided a high degree of precision in balancing these parameters, thereby enhancing the overall efficiency and reproducibility of the transformation process. The use of both GFP and GUS as reporter genes offered dual validation techniques, enabling real-time visualization of gene expression and enzymatic assays for deeper functional analysis. This dual approach not only confirmed the presence of the introduced genes but also allowed for the assessment of their active expression in the microalga. Such versatility in validation methods strengthens the platform's potential for broader applications in microalgal biotechnology. Molecular confirmation of successful transformation was achieved through PCR amplification of the GFP and GUS reporter genes, providing clear evidence of gene expression in *Chlorella* sp. (Figure 1 a-h). This molecular validation reinforced the robustness of the platform, offering a reliable and efficient method for genetic engineering in *Chlorella*, with potential applications in biofuel production, environmental monitoring, and other biotechnological fields.

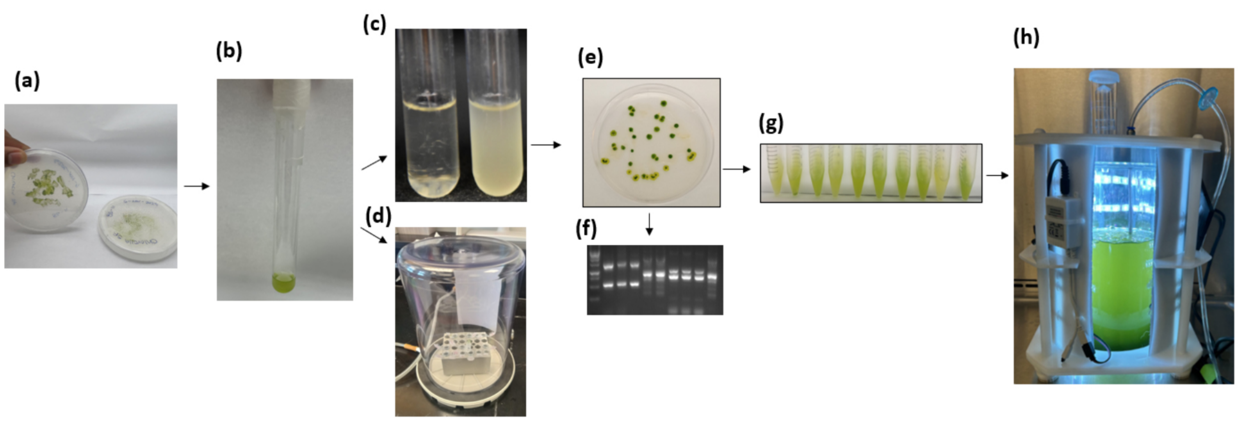

Figure 1 (a-h) illustrates the genetic transformation process of *Chlorella* sp. via *Agrobacterium*. Stages (a-b) culturing *Chlorella* sp. on BG11 medium for optimal growth, while (c-d) focus on culturing *Agrobacterium* as the gene vector. The vacuum infiltration method is used for gene delivery. In stage (e), transformed colonies are identified on selective media. Stage (f) confirms gene integration through PCR screening. Finally, stages (g-h) involve expanding the transformed colonies for further analysis and characterization.

OPTIMIZATION OF NUTRIENT CONVERSION AND RECOVERY IN MARINE AQUAPONICS

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Aquaculture production continues to increase and surpassed capture fisheries for the first time in 2022, accounting for 51% of global fish production. Despite this growth, aquaculture faces significant challenges. Wastewater from aquaculture contains high levels of nutrients, particularly nitrogen, phosphorus, and CO_2 emissions, contributing to ecological and environmental degradation. These environmental risks highlight the urgent need for sustainable waste management solutions. While aquaculture generates nutrient-rich wastes, this could be valorized and utilized for crop production through aquaponics.

Aquaponics, which merges aquaculture and hydroponics, offers a sustainable solution by creating a symbiotic relationship among plants, fish, and bacteria. However, most research focuses on freshwater culture, leaving the potential of marine aquaponics largely untapped. Marine aquaculture accounted for 55%, producing 71.7 million tons annually, while freshwater aquaculture contributed 45%.

This project integrates marine fish culture using hybrid striped bass and salicornia, a salt-tolerant crop with many uses and nutritional benefits in a closed-looped marine aquaponics system. The system is designed to convert fish waste for plant nutrition through nitrification in a biofilter tower. It also features CO_2 dispersion and extraction to enhance plant growth and development. The solid wastes are then repurposed for soil-grown or media-based plants, further reducing or eliminating nutrient discharge into the environment.

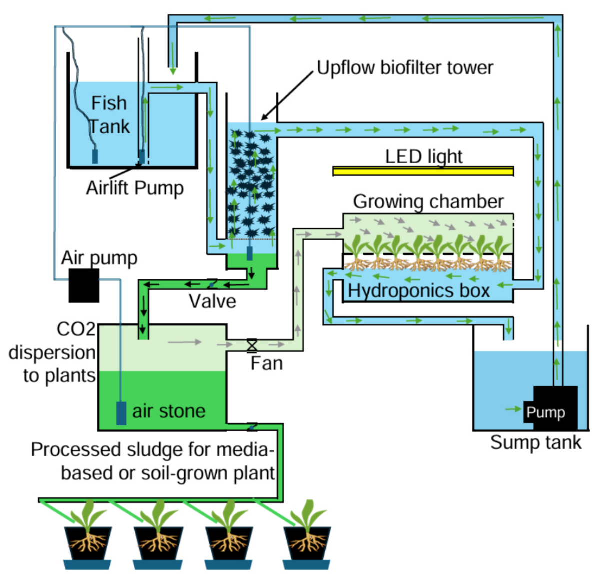

Figure 1. Conceptual design of the closed-looped marine aquaponics system for optimization of nutrient conversion and extraction.

PREVALENCE OF IRIDOVIRUS (AcIV-E) IN A STURGEON FARM IN NORTHERN ITALY: A STUDY FROM 2021 TO 2023

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Sturgeon farming is expanding in Europe, with Italy leading in caviar production. However, wild sturgeon populations have declined due to overfishing and non-native species. Aquaculture enhances caviar and meat output but raises concerns about disease management, especially viral infections like *Acipenser* iridovirus-European (AcIV-E). Young specimens and fry are particularly vulnerable, and diagnostic methods rely on real-time PCR (qPCR), as conventional virological testing is ineffective.

The sample included 482 sturgeon specimens with various health issues and tank mortalities, collected from eight farms in Northern Italy between January 2021 and December 2023. The fish represented *Acipenser* spp. and *Huso huso*, aged 12 months or younger, including *A. gueldenstaedtii* (n = 257), *A. stellatus* (n = 56), *A. baerii* (n = 50), *A. naccarii* (n = 47), *H. huso* (n = 33), *A. transmontanus* (n = 22), and *A. ruthenus* (n = 17). Samples were refrigerated during transport to the lab, where moribund specimens were euthanized with tricaine methanesulphonate (MS–222) following standard regulations. Each specimen then underwent necropsy, as well as bacteriological, parasitological, and virological assessments, to certify its health status. For the virological analysis, gills were sampled as they contain the highest concentration of the virus. The extracts were then tested using qPCR assay targeting the major capsid protein of iridovirus.

Of the 204 sturgeons that tested positive for AcIV-E, farmers observed symptoms such as anorexia and swimming ataxia, with affected fish exhibiting uncoordinated movements. *A. gueldenstaedtii* had the highest positivity rate, increasing from over 61% in 2022 to 72% in 2023 (average 63.81%). *A. naccarii* showed rates of 36% in 2022 and 54% in 2023 (average 40.43%), while *A. stellatus* had an average positivity of 23.21%. *A. baerii* and *A. ruthenus* remained below 15%, peaking at 20% in 2022, whereas *A. transmontanus* and *H. huso* tested negative throughout the study.

A three-year study (2021–2023) revealed AcIV-E infection as a significant health issue in sturgeon farming in Italy, with about 42% of the 482 tested specimens testing positive. *A. gueldenstaedtii* showed the highest positivity rate (63.81%), followed by *A. naccarii* (40.4%). The virus poses risks to natural populations, especially given the challenges in diagnosing AcIV-E and isolating the virus. The study highlights the economic threat of AcIV-E-induced mortality in aquaculture and its ecological risk to wild sturgeon populations, already endangered due to overfishing and environmental disruptions.

The inability to isolate the virus and lack of specific symptoms hinder timely diagnosis, making continuous health monitoring essential for sturgeon populations, both farmed and wild, to manage disease and ensure conservation.

EXPERIMENTAL INFECTION WITH *Lactococcus petauri* IN RAINBOW TROUT (*Oncorhynchus mykiss*): ASSESSMENT OF SERUM BLOOD PARAMETERS

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Lactococcosis is a serious bacterial infection that impacts both freshwater and marine fish species, known for its swift development and elevated mortality rates. The increasing prevalence of *Lactococcus petauri* infections in aquaculture poses significant challenges to rainbow trout health and production. Understanding the impact of this infection on serum parameters is crucial to understand the disease pathogenesis and for developing effective management strategies.

A total of 33 resistant (R) and 33 susceptible (S) rainbow trout individuals, weighing 450 ± 212.13 g, were anesthetized and injected intracoelomically with *L. petauri* (TURK72 strain) at a dose of 1×10^5 CFU/fish in 0.3 mL saline. Five S and five R individuals/time point were euthanized with MS-222 for blood and serum sampling, in accordance with current regulations (Legislative Decree No. 26/2014, which implements Directive 2010/63/EU, with permission for ethical statements No. 1012/2023-PR). Serum analyses were conducted at three different time points (T1-T4), comparing the results to baseline values (T0) as the pre-inoculation control. The analyses focused on total proteins, albumin, enzymes, lipids, metabolites, and minerals, while monitoring for mortalities was conducted.

Descriptive analysis of blood biochemical parameters in rainbow trout revealed variability across groups and time points. Urea levels ranged from 7 mg/dL (T4_S) to 10 mg/dL, while creatinine remained stable (0.14-1.09 mg/dL). Cholesterol and triglycerides exhibited significant variability, with cholesterol levels between 145 mg/dL (T1_S) and 624 mg/dL (T2_S), and triglycerides from 95 mg/dL (T1_S) to 640 mg/dL (T3_R). Significant fluctuations were observed in alanine aminotransferase (ALT) and alkaline phosphatase, particularly at T1. Particularly, phosphorus levels were significantly different in the resistant group at T1 ($p<0.05$), and total protein levels were higher in the resistant group at T1 ($p<0.05$). Iron levels increased significantly in both groups at T1 ($p<0.05$ for S, $p<0.01$ for R), indicating a differential response to *L. petauri* infection. Overall, certain biochemical parameters displayed significant changes post-infection, reflecting the varying responses between R and S trout.

The biochemical responses of rainbow trout to *L. petauri* infection indicate that specific parameters, such as phosphorus, ALT, calcium, and chloride, vary significantly between resistant and susceptible groups. These results highlight the potential of these biochemical markers for monitoring health status and responses to infection in farmed rainbow trout.

GLOBAL CHANGE AND ENVIRONMENTAL POLLUTION IN TROUT FARMING: EMERGING CHALLENGES

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Trout farming, as a key component of the Mediterranean aquaculture, faces escalating challenges from the combined impacts of climate change, water scarcity, re/emerging diseases, and environmental contaminants. These issues are of paramount importance to aquaculture research and innovation, necessitating advanced scientific and technological solutions. Climate change, notably through rising temperatures, poses severe risks to trout farming by affecting water quality, altering trout physiology and behavior, and intensifying disease pressures. Warmer waters disrupt the carefully managed conditions essential for trout, making them more vulnerable to disease outbreaks, reduced growth rates, and even mass mortality events.

Additionally, changing precipitation patterns worsen water scarcity, directly limiting the availability of high-quality freshwater critical for trout farm productivity. Reduced water supply and altered flow patterns challenge the maintenance of ideal rearing conditions, pressuring farms to optimize water.

A further pressing issue is the re/emergence of diseases exacerbated by environmental stressors. Effective disease management is essential for protecting trout farm productivity and maintaining ecological balance, as unchecked disease spread can lead to extensive stock losses and impact biodiversity in surrounding water systems. Meanwhile, the influx of emerging contaminants such as micro(nano)plastics, pharmaceuticals, and persistent organic pollutants presents additional risks to trout farming sustainability. These contaminants accumulate in aquatic environments, potentially compromising fish health and posing concerns for human consumers of trout products.

This presentation will examine innovative research strategies and technological advancements crucial for enhancing the resilience and sustainability of trout farming amid global change and pollution. Focus will be placed on the need for interdisciplinary collaboration and knowledge sharing to address these challenges comprehensively. By exploring current practices and future-oriented strategies, this talk aims to provide actionable insights for strengthening trout aquaculture's adaptability, ensuring its sustained role in global food systems despite increasing environmental pressures.

GENETIC AND GENOMIC RESISTANCE OF RAINBOW TROUT TO LACTOCOCCOSIS

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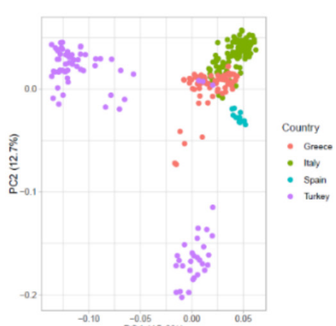
The management and containment of infectious diseases represent one of the main challenges for sustainable aquaculture development. Lactococcosis, characterised by high mortality rates, has a significant economic impact on both freshwater and more recently marine aquaculture production.

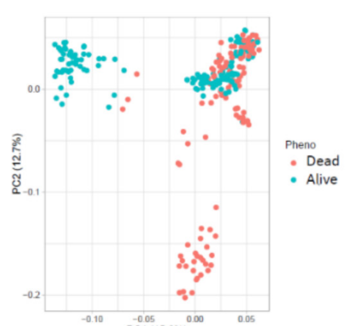
In the context of the European project SUPERTROUT selective breeding was proposed as control strategy for lactococcosis. Two different approaches were employed: a candidate gene approach based on the MHC class II gene, examining its involvement in resistance to lactococcosis, and a genomic approach aiming to investigate the involvement of different regions of interest across the genome.

This was made possible by selecting a farm with a high prevalence of lactococcosis, monitoring the progression of natural infection, and comparing the genetic and genomic profiles of dead and surviving individuals. Different MHC gene variants were found to be protective against lactococcosis in Italy, but only some of them have been confirmed as significant in other Countries partner of SUPERTROUT.

The study of 57,000 SNPs distributed throughout the trout genome, using commercially available systems, first revealed that the analysed populations (Italy, Turkey, Greece, and Spain) exhibited genetic differences among them; notably, the Turkish population was divided into two distinct groups, with deceased and surviving individuals separated within each group. However, the genomic association study did not identify markers significantly associated with disease resistance.

Figure 1: Trout population stratification.





DEVELOPMENT OF DIGITAL DROPLET PCR FOR THE DETECTION OF *Tetracapsuloides bryosalmonae* FROM WATER AND TISSUE OF PKD NATURALLY INFECTED RAINBOW TROUT

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Aim of the project RESILTROUT (Aquaculture resilient to global climate changes: research to support Italian rainbow trout production) is to perform a genome-wide association study (GWAS) to identify DNA markers for resistance to proliferative kidney disease (PKD) to be used in selective breeding programmes. GWAS will be conducted using a medium-density SNP array on dead and survived fish in natural outbreaks. Water samples were also collected to detect the presence of the *Tetracapsuloides bryosalmonae*, the causal agent of PKD.

A first screening on dead fish and water, for *T. bryosalmonae* detection, was performed using end-point PCR described by Kent et al. (1998) amplifying a 435-bp segment of the SSU-rDNA gene. Digital droplet PCR (ddPCR) was, instead, developed for absolute quantification and phenotype definition, establishing a ranking of infestation to support the role of *T. bryosalmonae* as primary cause of mortality. Specific primers and probes described for real-time PCR by Sieber et al. (2023) were used.

All dead fish analysed so far tested positive from kidney samples, with some spleen samples initially testing negative but confirmed positive by ddPCR. Water samples also tested positive for the pathogen. Figure 1 reports end-point PCR tested (spleen and kidney) while Figure 2 reports ddPCR plots for spleens negative in end-point PCR but confirmed positive, undiluted, with ddPCR (1S and 2S; B02 and G01); a kidney positive in end-point and confirmed diluted (1:100) in ddPCR (4K and F02); D01 was a kidney negative in end-point PCR tested undiluted in ddPCR and still negative. Results confirmed the high sensitivity of dd-PCR and its useful application for establishing a ranking of infestation.

Figure1. End-Point PCR.

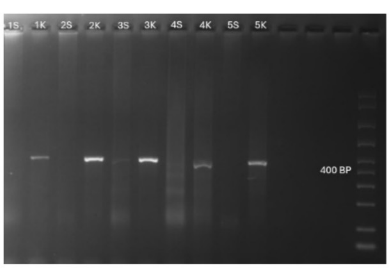
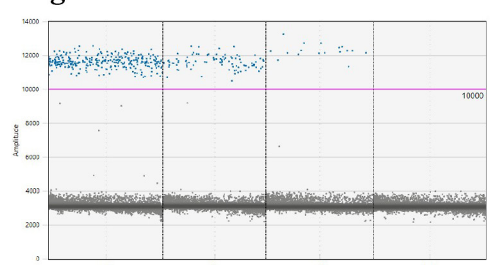


Figure 2. dd-PCR.



LACTOCOCCOSIS: A SINGLE DISEASE FOR MULTIPLE *Lactococcus* SPECIES

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Lactococcosis is a severe bacterial disease affecting both freshwater and marine fish, characterised by rapid onset and high mortality rates. Symptoms manifest early and include anorexia, melanosis, exophthalmia, and swim bladder ataxia. The bacterial species involved are *Lactococcus garvieae*, *L. petauri*, and *L. formosensis*, each display similar symptoms but variabilities in their genetic makeup and virulence have been detected. *L. garvieae* is the most common in Europe, while *L. petauri* is becoming more prevalent in Spain; *L. formosensis* has only been isolated in Southeast Asia.

In Italy, the situation is different, with *L. garvieae* still predominant in salmonid farms. In recent years, cases of lactococcosis have been reported in various species, including sea bass and gilt-head bream, and the disease shows high mortality rates (>50%), influenced by water temperature. Treatment is complex due to the rapid onset of symptoms and difficulties with antibiotics, which often lead to relapses, thereby complicating disease control efforts.

Effective disease management requires a robust prophylaxis strategy, including stringent biosecurity measures, optimised hygiene practices, and scheduled vaccinations. Although vaccination does not provide a definitive solution, it can be a valuable support in preventing lactococcosis, provided it is well planned and managed. Continuous monitoring of the pathogen prevalence and virulence, combined with the implementation of improved aquaculture practices, can enhance the resilience of fish populations against this emerging disease.

BUILDING A NETWORK FOR LONG SPINED SEA URCHIN *Diadema antillarum* PRODUCTION AND DISTRIBUTION IN FLORIDA

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Severe coral reef degradation throughout Florida's Coral Reef has resulted in >90% coral cover loss since the 1980s and actively threatens the identity, culture, and economy of this region. This reality has spurred the development and rapid expansion of in-water coral propagation and outplanting activities intended to restore live coral cover; unfortunately, restoration has been unable to keep up with the rate of ecosystem decline as evidenced by widespread coral bleaching and mortality earlier this year. Overcoming this immense problem requires an adaptive approach involving novel ecological intervention strategies.

One such strategy is re-establishing missing functional herbivory to prevent competitive overgrowth of corals by fast growing benthic algae. This presentation will detail the development of a network of collaborators making efforts to increase abundance of the long spined sea urchin *Diadema antillarum*, a formerly abundant keystone reef herbivore, through intensive aquaculture. Juvenile transport and grow-out investigations that occurred in both in-water and land-based enclosures will be discussed, along with future directions and opportunities for upscaling the program.

Figure 1: Transferring *Diadema antillarum* cultured in the Tampa Bay area directly to a vessel in the Florida Keys. These animals were placed in offshore cages designed for grow out prior to release on the reef.

AUTOMATING OYSTER AQUACULTURE WITH THE SUN

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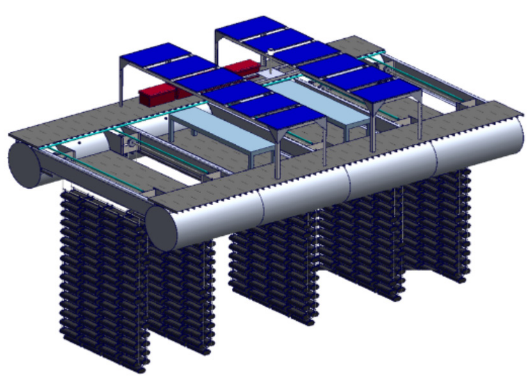
Oyster aquaculture technology has been relatively static in an industry needing to grow to meet global protein demand and advance restoration. Oyster aquaculture is very labor intensive, often growing less than a half million oysters per acre depending on gear types and site location. Floating and off-bottom gear is typically positioned in the near-shore environment or in the upper few feet of the water column, limiting overall growth and increasing conflicts with land-owners and other stakeholders.

The Solar Oysters Production System (SOPS) has been developed to take advantage of offshore space that is typically not used by other oyster aquaculture systems. The SOPS supports a series of cages in up to twenty feet of water and utilizes solar power to mechanically rotate the cage array through the water column and above the water, providing desiccation/air exposure and the opportunity for mechanized washing, which minimizes overall labor inputs. The rotation sequence can be programmed to meet the needs of the grower. The mooring system and robust design of the platform allows location of the SOPs in higher-energy environments, with integrated anti-poaching technology that allows units to be placed further offshore, potentially minimizing stakeholder conflict. SOPS can grow spat-on-shell oysters for restoration or from seed for market oysters. Approximately 150,000 oysters from seed can be grown on one 40’ x 25’ SOPS prototype (see below).

In the Chesapeake Bay watershed, nitrogen and phosphorus credits for oysters harvested from aquaculture operations can be traded on the nutrient credit market. Use of the SOPS for 1 acre of high-density oyster aquaculture may remove nutrients equivalent to treating stormwater runoff from over 100 acres of impervious surface.

A SOPS prototype was launched in October 2021 and loaded with spat-on-shell oysters in coordination with the Chesapeake Bay Foundation. For two years, growth was above average and mortality low.The diploid oysters were deposited on a reef at the entrance to the Baltimore Harbor in early November 2022. Triploid seed oysters were also grown effectively. In autumn 2024, SOPS was deployed onto an active oyster aquaculture farm in the lower Chesapeake Bay to gauge its effectiveness. SOPS will be commercially available in 2025.



MOORING STRUCTURAL LOADS ON A COMMUNITY-SCALE INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEM FOR THE GULF OF MEXICO

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This study focuses on the structural analysis of a sustainable Integrated Multi-Trophic Aquaculture system (IMTA), designed by the University of New Hampshire (UNH) for deployment in the Gulf of Mexico. Building on the IMTA system at UNH [1], this new system is intended for the growth of 4,000 red drum (*Sciaenops ocellatus*) as the fed species, alongside eastern oysters (*Crassostrea virginica*) and graceful red seaweed (*Gracilaria tikvahiae*) as the extractive species.

The floating structure utilizes 0.32-meter diameter high-density polyethylene (HDPE) pipes as concentric squares, forming two fish containment sections with a submerged volume of approximately 136 m³ each. Flotation is provided by the HDPE pipe with fitted expanded polystyrene (EPS) foam billets. The structure also includes transverse HDPE and longitudinal fiberglass beams, and support decking plates and stanchions. The design features an above-surface “jump net” (PET monofilament) and a submerged fish containment copper alloy mesh.

The mooring attachment loads were estimated using a dynamic, fluid-structure interaction software called Hydro-FE integrated with the Hexagon Marc solver [2]. Input parameters included waves and currents, associated with regular service and extreme hurricane conditions. The structural model was validated by comparing the results of the SolidWorks finite element analysis and Marc simulations for a reduced order model. The simulation results are being used to evaluate system stresses, appropriate safety factor values and to estimate the structure’s design life.

[1] Chambers, M., Coogan, M., Doherty, M., & Howell, H., 2024. Integrated multi-trophic aquaculture of steelhead trout, blue mussel and sugar kelp from a floating ocean platform. Aquaculture, 582, 740540.
[2] Knysh, A., Coyle, J., DeCew, J., Drach, A., Swift, M.R., & Tsukrov, I., 2021. Floating protective barriers: evaluation of seaworthiness through physical testing, numerical simulations and field deployment. Ocean Engineering 227, 108707.

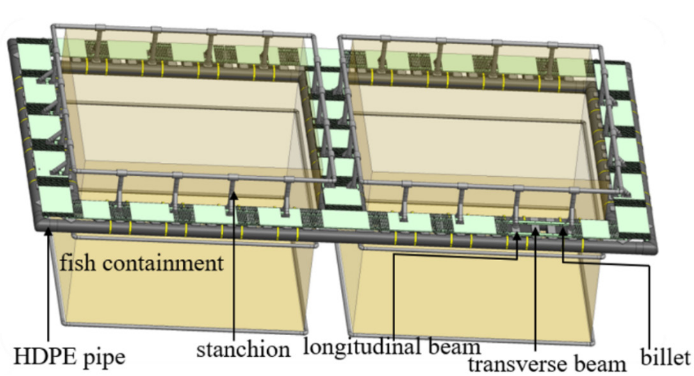

Fig.1: Integrated Multi-Trophic Aquaculture system

5
BUILDING A PIPELINE TO TRAIN AND RECRUIT INDIGENOUS STUDENTS IN COASTAL RESILIENCE AND SEAFOOD SAFETY

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Internships are incredibly popular for Indigenous undergraduate and graduate students, but many are advertised as all or nothing – an opportunity to learn, and engage, but a restrictive requirement to participate for the entirety of 8 (or 10) weeks. The summer months are times when school-age children are home, when canoe journeys are happening, and when students who have worked incredibly hard during the academic year may need intentional time to breathe. With this in mind, we have designed our Next Generation (NIFA-USDA, award #2023-70440-40154) internships to remove the bulk of conflict that comes from working with motivated aquaculture students who have cultural and family obligations during traditional internships.

In anticipation in meeting our Indigenous students' needs for an engaging summer of aquaculture research, we pivoted and changed our 8-week summer program to eight 1-week modules (Fig 1), which allowed flexibility not only for the students, but for the mentors and projects as well. The internships were place based at Northwest Indian College, in the Pacific Northwest, and the traditional lands of the Lhaq'temish People. Students were introduced to research concepts and practices of marine science, environmental water quality, freshwater nutrient loading, and oyster and clam toxicology (Fig. 2). A team of experts from academia, federal and state agencies, Indigenous organizations, and the commercial aquaculture sector supported the students. Students participated in a everything from basic concepts of oceanography and complex Indigenous management policies of shellfish and invasive species. After an introduction to summer aquaculture, our students will have the opportunity this academic year to present their research at scientific conferences, continued opportunities to engage with the aquaculture workforce, and support to apply for graduate schools.

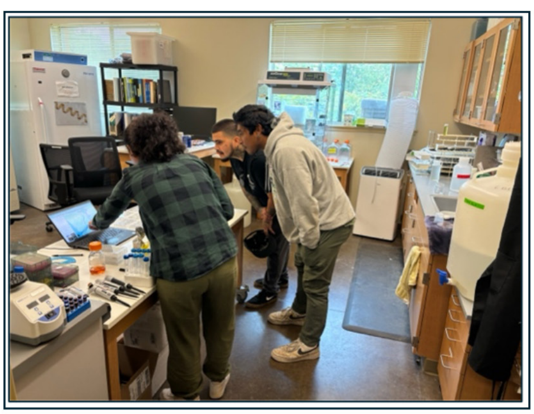

Figure 2. Students learn how to complete toxicology studies on larval Pacific Oysters.

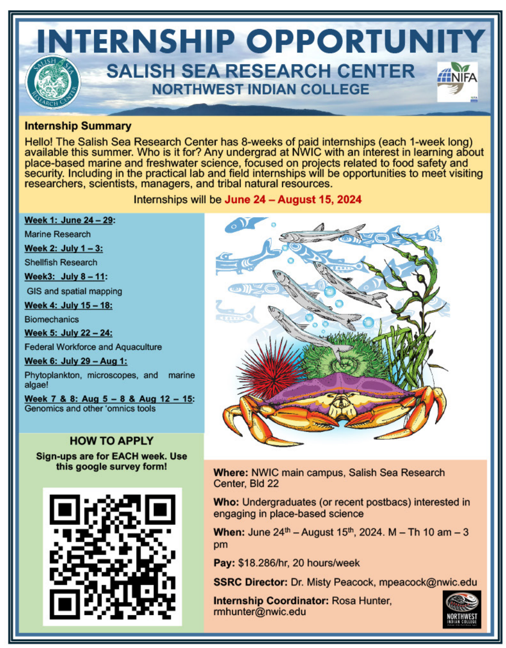

Figure 1. Summer internship flyer for the Indigenous program in aquaculture research at Northwest Indian College for 2024.

DEVELOPMENT AND VALIDATION OF A CHALLENGE MODEL USING GEOSMIN-SPIKED FEEDS TO OBTAIN OFF-FLAVOR COMPOUNDS IN THE TISSUES OF ATLANTIC SALMON *Salmo salar*

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Land-based aquaculture is a new and emergent way of producing seafood with minimal direct impacts on the marine environment and provides greater system control for farmers. However, fish filets derived from these systems often have flavors that are not desired by end-consumers, hereafter "off-flavor", which is largely attributed to the accumulation of geosmin (GSM) in fish tissues (Lindholm-Lehto & Vielma 2019). Research is needed to develop solutions for the avoidance or reduction of off-flavors when fish are grown in recirculating aquaculture systems (RAS). However, conducting these research trials currently requires the use of water-borne GSM which is problematic due to 1) the high cost of scientific-grade geosmin and 2) complications associated with GSM dosing and its interactions with life support systems. A previous study with trout showed that feeding GSM-spiked feeds to trout resulted in consistent GSM uptake in fish tissues and presents a potential tool in off-flavor research studies (Dupre et al., 2023); however, this approach has not been validated in Atlantic salmon (*Salmo salar*). The objectives of this research were to develop an open method for producing GSM-spiked feeds and validate the use of spiked feeds as a means of obtaining GSM in Atlantic salmon (*Salmo salar*) tissues.

In this study, we produced experimental feeds with five concentrations (10 ug/kg, 50 ug/kg, 100 ug/kg, 250 ug/kg, and 500 ug/kg) of GSM. Feed concentrations were validated using HiSorb™ GC-MS methods and showed a high correlation between targeted and measured concentrations of GSM in the feed (Linear regression, $R^2 = 0.96$). Benchtop trials were conducted to quantify the losses of GSM when feeds were exposed to water (leaching) and as a result of different methods of storage. In addition, a 4 week feeding trial with salmon, evaluating the relationship between feed and tissue GSM concentrations, was also conducted. The results of the benchtop and feeding trials will be reported and discussed. This research will provide a means to obtain consistent off-flavor compound concentrations in salmon tissues during research trials that aim to mitigate and eliminate these compounds in RAS.

RECENT ADVANCES AND APPLICATIONS OF PASSIVE ACOUSTIC MONITORING IN ASSESSING SHRIMP FEEDING BEHAVIOUR

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Passive acoustic monitoring (PAM) is a widely used tool for sound detection and assessing behavior of various aquatic species. Crustaceans have different mechanisms for emitting sounds, usually associated with specific situations or behaviors. The sound emission in shrimp occurs by the collision of their mandibles during food ingestion, generating a "click" sound that allows the association with the feeding activity. Therefore, PAM has been used to develop automated feeders in commercial farming, as well as represents a non-invasive alternative for studying shrimp feeding behavior in laboratory conditions. This presentation reviews the applications of PAM for evaluating shrimp feeding behavior in the laboratory, as well as introducing general concepts, terms and methodologies used in bioacoustics. Among the main contributions of PAM in laboratory studies with shrimp, the following can be highlighted: the acoustic characterization of clicks and mandibular structure associated with their emission for different species; detecting variations in acoustic parameters of clicks according to animal size and molt cycle, texture and size of feed pellets; and analyzing the effects on feeding behavior caused by shrimp size, stocking density and specific diet characteristics (texture, formulations, additives and pellet sizes). The PAM is an efficient ethological methodology to help improving our knowledge on the shrimp feeding behavior in laboratory conditions.

BUILDING A ROBUST PIPELINE TO AQUACULTURE CAREERS IN MAINE THROUGH WORKFORCE DEVELOPMENT & EDUCATION INITIATIVES

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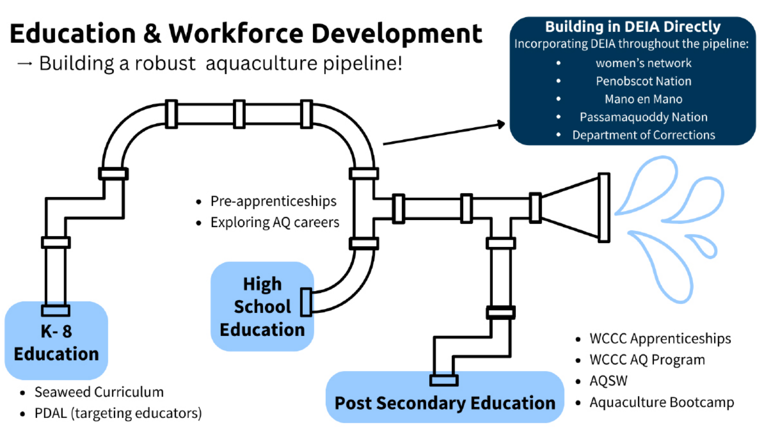
Climate change poses a major threat to the livelihoods of working waterfront communities around the world, prompting people to search for new career opportunities as many historical fisheries decline. In response to this vulnerability, aquaculture is emerging as an economic diversification strategy for working waterfront communities. With its ample coastline and access to resource-rich waters, Maine is a state that has long been reliant on wild-catch fisheries as an economic lifeline. Today, young people in the state find it increasingly difficult to enter wild-catch fisheries which has led members of the fishing industry to seek economic diversification in the form of aquaculture. However, as interest in Maine aquaculture grows, there is a need for strategic development of an aquaculture education pipeline that can support a skilled and reliable workforce. To create this pipeline, education and workforce initiatives must reach people at various stages of their education, from K-8 to high school to post-secondary.

Utilizing multiple funding streams to support a suite of projects, the Maine Aquaculture Innovation Center (MAIC) has worked with partners to create a strategic portfolio of aquaculture workforce and education initiatives that reach a wide range of participants. Such projects include: developing aquaculture curriculum resources for grades 4-6, working with high school students to deliver “Exploring Aquaculture Careers” classes, creating pre-apprenticeship opportunities for high school students to gain experience on aquaculture farms, and developing the first two-year Aquaculture Technology program in the state of Maine in collaboration with Washington County Community College. These initiatives are designed to be complementary and to target different age groups. To organize and execute these projects, MAIC works with partners across the state who are involved with aquaculture education and workforce development to ensure that programming meets state-wide industry and educational standards. Here we provide insight into the development of this strategic portfolio of aquaculture workforce and education initiatives, including the scope of each program/project. We also discuss how these initiatives are designed to support students along a pathway toward aquaculture careers while remaining flexible to the diverse needs and interests of various individuals.



IMMUNE GENE EXPRESSION AND THYMUS DEVELOPMENT AS INDICATORS OF IMMUNOCOMPETENCE IN LUMPFISH *Cyclopterus lumpus*

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Sea lice are a major threat to the financial and biological success of farmed Atlantic salmon, and are one of the top contributors to economic losses in the industry. The use of lumpfish, *Cyclopterus lumpus* L., as cleanerfish in salmon net pens has increased recently in Europe and Atlantic Canada, due to their demonstrated efficacy as a measure of sea lice control. To mitigate associated threats to fish welfare and biosecurity, aquaculture operations producing lumpfish must closely monitor the fishes' health and vaccinate them against harmful pathogens. An optimal vaccination regime has yet to be determined, as the understanding of lumpfish immune system development and adaptive immune response is still in its infancy.

This project seeks to establish when during early development lumpfish become immunocompetent through the expression of immunological gene markers, and the development of the lymphoid organ, thymus. A number of genes related to immune development and function, such as immunoglobulins M and D, T-cell receptor alpha, and major histocompatibility complex IIb, have been identified and Sanger sequenced to confirm identity. Additional immune genes are being tested and primer efficiency assays are underway. Once optimization has concluded, gene expression analysis using RT-qPCR will be conducted for each gene of interest spanning across developmental time points ranging from 28 days post fertilization to 34 days post hatch. Initial assessment of hematoxylin and eosin stained larval samples between 29 and 34 days post hatch show small clustering of lymphocyte-like cells on the medial side of each operculum, which may suggest the beginning of thymus formation. Histological examination of additional larval samples is underway. The intended outcome of this project is to inform lumpfish aquaculture operations of the developmental time point at which lumpfish are able to distinguish between "self" and "non-self" to ensure successful vaccination, avoid oral tolerance when fish are vaccinated, and minimize mortality caused by pathogens.

DOES NITROGEN, SUSPENDED SOLIDS, AND TURBIDITY PREDICT CHLOROPHYLL CONCENTRATION AND PRIMARY PRODUCTION IN CATFISH PONDS?

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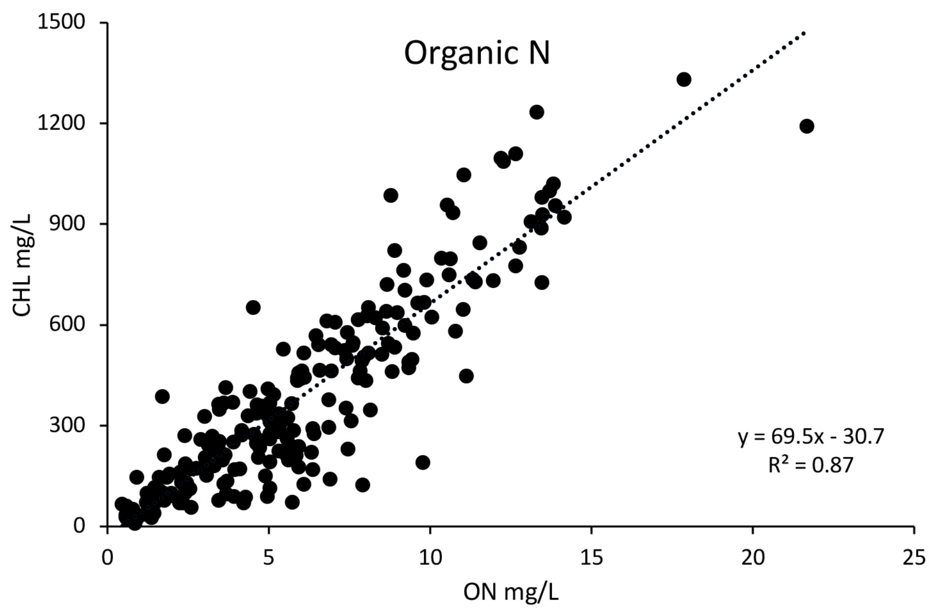
Phytoplankton play an outsized role in catfish pond ecology and are involved with ammonia removal, both dissolved oxygen concentration, and catfish off-flavors. Understanding factors influencing phytoplankton community dynamics will provide a better understanding of how major aspects of catfish pond production are regulated. Our goal was to evaluate the interplay between nitrogen, suspended solids, and turbidity as predictors of chlorophyll concentration, phytoplankton composition, and oxygen production through photosynthesis. Weekly water quality analyses were conducted with samples collected from 8 catfish ponds (0.25-acre) stocked with blue catfish (*Ictalurus furcatus*). Ammonia, nitrite, nitrate, and total nitrogen were measured using Hach TNT kits and a spectrophotometer, turbidity was using turbidimeter and suspended solids using standard methods. Photosynthetic production of oxygen and respiration by phytoplankton were measured in an illuminated incubator with shaker plates and changes in dissolved oxygen concentration were measured with a BOD probe. Using volatile suspended solids (VSS), turbidity, and organic N as predictor variables, total chlorophyll concentration was best predicted by organic N concentration ($R^2 = 0.81$). Organic N was also a good predictor of cyanobacteria ($R^2 = 0.71$), but not chlorophytes ($R^2 = 0.03$), likely because cyanobacteria tend to dominate as the phytoplankton community changes over the production season. Net oxygen production is best explained by VSS (R^2=0.48), followed closely by chlorophyll concentration (R^2=0.45). Organic N can reliably be used as a surrogate for total chlorophyll, but understanding other aspects of pond dynamics requires larger, more complex datasets.



EFFECTS OF BLACK SOLDIER FLY PROTEIN ON HISTOMORPHOLOGY OF INTESTINE AND LIVER IN JUVENILE RED DRUM (*Sciaenops ocellatus)*

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Fish meal is recognized for its excellent nutritional content and is a key element in many fish diets, however it takes up a larger part of overall cost compared to other ingredients. Additionally, fish meal market variability for aquafeed production confirms the need for an alternative protein source. Thus, it has been reported that incorporating Black soldier fish meal (BSFM) is a viable option to substitute fish meal for aquafeed production practices. Histological analysis of intestines in Red drum provides insights into the efficiency of nutritional absorption, immunity response and digestion processes. Similarly, histological liver analysis provides characteristics on lipid storage and lipid metabolism within the tissue. Thus, histological analysis is crucial to help identify potential impacts of experimental diets on the health and function of the liver and intestine when including BSFM as a fish meal substitute. Therefore, the objective of the study was to investigate the impact of BSFM on the liver and intestine morphology of Red drum. To this end, five experimental diets were formulated to meet nutritional requirements for Red drum. One control diet containing 100% fish meal and four with increasing BSFM protein levels to substitute fish meal (25%, 50%, 75%, 100%). A total of 400 fish were evenly distributed into 20 fiberglass tanks with a photoperiod of 12 hours light and 12 hours darkness. All experimental diets were randomly assigned to each tank with four replicates per treatment during an 8-week feeding trial. Our results suggest that fish fed with diets containing 50% BSFM protein inclusion presented increased muscle and villus thickness, with slight rise in goblet cells in intestine. Fish fed with 50% BSFM diet in the liver presented minimal changes in lipid deposition compared to the control. Fish fed with diet levels above 50% BSFM, presented noticeable signs of hyper-vacuolization and inflammatory responses in the liver.

EVALUATION OF BACTERIAL-BASED SINGLE CELL PROTEIN IN THE DIET OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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The worldwide expansion of aquaculture has resulted in a rising demand for fishmeal and fish oil for the manufacture of aquafeeds. However, relying on these resources is ecologically unsustainable. Therefore, the search for alternative sources of protein has intensified. One such alternative is bacterial-based single cell protein (SCP), which is considered sustainable because it can be produced using a variety of low-cost agricultural by-products and wastes, yielding a product that may contain high levels of protein and an attractive profile of essential amino acids. In the present study, the bacterial-based SCP MRD-Pro® was incorporated into diets for Pacific white shrimp (*Litopenaeus vannamei*) juveniles.

Based upon a control diet formulated with 21.4% fishmeal and containing 35% total crude protein and 6% crude fat, fishmeal was replaced with a single cell protein called MRD-Pro® (Meridian Biotech, LLC, Lexington, Kentucky, USA), produced by utilizing two different proprietary mixtures of organic coproducts and waste stream feedstocks as substrates. Meridian Biotech, LLC has developed a platform of sustainable and transformative technologies that repurpose low value by-products from multiple fermentation industries to produce value added, multifunctional feed ingredients for a variety of major and niche global markets including aquaculture.

For the first product, MRD-Pro® produced in Kentucky, USA (MRD-Pro®-Kentucky), 0%, 25%, 50%, 75%, and 100% of the fishmeal was replaced on a protein basis. For the second product, MRD-Pro® produced in India (MRD-Pro®-India), 0%, 25%, 50%, and 75% of the fishmeal was replaced on a protein basis. All diets were isoproteic and isolipidic.

Groups of twenty-five juvenile *L. vannamei* with an overall initial mean weight of 1.87 ± 0.02 g were stocked into 250-L circular tanks (71 cm diameter, 0.40 m^2 bottom area, filled to 200 L of seawater) in a recirculating aquaculture system (RAS). The RAS was equipped with aeration, sump tanks, biofilters, sand filters, UV light chambers and in-line heaters. Each experimental treatment was randomly assigned to five replicate tanks. Based upon pre-weighed feed rations, shrimp were fed to apparent satiation following visual cues to monitor consumed feed and adjust the ration daily, which was divided into three portions, administered at 08:00, 13:00, and 19:00 h.

The comparative feeding trial has just finished. The shrimp performance is being evaluated in terms of growth, survival, and feed utilization indices. Laboratory determinations, such as the *in vivo* apparent digestibility coefficients of the diets and the *in vitro* activity of trypsin and chymotrypsin from the digestive tract of shrimp in response to diet, are presently underway. In addition, the fatty acid composition of shrimp tissues and diets is being determined. Preliminary analysis of shrimp growth indicates that MRD-Pro® has a high nutritional value for *L. vannamei*.

ADVANCES IN THE CAPTIVE BREEDING OF *Chromis limbaughi* AND *Opistognathus rosenblatti*, TWO ENDEMIC SPECIES FROM THE GULF OF CALIFORNIA, MEXICO

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Limbaugh's damselfish (*Chromis limbaughi*) and the blue-spotted jawfish (*Opistognathus rosenblatti*) are aquarium fishes endemic to the Gulf of California, Mexico. In spite of being in the list of ornamental fishes with special protection status in the Mexican legislation for wildlife conservation (NOM-059-SEMARNAT-2001), they are among the five most heavily exploited fish species in this area. Such trade is based solely on the capture of wild specimens and the actual data on fish catches are unknown. Thus, there has long been strong suspicion of overexploitation, and possibly, species endangerment. Fortunately, greater awareness is currently raising worldwide among fish hobbyists about the environmental benefits of purchasing captive-bred and not wild fish. This presents a fantastic opportunity to lessen, and eventually eliminate, the current threat of fishing pressure on these species, since captive breeding may supply fish for the aquarium marine fish trade, and perhaps more importantly, for stock enhancement, which may be a key factor to their conservation. This study describes an ongoing investigation aiming at reproducing *C. limbaughi* and *O. rosenblatti* in captivity.

After obtaining fish collection permits (Permit No. SGPA/DGVS/00542/22 for *C. limbaughi* and SPARN/DGVS/04832/22 for *O. rosenblatti*) from the Mexican Agency for the Environment and Natural Resources (SEMARNAT), specimens of both species were captured in November 2023 in the waters adjacent to San Esteban Island in the Gulf of California, and placed, each species separately, in a recirculating aquaculture system -composed of glass aquaria, each with a capacity of 360 L- of the Kino Bay Experiment Station, University of Sonora (UNISON).

Fishes satisfactorily adapted to captivity, accepting foods in less than 48 hours after being captured. Foods offered include commercial flakes (Brine shrimp Plus Flakes, Ocean Nutrition), extruded feed (Gemma Diamond 1.5 mm, Skretting), pelleted feed made at the Aquaculture Nutrition Laboratory of UNISON (with fishmeal and krill meal as main sources of protein), as well as enriched (SELCO® Spresso INVE) on-grown, live *Artemia* sp., and fresh-frozen shrimp. The advances made so far are as follows. For *O. rosenblatti*, several spawns, in the form of egg masses, have been obtained, varying in size from 463 to 1,391 eggs. The eggs are circular in shape, with one oil drop, and a mean diameter size of 1,100 μm. Unlike other jawfishes, *O. rosenblatti* is not a mouth brooder and deposits the egg mass on the substrate inside a shelter. Unfortunately, none of the spawns contained fertilized eggs. For *C. limbaughi*, reproduction was achieved for the first time in captivity, with one pair of breeders producing four consecutive fertile spawns at intervals of approximately 18 days. Eggs are ovoidal in shape, measuring 862 ± 71.31 μm (length) × 619 ± 37.84 μm (width), with an oil globule of 200 ± 9.43 μm (diameter). Larvae were fed enriched rotifers (*Brachionus plicatilis*); however, they did not survive beyond day 6 of culture. Efforts are being made to further advance the captive breeding of both species.

USING POLAR BODY EXTRUSION TIMING TO OPTIMIZE PRESSURE SHOCK METHODS FOR INDUCING TRIPLOIDY IN BLUE MUSSELS *Mytilus edulis*

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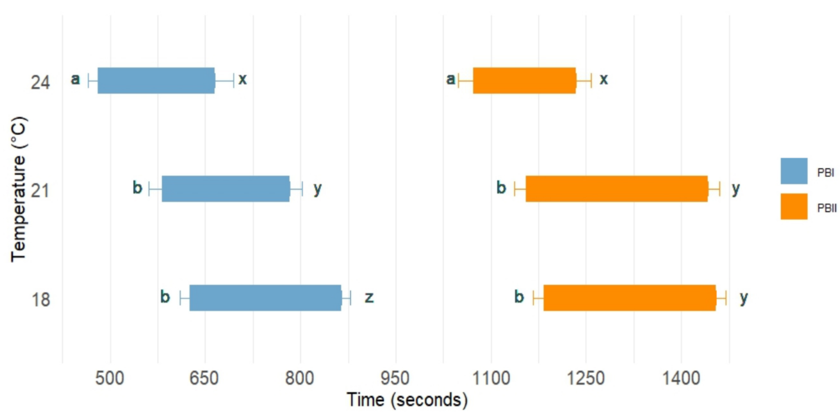
Triploid bivalves are increasingly favored in aquaculture due to their rapid growth and reduced investment in gametogenesis, which ensures steady market quality during the spawning season while minimizing ecological impacts on wild populations. The use of non-chemical methods to induce triploidy remains challenging, often resulting in low triploid yields and reduced larval survival. As consumer and industry preferences shift toward non-chemical treatments, refining these methods is essential for sustainable aquaculture. This study aimed to determine the optimal hydrostatic pressure shock treatment in the blue mussel (*Mytilus edulis*) to maximize triploidy induction while minimizing negative effects on larval survival and development. A series of experiments manipulating critical variables, namely time post-fertilization, pressure, and duration of the pressure treatment, were carried out using the timing of the polar body extrusion as a reference (Figure 1). Triploidy was confirmed via flow cytometry, and full-sibling diploid controls were produced for comparison. Survival and growth were measured every two days until competent larvae were observed (<28 days). Following this, experiments with different sources of mussels and temperatures were further carried out to examine the robustness of the optimal protocol (Figure 1). By refining the protocol for the hydrostatic pressure triploid induction method, this study provides valuable insights into the potential use of triploid mussels in aquaculture settings. The findings suggest that timing adjustments can significantly enhance triploid success, larval survival, and overall growth and ultimately offer a reliable technology for optimizing mussel production, benefiting both farmers and the industry.

FIGURE 1. Effect of temperature on the average timing (in seconds ± SE) of the first (PBI) and second (PBII) polar body extrusion in blue mussels (*Mytilus edulis*) from Prince Edward Island (Canada). Letters represent statistical differences between temperatures for each polar body (a,b,c for start time and x,y,z for end time).



IS DIRECT LOCAL MARKETING THE BEST STRATEGY IN EMERGING HALF-SHELL OYSTERS MARKETS?

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There has been marked growth in the local food movement. These markets are sometimes perceived as providing an opportunity for beginning small-scale farmers who cannot yet take advantage of economies of scale and have higher production costs. They often have low barriers to entry and consumers are often willing to pay premium prices. Local foods are sometimes promoted as helping local economies, and such initiatives are often supported with public funds. An important question is whether selling through local markets is indeed the most profitable and most economically sustainable option for growers. Some research finds that farmers choosing the strategy of sales only through direct-to-consumer outlets report earnings significantly lower than those from other strategies. Other research suggests that at the margin, relatively higher prices that farmers may receive from selling directly to consumers more than compensates for the increased marketing costs relative to selling through a distributor. Research also suggests that less experienced farmers are more likely to sell directly to consumers whereas more experienced farmers are more likely to sell through a distributor.

Small-to-medium sized oyster farms have two general strategies to get their product to market: selling directly into local farmed/branded half-shell market or selling dockside to a wholesaler. The direct-sale strategy provides farmers with the highest price potential but requires farmers to act as both farmer and distributor. It also limits sales to whatever the local market can absorb. Oysters that the local branded/farmed half-shell market cannot absorb will likely end up in the lower-value traditional half-shell market or the shucked-oyster market. The wholesale strategy allows the farmer to focus on production and to reach more distant markets, but reduces the price potential.

This analysis investigates the relative performance of these two marketing strategies using Monte Carlo simulation to estimate local market supply, demand, market shares, prices, and profit. It is particularly focused on emerging markets for farmed (off-bottom) oysters on the half-shell, that is, on markets where supply chains for locally farmed oysters may not yet be fully established. In the U.S., this means markets along the Gulf and southern Atlantic Coasts. The local market is modeled based on several factors, including the number and size of competing local oyster farms, the number of local buyers (restaurants), and number of oysters demanded per buyer. Results indicate that a marketing strategy focused on selling product at the farmgate to a wholesaler is likely to provide the greatest opportunity to be profitable relative to a strategy focused on selling directly into local markets. This result is due primarily to the inability of the local market to absorb all locally grown oysters. This result holds across a range of farm sizes, prices, growing conditions, transportation distances, number and size of competing farms, and number and demand of local buyers.

AN ASSESSMENT OF THE U.S. OYSTER MARKET

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U.S. oyster production has been in decline for decades, from the highs of the 1950s (80 million lbs. meat weight), to current production (25 million lbs). The value of production in real terms, however, is on par with that of the 1950s, at $219 million, implying that the real price of oysters has nearly tripled from $3/lb. in the 1950s to $8 today. The value of imports has nearly doubled, from around $50 million in the early 2000s to around $100 million today. U.S. consumption has increased as well, with total value increasing by half since the early 1990s, from about $200 million to over $300 million. Overall, domestic production has made up 80% of the value of domestic consumption, though that share has slipped to below 70% in the past few years.

One major change in domestic production has been the shift toward farmed oysters. NOAA estimates that U.S. farmed production has doubled since 2006, though FAO estimates indicate only a 25% increase. FAO estimates that farmed oysters account for 75% of domestic production compared to 2005 when it was last about 50/50. NOAA estimates farmed production at 42 million lbs., 1.7 times that of domestic landings. There may be some challenges, however, with these data. FAO appears to classify nearly all Atlantic Coast production as wild and Gulf Coast production as farmed. The Gulf Coast relies primarily on naturally spawned oysters grown on cultch, whereas much of the Atlantic Coast relies on hatchery-reared oysters. There remains a need for standardized definitions of what constitutes “wild” and “farmed”. As for NOAA data, it is not clear the extent to which landings data include farmed production.

Despite data limitations, some insights can be gained by examining implied prices. NOAA commercial landings over the past 10 years indicate the highest average price is among New England states ($39/lb), followed by Mid-Atlantic ($10) and South Atlantic ($7). The lowest prices are observed in the Gulf and Pacific ($6). New England has the lowest level of production, indicating production with hatchery-reared seed grown in containers bound for the half-shell market. Prices in the Mid- and South Atlantic indicate that both bottom and water-column production bound for both the half-shell and shucked markets. The Gulf leads all regions in production, where nearly all production is on cultch with naturally spawned oysters, much of it bound for the shucked market. The Pacific Coast is the second-largest producing region, relying primarily on hatchery-reared seed, but also likely sending many oysters to the shucked market.

More than half of the total value of imports over the past few years has been live/fresh oysters most likely bound for the half-shell market, 95% of which is classified as farmed and coming from Canada ($47 million in 2023) and Mexico ($11 million in 2023). Again, the implied prices may tell a story: Canadian farmed oyster price is $4/lb., whereas it is $2/lb. for Mexico. Canadian imports have surged of late, exceeding 10 million lbs., up from around 5 million lbs. prior to 2021. Mexican imports are also up, exceeding 6 million lbs. in recent years, up from less than 4 million lbs. prior to 2018.

A POTENTIAL MARINE PROBIOTIC STRAIN IN SHRIMP AQUACULTURE: NC201 *Pseudoalteromonas spp.*

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The genus *Pseudolateromonas* is known to have antibacterial activities which suggest that they could be potential candidates as probiotics in aquaculture. Several strains of bacteria have been selected in the surrounding marine environment of New Caledonia. After discrimination of the strains according to their antibacterial activities, one of them was selected and tested in larval rearing and during the juvenile phase in the blue shrimp *Penaeus stylirostris*. Called NC201, this *Pseudoalteromonas* showed inhibitory activities in vitro against various pathogenic *Vibrio* and more particularly those which affect local grow-out farms.

To understand the mechanisms that could explain these results, the NC201 complete genome was analyzed and more than 95% of identity was found with three other *Pseudoalteromonas* strains but 216 genes were specific to NC201 only. Two genes coding for proteins belonging to the LodA are involved in synthesis of antimicrobial molecules, *via* H_2O_2 production.

In vivo trials were conducted both in *P. stylirostris* larvae and juveniles to confirm the probiotic role of NC201. In larval rearings, animals which have received *Pseudoalteromonas* obtained higher survival rates than those with no treatment. The enumeration of the *Vibrio* load in the post-larvae showed a lower concentration in animals which received NC201 regularly. When administrated in juveniles, NC201 has improved survival rates in animals confronted to hypo and hypersaline stress as well as to a *Vibrio nigripulchritudo* infection challenge. Monitoring of hemolymph invasion by the pathogenic bacterium showed that NC201 slowed infection and could modulate the levels of several of immune and stress responses, thus, with an increase of the overall health status in the blue shrimp.

To allow a commercial outlet for this probiotic, freeze-drying trials were conducted in order to reduce the size of the strain's production and its ease of use. The NC201 strain was able to be concentrated at more than 10^{10} cfu/g and has been stored at -20°C for 4 years without loss of viability and alteration of its antibacterial properties <u>*in vitro*</u>. Further trials are now needed to confirm the probiotic properties of the lyophilized NC201 strains *in vivo* conditions in shrimp culture.

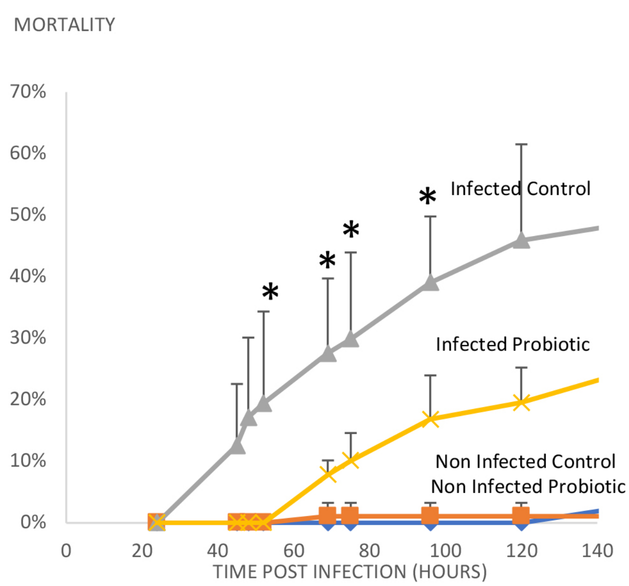

Figure 1: Cumulated mortalities in *Penaeus stylirostris* juveniles receiving or no probiotics when infected with *Vibrio nigripulchritudo*.

CHARACTERIZATION OF THE MOLECULAR DRIVERS OF PHYSIOLOGICAL TRANSITIONS IN PACIFIC SALMON

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Salmon and trout (salmonids) are important species for global seafood production and have great ecological and economic significance. Most aquaculture production of salmonids employs anadromous species that utilize both freshwater and saltwater environments.

Throughout their life salmon and trout undergo major physiological transformations to transition between hypotonic and hypertonic aquatic environments. Maturation and spawning is also associated with dramatic sexual dimorphic physical changes and systemic tissue degeneration. Coloration changes and loss of muscle quality are challenges for salmonid aquaculture, even in iteroparous species, like Atlantic salmon and rainbow trout, because it reduces market value of premature spawning fish.

We investigated the involvement of endocrine factors in Pink Salmon, *Oncorhynchus gorbuscha*; Rainbow Trout, *Oncorhynchus mykiss*; and Chinook Salmon, *Oncorhynchus tshawytscha* life history transitions using a combination of physiological analysis, CRISPR genome editing technology and whole transcriptome analysis. In adult Pink Salmon, the immune system appeared to be a key player in the degeneration of both somatic and reproductive tissues in the final stages of male maturation. Enhanced bone development was also important for the development of sexual dimorphic traits in these species, particularly in Pink Salmon for which the characteristic hump in spawning males is the partial result of increase bone growth. In larval fish, the loss of key endocrine pathways has important implications for the development of morphological traits and research is ongoing to understand the role of these pathways in the freshwater life stage. Our diverse findings shed light on several key aspects of salmonid biology which will add to the growing understanding of these important finfish species.

PAST SUCCESS AND FUTURE ROADMAP FOR GENOME EDITING IMPROVEMENTS IN AQUACULTURE

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The aquaculture industry is at the dawn of a new era in genetic improvement given the maturation of genomic selection and genome editing technologies. There is now more work on genetic improvement in aquaculture species than ever before. Genome editing has an important role to play in the future genetic enhancement of cultured aquatic organisms and there is a rich history of the industry being a leader in the use of biotechnology in animal agriculture.

How effective gene editing will be in improving aquaculture production and sustainability in the next decade will depend on the success of basic research and innovation in the aquaculture functional genomics and physiology communities. Advancing genetic enhancement will also require active and transparent engagement with public stakeholders as partners in progressing global food security through the development of resilient seafood supplies.

The talk will focus on where we are in the use of genome editing technology in aquaculture, and paths forward to overcome future barriers to its increased application in the industry. This includes three areas that need to be addressed. 1) Improving basic science at the interface of genetics and physiology, 2) Developing technical capabilities and genome resources for emerging species and complex traits, 3) Increasing workforce training and understanding of aquaculture biotechnology as well as open collaboration and sharing of edited germplasm resources, and finally 4) Communicating the benefits of genetic enhancement to meet the food security needs of a growing human population. These ideas will function as a platform for open dialog on genome editing technology and its ability to translate genomic discoveries into more productive and sustainable aquaculture stocks.

INTEGRATING MACHINE LEARNING DRIVEN PHENOMICS AND HIGH-THROUGHPUT CRISPR GENOME EDITING TO BUILD THE FOUNDATION FOR RAINBOW TROUT GENOME DESIGN

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Advances in genome editing have enabled new potential for improving the genetics of aquaculture species. For species such as salmonids where genome editing technology is highly effective and well-established, the ability to modify new production traits is limited only by our knowledge of salmonid molecular physiology. Knowing what to edit is a serious challenge since identifying genetic mechanisms underlying key production traits can take decades of basic research.

The explosion of the field of aquaculture genomics has dramatically increased our understanding of genome structure in many species, but significant work remains to exploit this information to define how the genome functions. To help address the daunting task of characterizing genome function in rainbow trout, *Oncorhynchus mykiss*, major efforts are underway to integrate genome editing capabilities with modern machine learning technology to rapidly assess the impact of gene edits on fish physiology. This ongoing Decode the Salmonid Genome Project is initially focused on genetic traits associated with growth and environmental resilience in trout but also is laying the foundation for investigating other key production traits. Currently, hundreds of unique genetic lines are being examined in mass phenomics trials (i.e., growth, thermal and hypoxia tolerance) to identify high performing genetics.

The information we have gained has provided new insights into potential mechanisms of enhancing production and sustainability in this species. This knowledge is beginning to define core biological mechanisms that could form the foundation of intelligent genome design of rainbow trout as well as other commercially valuable salmonid species. Our work demonstrates the power of high-throughput genome characterization as an underlying tool for promoting a future where genome designed aquaculture species are commonplace.

COMPARATIVE ANALYSIS OF PHYSICAL WATER QUALITY IN ADULT AND JUVENILE CATFISH PONDS

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Water quality is paramount for fish health and growth, with specific needs varying between life stages. Adult catfish thrive in temperatures of 65-80°F and prefer low turbidity and dissolved solids to minimize stress and support metabolic functions. Juvenile catfish, being more susceptible to environmental changes, require stable temperatures and minimal turbidity to ensure proper development and high survival rates. Understanding physical water quality is crucial as it directly influences fish metabolism, oxygen availability, and overall health. High turbidity can reduce light penetration and affect feeding behavior, while significant temperature variations can stress fish, leading to immune suppression and increased disease susceptibility. This study involves systematic sampling and analysis of water quality from both adult and juvenile catfish ponds located in Delaware State University Aquaculture Research and Demonstration Facility earthen ponds. This facility does not discharge water but recirculates through retention ponds to allow any potential sediments to settle and water to be filtered through submerged grasses to remove excess nitrogen and phosphorus build-up. Well water is the primary source for the facility. The ponds used in this study had 70x60dimensions. We had a total of 1000 juvenile catfish in each earthen pond. Water quality parameters consisting of water temperature, Dissolved Oxygen (DO), pH, and salinity were recorded using a YSI 556 Multiprobe DSS. Preliminary findings indicated that both the juvenile and adult catfish are living in suitable conditions for their given life stages. Future research will examine the long-term effects of physical water quality variations on catfish growth performance and physiological responses, further contributing to sustainable aquaculture management strategies.

SEA LICE *Lepeophtheirus salmonis* RESISTANCE IN THREE STRAINS OF ATLANTIC SALMON *Salmo salar*

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The USDA National Cold Water Marine Aquaculture Center (NCWMAC) has been evaluating families of North American Atlantic salmon (Salmo salar) for resistance to sea lice (Lepeophtheirus salmonis) since 2015 and are currently evaluating the second generation of families selected for sea lice resistance. With the spawning of the second generation in 2021, the USDA began several different efforts to evaluate the success of the selection program. One of the efforts was to identify if different strains of Atlantic salmon with discrete long-term exposure histories to sea lice had different levels of resistance. This effort was supplemented with fin and skin transcriptomic profiling of each strain at sites of infection and at parasite free locations to investigate what factors might explain phenotypic differences between the strains.

Three different strains of Atlantic salmon smolts were compared: the St. John River (SJR) strain of historical sea run Atlantic salmon with many generations of continual sea lice exposure and the primary commercial strain used in net pen culture in Maine; the Casco Lake (CAS) strain which has been historically land locked with no history of exposure to sea lice in modern history; and the Grand Lakes Stream (GLS) strain, which generally remains in freshwater lakes and rivers but does have access to the ocean where they could be exposed to sea lice. Smolts from all three strains were challenged in four replicate tanks with 30 fish from each strain per challenge tank. A two-way ANOVA accounting for strain and challenge tank showed a significant difference in lice density between strains with CAS fish being more susceptible to sea lice than either SJR or GLS. Transcriptomic analyses of skin and fin revealed that, although skin and fin had strikingly different transcriptomic profiles, sites with or without sea lice copepodid attachment were generally indistinguishable. This study indicates that selection for sea lice resistance can be made within Atlantic salmon; however, transcriptomic responses in all 3 strains indicated little to no parasite recognition, suggesting immune responsiveness to copepodid infestation may not be a significant pathway involved in observed variations in resistance.

PRODUCTIVITY IMPROVEMENTS FOR LONG-SPINED SEA URCHIN *Diadema antillarum* AQUACULTURE FROM SETTLEMENT THROUGH JUVENILE GROWOUT

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The long-spined sea urchin *Diadema antillarum* is a keystone herbivore historically responsible for reducing competitive algal overgrowth on Caribbean coral reef habitat. Limited natural population recovery following a mass mortality event in 1983-84 has inspired intensive aquaculture and restocking efforts to restore functional herbivory in support of ambitious coral reef restoration goals. *Diadema* is difficult to sexually propagate in captivity; culture bottlenecks during various stages of this species' biphasic life history challenge the viability of scaled production for restoration. Here we report findings from multiple replicated investigations towards improving 1) larval settlement rate, 2) growth and survival of newly settled postlarvae (< ~2 mm test diameter (TD)), and 3) prepared diet selection for intensive juvenile (> ~2 mm TD) growout.

In an initial study, larvae reared in a novel recirculating aquaculture system for 46-49 days were transferred to replicate petri dishes and exposed to a range of potential settlement cues. Significantly higher mean percent settlement (± SE) occurred in response to the calcareous macroalgae *Halimeda* sp. (58.0 ± 3.7) compared to naturally derived biofilm (30.0 ± 7.1). A subsequent diet study revealed significantly improved postlarval growth response to flocculated live microalgae (flocc; 0.027mm day^{-1}) compared to naturally derived biofilm (0.012 mm day^{-1}) and a benthic diatom *Navicula perminuta* (0.023 mm day^{-1}) over five weeks. A mixture of postlarvae and juveniles from the same cohort were then grouped by size and distributed to replicate bins containing either flocc or dried nori in a 2x2 factorial experiment. Results suggest that *Diadema* can be weaned onto prepared foods at ~2 mm TD. Lastly, we describe efforts to develop a water stable diet with high nutrient retention and juvenile growth response by combining blended dried nori with various concentrations (w/v) of alginate and gelatin binder. Experimental results from these efforts will be provided.

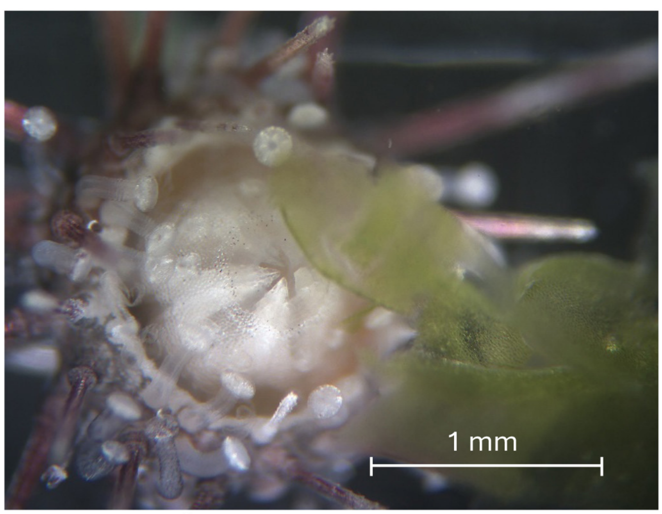

Figure 1: Oral view of juvenile *Diadema antillarum* consuming nori.

CONNECTING UNIVERSITY SHELLFISH AQUACULTURE RESEARCH FARMS

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As the shellfish aquaculture industry in the United States has grown, so has the need for applied research specifically focused on optimized production and farm efficiency. The United States Department of Agriculture supports state land-based agricultural experiment stations that investigate potential improvements to food production and agribusinesses. Much like land-based agriculture, there is need for country-wide applied research in shellfish aquaculture to advance the industry. Multiple land grant universities and public entities across the United States utilize shellfish leases, also referred to as farms, to conduct relevant research on shellfish aquaculture production; however, these entities often operate independently with minimal communication between one another, which hinders the advancement of the industry. Improving communication and establishing collaborative projects between research farms across the country would likely improve responsiveness to industry needs, open possibilities of standard data collection, and provide a comprehensive understanding of how issues affect different regions of the United States.

The focus of this project is to bring these institutions together to increase communication of current research focuses within applied shellfish aquaculture research and create a collaborative network. A comprehensive list of participating facilities in the United States will be published in the format of an ArcGIS Story Map, allowing colleagues to discover potential collaborators, and easily find current contacts for facilities. In addition to the comprehensive list, a “Shellfish Research Farms” networking group has been curated in Aquaculture Information Exchange and will be maintained to facilitate communication. This will serve as a catalyst for the network to expand, leading to increased support for the shellfish industry.

WORKFORCE DEVELOPMENT OPPORTUNITIES WITH THE VIMS COMMERCIAL SHELLFISH AQUACULTURE LAB & TEAM

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The Commercial Shellfish Aquaculture Lab & Team (CSALT) at the Virginia Institute of Marine Science (VIMS) is offering workforce development opportunities that complement existing programs and respond to shellfish aquaculture industry input.

In response to industry demand for individuals with grow-out experience, the shellfish research farm apprenticeship at VIMS is designed to train individuals to be well-rounded members of the shellfish aquaculture workforce. The learning objectives and goals are adopted from the Maine Aquaculture Association's Occupational Standards for Shellfish and Sea Vegetable Farms for the standards associated with "crew chief" (e.g., operations management, team management, and regulatory compliance). To learn these objectives the apprentice works one-on-one with the farm manager of the CSALT lab on a one-acre research farm in a six-month, full-time position. Throughout the process, crew chief skills are rehearsed and evaluated to ensure proper training. Ideally, trained apprentices are well-positioned for a position with a commercial shellfish farm. The condensed pilot program launched in August of 2024 to guide the program in 2025.

In addition to the apprenticeship, CSALT hosts professional Master's (MA) degree students through the Batten School of Coastal and Marine Sciences. While the MA program has students studying a variety of disciplines, the programs for students in the CSALT lab are tailored to prepare individuals for careers in shellfish aquaculture. Coursework includes experiential learning on commercial farms and modules in different aspects of bivalve shellfish aquaculture (biology & production, environmental interactions, and socio-economic considerations). Professional development is encouraged, including participation at industry association meetings.

Finally, VIMS students can participate in two-credit, semester long experiential learning opportunities of 'Shellfish Aquaculture Applied Methods'. These include learning opportunities in the hatchery, nursery, algal production and/or grow-out operations. These courses expose students to new career pathways and provide hands-on learning, framed again around the Maine Aquaculture Association Occupational Standards for Land-Based Shellfish Hatcheries.

CARBON-NEGATIVE SHELLFISH FARMING THROUGH ELECTRIFICATION AND RENEWABLES – AT SEA AND ON LAND

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As climate change increasingly threatens marine ecosystems, the shellfish farming industry faces challenges and opportunities. Shred Electric is at the forefront of addressing these challenges by pioneering carbon-negative shellfish farming solutions. In response to the urgent need for sustainable aquaculture, we have developed a suite of technologies that significantly reduce the carbon footprint of shellfish farming operations. These include advanced battery and renewable energy kits for on-farm equipment, swappable batteries for electric outboard motors, and ShredCube™ solar-powered refrigerated containers optimized for use with electric trucks and vans. This comprehensive approach to electrification drastically lowers the carbon intensity of farming, processing, and transportation.

Shred Electric's innovations, supported by the Maine Technology Institute, the National Science Foundation, the Island Institute, and the Gulf of Maine Research Institute, directly address the intersection of climate change and shellfish farming by offering practical tools for carbon footprint management. As natural carbon sequesters, shellfish help combat ocean acidification and climate change. By eliminating the carbon emissions associated with their farming, we contribute to a cleaner and more resilient marine environment.

Shred Electric developed solar-powered barge and float canopies, portable battery powered haulers, winches, and pumps, and self-powered refrigerated containers to decarbonize sea farm operations. These innovations offer scalable solutions that can be adopted across the industry, creating a pathway to genuinely carbon-negative aquaculture.
Our patent-pending ShredConnect™ system enables cloud-based monitoring and controls of sea farm power equipment, allowing farmers to focus on farming. Shred Electric monitors battery charge states, uses AI to analyze operations and power generation, and alerts partners when issues arise.

The growing awareness of aquaculture's role in addressing climate change underscores the importance of managing the carbon footprint of shellfish farming. By combining renewable energy systems, electric transportation, and sustainable farming practices, Shred Electric aims to reshape the future of shellfish farming, turning it into a carbon-negative, climate-resilient food production model.

HOW MANY MARKERS DO I NEED? OPTIMAL MARKER DENSITY AND GENOTYPING TOOL CHOICE FOR LONG-TERM GENETIC SUCCESS IN AQUACULTURE BREEDING PROGRAMS

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Achieving sustained genetic progress in aquaculture breeding programs is now commonly requiring the evaluation and implementation of genotyping tools. Selecting a tool with the optimal or minimum viable number of markers required for the successful long-term implementation of the chosen breeding strategy is key to the success of the breeding program. This presentation outlines and examines through two case studies the role that the number of traits, genome size, linkage disequilibrium (LD) breakdown, recombination rate, genotype by environment interactions (GxE), and the need for sustained precise selection over multiple generations has on the required number of markers.

The number of traits under consideration plays a pivotal role in marker density determination. A multi-trait breeding program demands a higher marker density to capture the genetic variation associated with each trait accurately. Different traits, such as improving growth rate, disease resistance, quality, and reproductive performance, all influence the required density and selection of markers to ensure all gains can be made across traits aligned with the program's breeding objectives.

Genome size affects the overall genomic landscape, necessitating adjustments in required marker density to adequately cover the entire genome. Linkage disequilibrium breakdown and recombination rate are crucial factors influencing marker spread and effectiveness. Understanding the dynamics of LD breakdown and recombination allows for the strategic placement of markers to ensure coverage of critical regions linked to the target traits. Balancing marker density to accommodate LD decay and recombination events is essential for maintaining breeding program efficacy over generations.

In conclusion, this presentation will underscore the importance of a comprehensive and adaptive approach to marker density planning and genotyping tool choice in aquaculture breeding programs. By considering the number of traits, breeding objectives, GxE interactions, genome size, LD breakdown, recombination rate, and the impact of selection over multiple generations, breeders can develop resilient strategies for sustained genetic improvement in aquaculture species through the appropriate choice of genotyping tools.

HARNESSING EFFICIENT GENETIC IMPROVEMENT TO MAXIMIZE ECONOMIC RETURN IN OYSTERS

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Selective breeding is the process of improving one or more desirable traits of a cultured species through the selection of superior parents for the next generation. Phenotypes that can be selected directly such as growth are relatively easy to measure and select for in most breeding programs. Indirect or complex traits such as robustness or selecting for a combination of important traits is more complicated both to measure and to fit into a statistical broodstock selection model. In this talk we will briefly discuss the general concepts and common strategies for breeding program management, from the simplest requiring the least amount of investment to the more complex, requiring greater investment but delivering greater genetic gains across more traits. More importantly, we will illustrate the use of the phenotypes and genotypes for a breeding program and how the breeding strategy should be designed to maximize economic returns by balancing input costs with the expected genetic and economic gains for a commercial aquaculture producer.

In summary, there are multiple options for enhanced selective breeding program management, each requiring different inputs and investment with varying potential returns and gains. Key to the design of a genetic improvement program is the consideration of each program's breeding goals, size, and available budget along with selection of appropriate tools to support such a design. Tools under consideration range from relatively simple mass selection plans, through sophisticated genomic selection strategies, to incorporation of new technologies like genome editing.

DEVELOPMENT AND TESTING OF A NON-LETHAL DNA BIOPSY METHOD IN EASTERN OYSTER *Crassostrea virginica*

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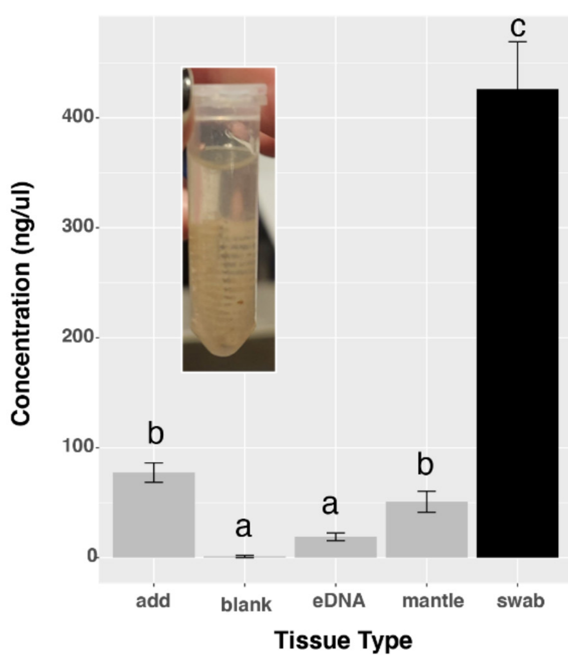
As cost-effective high-throughput genotyping tools become more widely available, the application of genomic selection in oyster breeding is becoming more common. A critical step in this process is the non-lethal DNA biopsy of candidate broodstock for genotyping and generation of genomic estimated breeding values (GEBV). However, non-lethal biopsy can be challenging because oysters must be 'relaxed' with MgSO4 to access tissues, and extraction of mantle or hemolymph can be time consuming and damaging.

Here we present results examining multiple non-lethal biopsy methods – mantle clipping, swabbing of viscera, and filtered culture water of individual oysters (eDNA) – for their effect on DNA concentration/quality and genotype call rate using the Eastern oyster 66K HD SNP array. Genotype correspondence was also assessed between non-lethal biopsy DNA and DNA extracted from the adductor muscle of the same individual. DNA yields were substantially higher from the brush swabs, followed by mantle clips, and eDNA extractions. Genotype call rates were >94% for all mantle and brush samples and not significantly different – call rates were lower for the eDNA extractions but still generally above 90%. DNA from swab samples (cytology brush, mascara brush, and cotton tipped swab) had similar and very high genotype correspondence with adductor-derived DNA (less than 1% genotype error) with cytology swabs providing the lowest error rate. Overall, non-lethal cytology brush biopsy DNA provides high quality SNP array genotype data, similar to mantle or adductor-derived DNA, and are easier and faster to implement when sampling.



USING A DYNAMIC ENERGY BUDGET MODEL TO ASSESS THE RISKS OF SHIPPING NOISE ON GIANT SCALLOPS BEDS IN THE GULF OF ST. LAWRENCE

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The physical characteristics of sound wave propagation in aquatic environments make it an efficient sensory cue for many aquatic organisms, providing a means for long-range directional perception. Since the industrial revolution, underwater acoustic environments have been significantly altered by human activities. Among these disturbances, shipping noise is the most widespread in both space and time, covering the broadest range of sound frequencies among anthropogenic noise sources.

In this presentation, we integrate laboratory-based behavioral data on the sound perception thresholds of two developmental stages of the giant scallop (*Placopecten magellanicus*), 1-year juveniles and 3-year adults, with a three-dimensional model that estimates marine traffic noise in the Gulf of Saint Lawrence, available on an annual scale with daily resolution (https://soundscape-atlas.uqar.ca). Our findings suggest that both juvenile and adult scallops experience an increase in valve movements triggered by navigation noise, affecting up to one-third of the scallop beds within our study area, particularly during late fall, winter, and early spring.

To quantify the energetic costs associated with these behavioral changes and their impacts on key physiological functions, we employed a Dynamic Energy Budget (DEB) model. This approach allowed us to estimate the energy expenditure due to increased valve movements, offering a deeper understanding of how shipping noise induced stress affects scallop health and population dynamics.

Building on these findings, combined with existing knowledge of giant scallop auto-ecology and acoustic modeling, we have mapped the risk areas for maritime traffic noise impacts on scallop fishing grounds in the Gulf of Saint Lawrence. These theoretical results lay the foundation for future *in-situ* experiments and will contribute to the development of management strategies aimed at protecting these ecologically and economically valuable marine ecosystems.

ECONOMIC EVALUATION OF PHOTOVOLTAIC ENERGY GENERATION AND USE FOR THE OPERATION OF A LABORATORY AND AN AQUAPONIC SYSTEMS MANAGED UNDER DIFFERENT CONDITIONS[1]

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The study aimed to evaluate the efficiency of photovoltaic energy in operating a laboratory and aquaponic systems managed under different conditions. For the phytotechnical performance analysis, curly lettuce (*Lactuca sativa* var. *crispa*) was used, while for zootechnical studies, lambari (*Astyanax lacustris*) with an average weight of 1.38g and 7g, respectively, was utilized. The experiment was conducted in 12 experimental units containing treatments defined as permanently coupled (PC) and on-demand coupled (ODC), both with and without heating.

The ShinePhone app (version 7.1.0) was used to monitor data on electricity generation, and at the end of the study, the amount of energy generated by the solar panels was compared to the energy consumed by electronic devices used in the aquaponics greenhouse and the Laboratory of Nutrition of Aquatic Organisms (LANOA). The results were subjected to analysis of variance (ANOVA) and Tukey's test ($P<0.05$).

A viral outbreak in the system led to total fish mortality three consecutive times. Consequently, this study presents only the phytotechnical and economic analyses of the experiment. The results revealed that in the ODC system, lettuce exhibited better development and higher averages for total weight, leaf length, leaf wet weight, root wet weight, and root dry weight, regardless of the presence of heaters.

In the photovoltaic energy generation analysis, the results indicated that all the energy required to maintain the aquaponics greenhouse during the 21 experimental days was supplied with only one day's generation from the solar panels. The total electricity consumption of the laboratory was supplied by approximately 50% from photovoltaic energy generation, demonstrating it to be an efficient and environmentally sustainable energy source.

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DETERMINING THE EFFECTS OF SEDIMENTS FROM STAMP SANDS ALONG LAKE SUPERIOR ON ZEBRAFISH *Danio rerio*

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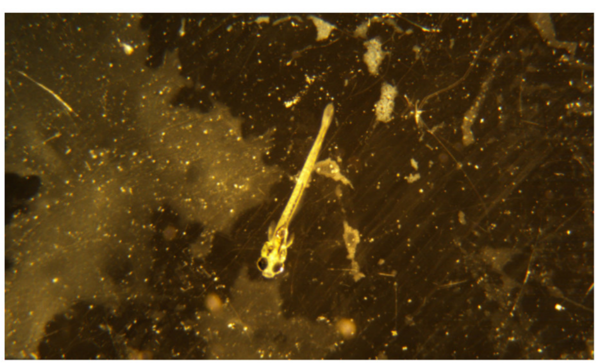
Zebrafish *Danio rerio* eggs, larvae, and adults can all be used as models for aquatic toxicology. Their small size, short life cycle, and sensitivity to pollutants make them an ideal organism to study the effects of contaminants. This study tests the effects of potential contaminants in stamp sands from a former copper mill in Freda, located in the Keweenaw Peninsula in Michigan's Upper Peninsula (UP). Stamp sands, a byproduct of copper mining, are a common contaminant along shorelines where they were deposited. They can lead to elevated levels of copper in the water, which has been shown to affect aquatic organisms. This study's objectives are to determine the effects of stamp sands from Freda on zebrafish at multiple life stages, determine how much copper and other trace elements are leached from the stamp sands into water, and determine if mortality rates of zebrafish differ between fish raised in water contaminated with stamp sands compared to water with sediment from a noncontaminated area and a control. This will provide information on the effects of stamp sands in Freda on sensitive organisms and how much copper from those stamp sands is leached into water.

Larval zebrafish were kept in beakers with 100 mL of water for one week. Five beakers per replicate were used; one beaker with 10 g stamp sand, one with 20 g stamp sand, one with 10 g beach sand (from a rural Eastern UP beach), one with 20 g beach sand, and one with no sediment. Water samples were collected on the first, third, and seventh days. Eggs were subjected to water exposed to the same concentrations of sediments as above for one week. Adult zebrafish were kept in tanks with a layer of stamp sand, beach sand, or no sediment for one week, then examined using uXRF spectrometer for elemental imaging and ICP-MS analysis for tissue element quantification. Water samples were taken at the conclusion of the experiment. All water samples were analyzed on an ICP-MS for copper and other metals. Mortality and hatch rates were compared with ANOVA and Tukey tests on R Studio.

Results will show if any differences in hatch rates or mortality rates occur between the stamp sands, beach sands, or controls. The uXRF imaging results will show where contaminants concentrate in zebrafish. The ICP-MS will show how much copper and other trace elements are present in the water and bioaccumulate in the fish. Further research on the effects of stamp sands from Freda on aquatic and terrestrial organisms is needed for a more complete understanding of how they impact the environment.



LAKE SUPERIOR STATE UNIVERSITY AQUACULTURE CLUB

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The aquaculture club at Lake Superior State University is currently 11 members strong and a proud student subunit of the U.S Aquaculture Society. Our club was formed in 2020 with 15 members. We have three deep water culture aquaponic systems, a recirculating aquaculture system, a vertical aeroponics system and zebra fish research tanks. Our aquaponic systems are growing Atlantic salmon, yellow perch, koi, and goldfish. We grow a variety of plants, anything from herbs and house plants to fruiting plants and trees.

The club puts on plant sales every month to have a continuous fundraiser. This schedule allows us time between sales to empty out the grow beds, clean and sanitize, before moving the next set of sprouts into the systems. We hold a yearly tie dye event where community and club members get to tie dye their very own fishman shirt or tote bag. The club has also hosted cooking demonstrations and soap making events.

We hold numerous tours of our facility throughout the year for K-12 groups, campus events, and community outreach. Our goals as a club are to educate the club members and community about aquaculture and aquaponic systems and practices. We also love to tour other aquaculture or aquaponic facilities. These activities allow the club members to gain knowledge about how others run their systems.

The club sends members to different conferences each year. Five members attended the Wisconsin/Minnesota Aquaculture conference in Bayfield, Wisconsin in February 2024. Two of those members had oral presentations and made posters, and all attendees made connections with local industry workers. The members also got the opportunity to visit the Red Cliff Fish Hatchery and Northern Aquacutlure Demonstration Facility before heading back to campus. Our advisor, two student sub-unit members, and one former sub-unit member gave talks at the San Antonio 2024 World Aquaculture conference. The opportunity to go to the conference was an eye opening and educational experience. The club looks forward to new members and new experiences each year.

EFFECT OF DONOR CELL TYPE ON CHANNEL CATFISH, *Ictalurus punctatus*, XENOGEN PRODUCTION

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Hybrid catfish (♀ channel catfish, *Ictalurus punctatus* × ♂ blue catfish, *I. furcatus*) account for ~70% of the catfish production due to superior performance compared to the parent species for several traits. Xenogenesis has been utilized to potentially produce hybrid catfish embryos more efficiently by transplanting unsorted gonadal cells from donor diploid blue catfish into triploid channel catfish fry. Then xenogenic channel catfish males are mated with normal female channel catfish to produce 100% hybrid progeny. The stem cells have not undergone meiosis and are isogenic. This offers an opportunity to conduct reciprocal recurrent selection and identify the absolute best individual female channel catfish and male blue catfish that have the best combining ability, resulting in the ultimate hybrid progeny. The gonads of these individuals can essentially be 'cloned' and multiplied into large populations through xenogenesis, ensuring perpetuity without inbreeding and maintaining consistent performance of hybrid progeny. An impediment to this approach is that the brooders with the best combining ability are not identified until they reach maturity, at which point the number of gonadal stem cells is low, allowing for the production of only a few xenogenic progeny. However, if the spleen and kidney cells have colonizing and proliferation abilities, potentially 400 and 800 fry, respectively, could be injected from these donor organs alone.

Triploid channel catfish surrogates were injected at 5 days post hatch (DPH) with either unsorted gonadal, kidney, spleen, or somatic cells (extracted from skin tissue) labeled with PKH26 fluorescence dye. PKH26 and PCR analysis indicated that 100.0, 90.9, 54.5 and 0.0 percent of fry injected with mixed gonadal, kidney, spleen and somatic (skin) cells were xenogenic. Theoretically, this would allow production of 1, 727, 218, and 0 xenogenic fry from gonad, kidney, spleen, and somatic cells, respectively from adult catfish. Colonization and proliferation of donor cells (predictors of future fertility) were evaluated using PKH26 by calculating percent cell (<150 µm^2) and cluster areas (>150 µm^2). At 45 DPH, gonadal xenogens had a larger cell area than the somatic (P = 0.004) and spleen xenogens (P = 0.031). By 90 DPH, gonadal (P = 0.003) and kidney surrogates (P = 0.029) had higher cell areas than spleen surrogates (P = 0.134). By 90 DPH, no fluorescent dye was found in somatic surrogates. Cell area for surrogates injected with gonadal (P < 0.001), kidney (P < 0.014), and spleen cells (P < 0.012) increased in size from 45 to 90 DPH. Cluster area also increased in size for surrogates injected with both gonadal (P < 0.001) and kidney cells (P < 0.002). Total cluster and cell area was highest for gonadal xenogens followed by kidney and then spleen xenogens. Kidney cells appear to be a viable option, and potentially spleen cells as well, for generating clonal populations of catfish to permanently fix maximum combining ability. Additional improvements could be achieved through cell purification.

AQUAUCULTURE OUTREACH AND EDUCATION AT AUBURN UNIVERSITY

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The United States Aquaculture Society (USAS) student subunit at Auburn University (AU) strives to promote aquaculture education, sustainability, professional development, and networking opportunities for students and the local community. Key initiatives include an annual shrimp sale, where fresh, head-on shrimp from AU's Gulf Shores facility are sold to the public to raise funds for the subunit. This fundraiser not only supports the subunit's activities and funds professional development activities for members but also helps bridge the gap between aquaculture research and the community. Additionally, the subunit organizes an annual student-professional mixer, providing students the opportunity to network with aquaculture experts and explore potential career paths.

The subunit's outreach efforts include a variety of family-friendly events throughout the year. These range from a catfish fry during AU's College of Agriculture family fun day to co-hosting International Fishing Day, where we teach novices how to fish. Our outreach is centered on education and community engagement, with environmental stewardship being another key goal. This goal is supported through activities such as roadside and stream cleanups.

This year, we are excited to launch a new project in collaboration with Auburn High School students, made possible by a USAS grant received last fall. Through this initiative, we aim to teach students the process of shrimp cultivation while emphasizing the importance of sustainable farmed seafood. Through these diverse activities, the AU USAS student subunit seeks to inspire the next generation of aquaculture professionals and strengthen connections within the aquaculture community.

QUALITIY SCORE SELECTION IMPACTS MICROBIAL ABUNDANCE AND DIVERSITY OUTCOMES IN AQUAPONICS MICROBIOME DATA ANALYSIS

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The decreasing cost of Next Generation Sequencing is allowing researchers to gain valuable information on microbial communities in unique and often complex environments. The microbial composition in aquaponic (AP) systems is a relative 'black box.' An increasing number of studies are providing insight into the role of microorganisms in nutrient cycling, plant productivity, and food safety of AP systems. One challenge is determining how to filter the data post-sequencing. In Illumina short-read sequencing, a per-nucleotide Phred quality score (Q) indicates the probability that the sequencer correctly identified the nucleotide at a specific position. Q filtering is essential in sequencing data analysis as it removes imprecise reads that would otherwise inflate microbial diversity estimates. Here we demonstrate the impact of Q selection on diversity metrics and taxonomic composition of plant-associated microbiomes in AP systems.

16S rRNA raw sequencing data (Illumina MiSeq) was obtained from research examining the effect of development stage on the composition of rhizosphere microbiome in bell pepper (*Capsaicin annum*) plants grown in AP systems. Sequencing data was analyzed with QIIME2, using Greengenes database for taxonomic identification. Nucleotide quality filtering was performed at High (H; >30), Medium (M; >20) and Low (L; >5) Q thresholds.

Quality score selection resulted in significant differences in alpha diversity, beta-diversity, and microbial abundance estimates. Retention of H quality nucleotides retained 50% of input data, while 25% and 10% were retained on M and L, respectively. Shannon diversity index was significantly higher in H-filtered samples (6.66 ± 0.72) than M (3.79 ± 0.98) and L (5.94 ± 0.77), which were significantly different from each other (Figure 1 [$p \leq 0.05$). In addition, the number of taxa identified varied considerably between Q, with H, M and L comprised of 296, 103 and 192 unique taxa, respectively. High and L Q resulted in similar pattern of dissimilarity on a principle component analysis plot, which differed considerably from M.

Results from this exercise highlight the importance of filtering reads in AP microbiome data. Retention of low-quality nucleotides can lead to sequencing errors being misinterpreted as unique taxa, thereby inflating diversity metrics, while H may eliminate rare but potentially important taxa altogether. Medium-quality filtering may inadvertently remove both genuine low-abundance taxa and higher quality reads that would contribute to the richness and evenness required for a higher Shannon index. Analysis of the microbial composition of APs systems may benefit from a multi-filtering approach, where H and L quality datasets are analyzed together. Rare taxa that appear consistently across both H and L filtering could be treated as part of the 'extended core microbiome', which may be functionally important despite their low abundance.

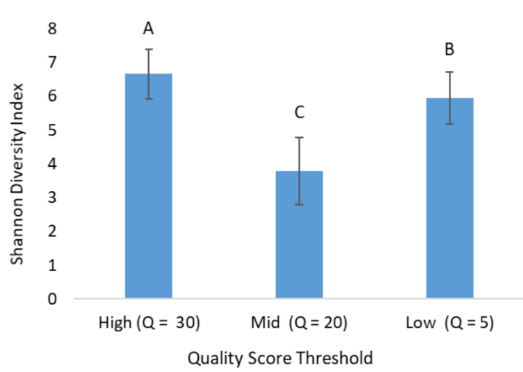

Figure 1. Effect of Quality Score Threshold H, M and L on Shannon Diversity Index

NOVEL APPLICATION OF BREWER’S SPENT BYPRODUCT SERVE AS AN EFFECTIVE FEED ADDITIVE TO RAINBOW TROUT *Oncorhynchus mykiss*

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Brewery byproducts, brewer’s spent yeast (BSY, *Saccharomyces cerevisiae*) and brewers spent grains (BSG), have little-to-no economic value, as they have little conventional usage outside of disposal; however, if a novel process is applied, the valuable nutritional properties of the byproducts could be utilized by the aquaculture industry as a beneficial feed additive. Specifically, the byproducts can serve to enhance the health and growth of rainbow trout (*Oncorhynchus mykiss*), which are both economically valuable and a model species for other *salmonids*. Application of brewer’s byproduct were performed via top-coating directly onto commercial feed, and both BSY and BSG underwent novel processing and were provided to this study.

The aim of this research was to determine if processed BSY, and black soldier fly larvae (BSF) cultured on BSG, could enhance the health and production of farmed rainbow trout in correlation to their intestinal bacterial community structure. Fourteen juvenile rainbow trout (28.6 ± 0.4 g, mean ± standard error) were stocked per polyethylene tank across 24 tanks (170 L each) in a single recirculating aquaculture system (RAS). The treatment groups for BSY were performed in quadruplicates at the tank level, while for BSF was performed in triplicates. Over the eight-week trial, compared to the control, all diets grew significantly ($P < 0.01$**, Table 1.) faster than the control while also having decreased feed conversion ratios (FCR). Moreover, there were no significant differences of intestinal bacterial community structure between treatment diets and the control, implying no dysbiosis due to the altered diets. Overall, the supplemented byproduct diets served as an effective growth additive for the trout without negatively impacting health.

Table 1. Trout production and biometric data [mean ± standard error].

Treatment	Overall Survival (%)	Weight Gain (g)	Percent Difference (%)	FCR
Control T2	98.2 ± 1.55	56.0 ± 2.5	NA	1.19 ± 0.05
LY	100	67.9 ± 0.5 **	20.8	1.01 ± 0.02*
HY	100	76.1 ± 0.4 **	35.4	1.04 ± 0.05
Treatment	**Filet Yields (%)**	**VSI**	**HIS**	
Control T2	50.4 ± 2.0	13.7 ± 0.9	1.68 ± 0.13	
LY	53.8 ± 1.8	13.9 ± 0.6	1.67 ± 0.37	
HY	52.2 ± 1.4	13.6 ± 0.6	1.70 ± 0.33	
Treatment	**Overall Survival (%)**	**Weight Gain (g)**	**Percent Difference (%)**	**FCR**
Control T2	98.2 ± 1.5	56.0 ± 2.5	NA	1.19 ± 0.05
BSF1	97.6 ± 1.7	75.8 ± 3.7 **	34.9	1.03 ± 0.01**
BSF2	97.6 ± 1.7	70.2 ± 2.7 **	24.9	1.09 ± 0.01**
BSF3	97.2 ± 1.7	72.4 ± 3.2 **	28.8	1.06 ± 0.01**
Treatment	**Filet Yields (%)**	**VSI**	**HIS**	
Control T2	50.4 ± 2.0	13.7 ± 0.9	1.68 ± 0.13	
BSF1	52.1 ± 2.0	14.1 ± 0.9	1.65 ± 0.50	
BSF2	50.5 ± 1.7	13.7 ± 0.8	1.68 ± 0.48	
BSF3	53.2 ± 3.3	13.6 ± 1.3	1.62 ± 0.17	

TEMPERATURE, MAXIMUM SIZE, AND THE ATLANTIC SURFCLAM *Spisula solidissima*: THE SENSITIVITY OF DEMOGRAPHICS TO CLIMATE WARMING, THE PERFIDY OF FOOD, AN A VIGNETTE ON SIZE-LIMITED MANAGEMENT IN BIVALVE FISHERIES

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A dominant theme in explaining intraspecific latitudinal gradients in size is the temperature-size rule which states that intraspecific growth rates decline but maximum sizes increase with declining temperatures over a species' geographic range. The temperature-size rule has accumulated considerable support in poikilotherms, yet amongst marine taxa, few reports issue from studies on the Bivalvia despite the underlying physiological conducement provided by the differential allometric scaling of respiration rate and filtration rate. Amongst the Bivalvia, the mactrid *Spisula solidissima* (Atlantic surfclam) is noteworthy for its size, the extensiveness of its latitudinal range, and its biomass dominance on the continental shelf. Here, the demographics of *S. solidissima* are evaluated with respect to the temperature-size rule, followed by contemplations on the impact of climate change on the species and its management. Analysis of size-frequency distributions from the southern range boundary near the Chesapeake Bay to Georges Bank, identify the anticipated trend of increasing maximum size with decreasing temperature. Application of a metabolic energetics model shows that the dramatic variations in size accrue from the direct physiological impact of geographic variations in temperature strongly modulated by variations in food supply. The influence of food likely explains the rarity of direct observations of the temperature-size rule in Bivalvia. Management measures based on the size frequency are discussed, and specifically regulatory size limits on landings are identified as incompatible with the temperature-size rule.

TRACKING OYSTER MORTALITY AND MSX DISEASE PREVALENCE IN THE EASTERN OYSTER *Crassostrea virginica* IN THE MARYLAND PORTION OF THE CHESAPEAKE BAY FROM 2023 TO 2024

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Multinucleated sphere unknown (MSX) is a disease of eastern oysters (*Crassostrea virginica*) caused by the spore-forming parasitic protozoan *Haplosporidium nelsoni*. MSX does not affect humans however it can cause high mortality in oysters. *H. nelsoni's* ability to infect oysters and the rate it causes mortality is heavily influenced by salinity. *H. nelsoni* needs a salinity of 15 ppt or greater to infect oysters. At a salinity of 10 ppt or lower though, oysters are able to expel *H. nelsoni* from their tissues. Using this information, salinity can thus be used as an indicator of whether MSX prevalences will increase or decrease in areas where salinity fluctuates around the 15 ppt threshold. The amount of mortality that results from MSX however is difficult to predict as there are many factors at play that influence this. To better understand the relationship between MSX and oyster mortality as well as the details of salinity's impact on MSX, oysters were sampled from 3 sites in the Maryland portion of the Chesapeake Bay. The sites were the Choptank River (Lighthouse bar), Choptank River (Royston bar), and Tangier Sound (Piney Island bar). They were sampled for MSX (n=30 per site) in Fall 2023, Spring 2024, Summer 2024, and Fall 2024 so the seasonal cycle of MSX could be captured, and effects of low and high salinity could be seen. These sites were also tested for Dermo disease so this could be taken into account as a possible source of mortality if present. Dermo is also harmless to humans though it can be lethal to oysters if infections are heavy. Dermo is usually less of a concern for oyster farmers than MSX though since oysters are usually harvested before dermo infections have time to become lethal. The data obtained in this study along with salinity data records will be helpful in predicting when a high MSX prevalence season for the Maryland portion of the Chesapeake Bay is likely ahead.

EXAMINING TAGGING METHODS FOR THE WARTY SEA CUCUMBER *Apostichopus parvimensis*

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The warty sea cucumber, *Apostichopus parvimensis*, is one of two commercially fished sea cucumber species along the California coast, ranging from Point Conception to Puerto San Bartolome in Baja California, Mexico. *A. parvimensis* is becoming a species of interest for aquaculture because of its high market value, over-fished status, and suitability for integration in multitrophic aquaculture systems. To facilitate aquaculture development (e.g. broodstock management) and research, there is a need for reliable, non-invasive, tagging of *A. parvimensis*. We therefore evaluated tag retention, growth, and survival in *A. parvimensis* using four different tag types.

Wild-origin adult *A. parvimensis* (52-178 g in size) were acclimated to captivity and randomly assigned to one of four treatment groups of 10 animals each, plus a control group. Treatments included: 1) a Floy tag inserted in the musculature band (FT-M), 2) a Floy tag inserted into a papillae, or "wart", (FT-P), 3) a Passive Integrated Transponder tag on monofilament fishing line threaded through the body wall (PIT), and 4) a Visible Implant Elastomer tag (VIE) injected subcutaneously into the upper dorsal surface. The Control group was handled and cared for in the same way but without any tagging. All individuals were assigned to their own tank with flow-through seawater under ambient conditions and were measured weekly. At the end of the 12-week experiment, FT-M, FT-P, and VIE had the highest retention rates of 60%, 50%, and 40%, respectively. All individuals in the PIT group lost their tags. In the treatments that retained tags, average body weight decreased between the start and end of the experiment; the control group increased in size by an average of 11.9g over the 12 weeks. Only one mortality occurred, coming from the FT-P group.

Our findings conclude that external tags are difficult to retain for long-term holding of *A. parvimensis* and may need to be monitored frequently and retagged as necessary. VIE tags using larger marks and focusing on color combinations (yellow and orange) that are most visible, is a possible future area of research, as this was the least invasive procedure.

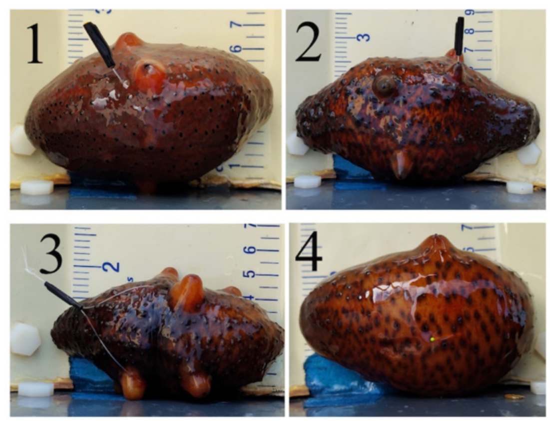

Figure 1. Tagged groups. 1) FT-M, 2) FT-P, 3) PIT, 4) VIE

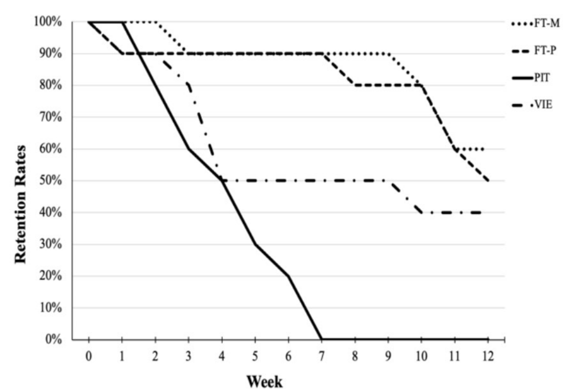

Figure 2. Tag retention rates for all treatments over twelve weeks

ALGAL SYSTEMS IN AQUACULTURE: A REVIEW OF WASTEWATER TREATMENT AND POTENTIAL APPLICATIONS

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Wastewater treatment is part of the ongoing post-processes that the Aquaculture industry must deal with as part of their routine practices. This is an aspect that needs continuous attention given the reinforcement of regulations. To attend to these continuous demands, algae cultivation systems have supplied proven and sustainable solutions in different aquaculture applications, for example, serving as feedstock for juvenile shrimp cultivation, or providing a natural stock for oxygen supply in fish cultivation. The current work intends to review a state-of-the-art application of algae systems in the aquaculture industry, emphasizing its benefit for wastewater treatments.

The utility of algae systems has been maximized when deployed as algae turf scrubbers (ATS). This technology based on periphytic filamentous algae can remove nutrients from water streams, serving in some cases as a better perfoming alternative to traditional filtration systems. The introduction of ATS in aquaculture cultivation has not only been demonstrated on fish with tilapia and catfish but also in cultivation of oysters. Their use in broader aquaculture applications has been limited, however, likely due to some operational constraints and confusion about their utility and application. This review will summarize past applications of attached algae systems in aquaculture production, and focusing on new opportunities for cultivation and sustainability approaches that will serve as a reference for specialists in this sector.

DIFFERENTIAL GENE EXPRESSION BETWEEN FEMALE AND MALE EASTERN OYSTER TISSUES

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The eastern oyster is the most valuable cultured mollusc in North America and the industry is increasingly reliant on seed produced in hatcheries. A critical step in hatchery production is the reproductive conditioning of broodstock. Throughout much of the species range, reproduction is seasonal, whereby germinal epithelia are resorbed during the fall months and develop anew in the summer. Environmental factors that promote reproduction (e.g. temperature and food availability) can be manipulated in hatcheries to accelerate and extend the spawning season but conditioning is a lengthy and expensive process. The lack of sexual dimorphism compounds costs because many more broodstock than necessary must be maintained to ensure the appropriate sex ratio for spawning is achieved. Therefore, the development of protocols that allow non-destructive sex identification early in the reproductive conditioning process would benefit the industry by reducing effort and cost.

Here we present the first step toward this goal by identifying genetic markers that differentiate female and male oysters. As with many marine invertebrates, little is known about the genes underlying sex differentiation in the eastern oyster. We compared global gene expression patterns in mature gonad tissue from 6 male and 6 female oysters. A total of 7,675 transcripts were differentially expressed between the sexes (3,936 and 3,739 up- regulated in males and females, respectively). Differentially expressed transcripts include those associated with sex in other invertebrate and vertebrate species such as Dmrt1, SOX-30, bindin, dpy-30, and histone H4 in males and foxl2, vitellogenin, and Bystin in females. GO terms associated with transcripts up-regulated in male gonad include protein modification, reproductive process, and cell projection organization, while RNA metabolic process and amino acid metabolic process were associated with transcripts up-regulated in females. Because gonad tissues are difficult to sample non-destructively, we also compared gene expression patterns in mantle tissues collected from the same 12 oysters. Far fewer transcripts were differentially expressed in this tissue; however, 41% of transcripts identified as differentially expressed between male and female mantle tissues were also differentially expressed between male and female gonads. This study represents the first characterization of differences between male and female eastern oyster transcriptomes and our results hold promise for the development of a non-destructive sex identification assay that can be applied to hatchery broodstock during the conditioning process.

TARGETED ISOLATION OF *Vibrio fluvialis* AND *V. mimicus* FROM OYSTERS AND SEDIMENT

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Although food safety efforts have focused on reducing *Vibrio parahaemolyticus* and *V. vulnificus* illnesses, other species are increasingly being recognized as causes of foodborne vibriosis, especially associated with consumption of raw bivalve molluscan shellfish. These emerging causes of vibriosis include *V. fluvialis, V. cholerae* (non-O1/non-O139), *V. mimicus, V. alginolyticus,* and *Grimontia hollisae*, that are naturally occurring in estuarine waters where shellfish are grown. In order to better understand the prevalence, distribution, and environmental drivers of these emerging human pathogens, appropriate methods are needed to detect, isolate, and identify these species from shellfish, water, and sediment.

This study aimed to specifically isolate *V. fluvialis* and *V. mimicus* from oysters and sediment. Oyster homogenate or sediment was enriched overnight in Alkaline Peptone Water (APW) at 35° and also 41° C for *V. mimicus*, then streaked to multiple chromogenic agars to target *V. fluvialis* and *V. mimicus*. Typical colonies were purified and further characterized by streaking to a panel of chromogenic agars and TCBS to determine phenotypic profile. Isolates with a typical *V. fluvialis* and *V. mimicus* profile were then analyzed with newly developed real-time PCR assays. Select isolates that were positive by real-time PCR were then confirmed by whole genome sequencing (WGS).

Of the 32 *V. fluvialis*-typical colonies (example in Figure 1), 20 were positive by real-time PCR. Four isolates were selected for WGS, and all four isolates were confirmed as *V. fluvialis.* Of the 39 *V. mimicus*-typical isolates (example in Figure 2), 28 were positive by real-time PCR. Ten PCR-positive and 11 PCR-negative isolates were sequenced, with 100% agreement between PCR and WGS. The results demonstrate the utility of this approach for isolating and identifying *V. fluvialis* and *V. mimicus*. Future work will include evaluating the real-time PCR assays for screening of enrichments to aid in detection and isolation of these emerging *Vibrio* spp.

Figure 1. Typical *V. fluvialis* colonies on (left to right): CHROMagar, TCBS, ChromoSelect, and HardyCHROM.

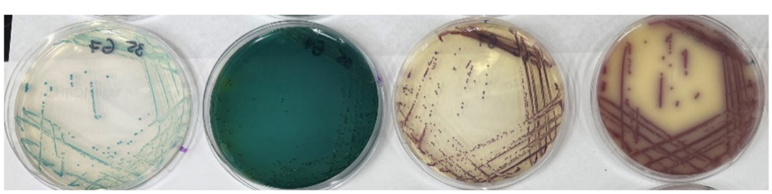

Figure 2. Typical *V. mimicus* colonies on (left to right): CHROMagar, TCBS, ChromoSelect, and HardyCHROM.

THE EFFECTS OF CHEMICAL INDUCERS ON LARVAL SETTLEMENT BEHAVIOR AND METAMORPHOSIS IN THE EASTERN OYSTER (*Crassostrea virginica*)

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Eastern oyster aquaculture involves the cultivation of *Crassostrea virginica* to support coastal economies through sustainable oyster production and to restore the ecological benefits of oyster reefs. Oyster hatcheries play a key role in the industry by producing juvenile oysters for aquaculture and use in restoration projects. Hatchery oyster production is often limited by the number of larvae that successfully undergo metamorphosis into juveniles, referred to as spat, which even under ideal hatchery conditions typically yields only 10-40%. Neurotransmitters, such as epinephrine, can induce metamorphosis in many bivalve species and are utilized to enhance commercial production of select shellfish species. However, the use of chemical inducers to increase spat production in *C. virginica* has not been well investigated. This project examined the potential use of chemical inducers (i.e., epinephrine, GABA, L-DOPA, lysine, arginine, tryptophan) to enhance *C. virginica* spat production by increasing metamorphosis at the larval stage. In 1-hour behavioral assays, we observed pediveliger larval behavior (i.e., swimming, foot extension, cilia beating) to determine which chemicals induced larval settlement responses, and their optimal dose. Next, we exposed competent *C. virginica* pediveligers to the active chemicals at the optimal dose for 48 to 72 hours to measure the percent that metamorphosed and assessed long-term spat survival. Overall, epinephrine and L-DOPA induced larval settlement behaviors but did not increase metamorphosis and spat production. Further research is needed to test additional potential chemical inducers and natural sources of settlement cues that may enhance larval conversion to spat in the hatchery or recruitment to restored reefs in the field.

FISH DISEASE CASES DIAGNOSED AT THE KENTUCKY STATE UNIVERSITY FISH DISEASE DIAGNOSTIC LABORATORY FROM 2022-20224

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The Fish Disease Diagnostic Laboratory (FDDL) at Kentucky State University Aquaculture Research Center diagnosed over 100 disease cases from Kentucky, Ohio, and surrounding states between 2022 and 2024. Working primarily with small scale farmers it is imperative that timely and accurate diagnosis of disease is completed. The goal of FDDL is to minimize mortality events, provide treatment recommendations, and minimize the spread of fish diseases between farms and state lines.

Under the direction of fish pathologist Dr. Robert Durborow, fish samples received were tested for bacterial, parasitic, and water quality causes of fish mortality. Species of bacteria and parasites contributing to mortality were successfully isolated and properly identified in order to provide expert treatment recommendations to farmers. All cases diagnosed at FDDL are uploaded into a database under each individual farmer and their production cycles. This database provides comprehensive access to farmer's disease case history, furthering the ability of staff to provide treatment recommendations. Disease cases involving *Aeromonas hydrophila, Flavobacterium columnaris,* and *Pseudomonas sp.* infecting Largemouth bath (*micropterus nigricans*), Rainbow Trout (*oncorhynchus mykiss*), and Channel Catfish (*ictalurus punctatus*), were especially prevalent. This comprehensive overview of disease cases at the FDDL at Kentucky State University Aquaculture Research Center shows the skill and accuracy of fish pathologists as well as the importance of Land Grant Extension initiatives.

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PROGRESS IN GENOME EDITING FOR PACIFIC WHITE SHRIMP *L. vannamei*

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Increasing ability to harness the power of genomics is forcing a rethinking of aquaculture genetic improvement strategies. Successful breeding programs will always be built on the careful selection of the next generation of broodstock, detailed record keeping, and accurate collection of phenotypic data. Genomics allows this base of phenotypic selection to be enhanced and ultimately accelerated to increase genetic gain per generation. This is currently done in shrimp at the most sophisticated level using Genomic Selection. However, another exciting technology is on the horizon that will fundamentally change how we deliver genetic improvement. This technology is Genome Editing.

With Genome Editing, genomic improvements can be accelerated to meet the growing needs of the aquaculture industry. While the agricultural and livestock industries have embraced Genome Editing, the aquaculture industry has fallen behind considerably in the application of these technologies. The complexity of farmed aquaculture species and the necessary support systems present a significant barrier to the application of Genome Editing, though this technology can offer major improvements in areas such as disease resistance, high growth, high yield, monosex culture, and environmental sustainability.

The implementation of Genome Editing in Pacific White Shrimp (*L. vannamei*), a major seafood species, will be important for the aquaculture industry. Several methods of gene editing have been reported in shrimp, with varying degrees of success. These methods are limited by the issues of the delivery of the gene editing enzymes and the survival of the larvae to adulthood on a commercially relevant scale. The progress in developing genome editing tools for *L. vannamei* remains limited.

Here, we will present our success in creating genome edits in *L. vannamei*, discuss the opportunities for genome editing to improve shrimp aquaculture, and highlight the challenges in the implementation of genome editing technologies in *L. vannamei*.

USING OMEGA TO MODEL GENETIC RISKS OF ESCAPED FISH ASSOCIATED WITH OFFSHORE FINFISH CULTURE: AN EXAMPLE USING THE PROPOSED PACIFIC OCEAN AQUAFARMS OPERATION IN SOUTHERN CALIFORNIA

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The Offshore Mariculture Escape Genetic Assessment (OMEGA) model was built with the purpose of simulating escapes from an aquaculture farm system to a natural population, and estimating the potential effect of escaped fish surviving to encounter wild conspecifics. The model is intended to provide an assessment of risk associated with aquaculture operations and aid in the development of strategies to reduce adverse effects from escapes through generating escape scenarios based on the planned project, and parameterizing the model with aspects of the species' biology, wild population dynamics, facets of the specific aquaculture operation, and by taking into account uncertainty around interactions between cultured and wild populations (Figure 1).

We provide a demonstration of OMEGA modeling and analyses based on the proposed Pacific Ocean AquaFarm's plan to cultivate California Yellowtail (*Seriola dorsalis*) in a moored net pen system in Southern California. The goals of the analyses were to evaluate ways fish may escape from the net pen system, the magnitude and age distributions of escaped fish under different production and escape scenarios, and the potential consequences of escapes surviving in the wild. This analysis also focused on potential magnitude of escape levels and consequences related to within-population genetic diversity and reproductive fitness of California Yellowtail.

For this project, OMEGA simulations were conducted for a range of escape scenarios under full- and half-scale production alternatives. Escape scenarios included assumed ordinary operational levels of escape (leakage and episodic losses) and large-scale escapes from rare, but severe, infrastructure losses. A subset of these simulations and results will be presented here to demonstrate the use of OMEGA in project level analyses, and how these results inform NEPA regulatory considerations.

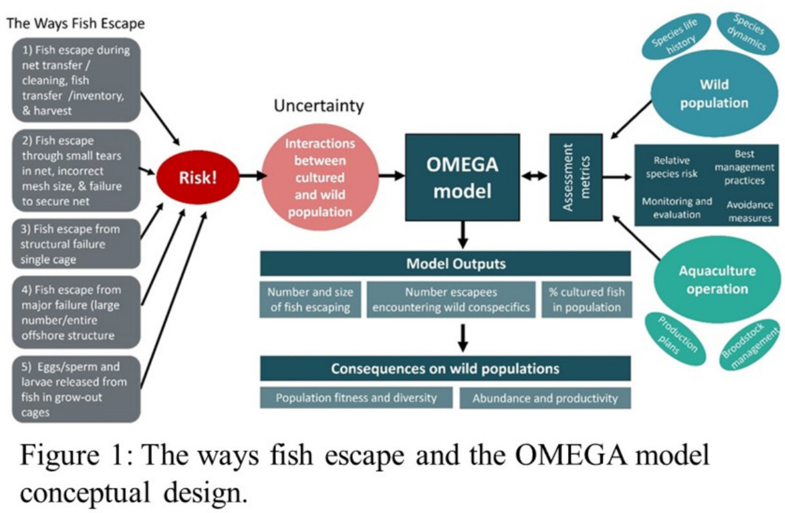

Figure 1: The ways fish escape and the OMEGA model conceptual design.

DETERMINING THE FATTY ACID PROFILES OF CONCENTRATED MICROALGAE IN COLD STORAGE OVER TIME

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Microalgae are essential for many hatchery operations, particularly in shellfish and finfish production. As reliance on microalgae increases, there is a growing need for more efficient production methods that not only maximize cell density and volume but also maintain nutritional value over time. Microalgae contain polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are crucial for the development and health of marine species. The nutritional quality of cultured microalgae can decline over time due to nutrient depletion and reduced photosynthetic activity. However, the effects of newer techniques, such as ultrafiltration and cold storage, on the degradation of PUFAs have yet to be thoroughly examined. This study aims to determine the impact of cold storage on the preservation of fatty acids in concentrated microalgae, identifying when stored cultures lose their nutritional value.

In this study, three microalgae species, *Chaetoceros muelleri, Tisochrysis galbana, and Tetraselmis spp.,* were concentrated using crossflow configuration ultrafiltration (CF-UF) and stored under aeration in a refrigerator for up to two months. Samples were periodically collected and frozen during this storage period. These samples were then processed and analyzed using Gas Chromatography-Mass Spectroscopy (GC-FID or MS) to assess their fatty acid profiles. Multivariate analysis was conducted to identify patterns and changes in the nutrient profiles of the different microalgae species over time.

Understanding the nutritional stability of concentrated microalgae during cold storage is key to improving hatchery outcomes. This knowledge enables the reliable use of concentrated microalgae stocks, reducing the time and effort required for culturing and harvesting while providing a safeguard against culture crashes. The ability to cold store live microalgae concentrates for extended periods will be extremely beneficial in the aquaculture and shellfish industries, ensuring a consistent, high-quality feed supply.

OVERVIEW OF THE OMEGA MODEL AND EMERGING TOOLS FOR ASSESSING GENETIC RISKS IN MARINE FINFISH AQUACULTURE

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The use of scientific-based assessments and tools has become increasingly important to address concerns and evaluate impacts from marine aquaculture development. A primary concern is the potential for negative ecological and genetic impacts when farmed fish escape and encounter wild stocks. NOAA and ICF jointly developed the Offshore Mariculture Escapes Genetics Assessment (OMEGA) model as a tool to assess risks from escaped cultured fish (ICF 2012; Gruenthal et al. 2014). OMEGA identifies and evaluates ways cultured fish associated with marine aquaculture operations, escape, disperse into wild populations, and potentially affect wild population fitness and viability. NOAA Fisheries is using the OMEGA model to identify and evaluate the risks associated with marine aquaculture operations, recommend management practices for responsible and sustainable aquaculture programs, explore the effects of regulatory and technical advances, and identify research priorities.

OMEGA is a mathematical model with inputs that include the size and growth characteristics of the cultured fish, the frequency and magnitude of escape events, survival rates of escapees in the wild, probability of escaped organisms encountering wild counterparts and interbreeding, and the dynamics of the wild population. Outputs from OMEGA describe the influence these aquaculture escapees may have on the fitness and population dynamics of the mixed population over time.

OMEGA analyses have been used to inform management decisions for several aquaculture projects (e.g., EPA response to Velella Epsilon, NOAA Biological Opinion consultation, NOAA Office of Policy NEPA EIS for permitting of a single operation). This presentation will provide a brief overview of OMEGA and other tools under development to support assessments of genetic risk for marine finfish aquaculture.

ASSESSING THE EVOLUTIONARY RESPONSE OF EASTERN OYSTER LARVAE TO EXPOSURE COASTAL ACIDIFICATION AND SEWAGE EFFLUENT: A CASE STUDY

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Although coastal organisms experience natural and anthropogenic stressors simultaneously across multiple spatial and temporal scales, the synergistic effects of multiple stressors are largely unknown. In urbanized estuaries, coastal acidification (CA) can be caused by eutrophication (i.e. nutrient loading). CA is strongly associated with a second anthropogenic stressor, sewage effluent (SE). SE can cause acidification locally by increasing nitrogen (N) loads and stimulating algal and microbial production of CO_2. Even treated SE can cause CA because excess N is often removed with microbial treatments, leading to effluent that is low in N but has decreased pH and increased concentrations of CO_2. The physiological effects of CA and SE have been well characterized separately but have never been examined together in early life history stages when organisms are most sensitive to stressors. Additionally, results from many studies have not been examined in a mechanistic framework, such as identifying the genes that provide resistance to multiple stressors. Here, we use multiple factorial exposures on eastern oyster larvae to characterize the effects of CA and SE on larval mortality and use expressed exome capture sequencing to detect which genetic variants lead to resistance and potential adaptation. Results indicate that CA, SE, and CASE induce clear changes in the allelic composition of larval pools and that the CASE treatment did not represent a composite of the CA and SE treatment. Higher gene ontologies for outlier loci appear to be related to chemical and stress response, supporting the possibility of adaptive resistance to multiple stressors.

WHAT IS ROTARY AND HOW DOES ROTARY WORK FOR SHELLFISH?

Christopher Puttock*

Rotary Action Group's Council Advisory Board Chair 2023-2025
Environmental Sustainability Rotary Action Group (ESRAG) Cofounder and Senior Advisor
www.esrag.org
Rotary International TRF Cadre of Technical Advisors for Water & Environment 2022-2025
Hawaii Conservation Alliance Foundation (HCAF) Founder & Director 2006+
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Rotary as an organization is an integral part of 36,000 local communities worldwide. Rotarians for the past 118 years have engaged with their local communities providing "service" and "doing good" wherever there is a recognized need with humanitarian and now environmental projects.

As a Rotarian or as a potential partner with Rotary, you will hear that The Rotary Foundation has spent more than $24 million on Environmental Projects worldwide since 2014. But how much or little has it been spent on shellfish restoration? I will give you the answers and how you can get involved in shellfish projects to increase efforts 100-fold.

I will also inform you about an oyster project started in Chesapeake Bay.

WHAT IS MEANT BY "MANGROVE FRIENDLY AQUACULTURE?"

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It is important today to clarify what is meant by the term "mangrove friendly aquaculture". Certainly, it is important to restore mangroves to areas where they have been destroyed or degraded. Mangroves provide many important benefits and services, such as shoreline protection from storms and erosion, enhanced wild fisheries, lumber and fuel wood, as well as carbon than other forest types. Mangroves also play a major role in providing for local communities' livelihoods and sustenance.

The concept of "mangrove friendly aquaculture" is based on the idea that implementation of small-scale local owner-based aquaculture for livelihood can be combined with re-establishment of mangroves in degraded areas. This may sound good in theory, but may fall short of its goals in practice, unless this practice somehow coincides with effectively establishing mangrove protected areas to ensure no further mangrove ecosystem losses to human development. Conservation of primary mangrove forest areas must go hand in hand with so-called "eco-friendly" development.

Utilizing already degraded or destroyed mangrove areas for partial recovery shared with some viable form of development enterprise which fosters human cultural and livelihoods needs makes sense, as long as we understand this to mean such areas of development so not often represent net gains in area of mangrove ecosystems and their derived full benefits and services. A shrimp pond is not a natural wetland, just as a system whereby mangroves are planted along bund walls of a shrimp pond are not an example of a restored mangrove ecosystem.

Globally, if there are extensive areas of abandoned or disused shrimp ponds, then we need to endorse restoring a good portion of these back to healthy, productive mangrove forest wetlands where possible. This is something worth working towards. Where such is not possible, promoting a partial recovery of some mangrove services, while promoting alternatives, such as small-scale, community-based aquaculture initiatives or other environmentally/community friendly alternatives, seems appropriate. A major challenge towards mangrove recovery involves land tenure issues.

Supporting such human-based development alternatives to full mangrove ecosystem recovery will encourage needed work to restore natural mangrove wetlands where possible. There must be some equitable give-and-take, even with nature. If we only take, and do not give back, the resources we take will soon run out.

One further thought: When a full recovery of a mangrove wetland occurs, this is not solely a loss of opportunity, but a real positive gain for the nearby communities who benefit by increased marine fisheries, an added buffer against coastal storms, erosion, and sea level rise, a counter to the climate crisis, etc. So, in this sense, bringing back a robust mangrove ecosystem necessarily augments anthropocentric benefits from such, but with much wider shared benefit implications for all affected.

FISH FARM TOURS AS A LEARNING TOOL

Herbert E. Quintero[1], Amy Shambach[2]

[1] The Ohio State University, Delaware, OH
[2] Purdue University, Illinois Indiana Sea Grant Program, West Lafayette, IN

A mixed group of experienced Ohio fish aquaculture producers and newcomers were exposed to two educational travel experiences to gain insight into the aquaculture industry in other US regions. The first educational travel experience took place during early Spring of 2024 focusing on gaining knowledge from commercial fish hatchery production facilities and research facilities in Arkansas. The second trip in the Fall of 2024 centered on aquaculture production systems both in academic and commercial settings in Missouri, Illinois, and Indiana.

A total of 11 fish farmers during both trips were exposed to farms culturing a variety of species included baitfish (minnows), goldfish, sportfish (hybrid striped bass, largemouth bass), rainbow trout, tilapia, and barramundi among others. Aquaculture production systems included hatcheries, outdoor land-based (earthen ponds, flow-through raceways), and indoor recycling (recirculating aquaculture systems (RAS)) and flow-though systems. Reflections on attitudes or beliefs, and perceived benefits gained from participation in the program were collected by performing pre- and post- surveys for both trips.

This activity was possible thanks to the Ohio Soybean Councill by the grant OSC 24-D-34.

HEALTH INVESTIGATION OF TILAPIA INDOOR BREEDING SYSTEMS

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Tilapia fish is the second farmed fish in the world. One of the most important features of tilapia is its greater resistance to diseases comparing to the other farmed fish. However, it should be noted that many infectious pathogens can affect the tilapia fish, which may lead to serious problems and losses. It is necessary to pay attention to the investigation of diseases and the basic planning of its prevention and control.

Aquatic diseases have damaged tilapia aquaculture industry in different areas of the world. Tilapia aquaculture does not have a long history in Iran and planning for hygiene observations and preventing of disease prevalence must be considered.

Tilapia hatchery and indoor systems of National Research Center of Saline water Aquatics in central Iran were monitored for infectious disease. Unusual locomotion/ feeding or uncommon signs in the shape or body surface of cultured tilapias, fries and eggs or exceed mortalities were mentioned for bacterial, fungal, parasitic or viral pathogens. Infectious agents in this study were bacteria *Streptococcus* and *Edwardsiella*, the fungi *Penicillium* and *Aspergillus* and parasitic monogeneans *Gyrodactylus and Dactylogyrus.* No viral agent was diagnosed.

Although recorded pathogens were rarely observed during the study, but much attention must be paid for hygiene monitoring and instruction performance. Key aspects of aquatic health management of tilapia breeding systems are reducing exposure to pathogens and reducing stressors that predispose fish to pathogen contamination.

CLIMATE-RELATED VARIABILITY IN PRECIPITATION DRIVES EARLY RECRUITMENT OF THE EASTERN OYSTER *Crassostrea Virginica* IN MISSISSIPPI

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In recent years, early oyster recruitment in Mississippi has been driven by climate-related variability in precipitation, as expressed by alternating periods of extreme wet and dry conditions. Since 2018, commercial oyster landings from western Mississippi Sound have ceased. The primary deterrent to early oyster recruitment shifted from substrate limitation to recruitment limitation in 2019, when oyster spawning stocks were completely decimated by unprecedented freshwater discharge from the Bonnet Carré spillway. This catastrophic event apparently hampered both the supply and survival of oyster larvae. Consequently, early oyster recruitment was effectively eliminated in 2019. Following low and regionally variable levels of early oyster recruitment in 2020, extreme low salinity conditions prevailed again in 2021 due to elevated regional rainfall throughout the oyster recruitment period. In 2023, early oyster recruitment returned to high levels in conjunction with a historic drought. The 2024 discharge regime is expected to be influenced by a La Niña pattern, characterized by dry and warm conditions in the southern US. Thus, for the past six years, early recruitment of oysters has been primarily regulated by climatic extremes. Spat settlement as well as growth and mortality of transplanted hatchery-reared juvenile oysters varied spatially and between years in conjunction with salinity. In addition to direct effects of salinity, covariation in associated biotic factors such as predation intensity, food quality, and disease also contribute to recruitment success. Extreme climatic events in conjunction with multiple stressors continue to limit the recovery of oyster resources in the Mississippi Sound estuary. The development of an adaptive oyster management strategy is essential in the face of current environmental challenges.

POPULATION CHARACTERISTICS ASSESSMENT ON *Callinectes sapidus* AND VIRUS IDENTIFICATION OF *Callinectes sapidus* REOVIRUS 1 (CSRV1) USING RT-QPCR ANALYSIS IN DELAWARE INLAND BAYS

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The blue crab (*Callinectes sapidus*) population in the Delaware Inland Bays (DIB) holds significant economic and ecological importance. *C. sapidus* is a keystone species and contributes to the top-consumed seafood in Maryland and Delaware. Within the state of Delaware, particularly in the Delaware Inland Bays (DIB), there is limited research on the abundance of *C. sapidus* and a notable knowledge gap regarding the presence and screening of *Callinectes sapidus* reovirus 1 (CsRV1). The primary objective of this study was to evaluate *C. sapidus* population characteristics and identify the prevalence of a pathogenic virus affecting *C. sapidus*. The study explicitly targets CsRV1, utilizing rt-qPCR (Reverse transcription-quantitative polymerase chain reaction) methods. Overall, there were six study sites distributed between the west and east sides of Rehoboth Bay. Based on distinct site characteristics, each side includes areas with oyster aquaculture, artificial reefs, and control sites (little to no structure). Crab abundance was estimated by deploying a variety of traps at each site in the Summer and Fall of 2022 and 2023. Data were collected on carapace length and width, sex, and maturity from 1,270 blue crabs in 2022 and 1,120 blue crabs for the 2023 field season. Artificial reefs continuously displayed the highest female and juvenile *C. sapidus* retention throughout all study sites. Additionally, CsRV1 was detected within crab samples from the DIB at a low prevalence out of how many samples you tested. Overall, this study aims to provide information on the knowledge gaps about *C. sapidus* population characteristics and CsRV1 presence/absence within the DIB. This study should continue as it will ultimately help the management of this important commercial species and this study serves a starting point for monitoring C. sapidus within the DIB.

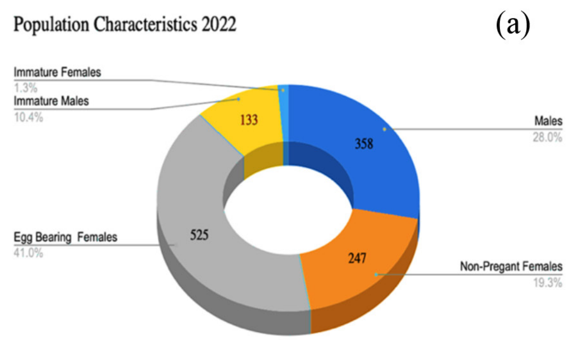

Figure 1 a-b. (a) *Callinectes Sapidus* population characteristics in Rehoboth Bay, DE, during the 2022 sampling season. Out of 1147 individuals around 525 females were egg-bearing. Roughly 787,500,000 eggs have been accounted for and released into Rehoboth Bay**. (b)** A picture of me processing blue crabs at our release and study site to minimize recapture likelihood.

CRYOPROTECTANT TOXICITY ON SPORES OF BROWN ALGAE *Macrocystis pyrifera* AND *Eisenia arborea*

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Cryopreservation studies are being conducted to conserve and restore populations of *Macrocystis pyrifera* and *Eisenia arborea,* two key brown algae species distributed along the Pacific coast of Baja California, which are impacted by climate change. Studies to determine the optimal cryoprotectant agents (CPAs) before cryopreservation are essential due to CPAs reduce ice formation in cryogenics temperatures. However, high concentrations of CPAs can produce toxicity and osmotic imbalance, potentially lethal to nonfrozen cells. Therefore, this study explores the cytotoxic effects of 3 cryoprotectant agents (dimethyl sulfoxide, ethylene glycol, and glycerol) at 2 concentrations (10% and 5%) and equilibration time for 10, 15, 30, and 50 minutes on the spores viability.

Before treatment, the percentage of spore survival and development into gametophytes and early sporophytes were recorded. Also, initial viability and oxidative stress was determined by flow cytometry. The results showed that some cryoprotectant concentrations affected spore development in both species. Spores of *Eisenia arborea* suspended in 5% glycerol remained in the spore germinate stage for 32 days, making them not viable for reproduction. However, 10% of dimethyl sulfoxide and ethylene glycol delayed the transition between stages and induced oxidative stress in the spores but did not affect the development of early sporophytes in both species. These findings underscore the potential toxicity of cryoprotectants and highlight the need for optimizing cryopreservation protocols for these important seaweed species.

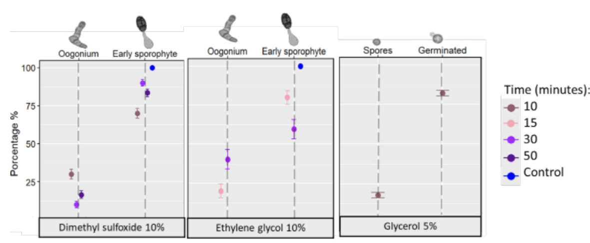

Figure 1. Percentage of gametophytes developing oogonia and early sporophytes after 32 days for *Eisenia arborea* following exposure to cryoprotectants at different times (10, 15, 30, and 50 minutes).

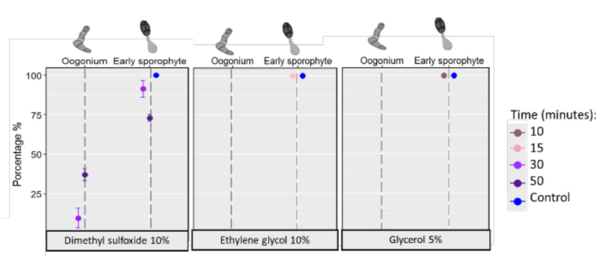

Figure 2. Percentage of gametophytes developing oogonia and early sporophytes after 32 days for *Macrocystis pyrifera* following exposure to cryoprotectants at different times (10, 15, 30, and 50 minutes).

FILLETCAM 2.0: ENHANCED AI TOOL FOR COMPREHENSIVE FILLET QUALITY AND DEFECT ASSESSMENT

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High-throughput and objective fillet color profiling and defect assessment are crucial for quality control and pricing benchmarks in the fish processing industry. Our research group has developed and tested a hand-held, smart device (FilletCam) that employs computer vision and artificial intelligence to enable real-time fillet color scoring. The initial research prototype demonstrated satisfactory performance with consistent accuracy and repeatability in color measurements; however, its functionality was limited to color-related quality assessments. Furthermore, the convolutional neural network (CNN) model was trained on a relatively smaller dataset collected under controlled lighting, which may affect its generalizability and adaptability to environments with variable or non-uniform lighting, such as fish processing plants and retail stores. This study aims to enhance the existing prototype by expanding its capabilities beyond color scoring to include fillet defect detection and color uniformity assessment. To improve model robustness, we are expanding and diversifying the training dataset with images captured under different lighting conditions and annotated for common fillet defects, including gaping, melanosis, blood spots, and scale residue. The dataset will be split into training (70%), validation (20%), and test (10%) subsets, and a custom model for defect detection and color profiling will be trained using Roboflow (Roboflow, Inc., Des Moines, Iowa, USA). The refined CNN model will be deployed on the upgraded FilletCam 2.0 hardware, and its performance will be evaluated in terms of mean average precision and F1 score. Predicted results will be compared against expert-annotated ground truth data, and findings will be presented.

THE EFFECTS OF AERATION RATES ON GROWTH, PRODUCTIVITY, NUTRITIONAL QUALITY AND NUTRIENT REMOVAL RATE OF *Ulva australis* IN AN IMTA SYSTEM WITH WHITE SEABASS (*Atractoscion nobilis*)

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Aeration is a critical component of the success of seaweed tumble culture, as it improves the growth and bioremediation potential of seaweeds. Specifically, aeration is used to tumble the seaweeds, thinning the diffusive boundary layer (DBL) and enabling seaweeds to absorb nutrients from the water while also fragmenting them. Optimizing aeration is important to the success of tumble culture, including the costs of operation. *Ulva* species have been considered ideal biofilters to remove dissolved nutrients in integrated multi-trophic aquaculture (IMTA) systems, and *Ulva* biomass has wide applications for human and animal food, biofuel, medicine, etc. Therefore, it is important to understand the effects of aeration on the performance of *Ulva* and associated cost : benefits in IMTA systems.

This study was conducted in an IMTA system at Hubbs-SeaWorld Research Institute in Carlsbad, California. White seabass (*Atractoscion nobilis*) were stocked in a 26,000 L raceway at a density of 20.0 kg/m³, with effluent gravity-fed into replicate 175L tanks containing *Ulva australis* stocked at 0.5 kg/m². Three aeration levels were set at 0.09 (low), 0.14 (medium), and 0.29 (high) L air/L seawater/min, with three replicates for each aeration level. *U. australis* was also cultivated in three tanks as that received raw seawater without nutrient supplementation and aeration set at medium. *U. australis* biomass was recorded and re-set to the original stocking biomass weekly. Seawater exchange rate was set at 63 vol./day. TAN, NO_2–N, NO_3–N, PO_4–P, and inorganic carbon (IC) were measured weekly. Photosynthetic parameters were assessed using a PAM fluorometer. Temperature, light intensity, dissolved oxygen, pH, and total dissolved gas were recorded daily. The study was run for four weeks.

Preliminary findings indicated that aeration levels did not significantly impact the specific growth rate (*SGR*) and productivity of *U. australis* (p=0.58 and 0.61) during the experimental period. *SGR* ranged from 20.89 to 34.89%/day, 25.70 to 32.83%/day and 17.71 to 34.20%/day for low, medium, and high aeration levels, respectively, and accordingly, productivity was in the range of 11.29-36.06, 12.96-37.85 and 8.55-39.94 g DW/m²/day. *SGR* and productivity also were not different between treatments in each sampling week (p=0.51-0.67 for *SGR*, 0.29-0.86 for productivity). *U. australis* had significantly higher *SGR* cultivated in effluent from *A. nobilis* tanks than raw seawater when aerated at low and medium aeration levels (p=0.020 and 0.016, respectively), but no difference in productivity (p=0.51 and 0.76). Under high aeration, *U. australis* did not show a difference in *SGR* and productivity (p=0.57 and 0.65). These initial findings suggest a potential cost reduction in the tumble culture of *U. australis* by lowering aeration levels to as low as 0.09 L air/L seawater/min. Results for water quality, nutritional composition, and photosynthetic capacities are still pending analysis.

EXPANDING COMMUNITY CAPABILITIES FOR DEVELOPMENT OF GERMPLASM REPOSITORIES FOR AQUATIC SPECIES THROUGH OPEN HARDWARE

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Establishing germplasm repositories for aquatic species on a global scale is challenging, largely due to the lack of standardized solutions. The Aquatic Germplasm and Genetic Resources Center (AGGRC, www.aggrc.com) launched its Fabrication and Design Program to develop open-source hardware, addressing the specific needs of aquatic species and supporting global communities to establish germplasm repositories. Conventional cryopreservation relies on costly commercial programmable freezers, often beyond the reach of resource-limited communities. To address this, a 3-D printable Positional Cooling Platform Device ("CryoKit") was developed to enable floating samples on liquid nitrogen and achieving multiple, reproducible cooling rates. Field-based cryopreservation, however, presents additional challenges as no commercial products are available. The Shipping Dewar Positional Cooling Device ("Cajun Ejector") was developed to address this, providing a 3-D printable, standardizable device for using commercial nitrogen vapor shipping dewars. These affordable solutions make cryopreservation accessible to a broader range of users.

The AGGRC team has also developed a series of open hardware that can support cryopreservation, animal care, sample collection and processing, and education for STEM programs. By integrating innovative tools with comprehensive support, a holistic approach can be created for a community effort in safeguarding valuable genetic resources of aquatic species. In addition, educational initiatives can foster awareness and inspire new generations of advances, empowering communities to take an active role in safeguarding aquatic species.

Figure 1. Examples of open hardware from the AGGRC (aggrc.com).

COMPARISON OF THE FIELD PERFORMANCE OF SELECTED LINES OF EASTERN OYSTERS IN U.S. NORTHEASTERN STATES

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Selective breeding programs spanning over 60 years of effort have supported production increases in the eastern oyster (*Crassostrea virginica*) culture industry in the Northeastern U.S. A major focus of the oyster selective breeding efforts in the region, as well as along the Atlantic and Gulf Coasts of the U.S., has been the development of disease-resistant stocks. Despite past success, shellfish breeding in the Northeast and beyond faces some significant challenges, not the least of which is that numerous estuaries along the mid-Atlantic and New England coasts where shellfish are cultured differ dramatically in habitat quality and disease pressure.

Recent advances in high-resolution and high-throughput genotyping of eastern oysters have provided an opportunity to use genomic information to estimate the breeding value of individuals in our broodstock programs. We genotyped live and dead oysters from a dermo challenge experiment with a 66K SNP array and predicted genomic estimated breeding values (GEBVs) for dermo resistance in a wild population from Delaware Bay. We produced two up-selected (based on two models), one down-selected and one average controls using oysters with highest, lowest and average GEBVs. Locally selected lines and their hybrids with Haskin NEH® were also produced and evaluated along with the four genomically selected groups over the past two growing seasons in several Northeastern states.

To date, we have observed sites-specific variation in the performance of these lines. While one of the up-selected and the average selected lines had higher yield in New Jersey, no significant difference in field survival has been observed between genomic selected and control lines probably because field survival is more complex than lab-based dermo resistance or genomic prediction is less effective across populations. Further, the triploid group had the highest yield, and the northern lines had lower survival possibly because they are less tolerant to MSX, dermo and/or heat stress. In NY, traditionally selected lines grew better while genomically selected lines suffered early high mortalities due to Roseovarius Oyster Disease (ROD, a.k.a. JOD). Similarly, in Maine, yield for the genomically selected lines from Delaware Bay wild lagged behind that of a traditionally selected line, though the difference was due more to growth rather than mortality. Our presentation will provide additional information on growth and mortality for these lines during the second field season for this ongoing project.

OLIVE FLOUNDER (*Paralichthys olivaceus*) PERFORMANCE IN RAS

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Olive flounder (*Paralichthys olivaceus*) have a long history of success in flow-through aquaculture production, starting in the 1960s in Japan where the species is commonly referred to as "hirame." Since that time, many challenges have been resolved such as breeding, first feeding, survival through the metamorphosis stage, and growout to food-size fish. The fish can reach a market size of 0.8 to 1.2 kg in 12 to 18 months, making them a fast-growing animal and market prices are very strong. As a flat fish, they have several key characteristics that make them ideal for aquaculture, including that they lay on the tank bottom most of the time, thereby conserving energy. This fact may also allow the fish to be cultivated in shallow tanks, and because they can lay several fish deep, high rearing densities are possible. The University of Miami (UM) has been cultivating them for nearly a decade in flow-through, partial flow through, and RAS systems. Over the last 14 months, Kentucky State University (KSU) has also developed RAS systems for them.

The opportunities for Olive flounder exist near coastal areas where native flounder fisheries have been decimated. Those opportunities extend well into interior locations where fresh seafood is very hard to obtain, but highly valued by chefs and consumers in all areas. Carefully designed RAS are the key to capturing both of these markets. Another industry that has seen growth in the United States and other regions is shrimp farming in RAS. Interestingly, this flatfish species requires the use of flat-bottom tanks like shrimp do, therefore some overlap may exist where a farm could explore the production of either species using the same production systems.

With regard to RAS design, because flounder lay on the tank bottom most of the time, the fish have a reduced oxygen demand while resting compared with other finfish species that may be swimming constantly. Another noteworthy issue is that the fish will tend to lay on top of air and oxygen diffusers, which can limit the dispersion of oxygen in the tank environment. This issue may be remedied by elevating one end of the diffuser slightly so fish can lie under and around it. The fish grow best at temperatures between 20 and 25°C, and have a high protein requirement of about 50% in their diet. These facts mean that biological filters must be larger than those of more tropical aquatic species that eat lower protein diets. The fish grow well in a variety of tank sizes; KSU was able to achieve biomass levels of 64 kg/m^2 with fish an average weight of 429 g in tanks that were only 1.2 m in size. Pilot-scale growout trials at UM in a variety of production systems and tank dimensions have been very successful as well over the years.

Ultimately, this fast-growing, feed-efficient species holds a great deal of opportunity for RAS production. Research at KSU and UM continues into genetic improvements, low salinity culture, growth in various salt mixtures, greenhouse gas capture, culture in IMTA systems, and other innovative areas.

FUNCTIONAL INTERROGATION OF THE DE-NOVO RAINBOW TROUT MICROBIAL GENOME ATLAS REVEALS LONGSTANDING SYMBIOTIC HOST-BACTERIA INTERRELATIONSHIP

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The critical role of the gut microbiome in supporting immune function, metabolic processes and somatic growth is well characterized in humans and terrestrial livestock species, however the role of the gut microbiome remains poorly elucidated in aquatic species. To this end we assembled the Rainbow Trout Microbial Genome Atlas (RTMGA) recovered from a highly domesticated farmed cohort (n = 118) originating from the USDA NCCCWA breeding program. A hybrid assembly approach combining 3.793 (Gb) of short, and long reads was employed, in tandem with in-vitro anaerobic culturing. A sum of 94 high quality bacterial genomes exhibiting a minimum completeness & contamination threshold of 70% and 10% respectively were taxonomically classified. We report 9 putative novel species identified, and 21 species, 8 genus and 53 family level taxonomic classifications, of which 7 genomes exhibited >99% completeness. Metagenomic functional profiling showed enrichment for short chain fatty acid production, acetate conversion to methane, and nitrogen metabolism in the anerobic gut macroenvironment. Comprehensive classification and functional interrogation of the Rainbow trout microbiome may assist in better capturing the breadth of commensal biochemical pathways underpinning commercially relevant phenotypes of interest.

STRIPERHUB: OVERVIEW OF STRIPED BASS (*Morone saxatilis*) AQUACULTURE

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StriperHub, coordinated by North Carolina Sea Grant, is part of a national initiative to streamline and expand striped bass (*Morone saxatilis*) and hybrid striped bass (*M. saxatilis* x *M. chrysops*) aquaculture in the US. It brings together Sea Grant programs across several states, industry partners, government agencies (NOAA, USDA), policymakers, and academic experts. The goal is to reduce the US seafood trade deficit, as over 80% of seafood consumed in the US is imported, contributing to an $18 billion annual deficit. StriperHub aims to develop striped bass as a viable aquaculture species and enhance hybrid striped bass farming to strengthen domestic seafood production and boost coastal and rural economies.

The initiative focuses on several key areas, including defining markets and production economics for striped bass, providing education and training programs, and clarifying regulatory and licensing procedures. Additionally, StriperHub seeks to increase visibility and outreach among potential producers and consumers, especially along the Eastern US Coast. As the aquaculture industry grows, StriperHub plans to bring in more partners from academia, government, and the private sector to expand operations nationwide.

Specific goals include improving seed stock production and distribution, refining economic parameters for striped bass aquaculture, developing marketing and business models, and enhancing communication and training efforts. Current efforts include optimizing broodstock and seedstock production, testing cost-saving feeding strategies, and synchronizing spawning for better seedstock yields. StriperHub also aims to expand the number of domestic striped bass producers to ensure steady commercial production. The F8 generation domestic striped bass were produced for the first time in 2024.

STRIPERHUB: BUSINESS MEETING AND CULTURE MANUAL DEVELOPMENT

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The initiative focuses on several key areas including the development of a revised striped bass aquaculture manual. The business meeting will focus on discussion between members of the audience representing academic, government and private sector partnerships to solicit input and feedback. Further, to discuss future directions of research and needs.

VITELLOGENESIS IN FISHES

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Higher-order teleosts (Acanthomorpha) express three distinct forms of vitellogenin (VtgAa, VtgAb, and VtgC) and these have been characterized in a number of fish species. The VtgAa and VtgAb are considered "complete" and contain five yolk protein domains (lipovitellin heavy chain, lipovitellin light chain, phosvitin, beta'-component, and c-terminal component), however the VtgC is "incomplete" and only contains the lipovitellin heavy and light chains. These egg yolk precursors are produced by the liver in response to estrogen, with gene expression mediated by estrogen receptor alpha. Vitellogenins released into the circulatory system are specifically taken up by growing oocytes via receptor-mediated endocytosis within clathrin-coated pits. Two vitellogenin receptors have been characterized in fishes: The Lrp13, which localizes throughout the zona radiata and granulosa cells and specifically binds VtgAa, and the LR8, which localizes to the oolemma and zona radiata interna and binds VtgAb. To date, no known lipoprotein receptor has been shown to bind VtgC in Acanthomorphs and it might enter oocytes through the endocytosis fluid phase or escorted by Y-box binding protein 2a, to which it binds. Additionally, VtgC localizes exclusively to lipid inclusions within growing oocytes, whereas VtgAb localizes to the ooplasm and yolk globules; VtgAa has not yet been evaluated in this manner. The VtgAb primarily enters growing oocytes during early- to mid-vitellogenesis, whereas VtgAa enters oocytes from mid- to post-vitellogenesis. The VtgAb is typically the predominant form in blood plasma and egg yolk. The VtgC is accumulated by oocytes beginning at pre-vitellogenesis and continues until post-vitellogenesis and its composition in the yolk can vary widely between species. In medaka, knocking out the *lr8* gene reduced VtgAb-derived yolk in eggs and increased VtgAa-derived yolk, compensating for the reduction without changing overall yolk protein levels per egg, although the survival rate of knockout-derived larvae was lower than in wild types. The overall proportional accumulation of different Vtgs within the yolk influences egg buoyancy. In higher-order teleosts, yolk proteins derived from VtgAa are degraded into peptides and free amino acids that drive oocyte hydration during ovarian maturation. A link between egg diameter and buoyancy were observed in striped bass, indicating that more buoyant eggs have a larger outer diameter due to hydration and a greater proportion of VtgAa yolk content. The proportional deposition of different Vtgs in this species was influenced by water salinity, such that eggs of the correct specific gravity were ovulated and tailored to the estuarine environment. Lower-order teleosts also possess different complete Vtg forms, although, with the exception the Ostariophysian fishes, they all appear to be functionally similar. These species also express VtgC. In salmonids, the complete VtgAs form binds both LR8 and Lrp13 receptors. In salmonids, the VtgAs and its derived lipovitellin (LvAs) are always predominant (95%) in the circulation and in the yolk of vitellogenic females. Understanding the functions of these multiple vitellogenins is relevant to egg quality, since yolk components not only provide nutrition to embryos and larvae at specific developmental stages but contribute to egg buoyancy as well.

THE NRSP-8 NATIONAL AQUACULTURE GENOME RESEARCH PROGRAM

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The NRSP-8 *Aquaculture Genomics Program* is a collaborative initiative within the broader NRSP-8 program (National Research Support Project 8), which focuses on improving animal genomics. This specific branch is dedicated to advancing genomic resources, tools, and research for the aquaculture industry. By focusing on genomics, the program aims to enhance breeding practices, improve disease resistance, optimize growth rates, and contribute to sustainable aquaculture practices.

The NRSP-8 Aquaculture Genomics Program advances aquaculture sustainability by developing genomic resources like reference genomes, genetic markers, and bioinformatics tools for key species such as salmon, trout, catfish, tilapia, striped/white bass, oysters, and shrimp. It promotes selective breeding to enhance growth, disease resistance, and adaptability, fostering collaboration across academia, government, and industry. By creating open-access databases such as AquaMine (https://aquamine.elsiklab.missouri.edu/), it provides valuable resources for researchers and practitioners, supporting the growth of sustainable aquaculture that minimizes environmental impact. The program outcomes enhance food security, reduce environmental pressures, and improve industry profitability by creating more resilient and productive aquaculture stocks.

The NRSP-8 Aquaculture Genomics Program is an essential driver for innovation in aquaculture, supporting both research and practical advances in sustainable food production and hosts an Aquaculture Genomics Workshop every year at the *International Plant and Animal Genome Conference* (https://www.intlpag.org/PAG32/).

The renewed *NRSP8: Genomic Capacity: Building Applied Genomic Capacity for Animal Industries* (Duration: 10/01/2023 to 09/30/2028) has three new objectives:

1. Extending genomics capacity to a broader range of Animal Science stakeholders.
2. Supporting capacity to integrate genomic and biological data.
3. Education, training and outreach to develop a data-savvy workforce.

BEST PRACTICES AND INNOVATIVE STRATEGIES FOR INCREASING SUSTAINABILITY AND PROFITABILITY IN AQUAPONICS

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Aquaponics is increasingly recognized as one of the more sustainable methods of food production, leveraging a circular symbiosis that enables plants to utilize the nutrient-rich waste generated by fish, rather than discarding it. While aquaponics is generally straight forward to implement on a small scale, transitioning to commercial operations introduces a range of challenges that must be addressed to maximize both plant yields and profitability.

Successful aquaponics requires a deep understanding of the specific plant needs, alongside effective solids mineralization practices to ensure optimal nutrient availability. Key factors influencing system performance include adequate aeration, appropriate lighting or shading strategies, efficient solids collection and carefully managed flow rates. Each of these elements plays a crucial role in maintaining a balanced ecosystem within the aquaponics system, thereby enhancing its sustainability and reducing the overall carbon footprint. Moreover, managing overall nutrients dynamics, as well as key parameters such as pH and EC is essential for maximizing crop yields and profitability in aquaponic production. By optimizing these variables, aquaponics can not only improve plant growth but also contribute to a more resilient and efficient food production system that can serve as a viable model for sustainable agriculture in the future.

DEVELOPING A FRAMEWORK FOR THE ASSESSMENT OF ADAPTIVE CAPACITY IN SALMON FARMING

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Climate change is already affecting marine salmon farming through impacts including rising water temperatures, lower oxygen levels, insecurity in the feed supply chain, and increasing incidence of algal blooms and jellyfish. These effects are creating challenges for aquaculture production, and sensitivity to climate change impacts is heightened in marine aquaculture operations where fish and farming infrastructure are exposed to the surrounding environment. Successful adaptation to climate change will rely on adaptive capacity, the ability of a system to prepare for stressors in advance or adjust and respond to the effects caused by those stressors. Adaptive capacity can reduce vulnerability to climate change impacts by modulating the relationship between exposure to climate change and sensitivity to climate change.[1]

Early work on adaptive capacity emphasizes the role of assets, specifically the availability of financial resources and technology. Several climate change adaptation responses have been proposed for salmon aquaculture ranging from on-farm solutions including early warning systems to transformative technologies such as offshore, submersible, and land-based farming systems. However, the availability of these technologies and financial resources does not guarantee adaptation. Increasingly, structural, social, and cognitive dimensions of adaptive capacity are also thought to be important in mobilizing adaptive capacity.[2]

Adaptive capacity is latent within systems, making it difficult to identify important dimensions of adaptive capacity until it is mobilized to address new challenges. However, climate change is not the first challenge encountered in salmon farming, and farmed salmon production has continued to grow over the past few decades despite these challenges, suggesting that adaptive capacity is present. Therefore, previous responses to system challenges provide an opportunity to identify adaptive capacity in action. Using a modified Delphi consensus building approach, we engaged a panel of industry experts in the development of an adaptive capacity framework. We engaged industry experts in discussions about challenges and responses to those challenges within the salmon farming sector, developed an initial adaptive capacity framework based on those discussions, and then used a questionnaire to validate the framework and gather information about the status of adaptive capacity within the salmon farming industry.

Preliminary results indicate that assets including financial assets, access to technology, and human resources are important dimensions of adaptive capacity. Social-cognitive dimensions including social organization, agency, flexibility, motivation, and knowledge were also mobilized towards addressing system stressors and will be important in adapting to climate change.

This work was supported by a UKRI Future Leaders Fellowship (MR/V021613/1).

[1]Engle, N. L. (2011). Adaptive capacity and its assessment. *Global environmental change*, *21*(2), 647-656.
[2]Cinner, J. E., & Barnes, M. L. (2019). Social dimensions of resilience in social-ecological systems. *One Earth*, *1*(1), 51-56.

HOW DOES AQUACULTURE ECO-CERTIFICATION SUPPORT SUSTAINABILITY ACROSS ECOSYSTEM SERVICES AND SCALES? AN ANALYSIS BASED ON THE ECOSYSTEM APPROACH TO AQUACULTURE

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Aquaculture eco-certification schemes offer the promise of improved aquaculture sustainability by providing market benefits to producers that meet a set of pre-defined sustainability criteria. These schemes operationalize sustainability through a set of written sustainability criteria and the process that producers must go through to obtain certified status. However, sustainability can have different meanings as illustrated by differences between eco-certification schemes. Where fisheries eco-certification schemes can rely on established concepts such as maximum sustainable yield to define sustainability, aquaculture eco-certification schemes must define sustainability for themselves; that is, they must determine what is/are the issues that eco-certification schemes should address. This has resulted in eco-certification schemes developing and implementing different assemblages of criteria that reflect divergent understandings and prioritization of social and environmental sustainability.

As eco-certification schemes have evolved, so have aquaculture practices and policies including the Food and Agriculture Organization's Ecosystem Approach to Aquaculture which calls for the integration of aquaculture within the wider ecosystem while promoting sustainable development, equity, and resilience of social-ecological systems. The Ecosystem Approach to Aquaculture provides an opportunity to analyse aquaculture eco-certification using a conceptualization of aquaculture sustainability developed outside of the structural and pragmatic requirements of eco-certification schemes. Therefore, the Ecosystem Approach to Aquaculture was adopted as a guiding framework in considering role of aquaculture eco-certification in creating desirable outcomes for the environment and society. Using marine salmon farming as an example, the role of eco-certification in aquaculture sustainability was explored through document analysis and interviews with people involved in the process of eco-certification. Results point to a need to recognize cultural and provisioning services within eco-certification criteria, challenges presented by a mismatch between the farm-scale application of eco-certification criteria and the improvement of ecosystem-level sustainability, as well as opportunities to address ecosystem-level sustainability throughout the process of eco-certification.

IMPACT OF BLACKBIRD DEPREDATION ON BAITFISH AND SPORTFISH AQUACULTURE IN ARKANSAS

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In Arkansas, Lonoke and Prairie counties produce 72% of the United States' total baitfish and sportfish sales. However, a substantial number (92%) of producers in this region consistently report that common grackles (*Quiscalus quiscula*; hereafter grackle) and other blackbirds often predate baitfish and sportfish when held in holding vats under sheds prior to shipment and on spawning mats in brood ponds. Despite predation loss due to blackbirds being a producer concern for decades, no studies have quantified the extent of these losses. Therefore, our objectives were to: 1) characterize the foraging patterns (e.g., frequency of visitation, timing, foraging success) of blackbirds within rearing/holding sheds and at spawning mats in brood ponds, 2) measure the amount of prey items consumed by these blackbirds, and 3) quantify the economic impact of predation by blackbirds.

During the 2024 field season, we conducted focal observations and camera surveys on ponds with spawning mats and vats under sheds from five baitfish and three sportfish farms between mid-March - July. Surveys were conducted biweekly, except during the peak of baitfish spawning season from mid-April to mid-May, when they were conducted weekly. Over the nine survey periods, actively foraging blackbirds were collected using air rifles and gavaged with 20 cc of phosphate-buffered saline to stop digestion for subsequent diet analysis. Stomach contents were sorted, identified to lowest classification level, dried at 60°C for 22-24 hours, and weighed to calculate diet type proportions. Twelve grackles were randomly selected for diet analysis through DNA methods.

Of the 111 processed grackles, 71 had consumed fish from ≥5 different species. No fish were observed in the six red-winged blackbirds (*Agelaius phoeniceus*) collected. Brood pond camera surveys documented nine other avian species, water snakes, raccoons, and mink predating fish on the spawning mats.

We will use multivariate analyses to compare aggregate percentage weight, frequency of occurrence, and prey richness by year, sex, and system (categorical; brood pond or fish shed). Results of this study will be used to guide best management practices for mitigating losses due to avian predation.

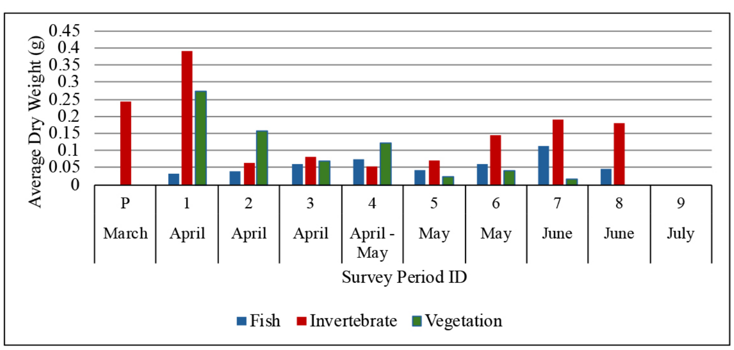

Figure 1: Survey period average of dry weight (g) by food type.

HARMFUL ALGAL BLOOM MONITORING IN THE FIELD AND SHELLFISH HATCHERIES USING FLOWCAM® TECHNOLOGY

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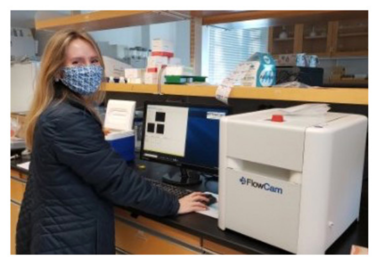
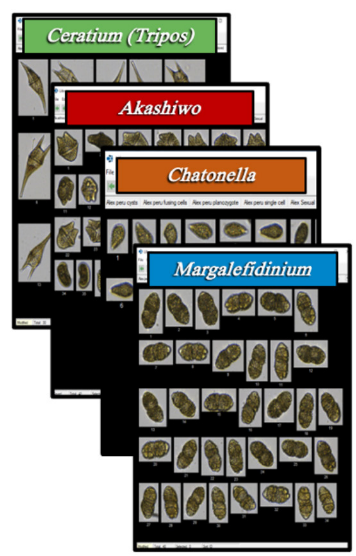
Monitoring shellfish growing areas for Harmful Algal Bloom species is essential to protect the health and safety of the consumer. Tidal waters in Virginia are home to a wide variety of naturally occurring, seasonally blooming dinoflagellates that have the potential to produce compounds which are associated with many shellfish syndromes. FlowCams (Yokagawa Fluid Imaging Technologies), laboratory benchtop high-throughput water analysis instruments that capture digital images of particles, are being used to facilitate harmful algal bloom (HAB) monitoring in lower Chesapeake Bay. The associated VisualSpreadsheet software is being used to classify key phytoplankton taxa, focusing on HAB species to create image libraries. Laboratory cultures of dominant bloom species including *Margalefidinium polykrikoides* and *Alexandrium monilatum* were used to expedite library construction. Field samples were then collected and run to add images of these species from their natural habitat, particularly during blooms throughout the bloom cycle when different life stages could be observed and documented.





To date, libraries are being made for several species including *M. polykrikoides*, *A. monilatum*, *Akashiwo sanguinea*, *Heterocapsa triquetra*, *Ceratium furca*, *Scrippsiella trochoidea*, *Chattonella subsalsa*, *H. rotundata*, *Prorocentrum cordatum*, *P. micans, Dinophysis* spp. and *Diplopsalis lenticula*. The FlowCam is being used to monitor water quality at the oyster hatchery facilities located at the Virginia Institute of Marine Science in Gloucester Point, Virginia. Water samples are collected at several stages in the hatchery to follow water quality condition with respect to phytoplankton presence and specifically to detect HAB contaminants and the algal food sources. These data are compared with larval growth and development from spawns. Some hatcheries are also using FlowCam images to follow oyster veliger growth, health and feeding efficiency. Field collections at growing areas monitored by the Virginia Department of Health will be used to validate these libraries with the intent to use the FlowCam as a screening tool for their monitoring program.

THE EFFECTS OF MARINE NOISE ON *Mytilus edulis* RESPONSE TO *Vibrio splendidus* INFECTION

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Blue mussels (*Mytilus spp*.) are a globally significant aquaculture species, cultivated across all continents except Antarctica. Both farmed and wild populations provide essential ecosystem services, including water filtration, nutrient cycling, and biogenic reef formation that enhances biodiversity and mitigates coastal erosion. However, mussels are increasingly threatened by stressors intensified by climate change, such as rising storm frequency, marine heatwaves, ocean acidification, irregular phytoplankton fluctuations, and emergent diseases. These challenges weaken mussel resilience and have been linked to mass mortality events in European populations over the past decade. Additionally, anthropogenic noise, known to harm various marine species and habitats, remains understudied in its effects on mussels. In this study, we investigated the influence of marine noise on infection outcome in *Mytilus edulis*. Mussels were exposed to simulated underwater ship noise for one week, followed by a bacterial challenge with GFP-expressing *Vibrio splendidus*. Although no significant transcriptomic response was observed to the noise alone, noise-exposed mussels exhibited higher levels of GFP+ *V. splendidus* and a suppressed transcriptional response to infection compared to controls. These findings suggest that noise exposure compromises the immune competence of mussels, impairing their ability to resist bacterial infection. This research highlights the potential impact of environmental stressors like noise on mussel health and resilience. Understanding such interactions could inform aquaculture management strategies, such as stress mitigation or site selection. However, further studies are required to explore the duration of noise-induced effects and whether mussels can develop tolerance to prolonged noise exposure.

FEED THE FUTURE INNOVATION LAB FOR FISH: INNOVATION AND TECHNOLOGY FOR SUSTAINABLE AQUATIC FOOD SYSTEMS

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The Feed the Future Innovation Lab for Fish, funded by the U.S. Agency for International Development (USAID), works to reduce poverty and improve nutrition, food security, and livelihoods in partner countries by supporting research on sustainable aquatic food systems. The Fish Innovation Lab is managed by the Global Center for Aquatic Health and Food Security at Mississippi State University. It is one of 16 Feed the Future Innovation Labs that leverage the expertise of U.S. universities and developing country research institutions to tackle some of the world's greatest challenges in agriculture and food security.

The Fish Innovation Lab supports research and capacity-building activities targeting three program areas: climate-smart aquatic system innovations, nutrition and food systems, and inclusive access to improved inputs. To enhance the development impact of Fish Innovation Lab research, each funded activity is also expected to incorporate cross-cutting themes related to gender equity and social inclusion, resilience, and capacity development. From 2018-2023, the Fish Innovation Lab supported 24 research activities in 10 countries: Bangladesh, Cambodia, Ghana, Kenya, Madagascar, Nigeria, the Pacific Islands, the Philippines, Peru, and Zambia. The program received a 5-year extension in 2023, and in 2024, it launched six 1-year Startup and Scaling Activities in Bangladesh, Kenya, Nigeria, and Zambia. These activities aim to scale up work funded during the first 5-year phase and identify new approaches to improve sustainable aquaculture and fisheries. Following a competitive selection process, the Fish Innovation Lab anticipates awarding 14 additional activities in 2025.

Fish Innovation Lab activities have addressed major issues in aquaculture and fisheries. In Bangladesh and Nigeria, teams have investigated different ways to improve aquaculture production and provide better quality fish products to consumers. In Cambodia, Ghana, Kenya, and Nigeria, teams have worked to improve the sustainability of local fisheries and provide nutrition training and information to promote consumption of aquatic foods for better nutrition amongst fishers, mothers, and children. Recently launched activities in Kenya will analyze fish health and antimicrobial resistance in aquaculture and use climate-smart solutions to integrate agriculture and aquaculture. Activities in Zambia captured a wide range of work, from fish vaccine development to reduce aquaculture losses to assessing population ecology and current distribution of introduced invasive crayfish. Additionally, ongoing work in Zambia developed and is now scaling a dried fish powder along with recipes for enhanced nutrition, particularly benefiting mothers and infants in vulnerable households.

To learn more about the activities of the Fish Innovation Lab, visit our website at www.fishinnovationlab.msstate.edu and subscribe to our newsletter at https://rb.gy/j17i6.

AQUACULTURE EXTENSION PROGRAMMING IN SOUTHEASTERN MASSACHUSETTS JOINTLY ADMINISTRATED BY BARNSTABLE COUNTY AND WOODS HOLE OCEANOGRAPHIC INSTITUTION SEA GRANT

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A marine extension position has existed on Cape Cod in excess of 50 years that has been responsible for a number of marine related topics including aquaculture as it developed. The past 30 years have involved a partnership developed between Cape Cod Cooperative Extension (CCCE) of Barnstable County and Woods Hole Oceanographic Institution Sea Grant (WHOISG). As currently defined in a Memorandum of Understanding between Barnstable County and WHOISG, the marine extension programs operate jointly and collaboratively to advance the efforts of marine extension services in the southeastern Massachusetts region.

Shellfish aquaculture has grown rapidly in Massachusetts over this time period and has been the focus of aquaculture extension efforts. The industry focuses on largely oysters and hard clams and now encompasses close to 400 farms and greater than $30 million in farmgate sales value. Extension efforts have focused on education, monitoring, and applied research to assist the growing industry.

COOPERATIVE RESEARCH IN MASSACHUSETTS; USING A RESEARCH FARM PLATFORM TO EXPLORE OPPRTUNITES FOR SHELLFISH GROWERS

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A research farm network (RFN) was established in 2005 with funding help from the Southeastern Massachusetts Aquaculture Center (SEMAC). The RFN program continues to generate valuable data regarding shellfish culture methodology and provides germane aquaculture research to growers. Working with shellfish growers throughout the state, the program explores challenges and opportunities through research with the farmers themselves but with scientific structure assistance provided by extension staff.

Research topics have varied but are generated from industry, often through an advisory group. Past projects have included research and culture of alternative species for shellfish aquaculture including surf clams, razor clams, and blood arks. Investigations of triploid oysters versus diploid counterparts, as well as biofouling organism settlement patterns and control methods. Growers involved rotate based on geographical area and interest. Challenges have included availability of funds but also the physical capacity to visit farm sites on a regular basis, especially if tide dependent.

PARTICIPATORY SCIENCE APPROACH TO MONITOR HARMFUL ALGAL BLOOMS, AND CHANGES IN ENVIRONMENTAL CONDITIONS WITH THE AQUACULTURE INDUSTRY

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The National Phytoplankton Monitoring Network (PMN) is a community-based network of volunteers monitoring marine and freshwater phytoplankton and harmful algal blooms (HABs). Formed in 2001, PMN enhances the Nation's ability to respond to and manage the growing threat posed by HABs by collecting important data such as phytoplankton species composition, distribution, and environmental conditions. HABs have been observed in every state resulting in over $1 billion in losses to communities that rely on recreation, tourism, and seafood harvesting. In the aquaculture industry, both shellfish and finfish, have experienced direct adverse effects of harmful algal blooms, both toxin-producing species and non-toxin-producing species. For the individual aquaculture farm, blooms of certain non-toxic phytoplankton are of paramount concern because they are known to cause the mortality of shellfish and finfish worldwide. Since these species do not cause human health concerns, most are usually not included in state-wide monitoring programs.

The Aquaculture Phytoplankton Monitoring Network (AQPMN) project expands the scope of the participatory science approach of PMN to include the partnership of aquaculture farms. The AQPMN will catalog existing and new phytoplankton species responsible for finfish and shellfish injury and establish a national monitoring platform operated by aquaculture farms, empowering farms to take action to protect or harvest. This presentation will cover the methods used to monitor aquaculture sites and progress made by AQPMN to establish a national participatory science approach to monitor ichthyotoxic HABs, co-developed by the shellfish and fin fish industries.

COMPARING AMMONIA AND NITRITE CONVERSION AMONG FOUR TYPES OF BIO-MEDIA

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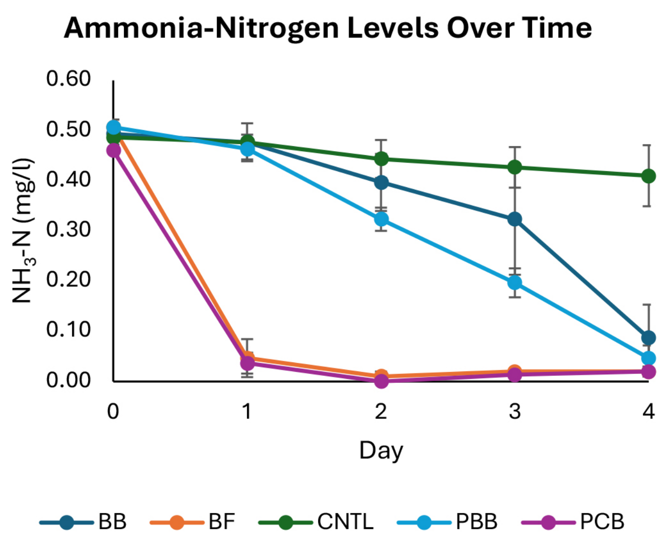
Ammonia and nitrite are significant concerns in aquarium environments, aquaculture, and larger aquatic ecosystems, as they can negatively impact water quality and the health of aquatic organisms. These nitrogenous compounds are toxic at elevated concentrations, making effective conversion and removal essential for maintaining aquatic systems. Currently, a variety of bio-media are available on the market, each varying in cost and effectiveness. Aquarists and aqua culturists are continually seeking the most efficient and cost-effective media for nitrogen conversion.

To compare the conversion effectiveness of four types of bio-media (porous ceramic blocks [PCB], bio-fill [BF], plastic bio-beads [PBB], and 1" bio-balls [BB] plus a control [CNTL]) under different environmental conditions, we conducted a series of short experiments. Prior to the trials, all media were conditioned for 21 days in a common RAS to seed them with nitrifying bacteria. For each trial, three 68-L tanks were randomly assigned to each media type, filled with 60-L well water and its assigned media. Then the addition of a single dose of ammonium chloride (NH_4Cl) was added to each tank to increase ammonia concentrations to 0.5 mg/L and baseline water samples were collected from each tank. analyzed for ammonia nitrogen (NH_3-N) and nitrite nitrogen (NO_2-N). Water samples were then collected daily to measure changes in NH_3-N and NO_2-N concentrations.

The results of this study will provide a comprehensive comparison of the ammonia and nitrite conversion efficiencies of each media type, contributing to the optimization of water quality management in both small-scale aquariums and larger aquaculture systems. This research will assist aquarists and aqua culturists in selecting the most efficient filtration media for their systems.



SEA OTTER INTERACTIONS WITH OYSTER FARMS

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Sea otters (*Enhydra lutris*) are considered a keystone species and can be found around mariculture oyster farms within Alaska. While oyster farms and sea otters have been coexisting in some locations, sustaining these farms, and the growing interest in expanding them, necessitates a comprehensive evaluation of potential interactions. For this study, sea otter interactions with oyster farms were assessed through behavioral observations (i.e., activity and foraging dives) in oyster farms, adjacent non-farm areas (controls), and bays with no farming activity (references). Behavioral observations, conducted through scan surveys, captured sea otter activities (e.g., resting, grooming, swimming, and foraging). Targeted foraging observations tracked foraging success and prey (species and count).

This study hypothesized that sea otters preferentially use oyster farms for foraging and resting activities compared to non-farm areas. Contrary to our hypothesis, sea otter activities showed no significant difference in these behaviors between oyster farms and controls. Similarly, foraging behavior, including success and prey diversity did not significantly differ among the areas. The dominant prey items in our study included clams (e.g., *Saxidomus gigantea*), crabs (e.g., *Cancer productus*), and mussels (e.g., *Mytilus trossulus*; Fig. 1). Notably, there were no observations of farm oysters being consumed by sea otters, which may be attributed to the type of gear used. There were significant differences in the average number of prey consumed per sea otter per dive between the control and reference areas, with the control area averaging 2.6 prey items per dive (SD = 3.6), and the reference area nearly doubling to 4.8 prey items per dive (SD = 7.3). These differences may be attributed to variations in prey biomass and environmental conditions. Our observations indicate that there are no discernible differences in overall sea otter activity or foraging behavior in the presence of oyster farms.

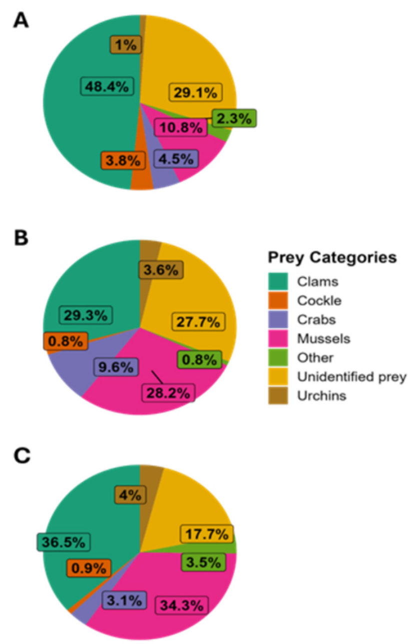

Figure 1: Pie charts illustrating the proportion of various prey items consumed by sea otters in the Kachemak Bay study areas, which include (A) the control areas, (B) the active oyster farm areas, and (C) reference areas. Prey categories were grouped by functional prey groups and color-coded. The "other" category were uncommon prey items (e.g., snails, worms, etc.).

MANAGEMENT OF BIRD-RELATED PATHOGEN RISK IN SHELLFISH AQUACULTURE

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The use of floating gear in oyster farming has gained popularity because mortality rates are reduced, product quality can be improved, and management of fouling is facilitated. Birds see floating gear as an attractive place to roost. Birds have been implicated in 11 outbreaks of Campylobacteriosis related to shellfish consumption nationwide since 2009.

The National Shellfish Sanitation Program (NSSP) describes the regulations for shellfish harvest, handling and distribution. The FDA, working with the Interstate Shellfish Sanitation Conference has devised regulations and guidance states designed to ensure that commercially harvested shellfish are safe and wholesome. The NSSP requires that if aquaculture gear may attract birds or mammals, operators must provide a written operational plan to describe how to address possible contamination of shellstock and potential adverse impacts to water quality.

The 2023 revision of the NSSP contains guidance on how states can address these concerns and the variety of approaches states can consider to minimize health risks to consumers. State authorities can consider tidal dilution and mandate resubmergence of impacted shellfish prior to harvest. Bird deterrents are mandated in many states and a variety of novel deterrent approaches are being tested. This presentation will describe some of the wide variety measures and regulations that state regulators have adopted to address public health concerns related to bird waste on the East Coast.

UTILITY OF 3D PRINTED ARTIFICIAL INSEMINATION DEVICES IN LIVE BEARING FISHES

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Open-source technology is advancing at a rapid pace. By using online resources such as Thingiverse, Blender and AutoCAD anyone in the makerspace can quickly download, model, and 3D print complex designs that would otherwise take specialized fabricating equipment to produce. In 2022, researchers at the Aquaculture Germplasm and Genetic Resources Center (AGGRC) of Louisiana State University created an open-source AI device. Their device was comprised of three parts, the delivery system, the user interface, and a foot pedal. The delivery system, or Standardized Artificial Insemination Device (SAID) is comprised of a linear actuator that controls the flow of fluid in a capillary tube. The User interface contains a potentiometer and OLED to allow the user to set the desired amount of fluid dispensed, and a foot pedal to control when the fluid is released. The goal of this study was to build-on the SAID system to make it more ergonomic and test the insemination efficiency with *Xiphophorus maculatus*.

I created a new device in in AutoCAD 2025 and printed using an Ender 3-S1 PLA Filament 3D Printer. This design combines all the parts from the SAID and fits it into a 130mm device. I redisgned the holder to make it more ergonomic similar to an oversided "pen" designed (Figure 1). Within the holder there is the injector that could inject volumes of ??? microliters.

This SAID "V.02" Incorporates the same user-friendly interface and design philosophy while taking up less space in the workspace. The SAID "V.02" will be used to inseminate 12 virgin females and 10 females of unknown virginity in late fall 2024.

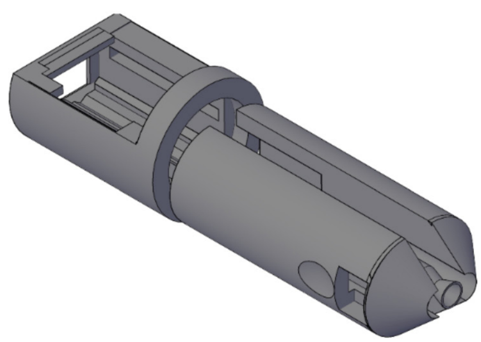

Figure 1. Standardized Artificial Insemination Device "V.02"

ASSESSING DEPURATION OF *Campylobacter spp.* FROM OYSTERS

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Floating oyster cages provide roosting opportunities for waterbirds. Roosting birds defecate and their feces may introduce human pathogens, such as Campylobacter into the oyster meat. These pathogens could have a human health impact if the oyster meat becomes contaminated and is consumed raw or undercooked, a common practice. Recently there have been foodborne outbreaks in Rhode Island (2021), Maine (2024), and Massachusetts (2024) caused by oyster meat contaminated with *Campylobacter spp*. These incidents have brought attention to the food safety risk from roosting water birds on floating gear. This is a concern for aquaculture growers, regulators, and consumers. The natural depuration of Campylobacter from oyster meats is not well known. This information would enable regulators and growers to make informed decisions regarding best management practices to reduce the risk of Campylobacter in oysters.

Oysters were artificially inoculated with clinical strains of 1) *Campylobacter jejuni*, 2) *Campylobacter lari*, 3) *Campylobacter coli,* and 4) a cocktail of the three strains. Inoculated oysters were marinated in inoculated tanks with aeration for 24 hours, then they were placed in raceways with flowing seawater (filtered, UV treated, and heated) drawn from Narragansett Bay. The oysters were sampled eight times over fifteen days. The analysis included determining the presence or absence of detectable Campylobacter, following FDA BAM methodology. We will discuss the depuration of the three clinical strains of Campylobacter by examining the detection of the viable pathogen.

DRAFT PROGRAMMATIC ENVIRONMENTAL IMPACT STATEMENT FOR AQUACULTURE OPPORTUNITY AREAS IN FEDERAL WATERS OF THE GULF OF MEXICO

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As directed by the Executive Order 13921, "Promoting American Seafood Competitiveness and Economic Growth" (May 7, 2020), the National Oceanic and Atmospheric Administration's (NOAA's) National Marine Fisheries Service (NMFS) Southeast Region is developing a draft programmatic environmental impact statement (DPEIS), in accordance with the National Environmental Policy Act (NEPA), to evaluate the potential adverse and beneficial impacts of identifying one or more Aquaculture Opportunity Areas (AOAs) in U.S. federal waters of the Gulf of Mexico (Gulf) and the potential impacts associated with siting future commercial aquaculture operations in those locations. The intent of this DPEIS is to support long-term planning for offshore aquaculture in the Gulf. The identification of AOAs does not support a specific regulatory or permitting action and does not authorize or permit any specific aquaculture-related activities or individual aquaculture operations.

This talk will provide an update on the progress that has been made to identify Aquaculture Opportunity Areas in the Gulf of Mexico, including the publication of the DPEIS and the next steps in the process.

The public can view the PEIS and related comments at https://www.regulations.gov/docket/NOAA-NMFS-2024-0135

PARASITISM CORRELATED WITH HIGHER SUSCEPTIBILITY TO REPEATED LOW-DOSE COPPER SULFATE TREATMENTS IN MARSH RAMSHORN SNAIL *Planorbella trivolvis*

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Mitigating *Bolbophorus damnificus* outbreaks in catfish aquaculture primarily consists of disrupting the parasite life cycle by targeting the snail intermediate hosts. Copper sulfate pentahydrate (CSP) is a common and effective molluscicide but can have adverse effects on pond and fish health. Previous studies have shown wild-caught snails to be more susceptible to CSP than lab-reared snails. This may be an indication of poor snail vigor from handling and transport, environmental stressors, or trematode parasitism. In this study, CSP toxicity was assessed in laboratory-reared Marsh Ramshorn snails *Planorbella trivolvis* and wild-caught individuals actively shedding trematode cercariae. Given the low prevalence of *B. damnificus* in snail populations (typically 1 - 5%), snails parasitized with a more common trematode, *Alloglossidium kenti*, served as a proxy for the effects of trematode parasitism on *P. trivolvis* exposed to CSP. Two trials were conducted, consisting of 4 weekly treatments of varying CSP concentrations. In both trials, survival curves and hazard analysis revealed that laboratory-reared snails were most resistant to CSP, followed by wild-caught non-shedding pond snails. Wild-caught snails actively shedding *A. kenti* cercariae were most sensitive to CSP treatment. Increased susceptibility of parasitized snails to lower CSP doses demonstrates a potential targeted approach selective against parasitized snails within catfish pond systems. Therefore, complete eradication of *P. trivolvis* from catfish ponds may not be necessary for effective trematode control, and lower doses than those previously reported may prove effective at mitigating trematode-associated losses in US catfish aquaculture.

COMMUNITY ACTION FOR FRESH WATER (CAFW): A ROTARY INTERNATIONAL (RI) AND UNITED NATIONS ENVIRONMENT PROGRAMME (UNEP) INITIATIVE TO RESTORE, PROTECT, AND SUSTAIN FRESHWATER ECOSYSTEMS

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Healthy freshwater systems are vital for sustaining all life, they are vital for flourishing and vibrant societies, but these systems are severely threatened by pollution, nature and biodiversity loss, and climate change.

The Community Action for Fresh Water (CAFW) is the flagship initiative of the strategic partnership between Rotary International (RI) and the United Nations Environment Program (UNEP). Announced in January 2024 (https://www.rotary.org/en/rotary-unep-partnership), the initiative focuses on restoring, protecting, and monitoring freshwater ecosystems globally in response to escalating challenges such as pollution, climate change, and habitat degradation. Through this initiative, Rotary and Rotaract members work closely with their communities to protect, restore, and sustain freshwater ecosystems in alignment with the environment area of focus.

The CAFW initiative incorporates, learns from, and expands upon the success of Rotary members who have been protecting and restoring freshwater ecosystems under the pilot program "Adopt a River for Sustainable Development" which began with UNEP and Rotary District 9212 (Eritrea, Ethiopia, Kenya, and South Sudan) in 2020 and has expanded worldwide. The partnership connects Rotary's member resources and expertise mobilizing volunteers with the technical expertise from UNEP to make even more of a sustainable, long-term environmental impact. UNEP is the leading organization within the United Nations system in the field of the environment with the global mandate for the conservation, protection, enhancement, and support of nature and natural resources, including biological diversity.

Projects can focus on improving water quality, mitigating pollution, addressing water scarcity, removing invasive species, and conserving and rehabilitating ecosystems. Your plan can include activities that need different levels of expertise and engagement. Projects can start at any size, scope, and frequency. After you've developed a plan, register your project with 'Community Action for Fresh Water' or reach out to cafw@rotary.org with any questions.

EFFECTS OF WATER PH BUFFERING ON *Crassostrea virginica* SPAT

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Ocean acidification has led to a decrease in seawater pH, which decreases aragonite saturation levels. Aragonite is the crystal form of calcium carbonate that shellfish use to build their shell especially within the larval stages. Seawater pH buffering using sodium carbonate or soda ash (Na_2CO_3) has commonly been used by shellfish hatcheries across the United States to increase aragonite saturation levels to improve larval growth and survival. This has been especially important for Pacific oysters (*Crassostrea gigas*), as they are more sensitive to aragonite levels, but is also used for Eastern Oysters (*Crassostrea virginica*). The effects of water pH buffering during the larval stages on long-term oyster health (e.g., oyster spat survival and growth) are understudied.

To investigate these impacts *Crassostrea virginica* larvae were raised to the pediveliger stage with and without soda ash buffering where 0.031g/L of Na_2CO_3 was added. Once the larvae reach the pediveliger stage they were set onto oyster shell to mimic oyster restoration reefs. The shells were outplanted in the Choptank River off the pier at the University of Maryland Center for Environmental Science Horn Point Oyster Hatchery in cages. Spat was measured for survival and size every three weeks with a total of 5 counts completed so far. This experiment was conducted at the UMCES Horn Point Oyster Hatchery starting in July 2024 and is still ongoing.

Preliminary results suggest that larvae treated with soda ash have decreased growth and slightly lower survival rate compared with the larvae now treated with soda ash (Fig. 1). Results will inform aquaculture and shellfish restoration success as the impacts of ocean acidification increase with climate change.

	Control	Experimental
pH	8.00	8.48
Salinity (ppt)	11.2	11.2
Temperature (°C)	25.9	25.9
Ω Ar	0.62	1.69

TABLE 1. Average control and experimental larval water quality conditions.

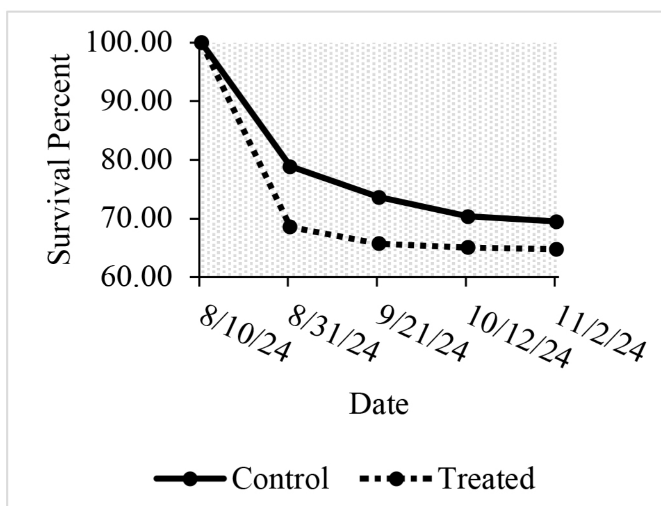

FIGURE 1. Preliminary spat survival percent over time.

STERILITY AS A SOLUTION: REDUCING ECOLOGICAL RISKS AND IMPROVING FARM MANAGEMENT

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Aquaculture is the fastest-growing food production sector globally, making the adoption of sustainable practices essential for both economic and environmental well-being. A major challenge to continued growth in this area is minimizing ecological impacts while maximizing production, particularly the risks associated with farmed fish, such as genetic introgression and ecological imbalances caused by escapes. One effective solution is the use of technologies to produce reproductively sterile fish.

Sterility ensures that farmed fish cannot reproduce, preventing accidental breeding or overpopulation, which can strain resources and space on a farm. It also reduces the risk of escapes by ensuring that accidental introductions cannot reproduce in the wild, thus protecting natural ecosystems. Additionally, sterility can be used to address regulatory inefficiencies and instill confidence in permitting and environmental review of aquaculture both onshore and at sea.

Sterile fish also offer significant benefits for farm management and productivity. By redirecting energy and nutrients that would typically go into reproductive development toward somatic growth, sterility can improve fillet quality or yield and overall farm productivity. Farms can more easily maintain and control desirable genetic traits without the risks of unplanned crossbreeding or genetic drift, ensuring consistent and high-quality product output. Moreover, sterility helps reduce the risk of disease transmission through breeding, enabling farms to maintain a healthier and more biosecure environment by preventing the introduction of new genetic material or pathogens through broodstock. It also simplifies farm management by eliminating the need for special husbandry practices to prevent maturation during the grow-out cycle.

Traditional methods like triploidy and interspecific hybridization are sometimes used to achieve sterility, but newer approaches aim for 100% sterility with fewer negative effects on fish performance. Advances in molecular and genetic technologies, such as gene silencing and genome editing, offer promising non-transgenic methods to induce sterility in some candidate species. These innovations, including immersion-based sterilization techniques, present new opportunities to enhance biosecurity and sustainability in aquaculture while protecting intellectual property and proprietary genetic resources.

A FOCUS ON THE FUTURE: PARTICIPATORY FOCUS GROUPS FOR ENVISIONING THE FUTURE OF OYSTER AQUACULTURE AND OUR COASTS

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Coastal communities in Maine, USA are facing rapidly changing social and ecological conditions that present unique stewardship challenges. A notable change across the coasts of Maine is the expansion of the aquaculture industry. The growth of oyster aquaculture, in particular, has highlighted the multiple values at play within coastal communities and the often-diverging visions concerning which human activities, marine industries, or ecosystem services should be prioritized. This leads to the question: *How can we identify diverse values and visions for the future, and subsequently best integrate this information into planning and action for our coasts?*

Participatory research approaches, like focus groups and interviews to document local knowledge and place-based visions, can support the ecosystem-based stewardship that is needed to address these challenges in coastal communities. We report on the results of a focus group analysis in the Damariscotta River estuary in Maine, USA. The Damariscotta is on the leading edge of many ecological, economic, and social changes that many Maine coastal communities are already experiencing or will experience in the future, including coastal development, the emergence of new species because of aquaculture – like wild oysters (*Crassostrea virginica*) – and conflict among co-occurring human activities.

Studying the visions for the future of the Damariscotta reveals that coastal stakeholders share foundational values that can be touch points for the bridging of differences – for example, shared views of the importance of water quality and the cultural and economic significance of the oyster. However, results also indicate that stakeholders envision diverse – and sometimes conflicting – scenarios for the future and identify phenomena that require further inquiry (Fig. 1). Importantly, our study highlights the value of seeking place-based perspectives of how changes are impacting coastal communities. Engaging community collaborators early and often through participatory research approaches can help elucidate the complex interactions driving change in our coasts and inform visioning and planning for thriving coastal communities in a future transformed by climate change.

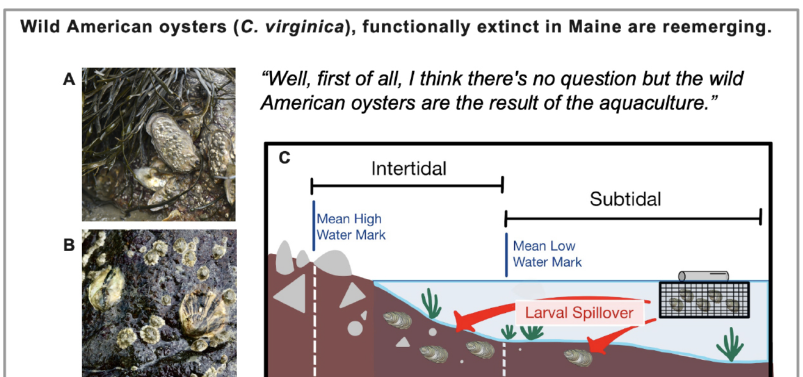

Figure 1: Local knowledge hypotheses and observations documented through local mapping and focus groups (quote above) highlighted the need for exploration into the linkages among emerging wild oysters (A. adult oysters, B. settled spat) and larval spillover from oyster aquaculture (C).

ASSESSING GLOBAL CLIMATE CHANGE EFFECTS IN CRAYFISH: DOES LOWERED PH INTERFERE WITH THE ABILITY OF *Faxonius Immunis* TO REPRODUCE

Rebecca Rivera*, Angel Colón-Zapata, Dennis McIntosh, Ph.D, Gulnihal Ozbay, Ph.D. and Grant Blank

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With global climate change occurring at a more rapid pace than ever, it is important to assess how those environmental changes affect known sentinel species. Crayfish, although abundant in overall species numbers in North America (300+), may be considered a sentinel species because they are often found in niche habitats where slight changes have large consequences. As an adult, crayfish must first molt into a reproductive state to mate and reproduce. Once reproduction is complete, they will again molt out of this reproductive state but are still considered adults. Because pH has previously been shown to affect molting in crayfish, we chose to evaluate the effects of lowered pH on adult female and male *Faxonius immunis* molting in and out of their reproductive state (♂= Form 1/Form 2: ♀= Mature/Immature). Once female and male *F. immunis* reach adult size we will use a Delaware State University (DSU) aquaculture rack system to house four separate crayfish groups at four different pH levels. Females and males will be stocked randomly across systems with pH values: 4.5; 5.0, 6.0 and 7.0 (control). Observations and data collected will include reproductive form, molt frequency, and any molt malformations. It is expected that with long-term exposure to lower pH, both adult female and male *F. immunis* will not molt into their reproductive form. It is also expected that *F. immunis* held at a neutral (7.0) pH will molt into their respective reproductive form. Additionally, water quality will be monitored from the experimental system daily (O^2, ^oC, pH) and analyzed weekly (NH_3-N, NO_2-N, calcium hardness).

Acknowledgements:
We acknowledge USDA NIFA CBG Award# 2023-38821-40369 “Providing Experiential Learning Opportunities and Extension Training in Aquaculture and Fisheries to Under-Served and Underrepresented Student Communities From 1890 Land Grant Institutions” for funding my internship and this project as part of the student experiential learning goal. We further acknowledge the Project Director, Dr. Gulnihal Ozbay for providing this internship opportunity and Dr. Dennis McIntosh for providing the space and resources at the DSU Aquaculture Research and Demonstration Facility to complete this project.

COMPARATIVE PERFORMANCE ANALYSIS OF WATER QUALITY TESTING DEVICES FOR AQUACULTURE IN MOUNT HOPE BAY, RHODE ISLAND

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Water quality testing provides essential data on chemical parameters critical for aquaculture, such as ammonia, nitrate, dissolved oxygen, and pH. Depth-specific samples yield different profiles, necessitating reliable devices for precise analysis. While many tools are beneficial, some stand out for their functionality and convenience. This study compares the DR1900 Portable Spectrophotometer and the Lamotte Water Link Spin Touch to determine the most accurate, cost- effective, and time-efficient device for aquaculture water quality monitoring.

This six-month study involved parallel testing of both devices on water samples collected from Mount Hope Bay (41.65092° N, 71.25615° W), evaluating key water quality parameters, including pH, dissolved oxygen, total ammonia nitrogen (TAN), nitrite, nitrate, and phosphate.

The DR1900's manual processes allow for more precise readings on complex parameters, such as nitrate and phosphate, providing greater confidence in its results. In contrast, the Lamotte Water Link Spin Touch's automated operation simplifies usage for individuals with limited experience in water quality testing, making it user-friendly and efficient with minimal effort. However, the Lamotte device's accuracy tends to fluctuate, particularly for complex readings compared to simpler parameters like pH and dissolved oxygen.

We hypothesize that the DR1900 will provide higher accuracy, particularly for complex parameters, while the Lamotte device will offer greater ease of use and time efficiency for routine aquaculture applications. These findings will guide aquaculture practitioners in selecting water quality testing tools that best meet their operational goals, balancing accuracy, cost, and ease of use for effective long-term monitoring. For efficient, user-friendly, and cost-effective testing, the Lamotte Water Link Spin Touch is ideal, while the HACH DR1900 is preferred for high-precision analysis, especially with complex parameters, despite its higher manual workload.

AQUACULTURE IN SHARED WATERS: 10-YEAR IMPACTS OF AN ENTREPRENEURIAL TRAINING PROGRAM

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Aquaculture in Shared Waters (AQSW) is a community-based aquaculture training program which offers trainings for fishermen and sea farmers based in Maine. Over the past decade, AQSW programs have trained over five hundred commercial fishermen, working waterfront professionals, and aquaculture entrepreneurs how to responsibly start and improve shellfish and seaweed businesses. Designed and taught by leading industry, regulatory, and scientific experts, AQSW programs cover technical, need-to-know topics for aquaculturists and offer hands-on opportunities, business planning, one-on-one mentoring, and access to financing to provide participants long-term support in building sustainable careers and farms.

Developments in this training program have paralleled the growth of aquaculture in Maine and across the U.S.–integrating the most current research and technology into the curriculum, expanding formats to serve new audiences, and building responsive programming that addresses the emerging needs and priorities of the industry. This work is supported by ten years of integrated social science research which provides insights into participants' motivations for seeking aquaculture training and evaluates program effectiveness to continually improve the learning experience for participants. Stewarded by several partner organizations AQSW has earned national recognition as a leading aquaculture training model.

In this presentation, we'll dive into the development and history of this long-standing program; chronicle its impacts; and discuss trends, changes, and lessons learned over the past decade. These insights will inform ongoing and future aquaculture training programs and reflect current needs in aquaculture workforce development.

MAINE'S SEAWEED SECTOR: INSIGHTS FOR SEAWEED FARMING IN THE U.S.

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Within the past decade, Maine has emerged as a leader in U.S. seaweed production, with a growing number of commercial farms, significant developments in supply chain and markets, and increases in landings, investments, and infrastructure. In 2010, Maine had a single kelp farm and an undeveloped supply chain, serving local markets and conducting preliminary investigations into value-added processing and products. Now, Maine has over 40 active commercial kelp farms, annual landings totaling over one million pounds, diverse and expanding processing operations, and a viable supply chain. This growth in Maine's seaweed sector has been driven by a number of factors–primary among them are a well-established maritime workforce, production efficiencies, value-added processors who contract directly with farmers, and investments in research, development, and extension. However, continued development of production systems, processing infrastructure and technology, and market expansion remain critical to advancing the seaweed sector in Maine.

In this presentation, we'll take an in-depth look at the status of Maine's seaweed sector across the supply chain–from nurseries, to value-added processors, and markets. Informed by interviews and input from Maine kelp farmers, processors, researchers, and decision makers, this work captures how the industry has evolved in Maine over the past decade and what priorities are on the horizon. These practical insights provide baseline knowledge to inform developing seaweed sectors in other states and reflect emerging priorities for seaweed aquaculture in the U.S.

LOUISIANA SEA GRANT OYSTER RESEARCH LAB AND OYSTER FARM IN GRAND ISLE, LOUISIANA

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Louisiana Sea Grant (LASG) has operated an oyster research laboratory on Grand Isle for 31 years. The research lab was originally started by LASG bivalve specialist, John Supan, in 1993. Since then, the research facility has had several foundations due to natural disasters, Hurricane Katrina (2005) and Hurricane Gustav (2008). In response to the Deepwater Horizon Oil Spill in 2010, the Louisiana Department of Wildlife and Fisheries (LDWF) built a new hatchery building, the Michael C. Voisin Oyster Hatchery (MCVOH; est. 2015). In support of that effort, LSG built a residential camp, dock, and a research oyster farm. Today, LASG is contracted by LDWF to operate the MCVOH in conjunction with LASG oyster research, extension, and education and outreach activities.

The LASG oyster farm is a 0.5-acre lease that sits adjacent to the MCVOH. The farm is currently comprised of 8 adjustable longlines (capacity: 432 hanging bags) and two, new floating cage lines (capacity: 30 floating cages). The front half of the farm is loaned out to visiting researchers. The back half of the farm and the floating cage lines are used to store hatchery broodstock and conduct internal and collaborative research projects. The farm houses broodstock from different bays across the state (Calcasieu Lake, Hackberry Bay (Barataria Bay), Vermillion Bay, and Sister Lake). In 2024, a real-time monitoring station (In-Situ Aqua Troll 800) was installed. The monitoring station collects data every 15-minutes. Parameters being measured include water temperature, salinity, dissolved oxygen, chlorophyll, pH, conductivity, turbidity, and blue-green algae.

Today, the LASG research farm is central to our research, extension, and education and outreach activities (Figure 1). LASG maintains an Alternative Oyster Culture (AOC; off-bottom culture) Extension Program to help assist new farmers entering the off-bottom industry. Future goals for our research farm moving forward includes the development of a workforce training program and an increase in research projects.

Figure 1: Participants of a LASG Teacher Workshop access the farm for a hands-on activity.

WALLEYE *Sander vitreus* REARING AT THE AQUACULTURE RESEARCH LAB AT PURDUE UNIVERSITY

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In 2020, the Illinois-Indiana Sea Grant (IL-IN SG) program formed a Walleye working group and held a workshop to determine the marketing and production barriers to a walleye industry. On the production side, the most pressing barriers were access to feed trained fingerlings and a reliable domesticated source of walleye brood stock. In years 2020-22 at the Aquaculture Research Lab (ARL), domesticated walleye held in ponds under natural conditions were spawned indoors and the resulting eggs incubated. Hatched fry was stocked into prepared earthen ponds feeding of planktonic organisms for approximately one month and harvested at approximately one inch in size. Fingerlings were stocked in a three-tank recirculating system and feed trained using Otohime® feeds before switching over to a commercial walleye diet. Fingerlings are generally sold at 6 months of age in the fall with a portion held back for future brood stock.

Procedures for the above production generally followed the protocols outlined the Walleye Culture Manual from the North Central Regional Aquaculture Center (NCRAC). Production of walleye fingerlings has been either pond based as described above or can be done wholly indoors using methods described in the University of Stevens Point Northern Aquaculture Demonstration Facility (UWSP-NADF) Walleye Culture Guide. In 2023, IL-IN SG funded research to determine if pond harvested fingerlings could be feed trained in pond-side tanks circulating pond water to remove waste products. Although survival was not as high (0-63%) when compared with indoor RAS systems (85-90%), there is significant saving on infrastructure costs.

In 2024, using eggs obtained from Genoa National Fish Hatchery and Minnesota Muskie, fry was stocked in ponds and two pond-side tanks. Pond-side tanks were reared under protocols similar to NADF with the exception that the turbidity was provided by natural pond water. Feeding commences one day post stocking with Otohime. Survival was relatively low at 10 and 37%. Progeny from this year class is to provide future brood stock the Blue Waters farm in Minnesota.

CURRENT KNOWLEDGE AND METHODS OF STRAIN IDENTIFICATION OF WALLEYE *Sander vitreus*

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There are two genetically distinct strains of Walleye (*Sander vitreus*) native to Kentucky watersheds: The Northern, or Lake Erie strain, and the Eastern Highlands strain. A genetically distinct subset of the Eastern Highland strain is known as Rockcastle River strain. Previously, stocking efforts in Kentucky caused Lake Erie strain to enter the Eastern Highland strain's native range, and their F_1 hybrids. Walleye is an extremely popular sportfish in Kentucky, as well as an important predator within Kentucky waterway ecosystems. It is critical that Walleye populations be efficiently managed for the health of Kentucky's watersheds and its recreational fishing industry. The goal of this work was to identify and compare different methods of strain identification for Walleye, so that stocking can better match the intended genetics of a population. The objectives were to: (i) review literature on molecular genetics and current knowledge for identification of Walleye strains, and (ii) discuss future work building on advancements in molecular genetics for effective management of native Kentucky Walleye populations. Microsatellite analysis has been the preferred method for identifying Walleye strains, but single-nucleotide polymorphism (SNP) assay was recently developed and used in strain identification (2023, 2024) that reduced the time needed to identify strain and that reported improved accuracy at identifying the two strains, and their F_1 and F_2 hybrids. The SNP method of strain identification may be at the forefront of genetic identification, but further studies are needed to evaluate capabilities and cost of microsatellite and SNP identification methods. Currently, broodstock used by Kentucky fish and wildlife were bred from Rockcastle River strain. A quick, cost-effective, reliable way to differentiate Lake Erie, Eastern Highlands, and Rockcastle River walleye strains would contribute to assessing and to better understanding of the Walleye populations in Kentucky, and increased efficiency in stocking and management efforts.

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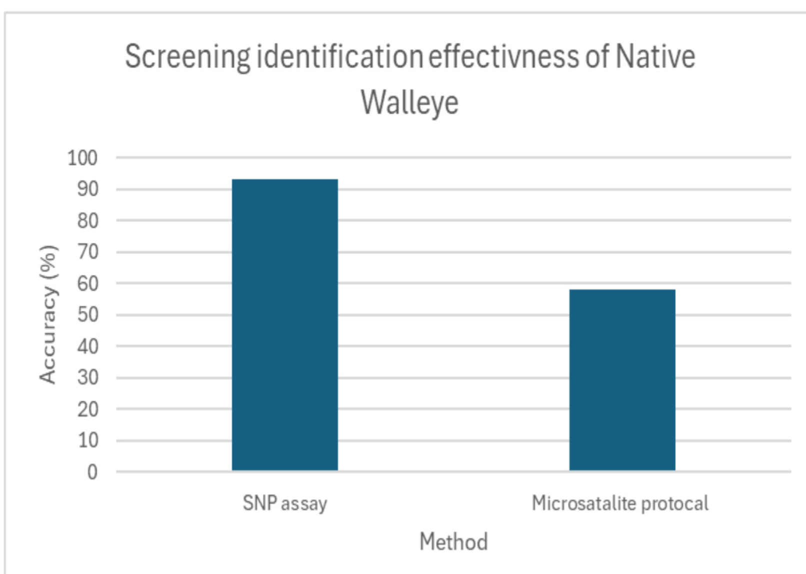

Figure 1: Differences in screening identification effectiveness of native walleye found in Johnson et. al, 2024

ADVANCES IN TRANSPOSABLE ELEMENT ANNOTATION FOR GENOME ANALYSIS IN AQUACULTURE SPECIES OF INTEREST

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Transposable elements (TEs) exert an increasingly diverse spectrum of influences on eukaryotic genome structure, function, and evolution. A deluge of genomic, transcriptomic, and proteomic data provides the foundation for turning any non-model eukaryotic species into an emerging model to study all aspects of organismal biology, ultimately shaping future directions for biomedical, environmental, and biodiversity research. However, in contrast to the progress in gene annotation achieved and standardized over the past decade, identification and annotation of the mobile genome component still need to catch up to the standards accepted for host gene annotation. New genome sequences and comprehensive transcriptome data can provide valuable resources for studying shellfish biology and improvement in essential aquaculture species.

While culturing new species in shellfish aquaculture, further understanding the role of epigenetic change within organism-environment interactions is necessary. When dissecting the epigenetic landscapes in novel genomes, a comprehensive description of its mobilome component must be provided in addition to the standard genic and transcriptomic datasets. Each step of TE identification, classification, and annotation should be focused on improving TE boundary designation, reducing identification error rates, and providing accurate information on the type and integrity of TE insertions. However, detecting and identifying TEs in newly sequenced species is still challenging and time-consuming, and different workflows have been proposed. I summarize some workflows and analysis pipelines for generating TE models in de novo assemblies for non-model organisms and, overall, improving their epigenetic landscape analysis.

DENGUE VECTORS CO-INFECTING SHRIMP VIRUSES: EPIDEMIOLOGICAL CONTROL CHALLENGES IN THE CONTEXT OF CLIMATE CHANGE AND CONTAMINATION BY INSECTICIDES AND PESTICIDES USED FOR COMBATING INSECTS SUCH AS GLYPHOSATE-BASED HERBICIDES

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Arboviruses are an important group of pathogens that cause diseases of medical and veterinary concern worldwide[1-14]. Dengue, an important mosquito-borne virus transmitted mainly by *Aedes aegypti,* is a major public health issue in Latin America and the Caribbean.[2-4] In Puerto Rico, the number of confirmed positive cases of Dengue in 2024 is 4,798.[2] In Ecuador, 50,408 confirmed cases of Dengue were reported in 2024.[3] The interactions of arboviruses with their host cells are complex, and frequently, the coexistence of two different viruses in the same cell results in the inhibition of replication in one of the viruses, which is a phenomenon called viral interference and can be exploited to develop antiviral strategies. The mechanisms responsible for viral interference have not been fully elucidated, but small RNAs could be involved.[1,5-13]

Persistent viral co-infections in arthropods have been studied by many researchers.[1-14] Kanthong et al. (2008)[7] produced stable, persistently infected C6/36 mosquito cell cultures by serial passage of exponentially growing whole cells infected with either a densovirus (AalDNV) or Dengue virus (DEN-2). Persistently infected cultures did not differ from naïve-cell cultures in growth rate and cell morphology. Immunocytochemistry revealed that 99-100% of the cells were coinfected and super-infection order had some effect on antigen distribution for the two viruses. Their results suggested that the capacity to support stable, viral co-infections may be a general phenomenon for arthropod cells, and such persistent infections would facilitate studies on interactions between co-infecting viruses. They also reported persistent, triple-virus co-infections in mosquito cells.

Anti-viral immunity in crustaceans and insects has been studied since 2004 at the laboratory of Dr. Tim Flegel in Thailand[5-9] His group reported that mosquito cells accommodate balanced, persistent co-infections with a densovirus and Dengue. He later proposed the hypothesis for heritable, anti-viral immunity in crustaceans and insects. [9,10] In Penaeid shrimp, the densovirus Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) causes IHHN and runt deformity syndrome and mortalities in *Penaeus stylirostris* but not in *P. vannamei.* Co-infection of IHHNV with White Spot Syndrome Virus (WSSV) showed the typical clinical symptoms of WSSV infection, but co-infected shrimps did not have any other external deformities.[11] Association of dual viral infection with mortality of *P. vannamei* farmed in India [12,13] and in Ecuador and Peru [14] have been reported. We present results of an in-depth review of scientific literature on molecular, genetic, and epigenetic mechanisms involved in densovirus-Dengue interactions in shrimp and address potential challenges for epidemiological control in the context of climate change and contamination by insecticides and pesticides used for combating pests and insects in crops of economic interest such as glyphosate-based herbicides.[15]

(Continued on next page)

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EFFECT OF STORAGE TIME AND TEMPERATURE ON THE MICROBIOLOGICAL QUALITY AND TEXTURAL PROPERTIES OF CHAME (*Dormitator latifrons*) MEAT: A COMPREHENSIVE STUDY ON STABILITY AND FOOD SAFETY

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Dormitator latifrons (chame) is a native fish of high nutritional value, particularly important along Ecuador's coast, especially in Manabí. However, its rapid spoilage creates challenges in preserving its microbiological safety and texture during storage. This study examines the effects of storage time and temperature (refrigeration and freezing) on the fish's quality. The goal is to propose improved preservation methods to enhance its food safety and texture.

A completely randomized 2x3 factorial design (CRD) was used to evaluate two temperature conditions (refrigeration at 3°C ±1 and freezing at -11°C ±1) and three storage times (24, 48, and 72 hours), resulting in six treatments (T1-T6). Additionally, a control treatment involved storage at 10-18°C for 24 hours. Male *D. latifrons* specimens (360-400 g) were washed, eviscerated, vacuum-packed, and stored according to these treatments. Microbial counts (mesophilic, *Escherichia coli*, *Staphylococcus aureus*) followed NTE INEN 1896:2013 standards. Texture profile analysis (TPA) assessed hardness, elasticity, and cohesiveness. Data were analyzed using ANOVA and Kruskal-Wallis tests ($p \leq 0.05$) with IBM SPSS Statistics 26 software.

Results on *Microbiological Stability* showed that freezing treatment at -11°C for 72 hours (T6) presented the best results in terms of microbiological stability, with a mesophilic aerobic bacterial load of 2.54 In (13 CFU/g), absence of *E. coli*, and a *S. aureus* load of 4.75 In (117 CFU/g), all within the limits established by the NTE INEN 1896:2013 standard. These results demonstrate that prolonged freezing is effective in preserving the safety of chame.

Data on *Textural Properties* showed that T6 treatment also showed the best preservation of texture, although a slight denaturation of proteins was observed. The values for hardness (1241 ± 137 N), elasticity (14.44 ± 0.29 mm), and cohesiveness (0.75 ± 0.16) were the most stable. However, some stiffness due to freezing was observed, slightly affecting the sensory quality of the meat.

Comparison of Treatments revealed that the refrigeration treatments at 3°C showed greater microbial proliferation and a faster degradation of texture. Shorter storage times, such as 24 hours, were insufficient to control microbial growth in meat stored under refrigeration.

THE MANGROVE EPIGENOME (MangroveENCODE) PROJECT OF THE FUCOBI FOUNDATION OF ECUADOR: A ONE HEALTH APPROACH TO CONSERVING MANGROVES BIODIVERSITY AND AVOID CLIMATE DISASTER

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Mangrove forests are natural laboratories for studying epigenetic and climate change. [1-4] Mangroves are salt-tolerant plant species that grow in coastal saline water and are adapted to harsh environmental conditions such as high ultraviolet light, low nutrition, and fluctuating salinity in coastal zones. They are highly productive and biologically diverse wetlands that serve as nurseries and habitats to many juvenile fishes, mollusks, and crustaceans like shrimps. Some mangroves began disappearing due to ozone depletion, freshwater diversion, ocean acidification, atmospheric aerosol pollution, and the introduction of exotic chemicals and modified organisms in the shrimp farms nearby mangrove habitats. Mangroves sequester large quantities of carbon that become significant sources of greenhouse gases when disturbed through land-use change. They also support diverse species of microorganisms such as fungi, and bacteria associated with biogeochemical transformations of nutrients. They trap sediments and assimilate nutrients along with associated sediment contaminants such as antibiotics and endocrine disrupting chemicals (EDCs) like metals, PCBs, PAHs, bisphenols, and glyphosate-based herbicides, among others. Mangrove species are known to have very low genetic diversity caused by their stressful living conditions, suggesting that epigenetic variation is likely a vital source for them to respond to environmental changes. The capacity to respond to environmental challenges ultimately relies on phenotypic variation which manifests from complex interactions of genetic and nongenetic mechanisms through development. While we know something about genetic variation and structure of many species of conservation importance, we know very little about the nongenetic contributions to variation. [1-4]

Epigenetic modifications, such as cytosine methylation, are inherited in plant species and may occur in response to biotic or abiotic stress, affecting gene expression without changing genome sequence. The long-term goal of the MangroveENCODE project of the FUCOBI Foundation of Ecuador is to study the epigenetic mechanisms associated with the interactions of CO2 uptake, EDCs in sentinel species (shellfish), and microbial communities considering environmental degradation-related health issues. The plan is to obtain baseline information for future studies to test mechanism-driven hypotheses to examine the interactions of CO2, EDCs, and microbial diversity using computational ecology tools. The short-term goals include characterization of the microbiome (bacterial communities), CO2 uptake, and EDC concentrations in mangrove sediment and shrimp. This mini review provides an overview of available studies on epigenetic regulation and adaptation of mangroves and summarizes (a) the best technologies to assess the microbiome and CO2 stocks from >1-meter-deep mangrove sediment, and (b) the genome sizes, microbiomes, and transposable elements of mangroves. For example, availability of the genome assembly and in natura epigenome analyses of *Bruguiera gymnorhiza*, one of the dominant mangrove species, allowed genome-guided transcriptome assembly for mangrove species. [1-2] The 309-Mb of the genome, predicted to encode 34,403 genes, has a repeat content of 48%. Depending on its growing environment, the natural *B. gymnorhiza* population showed drastic morphological changes associated with expression changes in thousands of genes. Moreover, high-salinity environments induced genome-wide DNA hypermethylation of transposable elements (TEs) in the *B. gymnorhiza.* DNA hypermethylation was concurrent with the transcriptional regulation of chromatin modifier genes, suggesting robust epigenome regulation of TEs in the *B. gymnorhiza* genome under high-salinity environments. The genome and epigenome data provided novel insights into the epigenome regulation of mangroves and a better understanding of the adaptation of plants to fluctuating, harsh natural environments.

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Rhizophora mangle is a foundation species that occurs in coastal estuarine habitats throughout the neotropics where it provides critical ecosystem functions and is potentially threatened by anthropogenic environmental changes. [3] Researchers studied (a) the levels of genetic and epigenetic diversity in natural populations of *R. mangle*, (b) how genetic and epigenetic variation are structured within and among populations, and (c) how faithfully epigenetic variation is inherited. They found low genetic diversity but high epigenetic diversity from natural populations of maternal plants in the field and epigenetic differences among offspring grown in common gardens were explained by maternal family. It shows epigenetic variation could be an important source of response to challenging environments in populations of this foundation species.

Laguncularia racemosa occurs in naturally contrasting habitats where it is subjected daily to salinity and nutrient variations leading to morphological differences. [4] Researchers unraveled how CpG-methylation variation is distributed among individuals from two nearby habitats, at a riverside (RS) or near a salt marsh (SM), with different environmental pressures and how this variation is correlated with the observed morphological variation. Significant differences were observed in morphological traits such as tree height, tree diameter, leaf width and leaf area between plants from RS and SM locations, resulting in smaller plants and smaller leaf size in SM plants. Genetic and epigenetic (CpG-methylation) variation in genomes from these populations revealed that SM plants were hypomethylated (14.6% of loci had methylated samples) in comparison to RS (32.1% of loci had methylated samples). Within-population diversity was significantly greater for epigenetic than genetic data in both locations, but SM also had less epigenetic diversity than RS, and significantly greater differentiation among locations for epigenetic than genetic data. Individuals with similar genetic profiles presented divergent epigenetic profiles that were characteristic of the population in a particular environment, suggesting that CpG-methylation changes may be associated with environmental heterogeneity, suggesting epigenetic variation in natural populations play an important role in helping individuals to cope with different environments.

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LIMITATIONS OF IMPLEMENTATION OF RNA INTERFERENCE (RNAi) IN SHRIMP AQUACULTURE

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Shrimp aquaculture is highly susceptible to viral diseases, including White Spot Syndrome Virus (WSSV) and Infectious Myonecrosis Virus (IMNV), which cause significant economic losses worldwide. RNA interference (RNAi) has emerged as a promising tool for disease control in shrimp farming, offering a targeted, environmentally friendly approach to suppress the impact of pathogens. RNAi works by introducing double-stranded RNA (dsRNA) molecules designed to silence specific pathogen genes, preventing their replication within the host. Despite its potential, the large-scale application of RNAi in shrimp aquaculture faces numerous technical and regulatory obstacles that limit its practical implementation.

Technically, RNAi in shrimp aquaculture is challenged by finding efficient and stable delivery methods ensuring RNA molecules are delivered to target cells. In addition, dsRNA delivery must ensure its integrity in aquatic environments where degradation rates are high. Various delivery methods are under investigation, including microencapsulation, yet these solutions remain in early development and are not cost-effective for widespread use. Additionally, concerns regarding the stability and efficacy of dsRNA in diverse water conditions further complicate their use in commercial aquaculture settings.

Regulatory challenges also pose substantial barriers to the adoption of RNAi in shrimp farming. Due to its genetic nature, RNAi-based treatments are subject to regulatory scrutiny in many countries, with concerns about biosafety, environmental impacts, and food safety. The potential for RNAi molecules to affect non-target species in the ecosystem has led to caution among regulatory bodies, which will lead to lengthy approval processes and the need for extensive environmental impact assessments. Furthermore, the limited regulatory framework specifically for RNAi in aquaculture will complicate compliance for producers (Fig.1). This lack of clear guidelines delays commercial development and may deter investment in RNAi technologies for shrimp aquaculture.

In conclusion, while RNAi holds promise as an innovative tool in shrimp aquaculture for disease management and increasing productivity, substantial technical and regulatory barriers must be addressed to enable its commercial implementation. Continued research, collaboration with regulatory bodies, and investment in RNAi technology will be crucial for overcoming these challenges and advancing sustainable shrimp aquaculture.

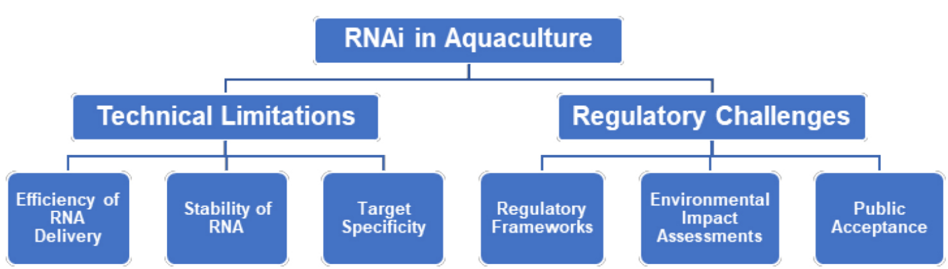

Fig. 1. Outline of the technical limitations and regulatory challenges of the implementation of RNA interference in shrimp aquaculture.

A TRANSPOSABLE ELEMENT–EPIGENETICS ONE HEALTH PERSPECTIVE TO UNDERSTAND ANTIMICROBIAL RESISTANCE (AMR) AND CONTAMINATION BY ENDOCRINE DISRUPTING CHEMICALS (GLYPHOSATE, METALS), MICROPLASTICS, BIS(2-ETHYLHEXYL) PHTHALATE (DEPH), AND PER- AND POLY-FLUOROALKYL SUBSTANCES (PFAS): ADAPTATION TO GLOBAL AND CLIMATE CHANGE

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Antibiotic-resistant pathogens are a public health concern, and better understanding of the underlying processes responsible for this continuous expansion is urgently needed. Here we discuss transposable elements (TEs) and epigenetic components in the context of ‘One Health’ to understand AMR and endocrine disrupting chemicals (EDCs) in soil, animals and people, and adaptation to global climate change via horizontal gene transfer (HGT) and horizontal transfer of transposons (HTT).

Heavy metal/biocides and antibiotic resistance genes (ARGs) co-selection has been suggested as one potential mechanism promoting the proliferation of antimicrobial resistance (AMR) in livestock farming. Metals are used as growth promoters and biocides as disinfectants with little restrictions on livestock farming. The interplay of metals and biocides was recently reported in pig farming, with pigs under continuous antibiotic exposure displaying the highest co-occurrence of ARGs and other genetic elements while the pigs under limited use of antibiotics still showed abundant co-occurrences (Li *et al*. 2022). Pathogens belonging to Enterobacteriaceae displayed increased co-occurrence phenomena, suggesting that this maintenance is not a random selection process from a mobilized pool but pertains to specific phylogenetic clades. These results suggest that metals and biocides displayed strong selective pressures on ARGs exerted by intensive farming, regardless of the current use of antibiotics.

Bis(2-ethylhexyl) phthalate (DEPH) is the most common member of the class of phthalates, which are used as plasticizers, and the most used for the widely used plastic polyvinylchloride (PVC) used in aquaculture. DEPH may increase the risk of cancer, birth defects or other reproductive harm. Exposure may harm the male reproductive system and child development during pregnancy. DEHP is also an antimicrobial species of the genus *Burkholderia*, filamentous bacteria like *Nocardia levis*, *Streptomyces sp.,* and other actinomycetes like *Saccharothrix sp.* (Bharti & Tewari, 2015). A review of the molecular mechanisms involved in TEs-epigenetics interactions associated with AMR is presented using glyphosate, metals-biocides, microplastics-phthalates, DEPH and PFAFs as examples. Urgent transgenerational epigenetic inheritance-One Health research is needed to address them and understand adaptation to global and climate change.

OPTIMIZING BAY SCALLOP GROWTH AND SURVIVAL: COMPARATIVE ANALYSIS OF GROW-OUT GEAR TYPES IN COLD SEASONS

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This study evaluates Bay Scallop (*Argopecten irradians*) performance across four grow-out gear types at an aquaculture farm. Conducted from October 2024 to February 2025 at Roger Williams University's 2-acre aquaculture farm in Mount Hope Bay (41.65092° N, 71.25615° W), the study compares four grow-out systems: bottom cages with bags (n = 6), double-stack bottom trays without bags (n = 4), floating boxed bags modified with side floats (n = 4), and floating plastic baskets modified with top floats (n = 4). The primary objectives are (1) to identify the grow-out gear that best supports scallop growth and survival in cold seasons, (2) to evaluate biofouling's impact on water flow and scallop health, and (3) to provide recommendations for sustainable aquaculture practices.

Scallops were sourced in August, sorted for uniform size in October, and initially measured for weight and length to establish baseline growth data. Monthly assessments include growth rates (weight and shell length), mortality, and Condition Index (CI), calculated as the ratio of meat to shell weight. Biofouling assessments record fouling organism type and extent monthly, with biweekly cleaning of gear to ensure optimal water flow and scallop health. Water quality is monitored hourly for temperature, light, pH, dissolved oxygen, and ammonia using Onset HOBO® sensors and a multi-parameter online analyzer.

We hypothesize that floating gear will exhibit improved growth and survival due to better water flow and less sediment exposure, while bottom-based systems may face higher biofouling and lower growth rates. We anticipate that floating systems, especially modified plastic baskets, will support higher growth and survival due to enhanced water flow and nutrient access, whereas bottom-based systems may encounter biofouling challenges affecting water quality and scallop health. These findings aim to guide aquaculture practitioners in selecting optimal gear for colder months, enhancing productivity, sustainability, and economic resilience in scallop farming.

INTRODUCING NEW LEAD SCIENTIST FOR THE WARMWATER MARINE FINFISH PROJECT AT USDA-ARS

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The USDA-ARS has recently appointed Nicholas Romano (Fish Biologist) as the new Research Leader to the National Warm Water Marine Aquaculture Unit (NWMARU), Ft. Pierce, FL. In this presentation, he will provide a brief overview of his professional background followed by some of his research visions for NWMARU with the goal of enhancing marine finfish aquaculture under Component 5: "Developing Marine Finfish Seedstocks" from the USDA-ARS strategic plan. Currently, the unit is still forming, and he will be recruiting new research scientists, technicians, as well as a postdoc to lead this research effort. The NWMARU will be working closely with faculty and staff at Harbor Branch Oceanographic Institute (HBOI) where there is a collaborative research agreement on marine finfish. The areas of research will focus on breeding/genetics, nutrition, and larviculture, and fish health. It is anticipated that by presenting some of the research directions and capacity for research-based solutions of NWMARU, this can foster open communication with stakeholders to develop ways to address their needs.

RESEARCH AND STATUS UPDATE ON THE USDA-ARS PROJECT ON WARMWATER MARINE FINFISH AT USDA-ARS

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The U.S. has tremendous capacity for meeting the domestic demand for seafood by expanding aquaculture in federal waters and land-based recirculating systems. As the largest importer of seafood products, expanding domestic production will reduce our reliance on imports and the trade deficit. This project will support the U.S. aquaculture industry by developing technologies that will ensure a steady supply of warm water marine fish seedstocks that are optimized for commercial production. To that end, the outdoor facilities used to hold broodstock as well as conduct selective genetics, marine larval culture experiments and nutritional trials have been renovated and expanded. Additionally, a Fish Health & Pathology Lab suitable for isolation, cultivation, and identification of common BSL-2 disease microorganisms in marine warm water finfish is currently in the final phase of becoming operational. Studies by the principal investigator at Harbor Branch, and other project collaborators, have been conducted that include responses and productivity of pompano at different salinities, evaluating live feed replacements, dietary approach to optimize egg quality, use of dietary probiotics/prebiotics, a transcriptome for Florida Pompano was completed based on whole-body juveniles and skeletal muscle transcripts, as well as nutritional studies to design more species-specific diets. With a newly arrived Research Leader at USDA-ARS, future research directions will be developed and team. Our focus will include selective breeding of marine fish, research towards creating sustainable species-specific diets, and ways to improve disease resistance with the goal of improving marine fish production in a sustainable manner.

BEHAVIOURAL METRICS OF INDIVIDUAL BROODSTOCK *Penaeus monodon* IN THE CONTEXT OF FEEDING

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Farming of *Penaeus monodon* continues to rely on wild-caught broodstock for seed stock production and the practice of eyestalk ablation to achieve satisfactory reproductive outcomes. However, the latter is facing increasing scrutiny due to welfare concerns. Assessing the welfare impacts of various husbandry interventions through behavioural profiling of this species may help mitigate these issues. To this end, our research group has identified specific behavioural metrics in individual broodstock *P. monodon* and refined those across several interventions, including feeding practices.

In prior work, we developed an ethogram based on known behaviours in other Penaeid species, which we further refined by identifying behaviours unique to *P. monodon*. Time budgets and diurnal activity patterns were categorised, encompassing behaviours such as walking, swimming, digging, feeding, grooming, eye beats, antennal scale flexion, forward antennal positioning, and spawning.

The experimental setup involved twelve aquaria with sand substrates, each with a custom computer module. Individual broodstock prawns (N=12) were monitored over 48 hours and fed twice daily (7:30 am and 2:30 pm) with fresh-frozen or live polychaetes at 1% body weight. Behavioural responses to different feed types were documented, including pre-feeding recognition, time to initiate feeding, quantity consumed in a single event, and feed attractivity. Notably, shrimp took longer to locate live polychaetes compared to fresh-frozen ones. In the afternoon feedings, animals detected and engaged with fresh-frozen polychaetes faster than live ones, though no such difference was observed during morning feedings.

This study offers valuable insights for future research in shrimp nutrition and feeding strategies, supporting the development of more responsible shrimp farming practices. Additional findings will be discussed in the presentation.

CIRCULARITY IN AQUACULTURE AT CSIRO, AUSTRALIA: R&D, TRENDS AND OPPORTUNITIES IN SYSTEMS AND NUTRITION

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Circular approaches in aquaculture are gaining momentum as industries strive for sustainable solutions that reduce environmental footprints and close nutrient loops. At the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, research efforts focus on advancing circularity in aquaculture production through innovations in systems and nutrition. Key areas include aquaponics, biofloc technology, and the integration of novel locally sourced and processing technologies in feed ingredients.

CSIRO's work in production systems primarily focuses on aquaponics and biofloc technology. Aquaponics research centres on efficient, close-to-market food production by leveraging aquaponics as a "bio-converter," which repurposes nutrient-rich effluents from fish farming for plant fertilisation. Since 2021, comparative trials have assessed the performance of aquaponics alongside recirculating aquaculture and hydroponic systems. Findings underscore the need for optimisation in configurations, feeding ratios, and nutrient supplementation, with the upcoming pilot-scale aquaponics facility set to support further research. The biofloc technology research, conducted through industry partnerships, centres on water reuse and nutrient recycling. Commercial-scale biofloc experiments in super-intensive shrimp farming in Asia have shown improved survival rates, productivity, and environmental sustainability, aided by advanced biosecurity, sensor-based monitoring, and data management systems.

On the nutrition front, CSIRO is exploring innovative ingredients and processes to enhance circularity. Research focuses on microbial biomass, value-added local ingredients, and food waste blends, with studies showing growth and health benefits in native fish and shrimp species. These efforts are supported by advances in processing and manufacturing technologies, including fermentation and novel formulation strategies, which improve the feasibility of replacing conventional imported ingredients.

These ongoing efforts at CSIRO underscore the importance of integrating knowledge and expertise in systems and nutrition to enhance aquaculture's circularity. This research domain aims to inform industry stakeholders and promote responsible growth in the Australian aquaculture sector while contributing to a global push for circular economy solutions. Detailed findings and insights into R&D needs will be discussed further in the presentation.

TOXIC METALS PRESENT IN BIVALVES AND FISH MOST CONSUMED IN QUITO-ECUADOR

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Foods of marine origin have been considered an excellent source of protein, vitamins, unsaturated fatty acids, and minerals. Unfortunately, the consumption of aquatic species is also related to the probability of ingesting contaminants such as toxic trace metals, which are persistent, and cause serious effects on human health, mainly in the medium and long term.

The main contaminant in fish is mercury (Hg), which in its organic form is highly neurotoxic. Benthic organisms such as bivalve mollusks can accumulate high concentrations of cadmium (Cd), lead (Pb), and chromium (Cr), which are also toxic to human health. These among other species are commonly consumed in coastal areas. Also, there is significant consumption in big cities such as the Metropolitan District of Quito and turns out to be necessary to evaluate how dangerous can they be when consumed, and how much can be safely consumed.

We analyzed Hg in croaker (*Cynoscion* spp.), snapper (*Lutjanus peru*), dolphinfish (*Coryphaena hippurus*), blue marlin (*Makaira nigricans*), and shark (*Mustelus mento*). Additionally, Cd, Pb, and Cr were determined in "concha negra" (*Anadara tuberculosa*) and "concha blanca" (*Anadara similis*), Hg was analyzed also in concha negra. The results of the studied species were used to calculate the health risk estimation indices, non-carcinogenic risk, carcinogenic risk, and recommended consumption dose (CRlim, g/week).

Specie	Metal	Children		Adults	
		Risk	CRlim	Risk	CRlim
Croaker (*Cynoscion spp.*)	Hg	No	132	No	636
Snapper (*Lutjanus peru*)	Hg	No	134	No	647
Dolphinfish (*Coryphaena hippurus*)	Hg	**Mid**	**31**	No	147
Blue marlin (*Makaira nigricans*)	Hg	**Yes**	**9**	**Yes**	**44**
Shark (*Mustelus mento*)	Hg	**Mid**	**29**	No	140
Concha negra *(Anadara tuberculosa)*	Cd	**Yes**	**25.9**	**Yes**	**125.0**
	Cr	No	>1Kg	No	>1Kg
	Pb	**Yes**	**47.1**	No	948.1
	Hg	**Yes**	**12.6**	**Yes**	**60.87**
Concha Blanca (*Anadara similis*)	Cd	**Yes**	**15.3**	**Yes**	**73.9**
	Cr	No	>1Kg	No	>1Kg
	Pb	**Yes**	**47.9**	**Mid**	**963.8**

Blue marlin, shark, and dolphinfish showed the highest Hg concentrations. The risk assessment presents no significant potential health risk for the exposed population over a lifetime for all the species analyzed, considering only one weekly intake. Based on our results, consumption of croaker and dolphinfish is recommended up to one serving per week, given the importance of essential fatty acids intake. Avoiding fish with elevated MeHg levels, such as blue marlin and shark is recommended. In the case of bivalves, the concentration of Cd and Hg exceeded the reference dose (RfD) for children and adults. In the case of Pb, the concentration values for children were above the reference values. Finally, in all cases, the frequent consumption of big portions of bivalves could imply a risk to health due to the presence of at least one contaminating trace metal.

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https://doi.org/10.3389/fenvs.2020.00134.

FISH WELFARE – A CASE STUDY: REVILING FOR THE FIRST-TIME SIDE EFFECTS OF VACCINATION IN EUROPEAN SEA BASS (*dicentrarchus labrax*) AND BARRAMUNDI (*lates calcarifer*) IN THE ISRAELI FISH FARMING

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Vaccination is an effective way to control many infectious diseases in fish. Israeli fish farming has successfully used two vaccines over the last 30 years and has no problem with the side effects of vaccination. However, after introducing new species, a new problem emerged: these fish, after vaccination, demonstrated peritoneal lesions such as granulomas. At the same time, the fish did not show retarded growth or suffering during the fattening period. This study was conducted to establish the connection between vaccination and the appearance of granulomas. Evidence drawn from this research work and comparing vaccinated and non-vaccinated fish confirms that intraperitoneal granulomas do not impact the growth, performance, or fish fillet quality at harvest.

COMMON AND NEWLY DISCOVERED PATHOGENS IN THE ISRAELI MEDITERRANEAN SEA MARICULTURE

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https://doi.org/10.46989/001c.35823

In the last few decades, farming different fish species in Israeli mariculture has gradually increased. This paper presents the results of the last 15 years of study that define the most significant fish pathogens and evaluate their seasonal or annual occurrence. During this survey, three new pathogens were detected, described, and entirely or partially characterized.

From its foundation, the Israeli fish farming industry chose intensification as its main development direction. Two factors influenced this decision: 1) the necessity of maximum land usage and 2) the shortage of water sources in Israel (Shilo and Sarig, 1989).

The results of the continuous intensification have shown a significant increase in average yield: 10-30 tons per hectare (data from the Association of Israeli Fish Breeders). Such achievement was a limiting factor for further development in conventional ponds and reservoirs. Because of this, the Israeli fish industry has invested in developing a marine culture in the Mediterranean Sea. The particular adaptation of offshore cage technology was used because of the very shallow Israeli coastline in the Mediterranean Sea.

Another advancement was establishing hatchery technology for larval production of various fish species (Gordin, 2003). The prevention and effective control of fish disease is significant for the success of Israeli marine culture.

We aimed to collect essential information about the most current health situation, study the spread and the prevalence of various agents, and evaluate their significance for developing a strategy to decrease the effects of the diseases. This report focuses on the most common pathogens (although three are reported for the first time in Israeli farms on the Mediterranean coast) in the different species examined in the Central Fish Health Laboratory in Nir David (CHFL) during a routine inspection or subsequent outbreaks. Finally, we have summarized the current situation.

Candidatus SCALINDUA, A BIOLOGICAL SOLUTION TO TREAT SALINE RECIRCULATING AQUACULTURE SYSTEM WASTEWATER

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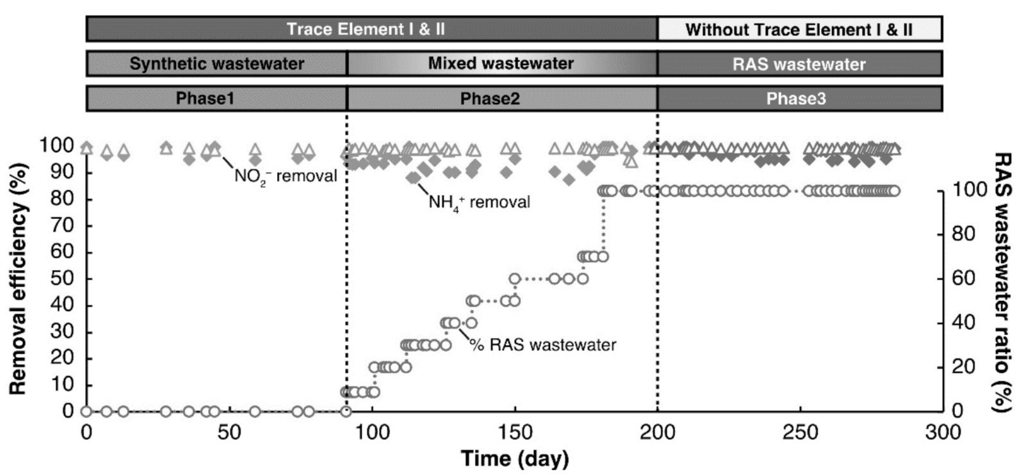
As the aquaculture sector development intensified, concerns regarding the impact caused by the high discharge of nutrients to the environment are rising. Recirculating aquaculture systems (RAS) allow high water reuse alongside a good control of the farming conditions. In RAS, ammonium (NH_4^+) is oxidized into nitrate (NO_3^-) via nitrite (NO_2^-) by nitrifying bacteria in aerobic biofilters. NO_3^- can later be removed from the system through anaerobic denitrification or by regular water exchanges. As an alternative, the anammox (anaerobic ammonia oxidation) process is a cost-effective and environment-friendly way to remove nitrogen compounds from RAS wastewater (WW), where NH_4^+ and NO_2^- are directly transformed into nitrogen gas (N_2).

We evaluated the potential of the marine anammox *Candidatus* Scalindua to clean RAS WW through a series of experiments in laboratory conditions. A sudden exposure to RAS WW, enriched in NH_4^+ (28 mg.L^{-1}) and NO_2^- (34 mg.L^{-1}), reduced its removal activity for these nitrogenous compounds, without impairing its relative abundance in the granule. To gradually expose *Ca.* Scalindua to RAS WW on the other hand resulted in a successful acclimation of the bacteria (Figure 1), even in the absence of trace element supplementation, while a slight decrease in relative abundance was observed. High NO_3^- concentrations typically encountered in RAS did not affect the removal rate of *Ca.* Scalindua for neither NH_4^+ nor NO_2^- but did reduce its relative abundance. Preliminary results suggest that *Ca.* Scalindua can maintain high removal (> 70%) for both NH_4^+ and NO_2^- rates when exposed to concentrations of NH_4^+ and NO_2^- commonly encountered in RAS (< 1 mg.L^{-1}). We conclude that *Ca.* Scalindua can be successfully used to treat marine RAS WW under laboratory conditions. Future studies need to validate this findings in a pilot RAS.

Figure 1. Anammox performance in the reactor.



VALIDATION OF THE HEMOCUE HB 801 PORTABLE HEMOGLOBIN ANALYZER FOR FISH BLOOD

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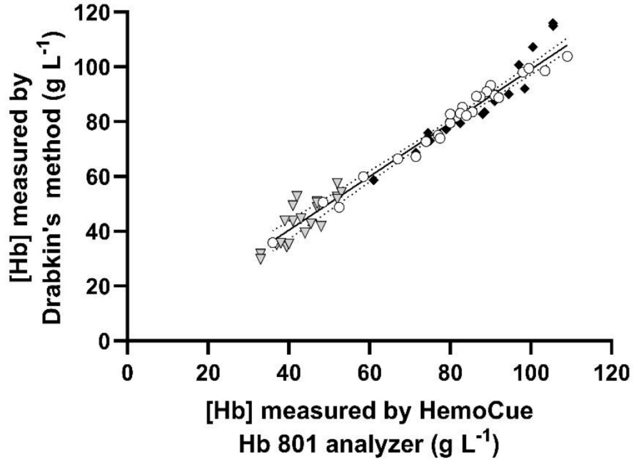
The assessment of hemoglobin levels (Hb) has become a routine measurement for the evaluation of the health and welfare status of farmed animals, including fish. The original method for measuring Hb, best known as the Drabkin method, is not well suited for work outside of a laboratory setting. It is time consuming, contains hazardous cyanide elements and requires specific laboratory material. As an alternative to the Drabkin method, portable analyzers have been developed to measure Hb in human blood. For example, in 1982, the Swedish company HemoCue developed a time-saving analyzer, the Hemocue Hb 201+, that measures human Hb within 10 minutes, using coated cuvettes. While this device was proven accurate and validated rapidly for mammalian blood, it was not until recently that this methodology was validated for fish blood. It has been shown that a correction factor is needed to account for the differences in blood composition between fish and human blood, when using this device to measure Hb in fish blood. In 2019, HemoCue launched a new portable analyzer, the HemoCue Hb 801. In comparison to the Hb 201+, this device uses uncoated cuvettes and takes less than 1 second to analyze Hb, making it even more efficient and suitable for field work.

In this study, the performance of the new HemoCue Hb 801 portable Hb analyzer was compared to the validated Drabkin method in three fish species important for the Swedish aquaculture industry (rainbow trout, *Onchorynchus mykiss*, Atlantic wolffish, *Anarhichas lupus* and Nile tilapia, *Oreochromis niloticus*).

Hb readings were not different between the two methods for any of the three species (Figure 1). Hence, we concluded that this new portable device can be measure Hb in fish blood. Unlike the previous model from HemoCue, the Hb 201+, the Hb 801 does neither need an incubation time nor a correction factor for fish blood. This represents a major gain of both time and precision, especially in settings outside of the laboratory.

Figure 1. Comparison of blood Hb measured by the Drabkin's method and the Hb 801 analyzer for rainbow trout (black diamonds), Atlantic Wolffish (grey triangles) and Nile tilapia (white circles). Curve for all fish (solid line): y = 0.9961x + 0.1012, R^2=0.971



INTEGRATING AQUAPONICS INTO SWEDISH 8^{TH} GRADE CURRICULUM: A PRACTICAL TEACHING TOOL

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Aquaponics, the combination of fish farming and plant cultivation in a symbiotic system, is a sustainable solution for food production and efficient resource use during the current climate crisis. It can be further used as a teaching tool to educate and empower students. Previous research has shown its advantages and potential of increased understanding of systematic thinking, which is required in sustainable development processes. Its utilization has been studied at different education levels in several countries, including Sweden. Nevertheless, there is a lack of literature on providing educational tools to teachers based on the Swedish syllabi and its educational framework. This study aims to lay a solid foundation of tools for 8^{th} grade teachers to implement an aquaponics system as a teaching tool in Sweden.

For this purpose, we designed and shared guidelines with recommended activities alongside practical explanations. An aquaponics system was built and maintained by 75 8th graders during the full academic year 2022-23, to test its pedagogical application within different subjects such as STEM, home education, Swedish, and English. Three rounds of semi-structured interviews were carried out with the teachers involved in this project to analyze and reflect upon the activities organized around the aquaponic system. The key findings on setting up this new aquaponic project were: 1) teachers lack the time to initiate new projects on their own without pre-made teaching materials, 2) improved planning for scheduling activities throughout the academic year, along with clear guidance for teachers starting from scratch are needed. From these findings, we have developed a practical guideline, in order to reiterate and expand on the activities suggested to embed aquaponics as a teaching tool on a deeper level within the Swedish educational framework.

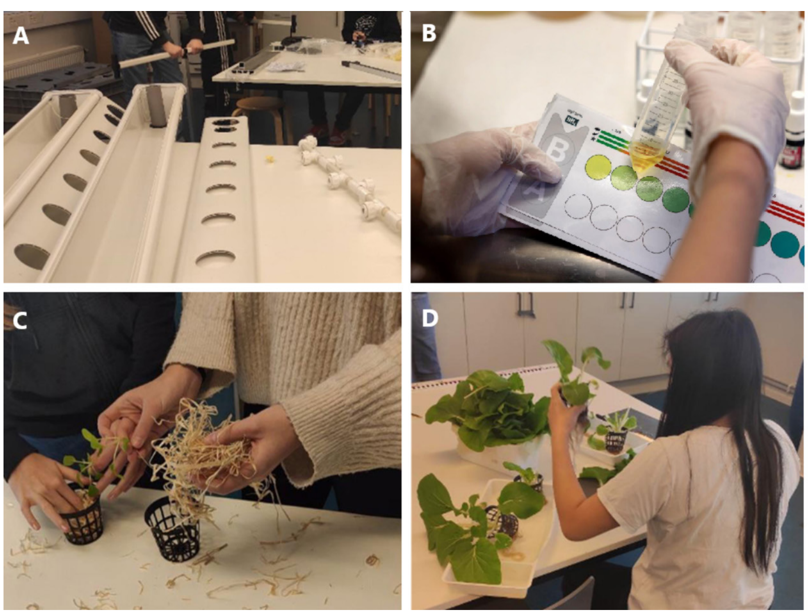

Figure 1. Building of the aquaponic system (A), weekly water quality check (B), seedling of the plants in hydroponic pots (C-D)

THE NORDIC MASTER'S PROGRAM IN SUSTAINABLE PRODUCTION AND UTILISATION OF MARINE BIORESOURCES: A MODEL OF SUCCESSFUL INTER- AND TRANSDISCIPLINARY COLLABORATION IN HIGHER EDUCATION

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In 2019, the Nordic master's program in Sustainable Production and Utilization of Marine Bioresources (MAR-BIO) was established by 4 Nordic universities: Gothenburg (Sweden), Nord (Bodø, Norway), Akureyri (left in 2022) and Hólar (both Iceland) in collaboration with the University of New England (Maine, USA). This unique trans- and interdisciplinary program aims to educate the next generation professionals in sustainable blue bioeconomy by combining natural sciences with environmental and social sciences, as well as economics and law. It introduces students to key aspects of the blue bioeconomy and its main industries in each partners' country. In Norway, focus is on salmon farming, in Iceland on the fishing industry and its sustainable use of natural resources and in Sweden on low-trophic aquaculture. All 3 countries jointly teach technical development of sustainable and innovative aquaculture systems, novel alternative feed ingredients and fish health and welfare in circular systems.

The program places strong emphasis on mobility and requires at least 30 ECTS to be earned outside the home university. The first course (15 ECTS) is a mandatory and hybrid course consisting of 3 modules of 1-2 weeks online, each followed by 1 week of on-site visits to each country. These visits include study tours to industrial partners and presentations of ongoing research (Fig.1). In the second semester, students apply for classes at 1 or more of the partner universities (15 ECTS) and the program is finalized by a 60 ECTS master's thesis course at any of the partner universities.

Now entering its 6th year, the master's program has grown from 5 students in the first cohort to 25. In this presentation, we will provide a detailed overview of the program, highlighting the transdisciplinarity and the future within this most rapidly growing and diversifying area of sustainable food production. We will show how the program offers a diverse network of contacts to the students both within the established industry, among innovative entrepreneurs and with authorities in the 3 partner countries. You will see the unique curriculum of the first mandatory course that lay the foundation for this successful cross-country collaboration in higher education, with potential to expand beyond the Nordic countries.

Figure 1. Examples of activities during the on-site week. (A) Visit of a fishing-trawler equipped with a super cooler technology (Iceland), (B) the fish auction in Gothenburg (Sweden) (C) a True Arctic salmon processing factory (Norway)

EVALUATING TUMBLE CULTURE AS A METHOD TO IMPROVE PACIFIC OYSTER *Crassostrea gigas* GROWTH AND LABOR EFFICENCY ON SOUTHEAST ALASKA SHELLFISH FARMS

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High water turbulence, tidal flux, and biofouling at Alaska's high latitude oyster farms create various challenges for growing marketable Pacific oysters (*Crassostrea gigas*). As oyster mariculture expands in this region, there is continued interest in developing efficient oyster grow-out methods that minimize farmer labor. Tumble culture oyster gear is configured to move with tide and wave action and be exposed during low tide, thus naturally tumbling oysters, deterring growth of fouling organisms, and reducing farm maintenance. To understand the efficiency of these tumble cages in creating marketable oysters in Alaska where this method of culture is not currently used, we stocked SEAPA cages with 500 oysters per cage, equipped the cages with HOBO temperature loggers and accelerometers, and deployed the cages in both the subtidal and intertidal at an oyster farm in Juneau, Alaska in early June 2024. After time 0 data collection in early June, subsets of 20 oysters were collected in late June, July, and September 2024, where we measured shell morphology (height, length, depth) and individual whole and wet meat weights to determine differences between the gear configurations per tidal region (intertidal cage pivot lines, subtidal cage stacks, control group of floating bags). Initial results indicate oysters from each treatment have comparable wet meat content and cup ratios (length: depth). Subtidal oysters have the highest shell growth, though growth is thin and uneven. In contrast, intertidal oysters have more rounded shell shapes which are desired for the half shell market, compared to the control oysters. Additional data on gear biofouling, water flux, temperature conditions, and labor between each tidal configuration will be quantified. Overall, these results can inform Southeast Alaska farmers about the best tumble gear configurations for sustainable, productive growth of oysters, providing opportunities for mariculture operations to expand in the region.

AERATION AND POWER REQUIREMENTS FOR CO_2-REPLETE TANK CULTIVATION OF THE RED SEAWEED *Devaleraea mollis* (PACIFIC DULSE)

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Land-based aquaculture of the commercially-significant red seaweed *Devaleraea mollis* (Pacific dulse) typically employs tumble culture systems, where compressed air is delivered to the bottom of the tank to provide gas exchange and promote mixing. However, aeration is a costly process because compressed air must be bubbled into the liquid, and power is required to drive the air compressor. In this study, fundamental relationships between aeration rate, biomass productivity, and aeration power consumption were developed for tumble tank cultivation of the red seaweed Pacific dulse under CO_2-replete growth conditions using CO_2 in air as the sole carbon source. Biomass productivity was measured over a range of aeration rates (0.086-0.684 L air L^{-1} liquid min^{1}) for fine-bubble (1-5 mm) and coarse-bubble (2-3 cm) aerators, and then correlated to the CO_2 transfer rate (CO_2-TR, mmol CO_2 L^{-1} h^{-1}) (Fig. 1). The CO_2-TR included the CO_2 partial pressure and the volumetric mass transfer coefficient (k_La) for CO_2, which lumped the boundary layer diffusion, volumetric gas flowrate, and gas sparger characteristics (bubble size, interfacial area) into a single parameter.

CO_2-replete biomass productivity required a CO_2-TR that was three times the CO_2 demand. For fine-bubble aeration, both CO_2-TR and aeration power consumption were higher than for coarse-bubble aeration over the range of aeration rates tested. However, when the aeration rate needed to achieve CO_2-replete biomass productivity was approached, fine-bubble aeration required less energy consumption due to lower aeration rate requirement, resulting in lower costs (Fig. 2). Overall, this study showed that aeration systems can easily limit biomass productivity and result in high aeration energy consumption if the aeration processes are not thoughtfully considered. The key tradeoff was that aeration energy consumption increased significantly as CO_2-replete biomass productivity was approached. Therefore, optimization would require the use of an aerator system that provides the best possible mass transfer coefficient (k_La) and lowest pressure drop at the lowest possible aeration rate.

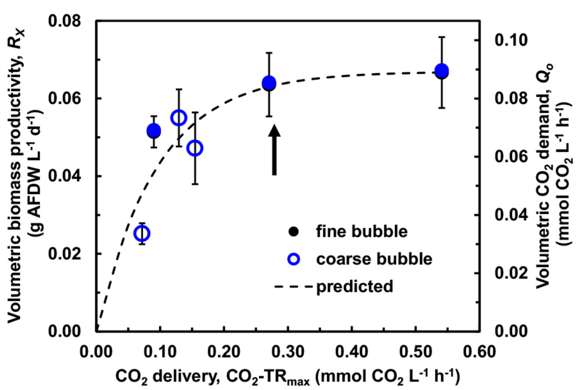

FIG. 1. CO_2-TR vs. volumetric productivity.

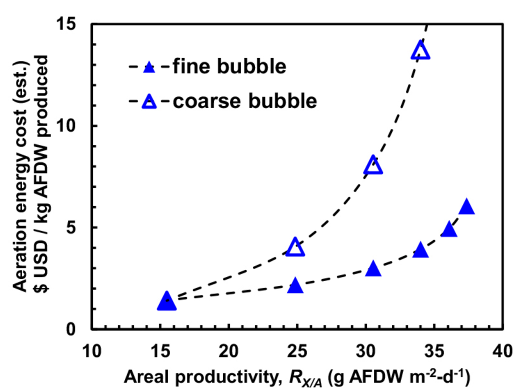

FIG. 2. Aeration cost vs. areal productivity.

A NEW ONLINE TOOL TO INTEGRATE NUTRIENT REMOVAL BENEFITS PROVIDED BY EASTERN OYSTER AQUACULTURE INTO THE AQUACULTURE PERMITTING AND REVIEW PROCESS

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Bivalve shellfish aquaculture can provide a variety of beneficial environmental services. Removal of excess nutrients from eutrophic coastal environments by eastern oyster farms has been formally recognized by multiple nutrient management programs in the Northeastern United States. However, the aquaculture permitting framework is currently focused on potential adverse impacts to the environment and society, and consideration of potential environmental benefits remains relatively unusual. Engagement with resource managers indicated the need for simple online tools, backed by robust science, to quantify beneficial effects of farms. Managers also expressed the need for an evaluation of variation in benefits provisioning over time, space, and common cultivation practices.

The newly released Aquaculture Nutrient Removal Calculator (ANRC; https://connect.fisheries.noaa.gov/ANRC/) tool uses eastern oyster size and harvest number to predict farm-scale nitrogen removal for US farms located from North Carolina to Maine. The literature review and synthesis has undergone peer review (https://doi.org/10.1371/journal.pone.0310062) and the data used in the tool have been publicly archived (https://doi.org/10.5281/zenodo.11966672). The methods used in the tool are highly transferable to other farmed bivalve shellfish species, and other regions, where sufficient data exist to support tool development.

The tool generates a report that was designed to integrate into the US Army Corps of Engineers public interest review process for new and expanding eastern oyster farms within the geographic region of existing data synthesis. Engagement is planned with the extension community, to increase awareness and use of the tool by aquaculture industry members.

THE TRANSFER OF FATTY ACIDS FROM BROODSTOCK DIET TO EGGS IN THE COMMON CLOWNFISH *Amphiprion ocellaris*

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Marine fishes such as the common clownfish, *Amphiprion ocellaris*, have been exhibited in aquariums for over 60 years. The industry has grown to more than 30 million marine organisms, such as fish and invertebrates, being imported into the United States annually to support the aquarium industry. Clownfish are an important component of the industry. Bottlenecks in commercial production, such as unpredictable hatch rates and juvenile deformities, reduce profitability of U.S. based production. One key factor affecting the success of clownfish aquaculture is the broodstock diet. This study aims to compare three different diets to determine their effects on spawn size, egg fatty acid profiles, and the health of hatched embryos. By optimizing broodstock diet, this project seeks to address commercial production challenges, increasing output and reducing deformities in aquacultured clownfish.

A. ocellaris pairs held in 20- or 30-gallon tanks in recirculating aquaculture systems at Roger Williams University will be fed three different diets (LRS® Fish Frenzy and PE® Mysis, fresh Quahog and Sea Scallop, and a custom gel diet composed of fresh clam, scallop, squid, and sardine, fish protein concentrate, gelatin, supplemental DHA, astaxanthin, taurine, and vitamin C). Each pair will be allowed to spawn three times per feed trial, with the third spawn from each pair collected for analysis. A subsample of eggs will be collected from each clutch at two timepoints, the day after spawning and the day before hatching, to be processed for fatty acid analysis. Remaining eggs will be hatched, and larvae will be raised on a standard larval reference diet to approximately 60 days post-settlement to determine the impact of parental diet of juvenile deformities. Hatch rates and larval mortality will also be recorded to help quantify the quality of a spawn.

It is anticipated that the different diets used in this study will result in different hatch rates, clutch sizes, larval survival rates, and differences in deformity prevalence and severity. This project also aims to identify the fatty acids most crucial to *A. ocellaris* broodstock. The data generated will contribute to advancing the profitability of *A. ocellaris* production.

ENGINEERED-AIRLIFT PUMPS CAN IMPROVE SUSTAINABLE OPERATION OF RAS SYSTEMS

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Recirculating aquaculture systems (RAS) are gaining popularity as a sustainable solution. One method through which fish farmers can reduce power consumption while improving water quality is replacing conventional technologies used for maintaining water quality with more energy efficient devices such as airlift pumps. This study was performed at the Ontario Aquaculture Research Centre (OARC) to evaluate the FloMov airlift pump technology compared to an optimized control tank equipped with aerators designed for land-based fish production. In the study, mixed sex Arctic char was randomly distributed in two production-scale rearing tanks (1500 fish/tank) where they were maintained using controlled tank aerators (control) or the FloMov (treatment). Measurements such as dissolved oxygen, water velocity, and total suspended solids were recorded through the length of the 3-month study. The results clearly demonstrate that the FloMov airlifts consistently achieved higher dissolved oxygen (DO) levels compared to traditional system. Additionally, measurements of total suspended solids (TSS) throughout the trial period also indicated better performance of the FloMov relative to the control tank. This is mainly due the enhanced water circulation, which also leading to the improved water quality parameters. Figures 1-3 display the above-mentioned results. The improvement in water quality parameters can significantly affect the fish productivity in a commercial RAS aquaculture setting for land-based fish production.

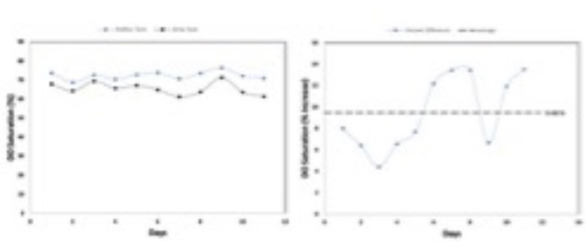

Figure 1: Dissolved oxygen (DO) saturation results

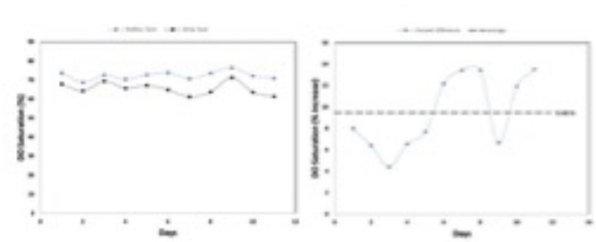

Figure 2: Total suspended solids (TSS) results

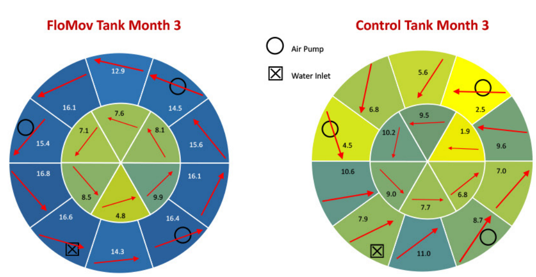

Figure 3: FloMov Tank monthly velocity map comparison with OARC Control Tank

VISUALIZING 20 YEARS OF CHANGE IN CHESAPEAKE BAY OYSTERS

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Understanding the dynamics of Chesapeake Bay oyster populations is critical for effective management and conservation efforts. This research analyzes long-term data from the Virginia Institute of Marine Science (VIMS) and Virginia Marine Resources Commission (VMRC) to investigate temporal trends in oyster populations across the Virginia portion of the Chesapeake Bay, using ArcGIS Pro and ArcGIS online software.

This research includes aggregating almost 20 years of fishery independent survey data, normalizing data using tools such as Model Builder, and presenting data in an accessible and visually compelling way. The final product is an online interactive map, designed to allow users to visualize temporal changes in oyster populations across varying spatial scales. This dynamic visualization tool facilitates a nuanced understanding of how environmental factors and management interventions influence oyster abundance and distribution.

By synthesizing complex spatial and temporal data into a user-friendly format, this project provides crucial insights for policymakers, researchers, and stakeholders invested in the sustainable management of Chesapeake Bay oysters. The findings underscore the utility of geospatial technologies in informing management and communicating to a public audience.

This work was supported by the National Science Foundation (NSF) Science Center for Marine Fisheries (SCEMFIS, www.scemfis.org) and a Research Experience Award to Rowe as the presenting author.

GROWTH AND WASTE REMOVAL CAPACITY OF THE WARTY SEA CUCUMBER (*Apostichopus parvimensis*) FED WITH WASTE FROM WHITE SEABASS (*Atractoscion nobilis*)

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The accumulation of nutrient-rich waste in aquaculture systems poses environmental challenges, particularly in monoculture setups. The warty sea cucumber (*Apostichopus parvimensis*, WSC) has shown potential for managing solid waste through integrated recycling. This study aimed to evaluate the growth, performance, and waste removal capabilities of WSCs when fed waste from white seabass (*Atractoscion nobilis*, WSB). The objectives were to determine the optimal feeding ration of WSB waste for WSCs and assess how WSC size influences growth, performance, and waste removal.

Three trials were conducted to address our objectives. In Trial 1, WSB waste nutritional quality was measured for changes over five days in seawater without WSCs. In Trial 2, 45 WSCs (100-150 g) were fed 1%, 2%, 3%, 4%, or 5% of body weight to determine the effects of daily ration on growth and waste removal capacities. In Trial 3, 45 WSCs were divided into three size classes (<100, 100-150, >150 g) and fed 4% of body weight to assess the impact of size on performance. Environmental parameters were monitored throughout the trials, with temperature ranging from 14.8°C to 23.4°C, pH from 7.82 to 8.30, dissolved oxygen from 7.76 to 11.16 mg/L, salinity from 30 to 35, and total ammonia nitrogen from 0.013 to 0.06 mg/L. All trials were conducted in 175 L tanks, run in triplicate, with regular measurements of waste, feces, and growth.

In Trial 1, the nutritional quality of WSB waste remained stable, with nitrogen content ranging from 2.96% to 3.5% and carbon content from 33.27% to 40.88%. In Trial 2, WSCs fed at 4% of body weight had a significantly higher specific growth rate (*SGR*) of 1.06% per day compared to other rations. Ingestion rate (*IR*) increased with higher feeding rations, ranging from 0.11 g DW/ind/day at 1% body weight to 0.58 g DW/ind/day at 5%. Fecal rate (*FR*) followed a similar trend, peaking at 0.30 g DW/ind/day in the 5% group. Apparent digestibility ratio (*ADR*) varied between 44.78% and 77.84%, with no significant differences between treatments. Waste removal efficiency (*WRE*) was highest at 4%, ranging from 28.75% to 44.05%. Standard length and width (*SLW*) measurements showed that WSCs fed at 4% had the highest *SLW* increase rate of 2.31% per day. In Trial 3, small WSCs demonstrated significantly higher nitrogen removal efficiencies, ranging from 42.67% to 48.71%, and carbon removal efficiencies from 34.95% to 48.16%, compared to large WSCs. The *IR* for large WSCs was 0.70 g DW/ind/day, significantly higher than the 0.30 g DW/ind/day for small WSCs. *FR* ranged from 0.12 g DW/ind/day for small WSCs to 0.29 g DW/ind/day for large WSCs, with *ADR* ranging from 57.10% to 59.84% across size classes. This study confirms WSCs as efficient waste removers, particularly when fed at 4% body weight. Smaller WSCs showed superior waste removal and growth, highlighting their role in optimizing bioremediation. Future research should explore long-term trials and scalability in larger commercial aquaculture systems.

A REVIEW OF BIODIVERSITY AND CHEMICAL CONTAMINANTS IN FISH FROM LAKE VICTORIA, UGANDA

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Lake Victoria connects the East African nations of Uganda, Kenya and Tanzania. We present here a review of the scientific literature on biodiversity and contaminants (pesticides, heavy metals) in fish from Lake Victoria on Jinja, Uganda. The review was performed using PubMed and PMC databases at the National Library of Medicine (https://www.ncbi.nlm.nih.gov/). Preliminary results will be presented.[1-12] Very limited information was found about chemical contaminants in fish of Lake Victoria in Uganda. The most common fish found in Lake Victoria, Uganda include, among others: Nile perch (*Lates nilotocus*), Nile tilapia (*Oreochromis niloticus*), dagaa (*Rastrineobola argentea*), catfish (*Xenoclarias eupogon*), elephant-snout fish (*Mormyrus kannume*), Nila killfish (*Micropanchax loati*), marbled lungfish (*Protopterus aethiopicus*) and cichlid fish (*Haplochromis thereuterion*).

Most of the publications were about pesticides and metals in Nile perch, *Lates niloticus*. For example, Ogwok *et al*. (2009)[6] analyzed pesticide residues and heavy metals in oil extracted from the belly flaps of varied sizes of Nile perch caught from Lake Victoria (Uganda). They found that the total residual concentration of dichlorodiphenyltrichloroethane, endosulfan, hexachlorocyclohexane, hexachlorobenzene, heptachlor, chlordane, endrin, aldrin and chlorofenvinphos increased significantly ($p < 0.05$) with fish size. Mercury and lead were detected in most samples while arsenic and cadmium were below detection limits. Nile perch may accumulate significant amount of chemical contaminants, but levels of contaminants in Nile perch oil were within limits considered acceptable by the German Food Law for human consumption.[6]

We will address the long-term health effects of low levels of pesticides and metals on human health[12], particularly considering the contamination by heavy metals in silver fish (*Rastreneobola argentea*) caught from Lakes Kyoga and Victoria, Uganda[8], the mercury concentrations in muscle, bellyfat and liver from *Oreochromis niloticus* and *Lates niloticus* consumed in Lake Albert fishing communities in Uganda[9], and mercury levels in Nile perch fillets in processing industries in Uganda.[11] The sociocultural factors associated with fish consumption in the fishing communities need to be addressed by the tree countries.[10]

Considering the new "Community Action For Fresh Water (CAFW) initiative between Rotary International (RI) and the United Nations Environment Program (UNEP) to restore, protect, and sustain freshwater ecosystems (www.rotary.org/en/rotary-unep-partnership), we plan to build collaborations with Rotaracts and Rotary Clubs from the three countries connecting Lake Victoria (Uganda, Kenya and Tanzania) to obtain preliminary results and prepare a global grant to perform studies on conservation of biodiversity and pollution in Lake Victoria to address food safety and food security issues.

(Continued on next page)

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SEEKIT™ WSSV FIELD KIT: ADVANCING RAPID AND RELIABLE DIAGNOSTICS FOR WHITE SPOT SYNDROME VIRUS IN SHRIMP

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White Spot Syndrome Virus (WSSV) poses a significant threat to shrimp. The virus spreads rapidly through aquaculture systems and leads to widespread mortality in shrimp populations. Early detection of WSSV is crucial for aquaculture operators to prevent the virus from spreading unchecked. Implementing timely diagnostic measures enables farmers to take swift action to contain the virus, minimizing its impact on both production and overall farm health. Currently, no field diagnostic test exists that can accurately detect WSSV earlier than 48 hours in an infection cycle.

To address the lack of reliable and rapid field testing for WSSV, Seek Labs innovated the SeekIt™ WSSV Field Kit, a rapid diagnostic kit that detects the presence of WSSV at only 24 hours post-infection, proving a strong alternative to commercial kits that rely on laboratory equipment and specialized training. Seek Labs completed an external validation study of the SeekIt WSSV Field Kit in collaboration with Florida Atlantic University's (FAU) Aquatic Animal Health Lab (AAHL). The study compared the SeekIt Field Kit's ability to detect WSSV in shrimp against AAHL's established laboratory protocol.

In the independent study, researchers at AAHL tested shrimp infection levels at various timepoints using the SeekIt WSSV Field Kit and AAHL's established laboratory protocol that relies on commercially available kits and qPCR. At only 24 hours post-infection, AAHL successfully detected WSSV with the SeekIt Field Kit, demonstrating SeekIt's underlying technology can detect viral loads early in infection lifecycles and as accurately as lab-standardized qPCR assays (see Table 1).

The results of the study highlight the efficacy of the SeekIt Field Kit in detecting WSSV without stringent laboratory conditions or equipment. This demonstrates SeekIt is especially suitable for pond-side diagnostics and can provide aquaculture stakeholders with a cost-effective and timely solution to manage WSSV outbreaks.

Shrimp #	Sample Type	SeekIt Test Call	Promega qPCR Call	Promega qPCR copies/100 ng DNA
1	Muscle	Positive	Positive	2.31E+05
1	Pleopod	Positive	Positive	4.51E+05
2	Muscle	Positive	Positive	1.48E+06
2	Pleopod	Positive	Positive	8.12E+06
3	Muscle	Positive	Positive	3.05E+02
3	Pleopod	Positive	Positive	2.18E+01
4	Muscle	Positive	Positive	6.07E+04
4	Pleopod	Positive	Positive	2.03E+05

Table 1. Comparison of average copy number/100 ng of DNA extracted from muscle (M) or pleopods (P) using SeekIt and Promega kits from WSSV infected shrimp at 24 hours post infection.

EGGS-PERIMENTS IN DISINFECTION: ELUCIDATING THE IMPACTS OF DISINFECTION METHODS ON THE MICROBIAL COMMUNITIES OF *Amphiprion ocellaris* EGGS

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Clownfish, particularly *Amphiprion ocellaris*, have been a staple in the aquarium trade for decades, with commercial producers supplying clownfish globally. However, producers face challenges that decrease profitability, such as unpredictable hatch rates potentially due to biofouling of spawning substrates or bacterial growth in larval tanks. To address these issues, it is common practice to disinfect eggs prior to hatching. Despite its widespread use, it is currently unclear how disinfection treatments directly impact the microbiome of clownfish eggs.

This study investigates the microbial and fungal communities associated with *A. ocellaris* eggs collected just prior to hatching, using metagenomic techniques to assess community changes in response to three commonly used disinfection treatments. Thirty eggs were harvested and frozen pre-disinfection at -80°C for baseline analysis. Eggs were divided into four groups: one control and three treated with either 1000 ppm hydrogen peroxide, 250 ppm povidone iodine, or 50 ppm formalin. Disinfectant concentrations were determined from a preliminary range-finding study that optimized survival rates and embryo hatch percentages. Following disinfection, each group was exposed to 12 hours of light in hatching tanks to inhibit hatching, after which 30 eggs from each group were frozen at -80°C for metagenomic analysis.

Microbial and fungal communities were characterized following established methods (Adamovsky et al., 2020). Samples were homogenized, and DNA was extracted using the ZymoBIOMICS DNA Miniprep kit. The 16S rRNA gene (V3-V4 region) and the fungal internal transcribed spacer (ITS) region were amplified using universal primer pairs (341F/806R and ITS1F/2), isolated with magnetic AMPure XP beads, and indexed with the Nextera XT DNA Library Preparation Kit and AccuPrime Taq DNA Polymerase. Libraries were pooled at equal concentrations, quantified using the NEBNext NGS Library Quant Kit, and sequenced on an Illumina MiSeq platform at the Interdisciplinary Center for Biotechnology Research, University of Florida.

This research aims to provide insights into the impact of disinfection methods on the microbial ecology of clownfish eggs, advancing our understanding of how microbial communities influence outcomes in clownfish aquaculture.

OPTIMIZING OYSTER FARMING: A YEAR-LONG INVESTIGATION INTO THE EFFECTS OF BAG TYPE, MESH SIZE, AND PLACEMENT ON AQUACULTURE CONDITIONS

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Oyster farming is a growing sector in aquaculture, valued for its ecological and economic contributions. However, optimizing growth conditions within oyster cages remains a challenge. Temperature and light exposure within grow-out bags can significantly impact oyster growth and health, yet little is known about how these conditions vary across different bag types, mesh sizes, and positions within a cage. Farmers often rely on trial and error to select setups, uncertain of the temperature dynamics within bags and their influence on oyster development. This study aims to fill this knowledge gap by systematically examining how environmental conditions fluctuate within oyster grow-out bags positioned at different cage locations. By analyzing the effects of bag type, mesh size, and placement on temperature and light levels, this research seeks to identify configurations that best support optimal oyster growth and farm efficiency.

This study, conducted at the FerryCliffe Aquaculture Farm at Roger Williams University, Rhode Island, investigates how bag type, mesh size, and bag placement within an oyster bottom cage influence water temperature and light intensity—two factors critical to oyster health and productivity. Using a 6-bag (2×3) oyster bottom cage, three 6-mm mesh bags were positioned on one side (top, middle, and bottom) and three 4-mm mesh bags on the other. HOBO® data loggers recorded hourly water temperature, and light intensity measurements were taken to assess environmental variation within the cage setup over a one-year period (March 2024–March 2025).

We hypothesize that temperatures will be higher inside the bags compared to outside, with the top bags experiencing the greatest warmth, especially in smaller mesh sizes due to limited water flow. Additionally, we anticipate light intensity to decrease with depth. By providing a clearer understanding of temperature variation within grow-out bags at different cage locations, this study will offer practical insights for farmers seeking to optimize oyster growth conditions.

The results of this study will inform best practices in oyster aquaculture by identifying optimal configurations that enhance productivity and minimize environmental stressors. By tailoring bag type, mesh size, and placement to meet specific environmental conditions, oyster farmers can potentially improve yield while supporting sustainable aquaculture practices.

COMPARATIVE ANALYSIS OF ACCURACY AND REPEATABILITY IN WATER QUALITY TESTING: EVALUATING PORTABLE HACH SPECTROPHOTOMETER AND LAMOTTE SPIN TOUCH SYSTEMS

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The advancement of aquaculture practices relies on the accurate and reliable measurement of water quality parameters, which are critical for optimizing growth conditions for aquatic species. This study presents a comparative analysis of two portable spectrophotometers—the HACH® DR 1900 and the Lamotte Spin Touch®—commonly used in aquaculture extension services. The analysis focuses on their performance in quantifying key water quality indicators, including total ammonia nitrogen (TAN), dissolved oxygen (DO), pH, and salinity.

To assess repeatability, multiple analysts performed identical tests on the same water samples using each device. Variability in results between analysts and repeated measurements was analyzed to identify any user-dependent variation or equipment precision issues. Additionally, the accuracy of both devices was evaluated using the spike-recovery method (Boyd and Tucker, 1992), where water samples with known TAN concentrations were spiked with 0.30 mg/L TAN and re-analyzed. The percentage recovery of the spike provided a quantitative measure of each device's accuracy, with recovery rates closer to 100% indicating higher accuracy.

Statistical methods, including standard deviation, coefficient of variation, and analysis of variance (ANOVA), were used to assess repeatability, variation, and differences in accuracy and time efficiency between the two devices. Lower variability and higher recovery percentages suggest greater repeatability and accuracy, respectively, while ANOVA results indicate if significant differences exist between device performances and among analyst results, thus evaluating user-dependent variability.

This study provides aquaculture practitioners with insights into the cost-effectiveness, time efficiency, accuracy, and repeatability of the DR1900 and Lamotte Spin Touch. By understanding the economic and practical aspects of water quality testing equipment and the influence of analyst variation, environmental managers and researchers can make informed decisions when selecting equipment for long-term water quality monitoring, ultimately contributing to improved standardization and reliability in aquaculture practices.

CAN WE PUT SCIENCE INTO REGULATION? A DISCUSSION

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The National Environmental Policy Act (NEPA) and state regulations for permitting aquaculture present significant challenges. At the core of this regulatory process is the requirement to use Best Available Science (BAS) and to meet the standards set forth by the Information Quality Act (IQA). The BAS principle requires agencies to use the most reliable and current scientific data in their decisions, especially when assessing environmental impacts. Meanwhile, the IQA focuses on ensuring the quality, accuracy, and integrity of all information used in government decision-making. The application of BAS is legally mandated, particularly in fields like environmental protection, health, and species conservation, to ensure agencies base decisions on the most up-to-date scientific evidence. The IQA applies broadly to all federal agencies, requiring them to follow internal data quality standards that ensure information accuracy and reliability. All agencies must align with these standards to issue reliable policies and regulations. Unfortunately, writers of NEPA documents need to be generalists due to the wide-ranging topics covered, yet each topic requires expert analysis and judgement. In addition, a significant challenge arises because peer-reviewed science and government publications that typically conform to BAS and IQA standards often do not align seamlessly with the needs of NEPA writers. This disconnect can complicate compliance, run up cost, and take significant time when preparing Environmental Impact Statements (EIS). To address this gap in other areas of government regulation, agencies frequently utilize Science Advice (SA) products—comprehensive reports developed by experts to synthesize scientific data according to IQA standards and the needs of the regulators. SA products are particularly valuable as they provide decision-makers and regulators with accurate, high-quality scientific analyses that align with regulatory requirements. However, the use of these products in areas like marine aquaculture remains underdeveloped, which hinders the efficiency of permitting processes. Developing more robust SA products that conform to legal and regulatory standards may prove essential in enhancing the speed and quality of producing NEPA documents.

PSP ALERT: AN INNOVATIVE, FIELD-DEPLOYABLE KIT FOR QUICK DETECTION OF PSP TOXINS IN SHELLFISH

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Paralytic Shellfish Poisoning (PSP), caused by saxitoxin (STX) and related toxins from harmful algal blooms (HABs), poses a severe public health risk, leading to paralysis, respiratory failure, and even death when contaminated shellfish are consumed. With the increasing frequency of HABs driven by climate change, there is a growing need for portable, efficient detection methods. Traditional PSP toxin detection relies on costly, time-consuming laboratory techniques like high-performance liquid chromatography (HPLC) and mouse bioassays (MBA), which are unsuitable for field use. To address this gap, Sensoreal developed PSP Alert, a rapid, on-site lateral flow assay (LFA) designed to detect PSP toxins directly in shellfish.

Compared to traditional detection methods, PSP Alert offers the following advantages. It is a portable and user-friendly tool that requires minimal training to operate, making it accessible to aquaculture farmers, shellfish growers, and researchers. The patent-pending conversion step in the sample preparation process, PSP Alert supports the detection of 12 common PSP toxins. Test results are available within 30 minutes of sample preparation, allowing for quick decision-making in the field. In addition to providing qualitative results, PSP Alert includes semi-quantitative capabilities, categorizing toxin levels as low, medium, or high. The device also features Bluetooth connectivity, enabling easy data storage and transfer for streamlined record-keeping.

Initial testing of PSP Alert on four shellfish species demonstrated a sensitivity of 100% and a specificity of 84%, outperforming several commercial alternatives. The device exhibited a false positive rate of 11.7% (Table 1) and a Kappa coefficient of 0.743, indicating a strong agreement between expected and observed outcomes. As Sensoreal continues to refine PSP Alert, the tool represents a promising, cost-effective solution for rapid, on-site detection of PSP toxins, reducing the need for expensive laboratory testing and improving public health protection.

Table 1: Precision metrics of PSP Alert

Parameters	Quantity
Number of tests	60
Number of shellfish species	4
False positive rates	11.7%
False negative rates	0%
Specificity	84%
Sensitivity	100%

OPTIMIZING A PILOT-SCALE AQUAPONIC PRODUCTION OF TILAPIA AND LETTUCE: A COMPARATIVE STUDY OF BATCH VS. STAGGERED CROPPING SYSTEMS WHEN STARTING NEW SYSTEMS

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When starting an aquaponic system, producers have two options with their plant harvest schedule; batch production or staggered. It is unclear if either approach impacts water quality more than the other. An experiment was conducted to investigate the comparative productivity of batch and staggered cropping systems of tilapia (*Oreochromis niloticus*) and lettuce (*Lactuca sativa* var. Muir) cultured in a coupled aquaponic production. Six systems consisting of a 350-L fish tank, a deep-water culture hydroponic subsystem (1.33m^2), and a 500-L sump were used. Treatments were randomly assigned to triplicate systems. Tilapia were initially stocked at a density of 19 kg/m^3. To optimize productivity, a high planting density of 47 plants/m^2 was used in both cropping systems. One batch harvest and three consecutive staggered harvests were conducted for a 23-day growing period each. At each harvest, the fresh weights of the plants were recorded and a total of twelve plants (four plants at each harvest of the staggered systems) were dried to quantify the dry mass. Fish feed input was 59.3 g/day/m^2 for batch culture and 61.3 g/day/m^2 for staggered production. At the end of the 49-day trial, tilapia were group-weighed, and mean final biomass (g), final fish weight (g), survival (%), weight gain (g), feed conversion ratio, and specific growth rate (%/day) were determined. There were no significant differences in any of the fish growth indices between treatments ($p>0.05$). Lettuce fresh weights harvested from the batch systems (111.9± 21.9g) were significantly higher than the ones harvested from the staggered production (102.3± 23.9; $p<0.05$). However, there was no significant difference in the lettuce dry weights between the two systems (3.8± 3.8 and 3.4 ± 3.4g, respectively). No nutrient deficiency symptoms were observed in either culture system. After the second plant harvest, total ammonia-nitrogen and nitrate values (2.1, and 62.9 mg/L, respectively) significantly increased in the batch production system compared to the staggered treatment (0.3 and 49.2 mg/L, respectively) and this elevation remained until the end of the experiment. The results showed that cropping systems have varying impacts on plant and water quality dynamics, emphasizing the importance of aquaponic system management and optimal nutrient cycling. Based on the current results, both plant harvest schedules had similar results, but a staggered production technique would be an economically viable option in pilot-scale aquaponic systems, especially for crops with short production cycles such as lettuce.

PHENOTYPIC CHARACTERIZATION OF F1 PROGENIES OF *clarias galmaensis* PRODUCED THROUGH HYBRIDIZATION AND GENOMIC DNA FROM *heterobranchus bidorsalis* USING SPERM MEDIATED GENE TRANSFER TECHNIQUE

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Studies on the phenotypic characterization of F1 progenies of *Clarias galmaensis* produced through hybridization and genomic DNA from *Heterobranchus bidorsalis* using sperm mediated gene transfer technique was carried out with the aim of determining the morphometric and meristic characters of the F1 progenies. Twenty one each of male and female *Clarias galmeansis* and three each of male and female *Heterobranchus bidorsalis* were used for the experiment. Female broodstock were induced with ovaprim and kept for latency period, after latency period, eggs from the females and testes from each male in each of the treatment were collected and weighed. Gonadosomatic index and fecundity rate were estimated. One gram (1g) of egg were collected from each fish and fertilized with 1mil of milt collected from the male broodstock. Treatment 1 to 5 involved introduction of genomic DNA of *Heterobranchus bidorsalis* to the milts at the concentrations of 0, 10, 20, 30 and 40µl before the fertilization while treatment 6 to 8 involved the use of hybridization between *Clarias galmaensis* and *Heterobranchus bidorsalis.* After fertilization, the eggs were incubated and reared for 30 days indoors. Thirty (30) fingerlings were collected in each treatment and reared for another 120 days outdoors for growth performance. After the growth performance, Three (3) samples were collected randomly for phenotypic examination. Data obtained from the phenotypic examination were subjected to analysis of Variance ANOVA. The results shows that the morphometric were mostly higher in treatment with crossing combination of ♀ Female *Clarias galmaensis* x ♂Male *Clarias galmaensi*s (♀Cg x ♂Cg) with 30µl of the genomic DNA. The meristic counts remain the same in all the crossing combination of ♀ Female *Clarias galmaensis* x ♂Male *Clarias galmaensi*s (♀Cg x ♂Cg) with 0, 10, 20, 30 and 40µl of the genomic DNA and also with hybridization combination of ♀ Female *Clarias galmaensis* x ♂Male *Clarias galmaensi*s (♀Cg x ♂Cg) with the absence of adipose tissue

DIETARY INCLUSION OF YUCCA EXTRACT AND BACILLUS SUBTILIS; A WAY TO MODULATE AMMONIA DISCHARGE, GROWTH PERFORMANCE, IMMUNITY AND CYTOKINES GENE EXPRESSION OF NILE TILAPIA (*Oreochromis niloticus*)

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The Nile tilapia (*Oreochromis niloticus*) is one of the most popular species worldwide. Nevertheless, intensive aquaculture systems is the most common fish farming systems generate a high amount of ammonia, a major metabolic waste product of fish, owing to fish waste and feed residue decomposition, increased organic matter deposition at the bottom of ponds, and a shortage of dissolved oxygen. On the other hand, plant extracts *Yucca schidigera* has been proven to be a potent, cost-effective agent for controlling ammonia. The inclusion of dietary *Bacillus subtilis* stimulates the immune system, promotes growth performance, and modifies the intestinal flora. Thus, evaluating the effect of using yucca and/or Bacillus bacteria on growth performance, immune status, and water quality of cultured Nile tilapia is the main objective of the current study.

Three diets were prepared to contain 0.2 g kg^{-1} of Yucca extract (Y), 1 g kg^{-1} of *Bacillus subtilis* (B), or a combination of Yucca extract and *B. subtilis* (0.2 and 1 g kg^{-1}) (YB), and the fourth diet (control) was left without an additive (CON). Nile tilapia (*Oreochromis niloticus*) fingerlings (13.54±0.32 g) were fed the test diets for 65 days.

As for the un-ionized ammonia (NH_3), CON gave a significantly higher value than the rest of the treatments; YB yields the lowest value of all. The highest growth performance and feed utilization were observed in the B treatment, followed by the YB treatment, while the control and Y treatments gave lower results. PA; PO; RB and ACH50 were showed the highest levels for YB-treatment with significant differences from the rest treatments; the lowest level was obtained for control (Table 2). LSZ lowest value was reported for YB-treatment which differs significantly from other treatments; the lowest value was for the control. The highest activity of SOD, GPx and GH were observed for YB- treatment, while the lowest activity was for the control (Table 1). Control showed the highest MDA result while the lowest result was reported for YB-treatment. The highest activity of Il-8 and Il-1 cytokines activity were observed for B- treatment followed by YB- treatment and control which possess the lowest activity. YB-treatment gave the maximum mean value of Tnf cytokine followed by B-treatment which higher than Y-treatment and control (Table 3).

Table 1. Effect of the experimental diets on the antioxidant parameters of Nile tilapia

	CON	Y	B	YB
MDA (U mg^{-1})	$4.07±0.15^a$	$3.27±0.15^b$	$2.40±0.10^c$	$1.03±0.15^d$
SOD (U mg^{-1})	$16.63±0.55^c$	$24.17± 0.25^b$	$25.30± 0.66^b$	$31.63±1.19^a$
GPx (μmol mg^{-1})	$0.230± 0.03^c$	$0.33±0.02^c$	$0.63±0.06^b$	$1.77±0.15^a$
GSH	$0.184±0.001^d$	$0.333±0.001^c$	$0.764±0.002^b$	$1.823±0.002^a$
TAC	$0.25±0.04^d$	$0.60±0.10^c$	$1.33±0.15^b$	$2.27±0.14^a$

Data are presented as mean values ± standard deviation of the mean. Data in the same row with different superscripts significantly differ ($P < 0.05$). malondialdehyde (MDA) superoxide dismutase (SOD), glutathione peroxidase (GPx) reduced glutathione (GSH), total antioxidant capacity (TAC).

Table 2. Effect of the experimental diets on the immunological parameters of Nile

	CON	Y	B	YB
PA	$3.93±0.47^d$	$6.67±0.59^c$	$9.60±0.36^b$	$12.27±0.25^a$
PO	$0.67±0.15^d$	$2.10±0.26^c$	$2.7667±0.15^b$	$3.93±0.35^a$
RB	$4.10±0.36^d$	$4.77± 0.15^c$	$5.53±0.15^b$	$7.77±0.21^a$
ACH50	$34.40±0.62^d$	$37.60±0.35^c$	$42.83±0.21^b$	$45.33±0.58^a$
LSZ	$82.67±1.53^d$	$87.00±1.00^c$	$114.67±2.08^a$	$95.00±1.00^b$

Data are presented as mean values ± standard deviation of the mean. Data in the same row with different superscripts significantly differ ($P < 0.05$). Respiratory burst (RB), Phagocytic activity (PA), Alternative complement activity (ACH50), Lysozyme (LSZ).

Table 3. Effect of the experimental diets on cytokines

	CON	Y	B	YB
Il-8	$1.20± 0.17^c$	$6.43± 0.05^b$	$8.23± 0.21^a$	$7.93± 0.15^a$
Il-1	$1.17± 0.15^d$	$2.85± 0.05^c$	$4.58± 0.07^a$	$3.14± 0.05^b$
TNF	$0.53±0.03^c$	$2.67±0.15^b$	$3.48±0.030^a$	$3.69±0.09^a$

Data are presented as mean values ± standard deviation of the mean. Data in the same row with different superscripts significantly differ ($P < 0.05$). Interleukin 8 (IL-8), Interleukin 1 (IL-1), and tumour necrosis factor (TNF).

PFAS IMPACTS ON ATLANTIC BLUE CRABS: CORRELATION BETWEEN *Hematodinium pereziand* AND PFAS LEVELS IN THE HEMOLYMPH

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Per- and Polyfluorinated Substances (PFAS) persist in marine environments through a variety of point and non-point sources. PFAS tend to persist in higher concentration in Atlantic Blue Crab tissue than the waters they inhabit. In the Maryland Coastal Bays (MCBs), *Hematodinium perezi* is a parasitic dinoflagellate that proliferates in the hemolymph of crabs, drastically impacting their survival. However, the relationship between high PFAS concentration and Hematodinium impacts have not been studied or connected. From the MCBs, 120 Blue Crabs samples were collected, and their tissue and hemolymph will be tested for PFAS levels and Hematodinium levels. Additionally, environmental parameters such as temperature, salinity, and water quality were also considered to evaluate potential associations with parasite prevalence and PFAS contamination. This study will provide valuable insights into the concurrent exposure of Atlantic Blue Crabs to parasitic infections and environmental contaminants in the MCBs. Thus, will contribute to our understanding of the ecological health of blue crab populations and inform management strategies aimed at conserving this economically important species. This is a current ongoing study, and results will be presented when analyzed.

EXPLORING PATHOGENIC AND PROBIOTIC BACTERIA FROM BIVALVES TO PREVENT LARVAL MORTALITY IN HATCHERIES

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Shellfish hatcheries are critical to bivalve aquaculture, yet frequent larval crashes significantly hinder seed production. While the exact causes of these crashes remain unclear, evidence suggests that the microbiome plays a key role. Understanding the interactions between pathogens and beneficial bacteria is essential for mitigating these crashes and improving hatchery production. To investigate this, we collected larval samples from bivalve hatcheries to isolate and screen for both pathogenic and probiotic bacteria. From March 2023 to July 2024, 381 isolates were identified from oyster, clam, and scallop larvae. The most abundant genera among the 35 identified were *Vibrio* (35%), *Pseudoalteromonas* (26%), and *Alteromonas* (12%). Isolates were screened for antimicrobial activity, hemolytic activity, quorum quenching, and biofilm formation. Several isolates were identified as potential pathogens, including *Vibrio neptunius* DEN11, *V. tubiashii* DEN41 and DEN12, *V. toranzoniae* CH7, *V. rotiferianus* CH3, *V. chagasii* CH4, *V. alginolyticus* CH1, *Tenacibaculum ascidiaceicola* Clam15, and *Cellulophaga lytica* CH30, which exhibited pathogenicity on either hemocytes or larvae, highlighting their threat to hatchery production.

Four isolates (*Algoriphagus winogradskyi* DEN5, *Marinomonas gallaica* Clam9, *Glutamicibacter soli* Clam16, and *Pseudooceanicola nitratireducens* NEH7) were selected for their probiotic potential and tested for immunomodulatory effects on larvae. Although no significant differences were observed between the probionts, larvae treated with these isolates and challenged with *Vibrio coralliilyticus* RE22 and *Aliiroseovarius crassostreae* (JOD) showed a 16-42% and 56-81% increase in relative percent survival (RPS), respectively. Larvae treated with *Mg*Clam9 (46%) and *Aw*DEN5 (52%) had higher RPS when challenged with *Vt*DEN41. Probiotic treatment did not improve survival (1-9% RPS) when larvae were challenged with *Vn*DEN11, as high mortality rates indicated that *Vn*DEN11 is a highly pathogenic strain (Table 1). A combination of all selected probiotic strains, along with *Phaeobacter inhibens* S4, resulted in better larval survival than *Pi*S4 alone (Table 2). This research highlights the importance of identifying pathogens and developing targeted probiotics to enhance larval resilience and support sustainable bivalve aquaculture.

TABLE 1. Relative Percent Survival (RPS) of oyster larvae pretreated with potential probiotics and challenged with different pathogens.

		PATHOGENS			
		*Vc*RE22	JOD	*Vt*DEN41	*Vn*DEN11
PROBIOTICS	*Gs*Clam16	16±12[a]	56±13[a]	31±5[c]	6±4[a]
	*Mg*Clam9	42±20[a]	81±7[a]	46±3[ab]	6±5[a]
	*Aw*DEN5	18±4[a]	60±21[a]	52±3[a]	1±2[a]
	*Pn*NEH7	35±6[a]	64±14[a]	32±4[c]	9±9[a]

TABLE 2. Relative Percent Survival (RPS) of oyster larvae treated with probiotic cocktails and challenged with *Vc*RE22.

COCKTAILS	RPS (%)
*Pi*S4	23±6[b]
*Pi*S4+*Mg*Clam9+*Gs*Clam16	25±15[ab]
*Pi*S4+*Mg*Clam9+*Gs*Clam16+*Aw*DEN5	27±6[ab]
*Mg*Clam9+*Gs*Clam16+*Aw*DEN5+*Pn*NEH7	39±9[ab]
*Pi*S4+*Mg*Clam9+*Gs*Clam16+*Aw*DEN5+*Pn*NEH7	50±15[a]

ASSESMENT OF CORN FERMENTED PROTEIN WITH YEAST AS A COMPLEMENT PROTEIN SOURCE TO IMRPOVE SOYBEAN MEAL FORMULATION IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei,* UNDER GREENWATER RECIRCULATION SYSTEM

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The use of corn-coproducts has become an efficient way to utilize ethanol co-products as concentrated sources of energy and nutrients in feeds. They are abundant in volume and processing technologies are well established, making them a good candidate to complement plant-based diets formulations. The corn fermented protein with yeast comes from a fermentation process, which increases the bioavailability of protein and reduces fiber and anti-nutritional factors (ANFs). The current study was conducted to evaluate efficacy of corn fermented protein with yeast that contains 50% of crude protein (CFP50Y), under green-water recirculation system. Four diets with 35% protein and 8% lipids extruded diets were formulated to partially reduce soybean meal as the inclusion of CFP50Y increase (0,5,10 and 20%). These diets were evaluated in 16 tanks of 800L, stocked at 34 shrimps $m^{-2}$with an initial weight at 0.19 ± 0.006 g, over an experimental period of 8 weeks. At the conclusion, no significance difference was found in feed conversion ratio, growth and survival. Whole body analysis did not reveal any significant difference among diets. The outcomes from this trial indicate that CFP50Y can be used up to20% of inclusion level in the diets of pacific white shrimp without compromising growth and health.

Growth Performance	Basal	CFPY-5	CFPY-10	CFPY-20	PSE[2]	p-value[3]
Survival Rate (%)	98.35	94.18	99.18	99.18	2.00	0.282
Final Average Weight (g)	20.08	20.59	20.02	20.01	0.286	0.452
Weekly Weight Gain (g)	2.49	2.55	2.48	2.48	0.035	0.404
Thermal-unit Growth Coefficient	0.38	0.39	0.38	0.38	0.054	0.440
Feed Conversion Ratio	0.98	1.00	0.98	0.98	0.021	0.902

[1]Mean

[2]Pooled Standard Error

[3]One-way ANOVA

THE USE OF CORN FERMENTED PROTEIN WITH YEAST AS A PROTEIN SOURCE COMPLEMENT WITH SOYBEAN MEAL IN PRACTICAL DIETS IN POND PRODUCTION OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Despite forecasts of low prices and higher input costs, shrimp production is forecasted to increase, driven by contributions from India, Ecuador and Vietnam. The feed industry has been supporting shrimp farmers by working to reduce or stabilize price increases through the use of lower cost feed ingredients and improved formulations. For that reason, the implementation of cost-effective ingredients that assure growth and health has demanded more attention in the past few decades, with ethanol industry leading the development of new corn co-products, such as corn fermented protein with yeast (CFP50Y) Contain 50% crude protein and less fiber, this is an attractive protein source in feeds. The current study assesses the efficacy of CFP50Y on pacific white shrimp under pond conditions. Four isonitrogenous (35%) and isolipidic (8%) extruded diets were formulated to partially reduce soybean meal as the inclusion of CFP50Y increase (0,5,10 and 20%). The pond trial was conducted in 16 ponds (0.1 ha) stocked at 0.032 g and 25 shrimp/m^2, over 82 day of culture period. The feed was delivered using passive acoustic feeders from AQ1. At the conclusion of the pond trial, whole body analysis revealed a significant increment in phosphorous retention at 20% of inclusion of CFP50Y, (P=0.009). There were no significant differences(P>0.05) in shrimp production across the dietary treatments. However, the feed cost was significantly lower at the highest inclusion level of CFP50Y.These results indicate that CFP50Y can be used up to 20% without adverse effects in growth performance of *Litopenaeus vannamei.*

	Basal	CFP50Y-5	CFP50Y-10	CFP50Y-20	PSE[1]	p-value
Weight (g)	35.12	36.43	35.14	35.38	0.697	0.522
Growth(g/week)	3.02	3.13	3.02	3.04	0.058	0.494
FCR	1.07	1.09	1.12	1.04	0.026	0.225
Survival (%)	97.67	88.75	91.95	97.23	2.720	0.108
Yield(kg/ha)	8,602.53	8,205.75	8,090.50	8,618.00	234.0	0.306
Phosphorous Retention (%)	22.18[a]	24.21[ab]	26.69[bc]	27.16[bd]	0.942	0.009
Feed cost$/kg Shrimp	1.36	1.34	1.34	1.18	0.031	0.004
Partial income ($/ha)	22,777.50	22,120.75	21,584.00	24,618.00	913.0	0.154

ASSESSMENT OF OXYGEN DYNAMICS IN A SHRIMP POND USING OPTICAL DISSOLVED OXYGEN SENSORS

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The application of technology to aquaculture has been increasing, allowing for improved growth and health in many aquaculture species. For shrimp, the implementation of passive acoustic feed management in conjunction with automated aeration controls tied to dissolved oxygen (DO) monitoring has led to major improvements in production. These technological tools aim to improve decision-making but are limited due to the per-unit cost of collecting data. Quite often, these environmental records are limited to a particular unique point (s) this limited data is then used to extrapolate the environmental conditions for the entire pond. For example, a single DO reading at the effluent of a 10 Ha pond may be used to “extrapolate” DO levels of the entire pond. Because of cost and/or labor requirements, little work has been done to evaluate diurnal trends in temperature and DO across a wider area of ponds to map shifts in temperature and DO. An alternative to DO recording using internet of things (IoT), independent DO and temperature loggers are available (e.g. miniDOT® logger), which are less expensive and can be deployed for monitoring purposes. Hence, in addition to continual DO monitoring slaved to aeration management we deployed in four ponds, one extra monitoring DO sensor that does not need loT infrastructure, placing the sensor in the shallow end of the pond. We monitored the oxygen and temperature trend in these four ponds over 60 days of culture. Then, four Mini Dots were then deployed in one of those ponds to monitor DO over 16 days before harvest to track D.O across pond locations. During the draining process for harvest, one miniDoT was placed next to the optical sensor to evaluate DO, right before harvest. The results indicated no significant difference in DO concentrations in the shallow end (p>0.05) compared to the optical sensor deployed in the feeding area. In the 16 days when four sensors were deployed in one pond, no significant differences were found across pond locations. However, when ponds reached 25% of the volume, the hypoxia conditions increased. In small ponds such as the 0.1 ha research ponds, one water quality sensor might be enough to understand oxygen dynamics for the entire pond. However, in large ponds, DO are more likely to vary across the pond area, as water mixing is likely insufficient.

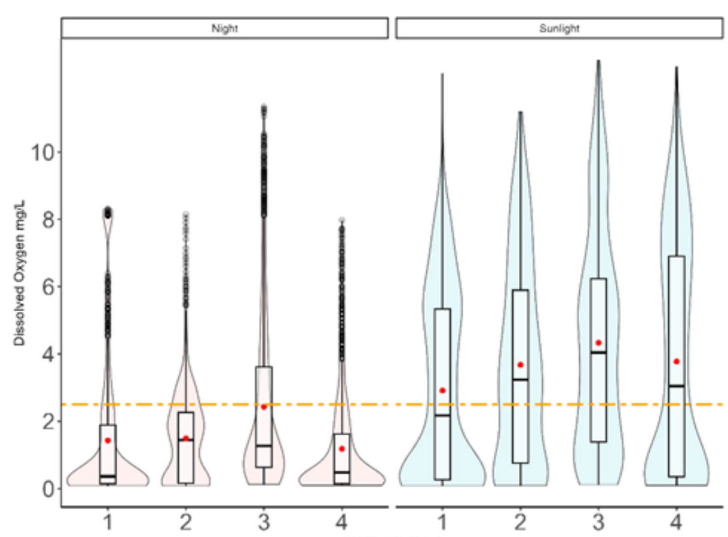

Figure 1. Distribution of dissolved oxygen across different location in one pond. Red dots represent the means.

SUSTAINABLE AQUACULTURE: STRATEGIES FOR COMPREHENSIVE IMPROVEMENT

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Aquaculture supplies more than half of food production in the fishing sector, which is why it has become one of the most important practices for the food industry worldwide. The need to produce food under strict quality standards and reduce the impact on the environment, leads to development of food industry, particularly aquaculture practices. Minimizing the impact of this industry implies the use of non-chemical antimicrobials and better practices for a sustainable management. Aquaculture biotechnology provides tools and processes that allow the development of pathogen detection and control strategies, improvement of genetic lines, improved performance (lower mortality and greater growth), and less impact on the environment.

Search for alternatives for crop improvement and protection against pathogens is of utmost importance to avoid production losses and dispense with the use of antibiotics. In traditional medicine, plants have been used ancestrally for therapeutic purposes, which is why their antimicrobial potential is well recognized. Medicinal plants offer a natural and sustainable alternative with high antimicrobial capacities, particularly if native species are used. Antimicrobial potential, in addition to antioxidant, which medicinal plants can contribute to aquaculture practice, implies the elimination of harmful antibiotics from the environment and contributes to the improvement of aquaculture practice and the reduction of its impact. The use of medicinal plants as antimicrobials in aquaculture systems requires the evaluation of the dose and method of application, since direct application can result in a certain degree of toxicity to organisms.

The use of yeasts and bacteria as probiotics has represented a highly effective strategy in: reducing pathogens by competition or by the production of metabolites such as bacteriocins, increasing feed digestibility by the production of digestive enzymes, increasing immune response, greater growth and lower mortality.

Plant extracts, together with microorganisms, can help minimize pathogens in the culture water, as well as reduce or eliminate contaminating compounds such as heavy metals, among others, facilitating the bioremediation of water and reducing the impact on the environment by eliminating the use of antibiotics and generating cleaner water that can be used in another system, integrating a multi-culture.

These and other strategies have been evaluated with different species of interest to aquaculture, particularly with white shrimp. The integration of these strategies can increase the productive performance of aquaculture systems and reduce the impact of the industry on the environment. A comprehensive system with best practices and innovative scientific and technological tools can boost the aquaculture industry and contribute to food sovereignty.

STATE OF AQUACULTURE AND PROSPECTIVE STUDY OF THE USE OF BIOTECHNOLOGICAL TOOLS TO CULTURE IMPROVEMENT

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Aquaculture activity is becoming increasingly important for the food industry since it allows us to relieve pressure on fisheries, but at the same time, generate quality food in strategic locations for its distribution. The high density that is managed in some crops, facilitates the spread of diseases caused by pathogens of viral and bacterial origin. The use of antibiotics is controlled and avoided due to the adverse effect on the environment and the consumer due to the accumulation of said chemicals. Aquaculture biotechnology seeks to generate solutions to control pathogens, to promote the growth of cultured organisms and to reduce the impact on the environment of aquaculture activity, along with the adoption of integrated cultures. Probiotics, medicinal plant extracts and biofilms for bioremediation are some of the strategies with the greatest biotechnological potential for aquaculture improvement. There are no strategies for real public policies that promote biotechnological development of native species with aquaculture potential, so the efforts made in this regard depend only of the disposition and work of the researchers in the different centers of aquaculture research in the country, including educational institutions as the University of Guadalajara. This work seeks to take advantage of this space where academia and the productive sector gather to collect information on aquaculture practices. Attendees will be asked to enter via QR code to participate in a survey that will allow them to obtain valuable information on: main consequences on aquaculture production due to the COVID19 pandemic, main pathogen mitigation strategies, knowledge and use of biotechnological tools, integration of scientific knowledge and technology in commercial farms, use and management of water and knowledge and use of integrated cropping systems. The presentation of this work and the participation of attendees will help integrate the aquaculture regulation of the state of Jalisco and the state of aquaculture activity in Mexico, in comparison with the rest of the world.

BIOPROSPECTING FOR LIPID PRODUCTION OF ELEVEN MICROALGAE STRAINS FOR SUSTAINABLE BIOFUEL PRODUCTION

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With the decline of fossil fuels and rising climate concerns, renewable energy sources have gained importance. Microalgae, a diverse group of organisms, offer promising applications in biotechnology and biofuel production due to their ability to convert atmospheric CO_2 and their rich biochemical composition. Despite their potential as biofuel sources, challenges remain in dewatering, growth rate optimization, and product synthesis. This study evaluates five microalgae species isolated from Baja California, Mexico, assessing their growth rate, biomass production, proximate composition, and fatty acid content to determine their suitability for biofuel production.

The growth rate ($p<0.05$), total dry weight ($p<0.05$), organic dry weight ($p<0.05$), ash content ($p<0.05$), and biomass productivity ($p<0.05$) differed among eleven microalgae strains. The proximate composition differed ($p<0.05$) between the eleven microalgae strains. The lipids contents were higher in *Cymbella* sp. (strain 2) (42.46±1.06%) ($p<0.05$). The carbohydrate content was higher ($p<0.05$) in *Aphanocapsa marina* (42.40±1.18%). The protein content was significantly higher ($p<0.05$) in *Chlamydomonas mexicana* (52.83±0.60%). The contents of saturated (SFAs) ($p<0.05$), monounsaturated (MUFAs), and polyunsaturated (PUFAs) ($p<0.05$) fatty acids among the eleven microalgae strains were different. The most important indicators of biodiesel properties are the cetane number (CN), iodine value (IV), and saponification value (SV). It was concluded that *Chlorella vulgaris* was an adequate strain to be used in the production of biodiesel due to their high amount of palmitic acid, oleic acid, and alpha-linolenic acid, by high values of IV (147.27 g I2 /100 g), and SV (210.96 mg KOH/g) and the highest value of CN (48.88). The diatom *Cymbella* sp. is a promising strain for biodiesel production due to its high values of lipid content (42.46%), lipid productivity (1.24 g/L/day), growth rate (0.67 divisions/day), and IV (197.93 g I2 /100 g), and by the lowest values of generation time (35.60 h) and SV (208.85 mg KOH/g). Another promising strain for biodiesel production is *Porphyridium cruentum* due its high values of biomass productivity (0.038 g/L/day), lipid productivity (0.80 g/L/day), CN (45.60) and IV (220.15 g I2 /100 g), and by the low generation time and SV (199.25 mg KOH/g).

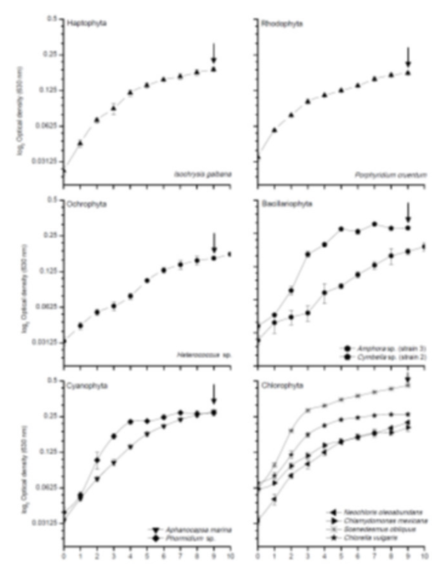

Figure 1. Mean (n=3) and standard deviation of optical density during the growth of eleven microalgae strains in batch cultures.

MICROPLASTICS AS VECTORS OF BACTERIAL PATHOGENS ALONG THE TUMBES COASTLINE, PERU: PRELIMINARY ASSESSMENT AND RISKS TO MARINE ECOSYSTEMS

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Plastic pollution, resulting from poor solid waste management, has become a serious problem for marine ecosystems. These wastes, originating from various activities such as fishing, agriculture, and tourism, can act as vectors for the transport of pathogenic bacteria, affecting both marine biodiversity and human health (Saldarriaga, 2007; Moran, 2017). The project "Microplastics and their Role as Vectors for the Transport of Bacterial Populations in the Tumbes Region, Peru: First Stage, Tumbes Coastline" aims to evaluate the relationship between the distribution and composition of microplastics and the transport of bacterial pathogens in coastal habitats of Tumbes, Peru.

The study was conducted along a coastal strip of Tumbes, covering key areas such as Puerto Pizarro, Playa El Bendito, La Cruz, Punta Sal, Canoas de Punta Sal, and riverbanks. Sampling was carried out in three specific zones along the tide line. Microplastics were collected by sieving the substrate and then classified and analyzed for identification . Additionally, metagenomic DNA extraction and bacterial isolation were performed using selective culture media. Preliminary results (Tabla 5), obtained by January 2023, show the presence of microplastics in all sampled areas. Bacteria associated with microplastics were isolated and purified from various beaches, including Canoas de Punta Sal and La Cruz. In the case of the islands of Puerto Pizarro, bacterial purity reached 78.6%, while in Playa El Bendito, purity was 44.4%. The identification of microplastics and the associated bacterial pathogens will help assess the risk of their impact on ecosystems and the local food chain.

In conclusion, microplastics along the Tumbes coastline not only represent a physical pollution problem but also have significant potential as vectors of pathogenic bacteria. This underscores the urgency of implementing effective waste management policies in the region.

Tabla 5. Resumen de resultados presentados

Procesamiento en el laboratorio hasta agosto 2022				
Categorización		**Playa de muestreo**	**Cantidad**	**Observaciones**
Microbiología	Bacterias puras	Canoas de Punta Sal	22 bacterias	Descrita microscópica y macroscópicamente, con registro fotográfico y conservadas en TBS con glicerol 15-20% a -20°C en el laboratorio
		La Cruz	7 bacterias	
	Bacterias para su reactivación y verificación su pureza	El Bendito (muestreo preliminar)	14 bacterias	Descritas parcialmente y se decidió conservar con glicerol a -20°C para luego su reactivación y verificación de pureza.
		El Bendito (2° colecta, 3°muestreo)	18 bacterias	
		Punta Sal	21 bacterias	
		Puerto Pizarro (1° maestro)	14 bacterias	
		Canoas de Punta Sal	2 bacteria	
		La Cruz	3 bacterias	
Clasificación de microplásticos	Descripción de los microplasticos y registros fotográficos	El Bendito (muestreo preliminar)	12 microplasticos	Fueron recuperados de los medios de enriquecimientos y de procedo de extracción de ADNm. Luego se lavaron con solución salina y agua destilada estéril y se almacenaron en microtubos de 1.5ml a temperatura ambiente
		El Bendito (2° colecta, 3°muestreo)	18 microplasticos	
		Punta Sal	17 microplasticos	
		Puerto Pizarro (1° maestro)	17 microplasticos	
		Canoas de Punta Sal	34 microplasticos	
		La Cruz	37 microplasticos	
Identificación polimérica de los microplasticos				
Lecturas en el FTIR		El Bendito (muestreo preliminar)	12 microplasticos	Obtención de los primeros perfiles, almacenados a temperatura ambiente.
Estudio molecular				
Extracción de ADN metagenómico		El Bendito (muestreo preliminar)	2 réplicas de ADNm	ADNm fueron almacenado a -20°C en el laboratorio
		El Bendito (3°muestreo)	2 réplicas de ADNm	
		Punta Sal	2 réplicas de ADNm	
		Puerto Pizarro (1° maestro)	2 réplicas de ADNm	
		Canoas de Punta Sal	2 réplicas de ADNm	
		La Cruz	2 réplicas de ADNm	

EXPERIMENTAL DEVELOPMENT OF AN IOT DEVICE FOR REAL-TIME MONITORING OF CO2 IN A RECIRCULATING AQUACULTURE SYSTEM

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In a recirculating aquaculture system (RAS) with high stocking densities, CO_2 tends to accumulate in the water to concentrations that can negatively affect both productivity and economics. Currently, it is a challenge to monitor and control the CO_2 concentration in an RAS in real-time, due to inefficient monitoring methods.

Based on the original design published by Pfeiffer et al., 2011, a system will be developed capable of recording, storing, graphing, and delivering real-time relevant information for comprehensive monitoring of CO_2 present in a recirculating aquaculture system using microcontrollers, sensors, cloud computing, and actuators, connected via the internet. The equipment will be mounted on an experimental structure simulating a desorption tower, using freshwater and gaseous CO_2 injected through a diffuser stone to condition the water in a controlled manner. The devices will also provide information on salinity, temperature, dissolved oxygen, and pH in the water, as well as temperature and humidity levels in the gas effluent.

This data will form the basis for quantifying CO_2 emissions into the atmosphere and its concentration in the water. This will enable the future implementation of actuators (pumps, blowers, solenoid valves) to automate control of an RAS, maintaining a CO_2 concentration between 8 to 10 mg/l (suggested by the salmon industry) and using equipment that increases the demand for electrical energy only when necessary. In addition, it will provide the necessary knowledge to establish a potentially productive use of CO_2 that is currently discarded, promoting a circular economy.

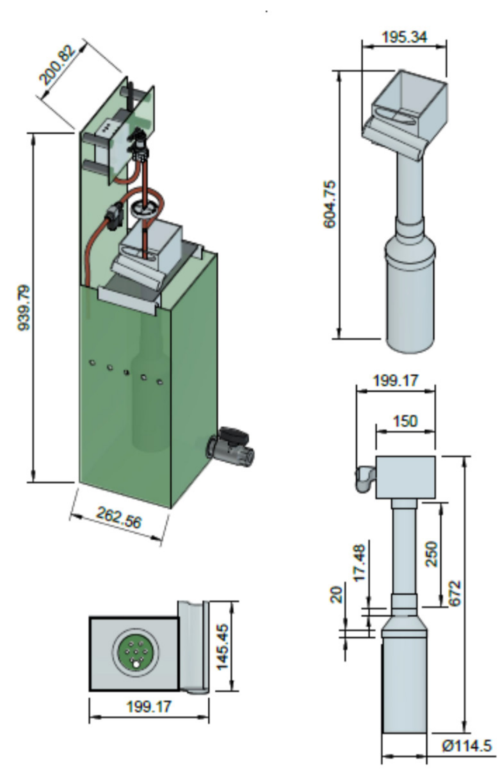

Fig 1. Dimensions in millimeters of the experimental prototype used to measure the partial pressure of dissolved carbon dioxide in water.

MICROBIAL CONSORTIUM AGAINST ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN *Penaeus vannamei* POSTLARVAE

Paola Lizeth Santos-Alfaro*, Sonia Araceli Soto-Rodriguez, Susana del Carmen de la Rosa Garcia, Sergio Alberto Gómez Cornelio

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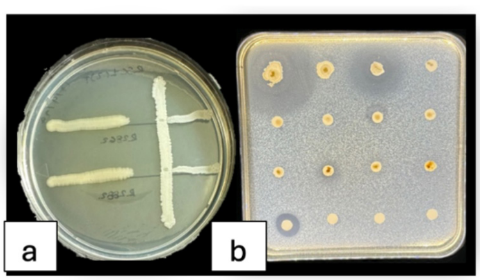
Aquaculture has been affected by the appearance of various diseases, mainly of bacterial and viral origin, and is considered the leading cause of loss of production in crops. In 2009, a disease of bacterial origin called "acute hepatopancreatic necrosis disease" (AHPND) was reported for the first time in China, capable of causing massive mortalities, up to 100% of production during the first 30 days of culture, significantly affecting the species *Penaeus monodon* and *Penaeus vannamei*. Different strains of *Vibrio* sp. were identified as the causative agent of this disease, which secrete a delta-endotoxin called PirAB, encoded by the *pirA* and *pirB* genes in a conjugative plasmid (pVA1).

The control of bacterial problems in shrimp farms has often relied on the use of antibiotics. However, these products' inappropriate and excessive use has led to health problems and the development of resistant bacterial strains. This misuse has also stimulated the transfer of resistance genes, exacerbating the issue.

Our study aims to design a microbial consortium that could be a significant change in the fight against AHPND in the Pacific white shrimp (*P. vannamei*) postlarvae. This consortium, if successful, could serve as a promising alternative to the use of antibiotics. The consortium will be carried out by purifying bacterial strains isolated from commercial probiotics and the digestive tract of healthy shrimp postlarvae. Pure isolates and their extracellular products were subjected to *in vitro* antagonism tests by means of various plate techniques such as well diffusion, disc diffusion, cross streak, and direct challenge by disc diffusion (figure 1), then the bacterial strains with the best antagonistic activity on AHPND-*Vibrio campbellii* strains will be selected. Mixtures of the potential probiotics will be used to evaluate them in an *in vivo* challenge using *P. vannamei* postlarvae infected with an AHPND-*V. campbellii* strain. Preliminary results will be presented.a

Figure 1. Antagonism techniques, cross streak (a), and direct challenge disk diffusion (b).



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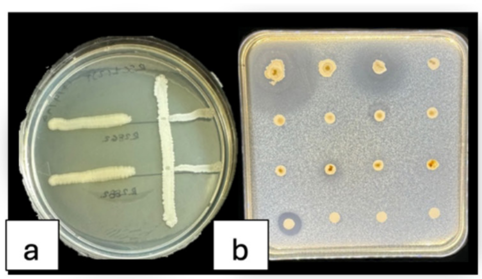

RASAGILINE AS A TREATMENT FOR MANGANESE NEUROTOXICITY OF THE DOPAMINERGIC CILIO-INHIBITORY INNERVATION IN *Crassostrea virginica*

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In humans, manganese is an environmental neurotoxin that when chronically inhaled can cause Manganism, a Parkinson's-like disease. The mechanism by which manganese produces this dysfunction is not fully resolved. Manganism involves disruption of dopamine neurotransmission, but it is different from Parkinson's disease. Current treatments for Parkinson's do not resolve the symptoms of Manganism. Previously, our lab determined that the bivalve *Crassostrea virginica,* when exposed to high levels of manganese, accumulated the metal in its tissues and demonstrated a dysfunction on the cilio-inhibitory dopaminergic innervation of the gill. Gill lateral cell cilia of *C. virginica* are innervated by serotonin and dopamine nerves from their ganglia. Dopamine is cilio-inhibitory, while serotonin is cilio-excitatory. Our lab has shown that acute and short-term manganese treatments selectively blocks the cilio-inhibitory effect of dopamine, but not the cilio-excitatory effect of serotonin. Our lab also showed that the drugs p-aminosalicylic acid and taurine reduced the tissue levels if manganese and alleviated the neurotoxic action of manganese on the dopaminegic system. Recently, reports suggest the drug rasagiline, a monoamine oxidase inhibitor (MAO-I) could protect against manganese induced reactive oxygen species, and offer a benefit to patients with Manganism. In this study we hypothesized rasagiline would reduce the neurotoxic actions of manganese on the cilio-inhibitory effects of dopamine in *C. virginica* gill. To test this, we conducted dopamine dose responses on excised gills of animals treated with manganese (10^{-4} M) for 5 days. Gill lateral cell cilia activity was measured by stroboscopic microscopy. Cilia of control lateral gill cells that were first activated by serotonin (10^{-5}M) responded normally to the dopamine (10^{-7} - 10^{-3} M) dose response with the appropriate decrease in cilia beating rates from about 20 beats/second to about 0. Manganese treated animals showed a statistically significant disruption of the dopamine cilio-inhibitory dose response. Applying rasagiline to the gills of the manganese treated animals did not significantly reduce the neurotoxic effect of manganese on the cilio-inhibitory actions of dopamine. This project shows that more studies need to be done with rasagiline to determine its possible effectiveness and therapeutic mechanism of action. Future experiments should be conducted with increased rasagiline doses and/or increase duration of drug exposure. The bivalve mollusc gill is a useful model to study regulatory mechanisms of ciliary activity as well as the pharmacology of drugs affecting biogenic amines in nervous systems. The information from this present study should be of interest to those studying the mechanism of action of manganese toxicity and others designing drug to alleviate the symptoms of Manganism.

This work was supported in part by grants 0537231071 of the CSTEP Program of NYSED, P120A210054 of the MSEIP Program of the DoEd, NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University, and CCUNY I-CORPS of NSF.

BENTHIC BIODIVERSITY UNDER MARINE AQUACULTURE: INTEGRATING META-ANALYSIS AND MODELLING FOR ADAPTIVE MEDITERRANEAN SEA MANAGEMENT

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The influence of marine aquaculture on benthic biodiversity has attracted considerable scholarly interest driven by the increasing demand for sustainable food production and the urgent need to conserve - and ultimately restore - marine ecosystems. This study presents a comprehensive meta-analysis encompassing 457 case studies drawn from 33 peer-reviewed articles, quantifying the effects of aquaculture operations on benthic biodiversity within the Mediterranean Sea. The evidence-based synthesis indicates that aquaculture exerts a moderate impact on biodiversity relative to undisturbed reference sites. Importantly, our meta-analysis reveals that the magnitude of aquaculture impacts is highly context-dependent, varying markedly across different Mediterranean eco-regions, habitat types and environmental conditions. For example, pronounced effects were detected in the Eastern Basins, whereas Central and Western basins experienced comparatively milder impacts. Additionally, habitat type was a significant determinant, with soft-bottom environments exhibiting more substantial impacts. A subsequent modelling exercise further identified the trophic status of cultured species and water temperature as critical factors; areas characterized by low chlorophyll-a concentrations and elevated temperatures were associated with more severe impacts. These findings have many implications and underscore the necessity for tailored, ecosystem-based and adaptive aquaculture management strategies that incorporate local environmental conditions to promote sustainability. Specifically, our findings emphasise the necessity of: (i) tailoring regulatory frameworks to specific eco-regions based on their vulnerability to aquaculture impacts; (ii) establishing mandatory buffer zones around aquaculture sites - designed according to local environmental conditions and incorporating biodiversity–habitat layers - to protect adjacent ecosystems from direct impacts; (iii) promoting the adoption of integrated multi-trophic culture systems to mitigate the adverse effects associated with single-species farming and to mainstream biodiversity considerations in aquaculture; (iv) enhancing environmental monitoring programs through the development of regionally standardized protocols that include key indicators, such as measures of benthic community biodiversity and functionality (encompassing both macrofauna and megafauna), chlorophyll-a concentrations and water temperature; (v) actively involving stakeholders in the design and implementation of monitoring plans and (vi) instituting adaptive monitoring cycles with periodic revisions, including performance indicators based on biodiversity responses that can be refined over time to inform management actions in the middle of rapidly changing global and local conditions. Future research should prioritize the integration of biodiversity as a critical response variable to advance our understanding and management of the ecological consequences of aquaculture.

TOWARDS SUSTAINABLE FISH-FREE AQUAFEEDS: EVALUATING MICROALGAL PRODUCTS FOR REPLACEMENT OF FISH MEAL AND FISH OIL IN AQUACULTURE DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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The expansion of commercial aquaculture over the past several decades has been crucial in realizing its potential to provide affordable and nutritious, high-protein food for the world's growing population. Ocean-derived small fish like anchovies and sardines for fishmeal (FM) and fish oil (FO) in aquafeeds, especially for salmonoids—the most significant users—has raised sustainability concerns. These concerns have driven the search for sustainable alternative ingredients for aquafeeds. Microalgae-derived protein and lipids have emerged as promising candidates to replace fishmeal and fish oil. We report our recent study aimed at developing a fish-free feed formulation for rainbow trout, which combines protein-rich, defatted biomass from *Nannochloropsis oculata* with either whole cells or oil derived from DHA-rich *Schizochytrium sp.*

We conducted an 89-day nutritional feeding experiment to determine the optimal level of FMFO replacement for growth and filet biochemical composition. We finally used the Cruz Aquafeed Sustainability Tool to dtect the diets' environmental impacts, including biotic resource use (BRU), global warming potential (GWP), water use (WU), land use (LU), marine eutrophication potential (MEP), and freshwater eutrophication potential (FEP). Growth study results showed that trout fed a fish-free diet by combining *N. oculata* co-product and *Schizochytrium sp* whole cells did not significantly differ from fish fed the reference diet in feed conversion ratio, growth, specific growth rate, and survival rates. The environmental impact analysis showed that BRU was significantly lower in the fish-free diet compared to the reference diet. WU, LU, FEP, and MEP were lower in the reference diet compared to other experimental diets, while GWP was not significantly different from the fish-free diet (NSW).

Thus, using *N. oculata* co-product with *Schizochytrium* sp. whole cells in trout feed can replace FMFO while maintaining growth, flesh composition, and GWP comparable to the reference diet.

TABLE 1. Experimental Diet Design

Growth Experiment
4 replicate tanks/diet
Reference diet (FMFO)
Nannochloropsis+Schizochytrium replaces 75% of FMFO (NSW75)
Nannochloropsis+Schizochytrium replaces 100% of FMFO (NSW100)
Nannochloropsis +*Schizochytrium* replaces 100% of FMFO (NSO100)

TABLE 2. Initial weight, final weight, weight gain, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and survival rate of rainbow trout fed experimental diets.

	Diet				
	Reference	NSW75	NSW100	NSO100	*P* value
Initial weight (g)	0.84 ± 0.03	0.83 ± 0.03	0.91 ± 0.06	0.84 ± 0.03	0.45
Final weight (g)	35.0 ± 0.26 ^{f}	33.0 ± 0.77 ^{fg}	34.3 ± 0.48 ^{fg}	32.9 ± 0.26 ^{g}	0.03
Weight gain (g)	34.2 ± 0.24 ^{f}	32.2 ± 0.77 ^{fg}	33.4 ± 0.51 ^{fg}	32.0 ± 0.25 ^{g}	0.03
FCR	0.88 ± 0.03	0.93 ± 0.03	0.89 ± 0.02	0.98 ± 0.03	0.09
SGR	4.1 ± 0.04	4.05 ± 0.04	3.99 ± 0.08	4.03 ± 0.04	0.52
PER	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.09
%Survival rate	100	100	100	100	na

FIELD PERFORMANCE EVALUATION OF SELECTIVELY BRED EASTERN OYSTER LINES IN NEW JERSEY AND RHODE ISLAND

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Eastern oyster aquaculture has increased steadily over the last three decades, but the industry has yet to achieve its full potential. In the Northeast USA, production is limited in part by a lack of high performing stocks suited to the geographically broad and heterogenous growing environment. For this study, hatchery lines from Maine, New York, and New Jersey as well as crosses between each hatchery line and the selectively bred Northeast high survival (NEH) line, were deployed at oyster farms in Rhode Island and New Jersey for performance evaluation. Replicates of each line were stocked and maintained according to standard husbandry practices. Oyster survival and growth were monitored for 15 months. At 4, 6, 11, 15 and 18 months post-spawn, live and dead oysters were counted in each replicate and shell height and total weight were measured for a subset of oysters per replicate to track survival and growth trajectories. In addition, during the second summer of the evaluation period, a subsample of seed from each line were tested for parasites *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) to provide context for observed patterns of mortality at the Rhode Island farm. All lines within each farm site will be compared to assess differences in performance. Line survival and growth between the two farms will be compared to identify genotype by environment (GxE) interactions. Based on previous studies, we expect that hybrid lines will outperform their purebred counterparts and significant GxE interactions will result in different performance rankings between farms. The side-by-side evaluation of local hatchery lines and their hybrids with NEH at representative Rhode Island and New Jersey oyster farms will contribute to the development of breeding strategies that effectively support the Northeast USA eastern oyster aquaculture industry.

EFFECTS OF TEMPERATURE ON BAY SCALLOP MAROSPORIDA (BSM) DYNAMICS IN *Argopecten irradians*

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The bay scallop *Argopecten irradians* has been an important species economically in New York since the mid 1800's, but has gone through some drastic population fluctuations, with the population and fishery impacted by eelgrass wasting disease, harmful algal blooms and more recently an apicomplexan parasite known as Bay Scallop Marosporida (BSM). Since 2019, the bay scallop population in New York has experienced mass summer mortality events, ranging from 90-99% reduction in adult scallop biomass. Along with being highly seasonal, these mortality events have been linked to increased prevalence and intensity of BSM infections.

To examine the impact of temperature on disease development and scallop survival, two experiments were designed. In the first experiment, scallops were maintained in a flow through system at ambient seawater temperature or adjusted to mimic past temperature (-3°C below ambient) and future temperature conditions (+3°C above ambient). Disease development was monitored every two weeks by quantifying BSM in scallop tissues. In the second experiment, scallops were maintained in a flow through system in ambient seawater temperature or adjusted to mimic future temperatures as well as a heat wave scenario. All scallops were initially acclimated at 20°C, then temperatures were altered over the next 26 days to implement the different temperature regimes. Samples were taken after the peak temperatures were reached for the different treatments including, 25°C, 28°C and 30°C. In addition, water and biodeposits were also collected in both experiments to quantify parasite release into the surrounding environment.

In both experiments, disease development and BSM quantification in scallop tissues, biodeposits and water were evaluated by histology, fresh kidney smears and qPCR. Data analysis is ongoing and anticipated results are expected to help understand disease development and BSM dynamics in a warming ocean.

CONNECTING SEA GRANT, THE NATIONAL CENTERS FOR COASTAL OCEAN SCIENCES, AND COASTAL-OCEAN COMMUNITIES TO IMPROVE SUSTAINABLE AQUACULTURE SITING AND DEVELOPMENT PROCESSES

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As aquaculture continues to expand in the U.S., it is necessary to ensure sustainability is at the core of all efforts, from planning to distribution, especially in crowded coastal and marine spaces. Careful farm siting and planning are one way to contribute to the sustainable growth of aquaculture in the U.S., and the National Centers for Coastal Ocean Sciences (NCCOS) have developed tools and resources that begin to address the complex needs of interested parties using coastal-ocean spaces. The planning tools and resources created by NCCOS rely on a science-based, community-led approach for continual feedback and development, ideally resulting in successful usage and, ultimately, the identification of optimal locations for aquaculture. Because coastal-ocean environments accommodate many activities that could overlap with aquaculture, it is important to deliberately connect and build capacity among local user-groups through conversations centered around aquaculture planning tools to improve sustainable aquaculture development.

To facilitate capacity building, Maryland Sea Grant and are partners are in the process of hosting six collaborative, regionally tailored workshops across the nation over the course of four years to connect the Sea Grant Network, NCCOS, aquaculture extension specialists, and other coastal-ocean groups. The workshop regions are as follows: Mid-Atlantic (Fall 2022), Gulf of Mexico (Winter 2023), California (Fall 2023), Pacific Northwest (Summer 2024), Pacific Islands (Early Winter 2025), and New England (Spring 2025). Through this project we aim to 1) extend NCCOS aquaculture planning resources, 2) increase connections and collaborations with diverse interested parties, and 3) advance aquaculture siting conversations more broadly with summary reports citing key themes and findings after each workshop.

After completing the first three workshops in the Mid-Atlantic, Gulf of Mexico, and California, we have begun to identify regional differences and similarities with respect to aquaculture siting tool use and perspectives among the various user-groups. Additionally, participants reflected on their present engagement with communities in coastal and marine spaces about aquaculture development to identify groups that need to be brought into the discussions. Prior workshop findings will partially inform the framework of the remaining three workshops and final project report.

EFFECTS OF HIGH AMMONIA AND DELAYED SAMPLING TIME ON NILE TILAPIA (*Oreochromis niloticus*)

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In aquaculture, fish are constantly encountering ammonia stress that negatively impacts growth and health. Ammonia is a toxic biproduct formed during protein degradation that is excreted as waste. When ammonia levels in the water get too high, it can cause several problems such as damaging the gills. Gill damage makes it hard for fish to breathe. High ammonia levels can also cause burns and sores on the skin and fins. In the wild high ammonia is not normally a problem due to bacteria found within the sediment that detoxifies ammonia. In captivity these bacteria must be introduced to negate ammonia stress. In this experiment, we wanted to investigate if high levels of ammonia cause physiological and immunological changes in tilapia. Tilapia were reared in high ammonia for four weeks and sampled to check for physiological and immunological status. To determine if delayed sampling time after capture is a factor that affects the quality of data, fish were caught and then held in a bucket for ten minutes before sampling instead of sampling within two minutes of capture. There was a total of four treatments: control, delayed, ammonia, and ammonia delayed. Length, weight, Fulton's condition factor, packed cell volume, blood glucose, serum lysozyme, and plasma protein were measured at the end of four weeks. Average packed cell volume was significantly different between control delayed and ammonia delayed treatments ($p = 0.035$). There was a significant difference in average blood glucose between control and ammonia delayed treatments ($p = 0.031$). Average weight was significantly different between control and ammonia and between control and ammonia delayed treatments ($p = 0.235$; $p = 0.001$). There was no significant difference between any of the treatments for the remaining parameters. The data shows that four weeks of high ammonia does not contribute to significant health problems, other than weight, within the fish. It also appears that delaying sampling by five minutes is an insignificant factor to data quality. If the fish were held in a high ammonia environment for a longer period of time the health of the animal may start to dwindle.

ENVIRONMENTAL MODELING FOR U.S. FEDERAL WATERS FINFISH AQUACULTURE-LESSONS LEARNED

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The NOAA National Centers for Coastal Ocean Science has ongoing collaborations with the U.S. Environmental Protection Agency (EPA) the NOAA National Marine Fisheries Service (NMFS) to provide depositional and water quality modeling products and science advice to support agency permitting and associated environmental reviews and consultations for finfish aquaculture projects proposed for U.S. federal waters. We will discuss the lessons learned from our coordination with EPA, NMFS, and project proponents focused on environmental modeling for individual projects proposed to be sited within U.S. federal waters across a range of environmental conditions and background data availability. This work serves as the foundation for the development of a decision framework that can be used to identify the appropriate level and type of modeling needed to inform management decisions for finfish aquaculture projects proposed in U.S. federal waters.

CRYOPROTECTANT SCREENING FOR HIGH THROUGHPUT MICROALGAL CRYOPRESERVATION

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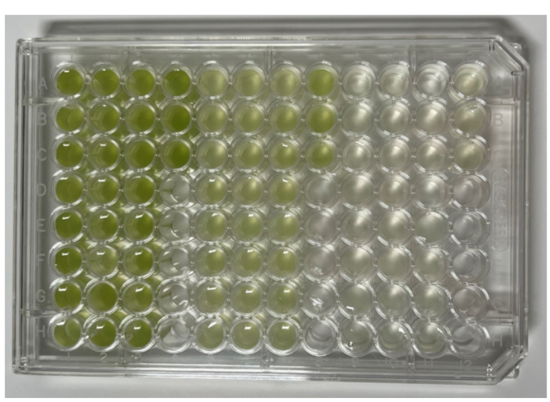
Microalgal strains have been traditionally maintained as live cultures. The time, cost and risk of maintaining these live cultures, has prompted culture collections to turn to cryopreservation to maintain strains that are less frequently used. The protocols used to this end, have been developed from those used for animal sperm preservation, and are not universally suited for microalgae.

Most microalgal strains preserved are obtained from active cultures and preserved in 0.5 to 1.0 mL containers. This small volume is enough to start cultures in small volumes (1-20 mL). The recovery of these cultures for aquaculture use, requires careful planning, to reach the needed volumes required for animal feeding. The variability on the cell counts in active cultures, increases the uncertainty of the time needed recover and expand the cultures for application as feeds. Higher concentration cultures may allow a faster recovery and expansion of the cultures. One of the variables that can impact the microalgal cryopreservation process, is the cryoprotectant type, equilibration time and the relation between the concentration of cryoprotectant and the cells concentration.

In this work, we explored the effect of cell concentration, equilibration time and ratio of concentration of cryoprotectant to cell numbers. Samples of microalgal cultures where concentrated by centrifugation in arrange of 1- 50X. Triplicate aliquots of each concentration were mixed with a solution of DMSO in culture media, in a ratio of 1:1. The samples were equilibrated for times of 5,10,15,20,25,30,35 and 40 minutes. Samples were inoculated in culture media and their growth monitored for 10 days or 5 days after start of the exponential growth phase.

For each culture concentration, the two treatments with the best growth rate were selected to test freezing rates from 10 - 40 °C. The samples were treated under the conditions selected. Triplicate samples of the selected treatments, without dilution, were placed in 0.5 mL straws, and frozen at each freezing rate to a temperature of -80° C and maintained at this temperature for 5 minutes. The samples were then placed immediately in liquid nitrogen. The samples were stored in dewars under liquid nitrogen vapor for at least 5 days. The samples were thawed, and their growth monitored as described above.

For all experiments, cell counts were performed on several quality control points, including the original culture, the culture after concentration (centrifugation), culture after cryoprotectant addition and equilibration (before freezing in the freezing rate experiment), and samples after inoculation in the culture media (thawed samples in the freezing rate experiment). Cell counts were done every other day thereafter for 10 days, or 5 days after the start of exponential growth. Results show that freezing rate can impact the survival of cryopreserved microalgae, while cryoprotectant stabilization times showed little to no impact.



EVALUATION OF EXEPTIONALLY EARLY PRESEASONAL SPAWNING IN PIKEPERCH (*Sander lucioperca*)

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At the Research Center for Fisheries and Aquaculture MATE AKI HAKI in Szarvas, Hungary protocols were refined for early hormonal stimulation of ovulation in pikeperch. In this region, the typical spawning period for wild pikeperch is April. For decades, pond-reared pikeperch have been successfully spawned upon injection in January. This study aims to produce high-quality gametes an additional month earlier, in December, thus "exceptionally early." Methodologies were developed to evaluate the oocyte maturation competence (OMC) in females based on the size of their oocytes and *in vitro* maturation dynamics. We evaluate exceptionally early-term ovulation stimulation in breeders grown in-pond conditions. Fish are transported from Fodina Fish Farm to MATE AKI HAKI in December. Fish are anesthetized and injected with 5 µg/kg of salmon gonadotropin-releasing hormone at water temperatures of 4-6 °C. At the time of injection, oocytes from each female are sampled using biopsy and photographed for size. One day after hormonal treatment, water temperature is increased by 1 °C each day until reaching 10 °C, when the final oocyte maturation (FOM) stage is evaluated. When fish oocytes reach germinal vesicle breakdown, the genital papilla of the respective female is sutured, and fish are moved to a 12 °C tank. This thermal transfer ensures ovulation in the following 24 h. Ovulation is evaluated at 9-, 15-, 20- and 24-hours post-suture. Non-synchronized ovulation is common in pikeperch but the described protocol enables high synchronization efficacy.

Fertilization takes place immediately after egg collection; egg adhesiveness removal is performed using a milk solution for 30 minutes, and finished with a kaolin solution bath. Egg volume is measured and stocked in the incubation jar. During incubation, eggs are cleaned from dead eggshells 2x per day. The volume of remaining embryos is again measured at 72 h post-fertilization to calculate the embryo survival rate, commonly used for the evaluation of the egg quality. One day after eye pigmentation, incubation water is hyperoxidized to force synchronous hatching. This study will determine the correlation between oocyte size at the time of hormone injection, latency time and embryo survival rate. Finally, feasibility of the artificial reproduction in the exceptionally early term will be discussed.

EFFICACY OF AN IMMERSION-BASED FORMALIN-KILLED VACCINE IN LARGEMOUTH BASS *Micropterus salmoides* AGAINST MOTILE *Aeromonas septicemia*

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Aquaculture expansion in the United States has brought novel challenges as producers look to magnify and diversify their operations. Recent data has indicated increased largemouth bass (LMB; *Micropterus salmoides*) production as culturists aim to capitalize on the high market value. While this lucrative opportunity has provoked much interest, production facilities must navigate LMB through their critical early-life stages. As one of the leading causes of mortality in warmwater culture, motile *Aeromonas* septicemia (MAS; including *Aeromonas hydrophila* and *Aeromonas veronii*) poses a significant threat to production facilities. Providing culturists with practical alternative tools to combat disease, such as autogenous vaccines, can be valuable for reducing mortality.

To determine the optimal timeframe for vaccinating LMB fingerlings, three experimental vaccination periods of 0-, 2-, and 4-months will be studied. At time 0 the fish will be ~5 g. For each experimental timepoint, formalin-killed cultures of *A. veronii* (ARS-LMB-32-2018) and *A. hydrophila* (ARS-LMB-2022-09) will be administered via a 30 min static immersion bath. The LMB will be monitored for 30 days post-vaccination (dpv) to allow the specific immune response to be generated. Thereafter, spleen, kidney, and distal intestines will be sampled at 0-, 15-, and 30 dpv for targeted gene expression to assess vaccine-induced immune responses. Microbial gut communities will also be characterized. At these times, sera will also be collected to evaluate *Aeromonas* spp.-specific antibody titers via ELISA. Additionally, morphometric analyses will be conducted to observe notable changes in response to vaccination. At 30 dpv, LMB will be challenged using a standardized fin-clip methodology and subjected to a 1 h static bath at ~2×10^{7} CFU mL^{-1}. Cumulative percent mortality will be monitored incrementally through the 7-day challenge duration.

With this long-term study, we aim to improve the understanding of LMB fingerling response to formalin-killed immersion bath vaccinations of *A. veronii* & *A. hydrophila* and the potential for mortality reduction. Data collection of 2- and 4-month timepoints is underway and future results will be discussed upon completion. Studies such as these will provide vital information to producers to reduce mortality, avoid antibiotic usage, and increase product security as the culture of the species expands for food-fish production.

THE EFFECT OF PREDATOR REMOVAL AND CAGE DISTURBANCE ON THE GROWTH AND SURVIVAL OF PLANTED SOFT-SHELL CLAMS *Mya arenaria*

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The soft-shell clam (*Mya arenaria*) is an infaunal, filter-feeding bivalve native to coastal and estuarine habitats between Nova Scotia, Canada, and North Carolina, USA. Commercial-scale aquaculture and harvest of the species is limited to the northern portion of its range, yet there are benefits to growing soft-shell clams in warmer climates; cultured clams grown over the winter and spring in the Chesapeake Bay exceed growth rates of 1 mm shell length added per week and reach market size of 50 mm in under a year. Growth and survival of soft-shell clams, however, are negatively impacted by recruitment of predators and elevated water temperatures (> 28 °C) over the summer in Chesapeake Bay. This project investigated whether grower interventions (i.e. removing sediments and recruited predators from cages) could enhance soft-shell clam growth and survival during the summer grow-out season. Between 15 May 2024 and 17 July 2024, 90 soft-shell clam aquaculture cages with ¼-inch flexible predator-deterrent mesh were deployed across three locations in the York River, VA. Clams were planted at a density of 1000 individuals per m^2 (66 clams per cage), and cages were assigned one of 5 treatment groups: weekly or biweekly sediment removal; weekly or biweekly predator and competitor removal; and an undisturbed control. Planting location and temperature were significant predictors of soft shell clam survival, but treatment group had no significant impact on survival. Treatment group was a significant predictor of growth, with significantly smaller clams in cages handled more frequently than the less-handled and control cages, suggesting that cage disturbance is detrimental to the soft-shell clam grow-out cycle.

EVALUATION OF TRANSLUCENT POST-LARVAE DISEASE (TPD) RESISTANCE IN VARIOUS FAMILY LINES OF PACIFIC WHITE SHRIMP, *Penaeus vannamei*

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Translucent post-larvae disease (TPD) is a recently emerged disease in shrimp that poses a major threat to hatcheries, and in grow-out farms, particularly in the first few weeks after stocking ponds with post-larvae. The disease was first discovered in China in 2020 and attracted significant attention due to its potential to cause a large-scale mortality. A highly virulent strain of *Vibrio parahaemolyticus*, known as *Vibrio parahaemolyticus* causing TPD (V_{pTPD}), was found to be etiologic agent of the disease.

Previous comparative studies on the pathogenicity of different V_{pTPD}-encoded proteins in post-larvae of *P. vannamei*, along with mass spectrometry, genomic analysis, epidemiological data and experimental bioassay, identified a novel virulence protein called *Vibrio* high virulent protein (*vhvp*), as a potential key virulence factors of the disease. The virulence factor is encoded by the *vhvp-2* gene, located on a 187,791 bp plasmid. Further genomic analysis revealed two additional potential virulence genes, *vhvp-1* encoding a protein larger than 100 kDa and *vhvp-3* encoding another virulent protein about 100 kDa. Both genes are located on the same plasmid.

For nearly four decades, the U.S. aquaculture industry has been the global leader in exporting high quality, healthy, disease-free broodstock and seed stock to aquaculture producers worldwide. Considering the threat that TPD poses to shrimp hatcheries in the US, there is an urgent need to develop TPD-resistant lines in *P. vannamei.* To investigate the genetic susceptibility of commercially available line of *P. vannamei* to the disease and explore potential to develop TPD-resistant lines, experimental challenges are being conducted using several family lines at an approximate growth stage of post-larval stage 20 (PL20). After receiving *P. vannamei* families from various hatcheries across the US, each family line is exposed to *V. parahaemolyticus* causing TPD via an immersion challenge route at 10^4 cfu/ml. Mortality data will be collected for each family lines and histopathology will be performed on representative samples. The bacteria load of *V. parahaemolyticus* in the target organ will be measured using a real-time PCR, targeting the virulence genes, *vhvp-1, vhvp-1* and *vhvp-3.* Comparison of these data among family lines with varying genetic background will help to determine the level of resistance and identify a genetic basis of resistance. The information will be valuable in developing TPD-resistant lines in *P. vannamei* shrimp by the US shrimp hatcheries.

THE GREAT LAKES AQUACULTURE COLLABORATIVE (GLAC) COMMUNITY OF PRACTICE

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The Great Lakes Aquaculture Collaborative (GLAC) was formed in 2019 to provide relevant, science-based initiatives that support an environmentally responsible, competitive, and sustainable aquaculture industry in the Great Lakes region. With support from the National Oceanic and Atmospheric Administration and National Sea Grant, our community of practice will continue through 2027 (8 years) and, if we remain relevant, beyond that. In this presentation we reflect on what has been effective in our community of practice, how it has evolved over the last six years, and what lies ahead.

The foundation of our effectiveness is that we built strong relationships with the aquaculture industry as the backbone of our work. We accomplished this through our industry advisory groups, one representing each Great Lakes state. We have convened these groups at least twice annually to understand the needs of the industry in each state and gain insightful feedback about GLAC's efforts. The members of these groups have changed over the years, but they keep us on course and do not hesitate to tell us when we are veering off into territory irrelevant to their needs.

GLAC maintains connections to aquaculture research. In the first iteration of the collaborative, research funding was built into the project with researchers as part of our collaborative working on questions relevant to our region. This was effective in training undergraduate and graduate students in aquaculture topics and publishing new research relevant to the Great Lakes region where aquaculture research was lacking. In subsequent iterations of our collaborative, funding did not support research, but we used our connections with our advisory groups and the biennial Sea Grant requests for proposals (RFP) from each state to ensure that aquaculture topics were included in research RFPs. So far this has resulted in three aquaculture research proposals that were funded for the 2024-2026 cycle.

Through listening to the needs of our aquaculture industries, we have turned most recently to aquaculture communication and literacy. We have expanded our audience from producers to adult consumers, K-12 students, fisheries managers, legislators, and other policy makers. Ultimately, the strengths of our collaborative are the relationships and trust we have built among Sea Grant aquaculture extension, researchers, and aquaculture producers across the Great Lakes region.

ADVANCING THE GREAT LAKES AQUACULTURE COLLABORATIVE (GLAC)

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The Great Lakes Aquaculture Collaborative (GLAC), one of the National Sea Grant supported Aquaculture Hubs, was formed to provide relevant, science-based initiatives that support an environmentally responsible, competitive, and sustainable aquaculture industry in the region.

We will provide updates about our most recent two years of GLAC activities including some of our outcomes and work that is still in progress.

1) We have strengthened GLAC's network leadership by maintaining collaboration among the Great Lakes Sea Grant programs and our state and regional advisory groups. In addition, we have directed aquaculture research in our region through contributing research topics to the Great Lakes Sea Grant programs' biennial requests for proposals. This resulted in three aquaculture research projects funded in our region during the 2024-2026 period.

2) We have supported our state aquaculture association annual conferences by assisting with planning, funding speakers, and supporting travel for underrepresented students to attend and speak. We have also supported students and early professionals with travel scholarships to attend both regional and national meetings.

3) We are continuing to develop education materials about aquaculture to deliver to our state agencies, policymakers, and legislators. Members of our collaborative have received separate funding from National Sea Grant to develop aquaculture tours for legislators and we are creating a tool kit for aquaculture producers in the Great Lakes region to help them engage with legislators about aquaculture in their state. Based on feedback from our GLAC state and regional advisory groups, this tool kit is something producers would value as they work with their state associations to raise the profile of aquaculture.

4) We are working with our state regulators to synthesize the existing aquaculture laws, regulations, and policies within the Great Lakes states and evaluate how regulatory agencies interpret and implement these rules with the industry. In addition, some state Sea Grant programs are working to provide simplified guides to aquaculture regulations within their states because one common issue we hear from our producers is that regulations are overly complex, not well organized and can be confusing.

Finally, we have received funding for an additional three years of our hub and we will preview our objectives to advance aquaculture communication and literacy in our region.

INCREASING GOLDEN SHINER *Notemigonus crysoleucas* PRODUCTION FOR BAIT IN MINNESOTA

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Over the last twenty years the supply of Golden Shiner in Minnesota has decreased while demand by anglers for use as bait has increased and far exceeds in-state production. Minnesota reported $101,000 in sales of Golden Shiner in the 2018 Aquaculture Census which was about 58% of the $175,000 reported in the 2013 census and 24% of the $425,000 reported in the 2005 census. Recent projections estimate a deficit of more than 10,000 gallons of Golden Shiner annually. Consequently, there is pressure from some anglers, bait dealers, and legislators to import Golden Shiner from other states, though this is currently prohibited. The primary concerns are that importation can introduce aquatic invasive species, disease, and parasites that may negatively impact native fish communities. Recreational fishing is one of Minnesota's largest industries, with an estimated direct economic value of $5.9 billion. Our study explores strategies to increase Golden Shiner abundance in Minnesota as an alternative to importation.

Production of Golden Shiner is limited due to slow growth rates in Minnesota where it takes two years for Golden Shiner to reach market size (8-12 cm) in natural ponds. We are exploring four different strategies to grow Golden Shiner to market size in Minnesota within one growing season (May-October). Our strategies include: 1) intensive indoor rearing of Golden Shiner using a recirculating aquaculture system (RAS); 2) growing Golden Shiner in an indoor recirculating aquaponics system; 3) stocking outdoor, constructed ponds with newly hatched Golden Shiner sac-fry (0.5-1 cm); and 4) producing feed-trained Golden Shiner frylings (2-3 cm) indoors before stocking them into outdoor constructed ponds.

Year one (2022) of our project produced optimistic results and provided future areas of research to pursue. Year two (2023) built upon year one results and we saw increased growth in most strategies. The production and growth of Golden Shiner in ponds stocked with both sac fry and feed-trained fry was variable but, in most cases, growth exceeded that of year one. Development of the aquaponics strategy was delayed in year one but was successfully implemented in year two. Unfortunately, we ran into an issue in our year two RAS strategy. We will summarize total production, growth and size structure at harvest for each strategy.

A major benefit from this project has been to develop strong working relationships among those in the aquaculture industry, state government and academia. The project has drawn high interest from bait dealers, aquaculture producers, the Minnesota Department of Natural Resources and the media. If successful and cost effective, these strategies could become a new model for production of a variety of minnow species used as bait in Minnesota and other northern climates.

DUKE AQUAFARM: A DEMONSTARTION SITE FOR OYSTER AQUCULTURE RESEARCH, EDUCATION, AND COMMUNITY ENGAGEMENT

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The Duke Aquafarm was established in 2018 as a demonstration site for shellfish aquaculture. The overall objective was to expose students to oyster aquaculture by providing immersive experiences in the marine environment where they work to produce food they can enjoy themselves. The Duke Aquafarm is a 0.6-acre farm in Tar Bay (Atlantic Beach, NC) nestled in the salt marshes adjacent to Fort Macon on the Beaufort Inlet. The farm is a grow-out site consisting of approximately 200 floating bags. Labor for the farm is based on student volunteers. Students sign up for weekly workdays (or rather "fun-days") where they take a short boat ride from the Duke University Marine Lab (DUML) to Tar Bay, get in the water to work on the farm for a couple of hours, and then take a short boat ride back to DUML. Most of the work is not strenuous and includes weekly flipping of the bags, cleaning and repairing gear, and splitting oysters into multiple bags when the bags become too heavy.

Since its establishment, the research and educational value of the Duke Aquafarm has expanded to support many courses as well as student research projects. For example, the Biodiversity of Marine Invertebrates course routinely collects invertebrates from the farm for their labs, and the Aquaculture and the Environment course takes field trips to the farm every semester. Student research projects conducted using the Duke Aquafarm include analyzing microplastics at the farm, evaluating the fate of ingested microplastic particles, monitoring spat recruitment, population genomics of wild oysters, developing sensors to monitor oyster gaping, and environmental monitoring (temperature, salinity, DO, pH, and depth). Future research projects supported by the Duke Aquafarm include continued environmental monitoring with a goal of understanding potential stressors, performance of genetic lines under these environmental stressors, genetic/genomic analyses of local wild oysters, and hatchery production of oysters for both the farm and lab/field-based experiments.

Student response to the Duke Aquafarm has been overwhelmingly positive with hundreds of students visiting the farm every year since its inception. The farm has also served as a source of community building for the university at large, providing student-grown oysters to many oyster roasts on the DUML campus. This engagement has extended beyond Duke as well, with an increasing number of public groups (e.g., local Boys & Girls Clubs, Scouting America) having visited and learned about the operational and research activities of the Duke Aquafarm.

VIRGINIA SEAFOOD AGRICULTURAL RESEARCH AND EXTENSION CENTER - PROGRAMMATIC OVERVIEW

Michael H. Schwarz*, Jonathan van Senten, Yiming Feng, Katheryn Parraga-Estrada, Mohammad Zarei, Fernando Goncalves, Wendy Stout, Taozhu Sun, Stephen Urick, Ethan McAlhaney, Keri Rouse, Tiffany Wood, Noah Boldt, Charles Clark, and Jireh Clarington

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The Virginia Seafood Agricultural Research and Extension Center of Virginia Tech is dedicated to producing applied research that addresses the needs of both industry and the community. Our core research and Extension programs focus on seafood safety and the quality of wild-caught and cultured animals and products, providing business and marketing support for the commercial fisheries(?) and aquaculture industries. We also specialize in engineering, thermal processing, bioprocessing, intensive recirculating aquaculture, aquaculture nutrition, and education/outreach for both industry stakeholders and consumers. Our expanding programmatic thrusts include sustainable Food Production Systems, Alternative Feeds and Foods, Cellular Agriculture, AI, Machine Learning and Robotics, Economics and Marketing. We assess policy and regulatory impacts, farm production economics, economic impact analysis, financial benchmarking, and the integration of automation and robotics. Additionally, we are committed to K-12 Outreach and Experiential Learning and enhancing Coastal Resilience.

LESS IS MORE: SMALLER LOT SIZES IMPROVE TRIPLOID INDUCTION AND HATCH RATES OF WALLEYE *Sander vitreus*

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Utah Division of Wildlife Resources (UDWR) has sought to improve triploid induction and hatch rates of Walleye *Sander vitreus*. To induce triploidy, fertilized Walleye eggs are first subjected to an anti-adhesion treatment before receiving a pressure shock. Historically there has been a desire to treat as many eggs as possible at a time to maximize output. However, this can result in poor induction rates and low egg survival. Over several spawning seasons, UDWR experimented with multiple egg adhesion and pressure treatments and found that smaller lot sizes greatly increased both triploid induction as well as hatch rate of pressure treated eggs.

PIONEERING AQUACULTURE STUDIES IN MICROGRAVITY FOR BENEFITS ON EARTH

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Microgravity research has already provided insights into terrestrial agriculture, enhancing our understanding of phenomena like plant gravitropism and stress responses. However, aquaculture, a critical and growing food source, has not yet received similar attention. Aquaculture research in space has been limited since the 2012 retirement of the Aquatic Habitat (AQH) onboard the International Space Station (ISS), which was developed by the Japanese Aerospace Exploration Agency (JAXA). Monolith LLC is proposing the development of a new aquaculture facility to fill that gap.

Aquatic biology research in space has been centered around small, model organisms like *zebrafish*, leaving true aquaculture organisms (oyster, shrimp, algae, etc.) largely unexplored. A newly proposed facility will take lessons learned from the AQH and provide more capability for research. The Monolith facility will feature: 30L usable volume, Variable Day/Night Cycles, Automatic feeding/circulation, Command and Data Handling (Imagery, Temperature, Salinity, pH, etc.).

The first mission will be performed to study the growth and physiology of oysters in the absence of gravity. There is currently limited to no research performed on mollusks in space. Terrestrially, the oyster is a critical organism for the remediation of salt and brackish water. Understanding oyster physiology in microgravity may uncover methods to reduce mortality rates, which is critical for the health of terrestrial coastal ecosystems. Furthermore, research and public interest in the oyster will increase awareness of the ongoing efforts to restore oyster populations in coastal ecosystems. Insights gained from this research will lay the foundation for future breakthroughs in aquaculture across species, fostering more sustainable food sources on Earth.

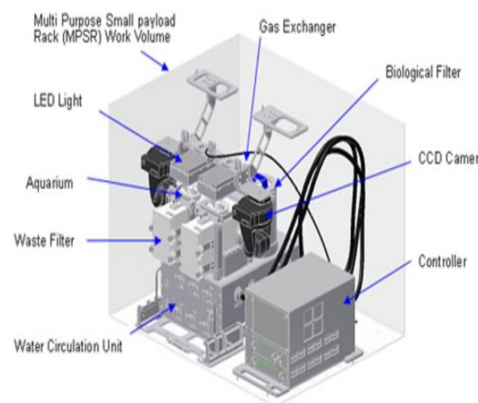

Figure 1: JAXA Developed AQH

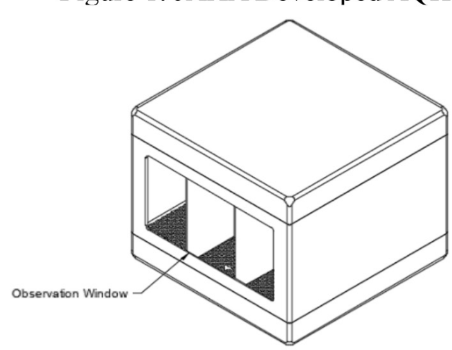

Figure 2: Monolith Blue

A new dedicated aquaculture facility will act as a bridge between ground-based research and flight-based research. This facility will provide both terrestrial biologists and astrobiologists the ability to perform research on aquatic organisms and systems in a space environment. The goal is to provide tangible scientific breakthroughs supporting terrestrial aquaculture and aquatic habitat conservation.

Future missions will study a variety of critical organisms such as shrimp, fish, algae, and kelp, laying the groundwork for more complex, larger habitats with diverse ecosystems, paving the way for breakthroughs in sustainable aquaculture on Earth.

LONG-TERM SHIFTS IN THE SEASONAL PHENOLOGY OF *Perkinsus marinus* IN DELAWARE BAY

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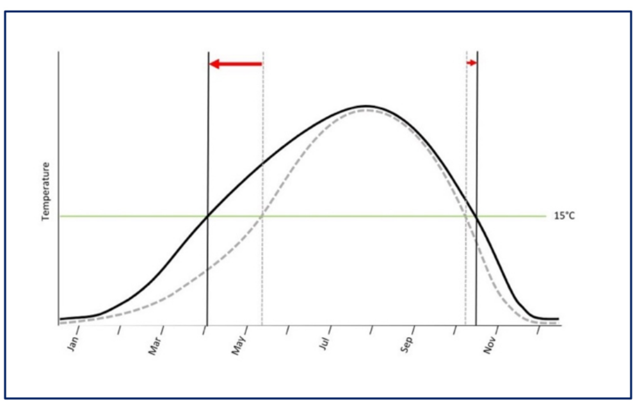
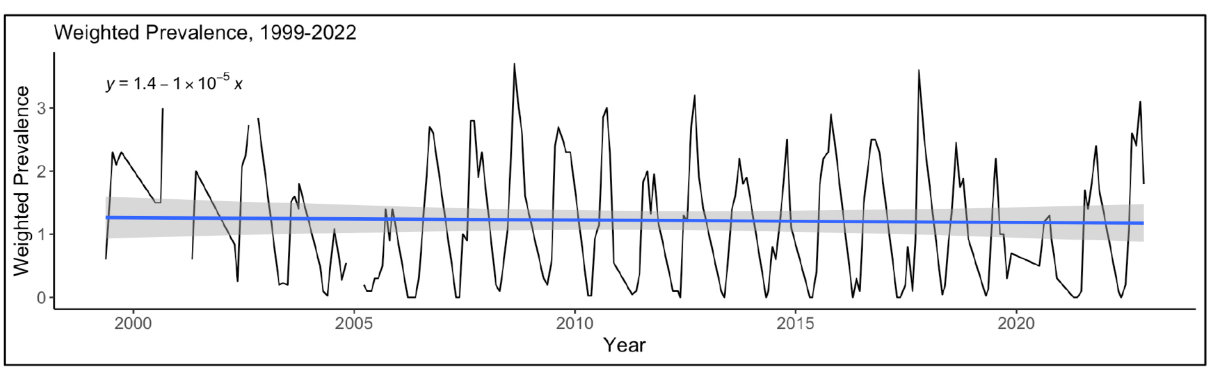
Perkinsus marinus (dermo) is a protozoan pathogen of the eastern oyster, *Crassostrea virginica*. Infection is often fatal and can result in mass mortality. Temperature and salinity are the primary drivers of disease patterns. The disease extends from the Gulf of Mexico to the coast of Maine, which contains latitudinal variations in temperature and salinity that influence the seasonal patterns of infection. In the northern end of the range, dermo prevalence and intensity are depressed during winter with a large surge in late summer and fall, whereas south Atlantic and Gulf populations show flatter seasonal patterns with disease prevalent throughout the year.

Dermo became endemic in the Delaware Bay in the 1990s following a period of extended warm temperatures. Long-term patterns indicate the infection and remission season begins and ends as temperatures reach then fall below about 15°C. The number of days above 15°C has increased by approximately twenty days since 2004. Temperatures above this threshold are moving earlier into spring at a faster rate than they are moving later into fall, indicating an asymmetrical widening of the dermo infection season. Interestingly, weighted prevalence (ie, infection intensity) has not increased over that time period, which defies what is expected from the typical positive correlation between temperature and dermo infections. This raises questions about whether mortality has become decoupled from infection intensity, and whether dermo infections in the Delaware Bay are shifting to a seasonal cycle similar to that found in lower latitudes.





TIGHT JUNCTION, HYPERPLASIA, AND IMMUNE/STRESS RESPONSE RELATED GENES ARE MODULATED IN MOZAMBIQUE TILAPIA EXPOSED TO TIDALLY-CHANGING SALINITIES

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Growth in tilapia, one of the main finfish cultured worldwide, is susceptible to changes in salinity. Specifically, the Mozambique tilapia, *Oreochromis mossambicus*, a species that can tolerate marked changes in salinity, is employed as a model to elucidate how gene transcriptional patterns that underlie growth and osmoregulation respond to salinity challenges. As the primary site for the active transport of Na^+ and Cl^-, the gill is critical to maintaining osmoregulatory balance of the whole organism. Here, we investigated the branchial expression of genes associated with tight junctions, hyperplasia, and immune/stress responses in tilapia residing under tidally-changing salinities.

Mozambique tilapia were reared in either fresh water (FW) or seawater (SW) and then transferred to a tidal regimen (TR), characterized by a change in salinity from FW to SW every 6 h for 15 days. Fish exposed to a TR were sampled at the end of either the FW- (TF) or the SW-phase (TS) of the tidal cycle. Using RNA-Seq, we then compared the gills of fish sampled in TF and TS versus those acclimated to steady-state FW and SW. Transcripts with the highest differential expression between salinity regime groups were followed up with qPCR analyses.

While plasma osmolality was inversely related to environmental salinity in fish acclimated to both steady-state and tidal salinities, plasma glucose was higher in fish at TF compared with all other salinity groups. The most abundant and differentially expressed tight junction protein (TJP) transcripts included claudins (*cldn-4 like*, *cldn-like ZF-A89*, *cldn7b*, and *cldn23a*), occludins (*oclna* and *oclnb*), and *tjp1a* and *tjp3*. In general, the expression of TJP genes varied to a greater extent between the FW- and SW-phases of the TR (TF and TS, respectively) than between steady-state FW and SW. A similar pattern was observed with the stress/immune response-associated branchial transcripts, *il1b*, *gstr*, and *hsp40*, but not *cmc20*. Lastly, in fish originally reared in SW, the hyperplasia-associated transcript, *pcna*, showed differential expression between TF and TS alongside an overall downregulation of *npcc*, *mk167*, and *ets1* in fish sampled in both TF and TS. Taken together, these results indicate that branchial responses differ between tilapia residing in steady-state versus tidally-changing salinities and thereby provide insights into how cultured fish are impacted by marked and frequent salinity changes.

[Supported by HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), and NSF (IOS-1755016 and -1755131)]

SUPPLEMENTATION WITH NUCLEOTIDES AND A BIOACTIVE OLIVE EXTRACT ENHANCES GROWTH PERFORMANCE IN PACIFIC WHITE SHRIMP

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The inclusion of functional ingredients in feed for Pacific white shrimp (PWS) can improve their growth as well as their overall health. Nucleotides have been shown to positively impact PWS health by modulating their immune response. Fruits and leaves of the olive oil tree contain bioactive compounds with anti-inflammatory, antioxidant, and antimicrobial effects. The objective of this trial was to evaluate the effects of the combined use of nucleotides and a bioactive olive extract (OE) on PWS performance.

The following products were used: a yeast extract containing a high amount of nucleotides (NU; Nucleoforce®, Bioiberica S.A.U., Spain) and an OE (Aquolive®, NATAC Biotech SL, Spain). After acclimatation for 2 days, 1,000 specific pathogen-free (SPF) PWS *Litopenaeus vannamei* (1.23 ± 0.23g) were classified into 4 groups (5 replicates/group; 50 PWS/500L-tank) and received different diet supplementations: 500ppm NU (NU500), 500ppm OE (OE500), 500ppm NU with 500ppm OE (NU500OE500) or no supplementation (Control group).

After 56 days, all supplemented groups showed significantly higher ($p<0.05$) final mean weight, mean weight gain and average daily gain (ADG), compared to the control group. Additionally, significant improvements ($p<0.05$) in final biomass, specific growth rate (SGR), feed intake and feed conversion ratio (FCR) were observed in the NU500 and NU500OE500 groups, compared to Control. Remarkably, PWS in the NU500OE500 group achieved the best production efficiency, with an FCR significantly lower ($p<0.05$) than that of the Control and OE500 groups (Table 1).

In conclusion, our data shows that the tested NU and OE have a positive impact on PWS growth performance. Moreover, our results also indicate that the 1:1 NU/OE combination leads to a greater performance benefit, especially seen in feed efficiency. The bioactive compounds provided by the combined use of such nucleotide-rich yeast extract and OE might therefore become a convenient tool to be used in feed for farmed PWS to optimize their growth performance.

Group	Initial mean weight (g)	Final mean weight (g)	Final biomass (g)	Mean weight gain (g)	ADG (g/day)	SGR (%/day)	Feed intake (g)	FCR
CONTROL	1.23 ± 0.05^{a}	22.03 ± 4.25^{a}	858.80 ± 23.59^{a}	20.80 ± 0.55^{a}	0.37 ± 0.01^{a}	5.15 ± 0.09^{a}	$1{,}032.16 \pm 10.91^{a}$	1.30 ± 0.05^{a}
NU500	1.21 ± 0.05^{a}	23.43 ± 4.23^{b}	988.04 ± 26.71^{bc}	22.22 ± 0.89^{b}	0.40 ± 0.02^{b}	5.29 ± 0.11^{b}	$1{,}052.27 \pm 0.42^{b}$	1.13 ± 0.03^{bc}
OE500	1.22 ± 0.03^{a}	23.06 ± 4.90^{b}	922.44 ± 78.75^{ab}	21.85 ± 0.64^{b}	0.39 ± 0.01^{b}	5.25 ± 0.06^{ab}	$1{,}057.54 \pm 5.69^{ab}$	1.24 ± 0.11^{ab}
NU500OE500	1.22 ± 0.03^{a}	23.74 ± 4.77^{b}	$1{,}014.43 \pm 21.83^{c}$	22.51 ± 0.72^{b}	0.40 ± 0.01^{b}	5.29 ± 0.05^{b}	$1{,}058.80 \pm 13.21^{b}$	1.09 ± 0.05^{c}

TABLE 1. Performance parameters of shrimp after 8 weeks. Different superindex letters indicate statistically significant differences ($p<0.05$).

RESTORATION AQUACULTURE FOR SOFT-SHELL CLAMS, *Mya arenaria*, IN VIRGINIA

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Soft-shell clams (*Mya arenaria*) are suspension-feeding, infaunal clams that have a native range on the North American east coast from Canada to Georgia. They are ecologically as well as commercially important in many benthic ecosystems. Natural populations of soft-shell clams have persisted in Virginia, and soft-shell clams have been cultured for many years in Maine. They have a wide environmental tolerance for temperature (-2 – 28 °C) and salinity (4 – 35 psu), which allows them to grow in many locations. *Mya arenaria* is a broadcast spawner with high fecundity and planktonic larvae that allow wide dispersal, but postsettlement mortalities are typically high. Population abundances in Virginia are currently low, which has led to recent efforts and discussions regarding conservation and restoration to raise populations to an alternative stable state.

We have been able to spawn *Mya* in VA, and there is potential capacity to grow them to seed size for transplanting for restoration if we can overcome temperature extremes and predation. Growth is quick in VA, as soft-shell clams can add >1 mm shell length/week when grown on-bottom from the fall to spring seasons. In Virginia, substantial mortality of *Mya* occurs when summer temperatures rise above the clam's thermal tolerance of 28°C. Predation is also a key determinant of *Mya* survival. Current populations of soft-shell clams in Virginia have been kept at a low stable state through predation, mainly from blue crabs. In addition, clams persist only in structured habitats, and sources of *Mya* broodstock likely reside in habitats such as seagrass beds and shelly habitats. Restoration of this species would need to focus on saturating structured habitats with high densities of seed in early fall to allow grow-out to sexual maturity (> 40 mm) and spawning before high temperatures hit in the summer. Experiments to determine the feasibility of restoration are underway and are showing promising results.

Figure 1. Soft-shell (*Mya arenaria*) juveniles from the VIMS Acuff hatchery.

THE APPLICATION OF KAOLIN CLAY IN TROUT HATCHERIES TO MINIMIZE *Flavobacterium* INFECTIONS AND MORTALITY IN TROUT FRY

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Flavobacterium spp. infections (columnaris, bacterial coldwater disease, bacterial gill disease) can be devastating for aquaculture businesses. *F. columnaris* alone caused $40-50 million in losses to aquaculture operations in 2015. The typical treatment methods include antibiotic feeds for internal infections and applications of potassium permanganate, hydrogen peroxide or chloramine-T to combat infections on the outside of the fish.

Past research has indicated that bacteria can bind to inert particulates. In 1915 kaolin clay was tested as a treatment for removing bacterial from the throat and nose. More recently kaolin clay has been researched for removing *flavobacterium* from fish culture tanks, with promising results.

Starting in June of 2024, weekly kaolin clay treatments of 1 g/l for one hour were added to trout fry tanks in a hatchery to gauge; 1) the physical tolerance of small trout (three-week old, 1500/lb) to kaolin clay, 2) if there is a hindrance to growth, and 3) anecdotally evaluate if there is a reduction in fry mortality while fish are in the hatchery.

GROWTH PERFORMANCE AND HEALTH OF FINGERLING LARGEMOUTH BASS *Micropterus salmoides* FED FERMENTED CORN PROTEIN

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Largemouth bass (LMB) are a popular sportfish in the U.S., and increased attention has given promise for this species to be cultured as a food fish. The aquafeed industry relies on animal protein as the primary ingredient in dietary formulations. Due to increased consumer demand, price volatility, and the risk of exploitation of aquatic species, the industry has pushed for a more sustainable approach to dietary formulation. New alternative protein sources such as poultry-by-product (PBP) and soybean meal (SBM) have displayed the potential to serve as replacement protein sources in formulated diets to influence the growth performance and health of several aquaculture species. Currently, the price and supply of SBM have become highly unstable due to global conflicts, which have caused turmoil in the supply chain. The search for a new alternative protein source is dire to keep aquafeed production profitable yet sustainable. The U.S. is one of the biggest producers of ethanol and fermented corn protein (FCP), which is one of the by-products of the ethanol industry. The CFP has served as an excellent protein source in feed production for the agriculture industry.

To evaluate the potential replacement of SBM with FCP, this study investigated growth performance health in LMB fingerlings. Twenty individual tanks, within a recirculating aquaculture system (RAS), were each stocked with twenty LMB fingerlings (21.76 ± 0.13 g). The fish were fed one of the four experimental (5, 10, 15, and 20% FCP) diets or the control (0% FCP) diet for 12 weeks. This trial is currently ongoing. No differences in tank biomass (P = 0.279), average individual weight (P = 0.086), FCR (P = 0.199), tank biomass gained (P = 0.144) have been detected after four weeks. On the contrary, differences in weight gain (%; P = 0.018) and thermal growth coefficient (TGC; P = 0.025) have been observed. At the conclusion of the growth trial, fish will be sampled for whole-body proximate analysis, blood chemistry (including serum lysozyme), intestinal and liver gene expression, and histological analyses. Similar to soy-based proteins, FCP could provide benefits to growth performance all while reducing LMB production costs. Furthermore, this ingredient may provide potential health benefits to increase production efficiency.

USE OF RELATIVE GROWTH TEMPERATURE INDEX (RGTI) TO ESTIMATE POTENTIAL GROWTH AND DAILY RATION FOR LARGEMOUTH BASS ACROSS BOTH WATER TEMPERATURE AND FISH SIZE

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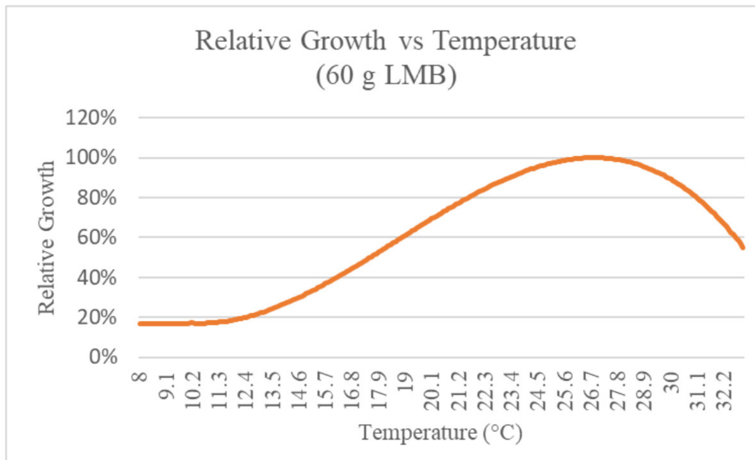
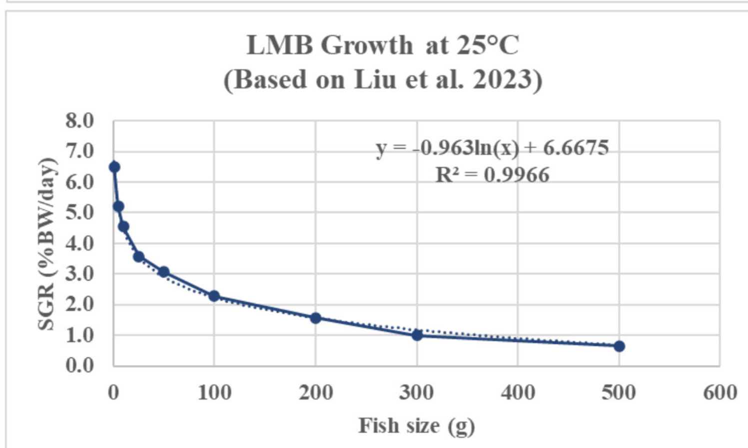
Underfeeding and overfeeding fish in aquaculture are issues facing production managers, especially those growing Largemouth Bass. As a guide, it is useful to have a tool to estimate the daily ration associated with growth potential across both fish size and water temperature to capture growth opportunities through the temperate growing season. Unfortunately, fish growth is not linear, and condition factor may change with fish size. This presentation will describe development of a tool to estimate daily ration associated with potential growth using RTGI for largemouth bass. The method is based on weight rather than length.

Both daily ration and potential growth may be expressed as % Body Weight/day. Potential growth equals the product of daily ration and feed efficiency. Based on this relationship an estimate of potential growth, and an estimate of feed efficiency can be used to generate a value for daily ration.

An estimate of potential growth across fish size and water temperature can be generated with two functions. The first is RTGI and the second is based on growth at a constant temperature. Both relationships are illustrated in the accompanying figures.

These relationships may be used to create a worksheet tool to construct a production plan estimating daily growth for a population of LMB. Fish number, total weight, water temperature, and target feed efficiency are necessary inputs. This tool may be applied during feed training, production of stockers, and grow out.





THE KENTUCKY STATE UNIVERSITY AQUACULTURE PROGRAM

Ken Semmens*, Andrew J. Ray, Robert M. Durborow, Noel Novelo, Waldemar Rossi, Janelle Hager, et al.

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The Kentucky State University (KSU) Aquaculture Program started in 1983 with Extension work to support Kentucky (KY) fish farmers producing catfish and trout. Early research activities focused on topics that included catfish, paddlefish, nutrition, and feed development.

The Aquaculture Research Center is located on the campus of Kentucky State University and is equipped for all aspects of aquaculture research & production. This 14-acre aquaculture campus includes 33 research ponds, a hatchery and multiple laboratories including genetics, shrimp production, aquaponics, nutrition, and disease diagnostics. A 14,400 square foot Indoor Aquaculture Production & Technologies Building houses state of the art marine freshwater RAS systems. In addition, a floating raceway facility is located at the Harold R. Benson Research and Demonstration Farm.

The School of Aquaculture & Aquatic Sciences offers many courses virtually for students of all kinds, including current employees with continuing education needs and students currently enrolled in other universities wishing to integrate aquaculture into their curriculum. Specific degree options include: a Certificate in Aquaculture and Aquatic Science, a Minor in Aquaculture and Aquatic Science, a Bachelor Degree in Agriculture, Food, and the Environment with an Aquaculture option, and a Master of Science in Aquaculture and Aquatic Science.

<u>Research teams are led by individual faculty:</u>

Aquatic Animal Health, Dr. Robert Durborow
Aquatic Animal Nutrition, Dr. Waldemar Rossi
Recirculating Aquaculture, Dr. Andrew Ray
Floating Raceways, Physiology, and Reproduction, Dr. Kenneth Semmens
Genetics and Reproduction, Dr. Noel Novelo
Aquaponics, Janelle Hager

The Aquaculture Extension program at KSU is primarily dedicated to serving small, limited resource, and minority farmers. Extension staff support KY farmers through development of factsheets, newsletter, farm visits, collaborative research, workshops, seminars, the diagnostic lab, and sharing relevant information via a stakeholder listserv.

EVALUATING WATER QUALITY ISSUES IN AQUACULTURE AREAS TO ENSURE OPTIMAL CONDITIONS FOR AQUATIC SPECIES: CASE OF THE VAAL RIVER SYSTEM IN GAUTENG, SOUTH AFRICA

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Aquaculture can be defined as the practice of raising aquatic organisms for food or other commercial uses. The health and development of aquatic organisms in aquaculture systems are significantly influenced by the quality of the water. The farmed species may experience stress, disease outbreaks, or even death as a result of poor water quality. Therefore, common water quality issues need to be addressed to ensure optimal conditions for their aquatic species. This study focused on the assessment of water quality issues in aquaculture at Vaal River system in Gauteng, South Africa to ensure optimal conditions for aquatic species.

In this study, the main stream of the Vaal River system was sampled. Most analytical methods used in this study follow the standard methods for examination. The following fundamental physico-chemical and biological parameters were assessed according to the standard methods for the examination of water: ammonia, total alkalinity, dissolved oxygen, sulphate, chloride, conductivity, total dissolved solids (TDS), phosphate, pH, temperature, E-coli, and algal pigments. These parameters were chosen because the constitute the common parameters in water quality analysis that should be frequently monitored to ensure optimal conditions for aquatic species.

The results showed the high TDS concentration (~177 mg/L) in the main stream of the Vaal river system is a potential threat to the agricultural sector, as it could be impacting on crop and aquacultural production. Oxygen concentrations are not regularly measured in the Vaal River, however, indications are the concentrations are usually relatively high (>60%). The concentrations of metals in the Vaal River is generally high, leading to frequent fish kills. Furthermore, the high TDS concentrations in the Vaal River evidently influence the turbidity of the water. And the higher pH values (6.3-8.7) in the Vaal River can be ascribed to higher algal concentrations. The high concentrations in E-coli (240 counts/100 ml) and algal pigments (69 mg/L) constitute a high risk the aquaculture production in the Vaal River system. A range of water related problems are currently being experienced by water users in the Vaal River system due to the current water quality status that prevails.

For the purposes of this study, the water quality issue needs to be assessed and addressed by re-evaluating new management options and/or application of treatment technology. The results would be helpful for the relevant authorities to select water quality monitoring parameters in the aquaculture areas.

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Aquaculture can be defined as the practice of raising aquatic organisms for food or other commercial uses. The health and development of aquatic organisms in aquaculture systems are significantly influenced by the quality of the water. The farmed species may experience stress, disease outbreaks, or even death as a result of poor water quality. Therefore, common water quality issues need to be addressed to ensure optimal conditions for their aquatic species. This study focused on the assessment of water quality issues in aquaculture at Vaal River system in Gauteng, South Africa to ensure optimal conditions for aquatic species.

In this study, the main stream of the Vaal River system was sampled. Most analytical methods used in this study follow the standard methods for examination. The following fundamental physico-chemical and biological parameters were assessed according to the standard methods for the examination of water: ammonia, total alkalinity, dissolved oxygen, sulphate, chloride, conductivity, total dissolved solids (TDS), phosphate, pH, temperature, E-coli, and algal pigments. These parameters were chosen because the constitute the common parameters in water quality analysis that should be frequently monitored to ensure optimal conditions for aquatic species.

The results showed the high TDS concentration (~177 mg/L) in the main stream of the Vaal river system is a potential threat to the agricultural sector, as it could be impacting on crop and aquacultural production. Oxygen concentrations are not regularly measured in the Vaal River, however, indications are the concentrations are usually relatively high (>60%). The concentrations of metals in the Vaal River is generally high, leading to frequent fish kills. Furthermore, the high TDS concentrations in the Vaal River evidently influence the turbidity of the water. And the higher pH values (6.3-8.7) in the Vaal River can be ascribed to higher algal concentrations. The high concentrations in E-coli (240 counts/100 ml) and algal pigments (69 mg/L) constitute a high risk the aquaculture production in the Vaal River system. A range of water related problems are currently being experienced by water users in the Vaal River system due to the current water quality status that prevails.

For the purposes of this study, the water quality issue needs to be assessed and addressed by re-evaluating new management options and/or application of treatment technology. The results would be helpful for the relevant authorities to select water quality monitoring parameters in the aquaculture areas.

DEVELOPMENT OF A TGC-BASED GROWTH MODEL FOR THE OLIVE FLOUNDER, *Paralichthys olivaceus*, AND ITS APPLICATION IN DEVELOPING A FISH GROWTH SIMULATOR ARCHITECTURE

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Temperature is one of the main factors affecting fish growth. Many studies have proposed a fish growth model considering the effect of temperature. Among these models, the TGC (thermal growth coefficient) model which digitized the influence of temperature on fish growth is the most notable. The original TGC model was made in the form of applying 1/3 as an exponent, but subsequent studies have shown that it is necessary to apply different exponent value or other constant depending on the dynamics of growth.

In this study, the original TGC model using 1/3 as an exponent and the new model using 2/3 as an exponent were compared for olive flounder. The seasonal temperature function under the conditions of the flow-through system was applied and the transition point of change in the growth dynamics was obtained by comparing the instantaneous growth rate of the two TGC models. Around 541 g of the transition point was obtained, and a combined TGC model was presented that integrated the two models (Figure 1). However, the growth prediction model based on these statistical techniques does not reflect real-time changes in each parameter and requires academic knowledge, making it difficult to use in the actual field. Recently, as the smart industry has grown rapidly, many solutions such as fish growth prediction simulators using statistical growth models have been developed. Therefore, in this study, input and output variables were classified and software architectures were presented so that the statistical form of fish growth model using TGC derived above could be applied when developing a fish growth prediction simulator (Figure 2). Deriving these growth models and interpreting them into languages in the field of ICT will enhance the field applicability of academic research results as a part of smart aquaculture technology.

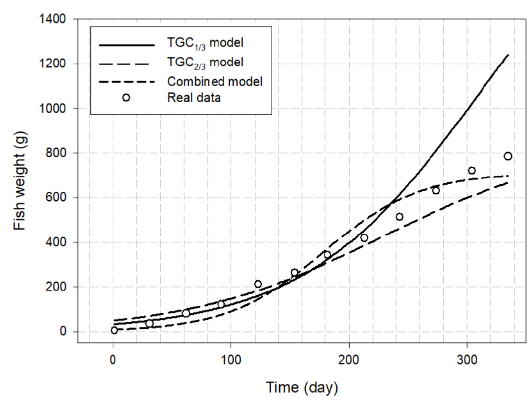

FIGURE 1. Regression graph for the three models.

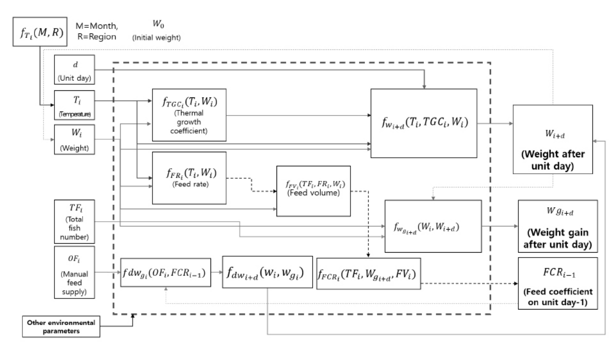

FIGURE 2. Schematic of the fish growth simulator software architecture.

K-12 PROGRAMMING AS A FIRST STEP TOWARD WORKFORCE GROWTH

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One aspect of the U.S. aquaculture industry-identified challenges and requirements for industry growth include labor cost and having adequate workforce. Among the strategies being adopted are developing workforce pipeline programs through K-12 school programs, internships, partnerships that introduces young people to aquaculture as a potential career pathway. These programs are designed to build a skilled and diverse workforce through education, training, and real-world experience to students and young professionals for aquaculture jobs. In 2019, Illinois Indiana Sea Grant (IISG) began working with school educators interested in teaching STEM through the lens of aquaponics, a branch of aquaculture that is well suited for introducing students to aquaculture and experiential learning. Since the formation of the Aquaponics in Schools program, 23 high schools in Illinois and Indiana have expanded their sustainable aquaponics and aquaculture educational programming, 10 school systems have been set-up/improved, 19 educators have received water quality training, \$2.7K in grants funds have been awarded for educators to purchase equipment, 745+ students have learned about aquaculture, teaching materials have been developed (e.g., Aquaponics: Farming Fish, Growing Greens curriculum), and water quality testing kits have been made available (Know Your H_2O – KYH2O) on loan to schools. The KYH2O program provides educators the opportunity to borrow commercial type water monitoring equipment to be used by students for collecting and analyzing real-time water quality data to reinforce water chemistry concepts in the classroom. The outreach and engagement program has taken some time, strategic planning, financial support, strong partnerships, and persistence. Using a stepped approach, IISG is looking at sustaining current programming and expanding its offerings to provide new opportunities for students to be introduced to aquaculture at younger ages and in different settings (e.g. 4-H).

ARTIFICIAL REEFS- USING PARAMETERIZED DESIGN FOR CORAL AND FISH SHELTERS

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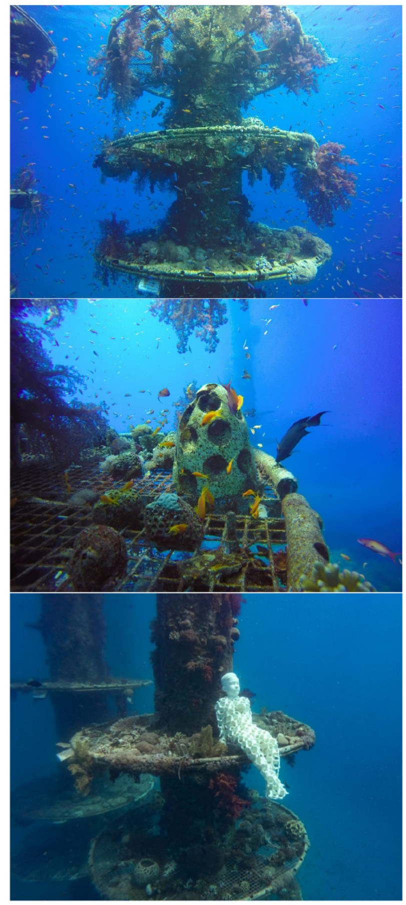
Artificial coral reefs have become an integral practice in conservation, tourism, and research of coral reefs, being used by a range of agencies worldwide. Originally relying on sunken ships and on secondary use of different objects, the trend is shifting towards purpose-designed structures, including statues and art. However, it is often difficult to identify, evaluate, and replicate the principles used in these designs, as they are not mathematically defined. Further, it is rare that designs are focused towards coral or fish, and hence it is hard to apply them in the aquaculture trade.

In our study, we sought to address these challenges by employing spatial and geometric principles in the design of 3D objects in artificial coral reefs, specially tailored towards fish recruitment and coral growth. Using a small number of well-defined parameters, we were able to control shelters for fish, spaces for invertebrates, connectivity between shelters and more. The designed structures were then 3D printed and subjected to comprehensive testing, including assessments of hydrodynamic flow and evaluation of their acceptance by fish. The results demonstrated the efficacy of our approach in creating artificial reef structures that were accepted as shelters to a diverse reef community.

By applying spatial and geometric principles, our method allows for the development of intricate and tailored structures capable of providing optimal conditions for various marine species. This advancement holds promise for the future of in-situ and ex-situ artificial coral reef design. It offers a systematic and replicable approach to examine, test and produce structures that are needed to enhance ecological functionality along with visual appeal, and to contribute to the sustainability of marine ecosystems.



Top- an artificial reef created around an abandoned oil jetty. **Middle-** experimental unit, testing parametric design, is readily accepted by fish as their home. **Bottom-** a child's statute made by parametric design, becomes a fish and tourists' attraction.

Photographs: Dr. Jenny Tynyakov- Shashar

“According to MarTech, a digital marketing provider, the world will spend $4.7 trillion on marketing by 2025. According to the Food and Agriculture Organization of the United Nations (FAO), aquaculture production in Nigeria has been growing at an average annual rate of 10% over the past decade. In 2019, Nigeria produced an estimated 136,000 tonnes of fish from aquaculture, making it one of the largest producers of farmed fish in Africa. The majority of the aquaculture production in Nigeria is based on tilapia and catfish, which are the most commonly cultivated species in the country. Other species farmed in Nigeria include Nile tilapia, Clarias Garie Pinus (African catfish), and Heterotis niloticus (Nile perch). The growth of the aquaculture sector in Nigeria has been supported by the government and development partners, who have provided infrastructure, training, and support to farmers. The government has also implemented several policies and programs aimed at promoting the development of the aquaculture sector, such as the National Aquaculture Development Plan (NADP) and the Agricultural Promotion Policy (APP). Additionally, the Nigerian government has provided support for research and development in the sector, which has led to the introduction of improved technologies and practices.

This paper focuses on the potential of digital aquaculture marketing and its potential in Nigeria.

PRELIMINARY WORK TO CAPTURE AND TRANSPORT, AND TO UTILIZE CONTROLLED RECIRCULATING MATURATION SYSTEMS TO MAINTAIN AND SPAWN STRIPED MULLET *Mugil cephalus*

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Globally, striped mullet, *Mugil cephalus*, are a commercially important fish in both wild fisheries and aquaculture. Striped mullet aquaculture began in Taiwan in the 1970's and has since been employed worldwide. Egypt leads mullet culture with 182,540 t of annual production from 2003-2012. Wild fry are often used in mullet aquaculture due to technological limitations that hinder captive breeding at commercial levels. Although popular abroad, striped mullet has received limited attention in the United States despite the potential for additional markets. These markets include feed in the aquarium industry, sale in the baitfish industry, stock enhancement in support of sustainable wild fisheries, and as a supplement for high-quality fish meal. The focus of this study was to initiate captive maturation research by starting two broodstock populations held in our photothermally controlled, fully recirculating, 28 m^3 maturation systems.

To obtain the wild adult mullet, a commercial fisherman was hired to capture fish with cast nets. Early efforts to transport the fish back to the lab resulted in high losses. Fish were transported in 450 L totes supplied with pure oxygen for ~40 mi over land (~60 min). In the first attempt fish transported with no water treatment resulted in 20% survival. A following attempt utilized Aqui-S 20e at 10 ppm resulted in 9.1% survival. Using a product called stress coat at ~130 ppm resulted in 25% survival. We finally achieved high levels of survival (85%) by using MS-222 at 14 ppm buffered with sodium bicarbonate. This transport method allowed us to establish two populations of mullet, population one at 2.3 females to 1 male and two at 3 females to 1 male.

To attempt to mature the fish, both broodstock populations were held at their approximate photothermal maturation conditions of 22°C and 12 hr of light for three months before they were sampled. No males were implanted/injected at either sampling/spawn. At the first sampling, population one was sampled and 5 of the 12 mature females, those with oocytes presenting at SGfg, were implanted with mGnRHa at a dose of 50 ug/kg. No eggs were harvested following this procedure. The second population of mullet was sampled the following week. Again, 5 of the 16 mature females were implanted with mGnRHa, this time at a dose of 100 ug/kg. Following the implantation of this second population, eggs were harvested 2- and 3- days post-implantation. The first release resulted in ~1.6 million eggs harvested and the second had ~1.3 million eggs; unfortunately, none of the eggs were fertilized. Once the fish were induced, they were phase shift out of maturation (30°C and 15 hr of light) to rest and given a hyposalinity treatment of 5 ppt for 45 days to combat the *Argulus sp*. found on the fish at the sampling. Future maturation work will be directed at incorporating mGnRHa dosing for the males, as well as the females, in hopes of achieving not only egg releases, but viable eggs from these inductions.

AQUACULTURE GRANT ACTIVITIES: UPDATE FROM SANTA FE COMMUNITY COLLEGE'S CONTROLLED ENVIRONMENT AGRICULTURE PROGRAM

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Santa Fe Community College's Controlled Environment Agriculture program (SFCC-CEA) trains students in aquaponics, hydroponics, algae cultivation and CEA operations and management. Students maintain culture systems in a 1,115m^2 off-grid greenhouse, with energy provided and stored using a photovoltaic solar array and lithium batteries. Students learn on large, semi-commercial scale systems where crops are produced year-round. Grants allow the program to grow while focusing on different areas of aquaculture. Our NSF ATE 3-year grant provides college level courses to high school students through dual credit offerings, using a blending teaching modality. Students earning college credit have an incentive to continue their college pathway at SFCC to finish their certificate or AAS degrees in CEA. A pipeline has developed, and students are moving from high school to college to study aquaculture. A NIFA Tribal College Research grant was awarded last year to Navajo Technical University (NTU), with SFCC receiving a subaward. The work focuses on hydroponic food production at NTU and the local Navajo Chapter House. SFCC is also collaborating on another NIFA grant awarded to the University of Arizona and New Mexico State University. This grant provides outreach to Hispanic and underrepresented high school students and scholarships for underrepresented college students pursuing careers in Fisheries Science. Texas State University was awarded a NIFA-AFRI grant to study novel fish species in aquaponics systems. SFCC-CEA is hosting a graduate student from Texas State for two summer sessions, with the research being conducted using the SFCC aquaponic systems. This grant is testing the impacts of Hydrogen Peroxide on fish welfare and determining the impacts on crop production in a coupled aquaponic system. Year one is complete and plans for the second and final year are currently being made testing novel fish species for aquaponic producers to consider. Finally, our Algae Cultivation program supports students through funding from the Algae Foundation. SFCC is the lead institution in curriculum and microcredential development for the Algae Technology Educational Consortium funded by a Dept. Of Energy contract to the Algae Foundation. Other grant work using hydroponic systems will be discussed, including an EPSCoR track II grant that introduces CEA and hydroponics to schools and tribal communities. Once hydroponics is established, it opens the door to move into more aquaponic production trails.

EVALUATION OF JUVENILE BLUE TILAPIA *Oreochromis aureus* FED PRACTICAL DIETS SUPPLEMENTED WITH BLACK SOLDIER FLIES *Hermetia illucens* REARED ON ALGAL TURF SCRUBBER BIOMASS

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Tilapia is one of the most widely cultured species of fish in the world for human consumption, as a forage for sport fish, and for biological control of nuisance vegetation. To keep pace with their rapid production, tilapia aquaculture should rely on sustainable practices. Since feed constitutes a major operating expense, cost effective sustainable ingredients should be utilized in their diets. Black soldier flies, *Hermetia illucens*, are found worldwide and can be economically and sustainably produced using waste products such as food remains and manure. Black soldier fly meal has been successfully incorporated into aquaculture diets at varying levels. Since the protein, fat, and ash content of the black soldier flies has been shown to be affected by the feed source they consume, care needs to be undertaken with their diet. One potential source of black soldier fly feed may be obtained from harvested algal turf scrubber biomass. Algal turf scrubbers are a simple system which pulses nutrient rich water over a sloped flow-way to produce an algal biomass. The biomass is harvested periodically to maintain system performance. Harvested algal turf biomass has previously been used for sea urchin feed as well as a supplement in potting soil. This study was conducted to determine the suitability of algal turf scrubber biomass fed black soldier flies as a dietary ingredient in practical tilapia diets.

Algae turf scrubber biomass was harvested weekly from a 9.14-meter algal turf scrubber placed at the outflow of the Statesboro wastewater treatment facility. The turf scrubber biomass was utilized as the sole feed source for black soldier fly larvae which were reared based on established protocols. Black soldier fly larvae were processed into a meal which was incorporated into the soybean meal dominant tilapia diets. Four diets were formulated to include a control diet (6% fishmeal), 50 and 100% fishmeal replacement with our black soldier fly meal, and a 100% fishmeal replacement with a commercial black soldier fly meal. These were tested along with a commercial tilapia diet. Juvenile blue tilapia were stocked into thirty 60.6-liter polyethylene tanks connected by a recirculating water filtration system to provide six replicates per treatment with six tilapia per tank. Tilapia were fed twice daily for the duration of the 6-week feeding trial. At the end of the growth trial an in-vivo digestibility trial was done to assess the digestibility of our and the commercial black soldier fly meal.

After 6 weeks, blue tilapia fed the 5 different diets showed no differences with respect to growth, survival, hepatosomatic index, visceral index, intraperitoneal fat, or muscle mass. There was also no significant difference in protein digestibility between our and the commercial black soldier fly meal. This study demonstrated a sustainable solution to utilize algal turf biomass in tilapia diets.

INTERAGENCY ENGAGEMENT IN THE AOA PROCESS

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The Environmental Protection Agency and the U.S. Army Corps of Engineers are cooperating agencies on the programmatic environmental impact statements being prepared in support of aquaculture opportunity area identification in southern California and the Gulf of Mexico. This presentation will describe the agencies' respective engagement in the process and how they plan to use the outcomes of the process to support their permitting activities.

HOLDING WILD SNOW CRAB, *Chionoecetes opilio*: EFFECTS OF STOCKING DENSITY AND FEEDING ON SURVIVAL AND INJURY

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Introduction

Snow crab (*Chionoecetes opilio*), has become a popular food product among consumers in recent years. The meat of cooked snow crab is white, flaky with a sweet taste and the product is exported to Europe, USA, Canada as well as to Japan and South Korea. The snow crab is an invasive species in the Barents Sea, where it was first observed in 1996. Snow crab occurs mainly in the eastern Barents Sea, but animals have also been recorded in the western parts of the Barents Sea. Commercial fishery in Barents Sea is performed by using crab pots that are baited with herring, mackerel or squid, and the species is generally harvested from June until April. The snow crab seems to be permanently established in the Barents Sea, as illustrated by the commercial landings of snow crab of 6302 tons in Norway representing an export value of approximately NOK 771mill. The snow crabs are either processed after landing or exported alive. The development of live holding and export of snow crab from Norway depends on both a reliable and consistent supply of crabs and the ability to hold and transport these, in good condition, to overseas markets. Capture-based aquaculture of snow crab is developing in Norway and effort has been put into establishing small and medium-sized enterprises using intensive culture systems for live holding snow crab. Knowledge of optimal live holding condition for snow crab is missing and there is a need to develop and improve the techniques for long-term storage to obtain higher value products. This study was therefore undertaken in order to describe the effects of stocking densities on survival, injuries and weight changes of wild caught male snow crab held in captivity for 1 month post capture.

Results

In this study the effects of different stocking densities on survival, injury and weight of captive male snow crab were examined. The first experiment (I) was carried out in square plastic tanks (700 L) with stocking densities of 100 (L), 150 (M) and 200 kg m3 (H) for 30 days. In a second experiment (II) snow crabs were kept at a stocking density of 50 kg m3 and were either fed (F) or not fed (S) for the same period of 35 days. The last experiment (III) was carried out with stocking densities of 25 kg m3 for 21 days with two groups, one with inactivated claw and one without rubber bands, with three replicates per treatment. In the first experiment mortality (H = 27, M = 26 and L = 36%) and occurrence of injuries (H = 27, M = 20 and L = 16%) were high in all groups. The weight loss during the experimental period was H = 15.3, M = 10.9 and L = 15.5 g, and was not significant different between the groups. In experiment II the mortality (F = 13% and S = 14%) and injuries were lower (F = 12% and S = 17%). The average weight increased in the fed treatment and decreased in the starved treatments. In the last experiment there was no mortality in any of the groups and the levels of injury were low (5% and 7%). The results show that adult male snow crab cannot be stored at densities equal to or higher than 25 kg m3 for 3 weeks without risk of mortality.

DIETARY SUPPLEMENTATION OF IRON NANOPARTICLES AND CALCIUM PROPIONATE FOR CHANNEL CATFISH *Ictalurus punctatus*

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Microminerals are essential nutrients for fish and play key roles in their normal metabolism. Among these elements, iron is related to several biochemical processes including oxygen transport, electron transfer, and energy metabolism. As an alternative approach to the use of bulk minerals, the use of elemental nanoparticles in animal nutrition has gained attention due to their increased surface area, which can potentially improve their bioavailability. In addition, acidifiers are commonly supplemented in fish feeds to improve weight gain, and they can synergistically interact with minerals, thereby enhancing their bioavailability. Commercial catfish feed mills have been supplementing feeds with high iron concentrations to mitigate idiopathic catfish anemia. Therefore, the aim of this study was to better understand the effects of high iron concentrations in catfish feed, the supplementation of iron nanoparticles, and the possible interaction between the nanoparticles and calcium propionate on catfish growth and health parameters.

Two different sources of iron, bulk iron ($FeSO_4.H_2O$) and nanoparticles (Fe 60 to 80 nm, 99.7% purity) at 1000 mg/kg, and two levels of calcium propionate supplementation (with and without calcium propionate, at 0.25%) were evaluated using a 2x2 factorial design. An additional control diet without iron or calcium propionate supplementation was included in the feeding trial which was conducted in a recirculating aquaculture system for 9 weeks. A total of 750 catfish fingerlings (average weight 1.9 ± 0.1 g) were distributed in 25 aquariums (110 L; 30 fish/tank). At the end of the feeding trial, growth performance, whole-body proximate, blood parameters, intestinal microbiota, and intestinal histology were evaluated. The remaining fish were subjected to a bacterial challenge using a virulent strain of atypical *Aeromonas hydrophila* (ML09-119) and survival was monitored for 72 hours. A separate digestibility trial was carried out for 5 weeks. Three hundred juvenile catfish (average weight 50.3 ± 2.3 g) were distributed in five tanks (400 L), operating as a recirculating aquaculture system. Each experimental diet was assigned to one tank for one week, and the assigned experimental diet of each tank alternated every week until all tanks receive all experimental diets. Fecal material was collected using a modified Guelph method. For the growth study, calcium propionate supplementation negatively impacted fish weight gain, and the interaction between nano iron and calcium propionate reduced catfish RBC. Hematocrit was significantly affected by dietary iron sources, where fish fed bulk iron (43.7 ± 3.6%) had higher values to fish fed nano iron (40.6 ± 2.3%). The intraperitoneal fat was higher in fish fed the nano iron-containing diet (3.4 ± 0.4%) compared to bulk iron (2.9 ± 0.2%). There were no differences in iron digestibility, whole-body proximate composition, protein conversion ratio, intestinal microbiota, or survival after bacterial challenge. In conclusion, dietary calcium propionate negatively affected the weight gain and RBC of channel catfish. Furthermore, iron nanoparticles decreased the blood hematocrit, negatively interacted with calcium propionate for RBC, and increased the visceral fat index. The histology of the intestine is currently being analyzed and will be presented during the conference.

BETA GLUCAN INDUCED TRAINED IMMUNITY IS CORRELATED TO VARIABLE DISEASE RESISTANCE AGAINST *Aeromonas hydrophila* AND *Flavobacterium covae* IN CHANNEL AND HYBRID CATFISH

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Trained immunity is a rapid innate defense mechanism that enhances the immune system's overall readiness to deal with various infections. The innate immune system can retain a memory of previous encounters with pathogens or certain stimuli. Beta glucan induced trained immunity was correlated to increased survival during *Edwardsiella ictaluri* and *E. piscicida* infections in channel catfish. This study evaluated beta-glucan induced trained immunity on disease resistance to *Aeromonas hydrophila* and *Flavobacterium covae* in channel and hybrid catfish. Fish were intraperitoneally (IP) injected with 100 mcg/g body weight beta 1-2 glucan linear structure (Calbiochem, CAS 9012-72-0, derived from *Saccharomyces cerevisiae*) in 0.8 mL physiological saline. Control fish were IP injected with 0.8 mL physiological saline. Fish were fed 32% protein commercial catfish feed at 3% BW per day. After a 30-day waiting period, anterior kidney tissues from control or beta glucan-exposed fish were dissociated and leukocytes isolated by Histopaque gradient. Leukocytes were incubated with either *F. covae* or *A. hydrophila* or physiological saline, and Reactive Oxygen Species (ROS) and Nitric Oxide Synthase (NOS) assays (Invitrogen EEA019 and G7921, respectively) were performed. The adherent cell populations were considered macrophages, and non-adherent cells were considered neutrophils and Natural Killer cells. Significant differences were found in ROS and NOS production of adherent and non-adherent cell populations in hybrid and channel catfish. Trained hybrid macrophages demonstrated significantly higher ROS and NOS values than trained channel catfish macrophages, control hybrid, and control channel catfish macrophages. However, there was no significant difference in NOS between control hybrid macrophages and control channel catfish macrophages. When macrophages from each group were co-incubated with *F. covae,* only trained hybrid macrophages produced significantly higher NOS values than control hybrid macrophages. Both trained and control hybrid macrophages produced higher ROS and NOS than trained and control channel catfish macrophages, respectively, when co-incubated with *F. covae.* For the non-adherent cell populations, trained hybrid neutrophils had NOS values significantly higher than trained channel catfish neutrophils, and this significance remained after co-incubation with *F. covae.* The trained hybrid neutrophils had NOS values significantly higher than the control hybrid neutrophils and they responded to a higher level than the untrained neutrophils after exposure to *F. covae*. However, this was not observed with the channel catfish. Also, there were no significant differences in the ROS responses of the neutrophils when comparing trained vs untrained before and after bacterial exposure. For the *Aeromonas* experiment, only hybrid catfish were available. Trained macrophages and neutrophils produced significantly higher amounts of ROS and NOS than control macrophages and neutrophils. Tank survival trials are currently being performed. The current study demonstrated that trained immunity differentially regulates leukocyte responses and disease resistance in hybrid catfish (*Ictalurus punctatus* x *I. furcatus*) and channel catfish *(I. punctatus*). Trained immunity is an exciting and evolving area of immunology and can transform aquaculture to become more efficient and resilient to disease.

THE ESTIMATED MARKET POTENTIAL FOR A COMMERCIAL SHELLFISH SEED HATCHERY IN NORTH CAROLINA

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The number of shellfish leases in North Carolina has increased rapidly in the past 10 years primarily because of availability of highly efficient gears and growth of the oyster and hard clam markets. This growth has enabled existing businesses to expand, new businesses to be created, and operations to move towards even higher efficiency for a given acreage. The industry has, however, met a stifling factor; North Carolina lacks a single commercial shellfish hatchery that is aimed at supplying shellfish seed to growers. The only remaining method for growers to acquire shellfish seed for their farms is to import it from other states, which requires permitting and additional shipping and pathology testing costs. Additionally, farmers in North Carolina have developed methods to grow bay scallops successfully and have begun building a market for farm raised scallops as well. Bay scallops add an additional difficulty for growth as few hatcheries in the US grow and have available seed to import into the state.

The North Carolina Division of Marine Fisheries (DMF) permits the importation of all estuarine shellfish seed used for aquaculture. In an effort to determine the market potential for a commercial shellfish hatchery in the state, the DMF has compiled and estimated the annual shellfish seed costs from import permits over the last 5 years by species (Figure 1). The DMF has also compiled averages of shipping and pathology testing fees to determine how much the lack of an instate hatchery is costing the industry in total. The results of this analysis show the size of the shellfish industry ad its continued expansion make it a viable area for a private shellfish hatchery.

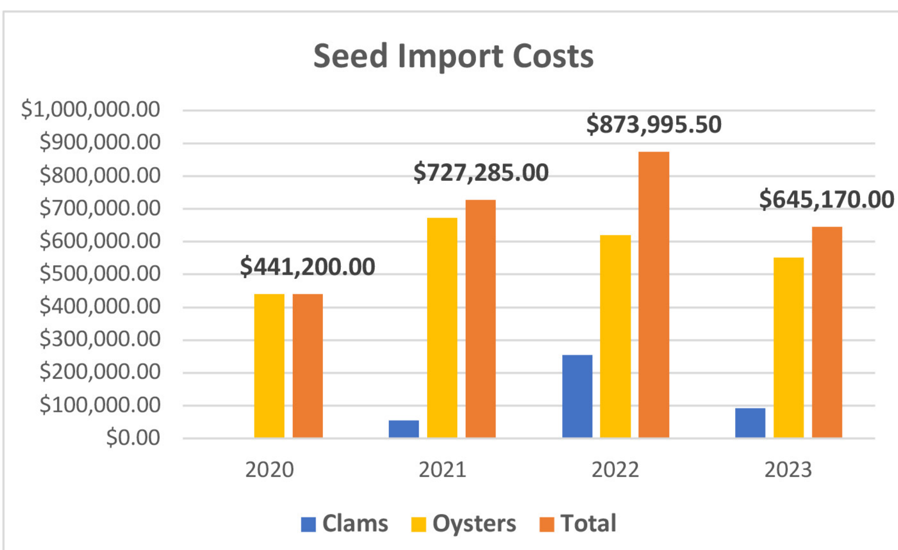

Figure 1: Species and Total Shellfish Seed Costs from Out of State Suppliers

ECO-FRIENDLY AQUACULTURE PRACTICES IN THE BRAZILIAN AMAZON

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Aquaculture has emerged as an activity of substantial economic and environmental significance, contributing over 90 million tons of global production annually. However, the rapid expansion of aquaculture has intensified ecological challenges, particularly regarding effluent management. When effluents are inadequately treated, there is a risk of pollution and eutrophication of aquatic ecosystems, leading to degradation of biodiversity and posing threats to public health. Among these challenges, the efficient treatment of aquaculture effluents and adopting sustainable farming practices are critical for mitigating negative impacts and enabling eco-friendly development in the sector. This study evaluated the feasibility of cultivating tambaqui (*Colossoma macropomum*) in a recirculating aquaculture system (RAS) integrated with aquatic macrophytes.

The focus was on assessing the water treatment capacity of macrophytes and their role as biofilters, alongside monitoring water quality parameters and fish growth performance. The experimental system was installed at the Novo Paraíso *Campus* and designed as a wetland-based RAS. The setup comprised a circular tank (volume = 26.2 m^3) to house the fish, four rectangular tanks (volume = 1.69 m^3 each) for the cultivation of aquatic macrophytes, and an additional rectangular tank (volume = 6.89 m^3) filled with gravel to serve as a mechanical filtration unit. Tambaqui juveniles were stocked in the system on April 4, 2024. Throughout the experiment, biometry was conducted biweekly to track the growth performance of the fish. Key water quality parameters such as total ammonia nitrogen (TAN) and nitrite concentrations were regularly monitored to evaluate the system's performance. Results showed that TAN concentrations ranged from 0.25 to 3.5 ppm, with a stabilization and subsequent reduction trend over time, likely attributable to the biofilter maturation. Similarly, nitrite levels ranged from 0 to 2.8 ppm, following a comparable stabilization trend.

Regarding tambaqui growth, the initial average weight of the juveniles was 7.0±4.0 g in April, increasing progressively to 216.5±66.5 g in June, 326.0±81.3 g in August, and reaching 437.0±118.4 g in September. After seven months of rearing, the fish achieved a weight gain of approximately 430 g, nearing the commercial size threshold of 500 g for juvenile tambaqui "curumim". The findings highlight the viability and efficiency of a RAS integrated with aquatic macrophytes as a sustainable alternative for tambaqui aquaculture. The system supports the production of marketable fish size within a reasonable timeframe and demonstrates effectiveness in mitigating environmental impacts through improved water quality. Using natural biofilters, such as macrophytes, represents a promising strategy for managing aquaculture effluents while fostering the sector's transition toward more sustainable practices.

ADVANCES TOWARDS THE CAPTIVE REPRODUCTION OF THE AMAZON *Arapaima gigas* (OSTEOGLOSSIDAE)

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The Amazon air-breathing fish *Arapaima gigas* (Pirarucu) is a threatened emblematic species of the Osteoglossidae family, recognized for its gigantism and prized meat both in South America and abroad. Despite its commercial potential, aquaculture of *A. gigas* has been constrained by challenges in achieving controlled reproduction in captivity. This report presents a series of studies aimed at advancing our understanding of *A. gigas* reproductive biology. First, to develop tools for identifying fish sex and assessing female maturation stages, we used an ureterorenoscope to examine and understand female gonadal anatomy. This enabled, for the first time, the development of the cannulation technique for *in vivo* analysis of oocyte maturation in *A. gigas*, and histological descriptions of oocytes from primary growth to ovulation. Subsequently, we monitored (year-round) ovary development in different captive broodstocks using cannulation, finding that females paired with males in earthen ponds reach final maturation but often fail to ovulate or synchronize with males. We then applied hormonal therapies in different trials, testing slow-release GnRHa implants (Evac) in single (Trial I) and double doses (Trial II), as well as intraperitoneal (IP) applications of GnRHa (Trial III), carp pituitary extract (CPE) and prostaglandins (Trial IV), to either stimulate reproduction in ponds or to attempt collection of eggs and semen. Although direct reproduction could not be observed in these different trials, slow-release GnRHa implants proved safer for *A. gigas*, while IP applications showed potential risks. In these trials, we evaluated the impact of therapies on plasma sex steroid levels, oocyte development, changes in fish color pattern, and nesting behavior. Additionally, the inclusion of *Spirulina* sp. in broodstock diets was found to improve oocyte quality (Trial V), with effects seen in oocyte color and lipidic index. Finaly, we recently demonstrated for the first time the feasibility of *in vivo* semen collection in *A. gigas*, providing a detailed description of spermatozoa using scanning and transmission electron microscopy (SEM and TEM). These findings provided novel tools for identifying fish sex and maturation stages, enabling hormonal therapy trials and offering critical insights into *A. gigas* reproductive biology. This lays the foundation for more effective approaches to controlling reproduction, advancing sustainable aquaculture and conservation efforts.

DEVELOPMENT OF A LONGLINE CULTIVATION SYSTEM FOR THE WINGED KELP *Alaria maringata* IN ESTUARINE SYSTEMS

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Macroalgal aquaculture is a priority in the blue economy for its potential to enhance local economies, provide food security, and development nutrition and biotechnical products. Since 2016, the seaweed farming industry in the United States has expanded by a factor of 40, growing from 18.5 tons to 740 tons. Despite this growth in the US, farming protocols and methods are still in development, particularly when catering to diverse environments and target species. This project aims to establish a robust cultivation protocol for the winged kelp *Alaria marginata* by comparing multiline and single longline systems along a depth gradient in an embayment. Estuarine systems such as Humboldt Bay offer a more protected environment for kelp aquaculture in comparison to more exposed environments and may provide more stable nutrient levels due to coastal proximity. However, they can also experience more fluctuations in salinity and temperature. Insights from the first year and comparative analyses will inform the second year's strategy, aiming to maximize biomass production and optimize cultivation protocol within the best growing season.

A. marginata sporophylls were collected from local populations and spawned in a controlled environment. Spores were settled on Matsuura Cremona twine spooled around 2-inch PVC to seed farm lines. Two farm systems were used: 1) a single-long-line system, where there is a single 107 m length of grow line attached to buoys, and 2) a multi-long-line system, where five 61 m grow lines were spaced 0.76 m apart using a 3.05 m aluminum spreader bar. The multi-long-line system allows the assessment of growth of more biomass in a smaller area while also allowing for a depth analysis. Biweekly measurements for blade length and width were taken randomly along the length of each grow-line. Water quality measurements were also taken.

In the first targeted growing season (July 2024 to September 2024), mortality occurred over time; By the sixth week, only two individuals remained on the entirety of a 107-meter grow line. Temperatures of 18 °C, a maximum of 0.1 nitrates ppm, and a maximum of 1.0 phosphates ppm likely limited growth and survival. Gametophyte banking will be utilized to outplant during times of the year when naturally occurring sporophylls are not present so that optimized growing season can be determined.

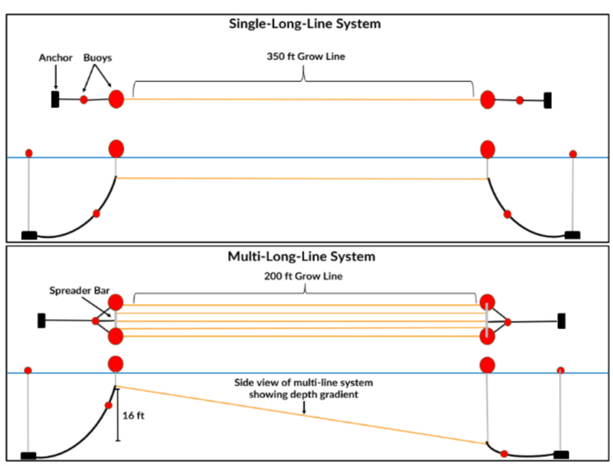

Figure 1. (Top) Top-down and side view of Single-Long-Line System. (Bottom) Top-down and side view of Multi-Long-Line System

CHARACTERIZATION AND MOLECULAR IDENTIFICATION OF *Vibrio parahaemolyticus* ISOLATED FROM *Litopenaeus vannamei* FOLLOWING AN OUTBREAK IN A LOW SALINITY RECIRCULATING SYSTEM

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Vibrio parahaemolyticus (Vp) is a gram-negative bacterium commonly found in estuarine, marine, and coastal environments and is known to induce disease throughout the global shrimp aquaculture industry. This pathogen is the causative agent of acute hepatopancreatic necrosis disease (AHPND) or early mortality syndrome (EMS) in Pacific white shrimp, *Litopenaeus vannamei*. This study describes the characterization of a Vp isolate (ARS-2-2024-GL) obtained from a naturally occurring outbreak in shrimp reared within a low salinity recirculating system (RAS; 363 L system). The RAS comprised 16 tanks (23L) maintained at 29 °C, each stocked with 10 shrimp (average weight of 2.63 g). Salinity at stocking was 9.5 ppt and was decreased approximately 2 ppt/day. During acclimation, a disease outbreak occurred. Affected shrimp displayed clinical signs of pale and watery hepatopancreas, alongside lethargy.

The moribund and deceased shrimp were collected and necropsied, and microbial samples were aseptically collected from the eyes, hepatopancreas, and gastrointestinal tract. The samples were initially cultured on marine agar for the general isolation of bacterial samples and CHROMagar™ Vibrio for the isolation and detection of *Vibrio* spp. and results were positive. Biochemical and phenotypic profiles were developed using the API 20 E strip system, Biolog Gen. III ID microplate identification system, and Fatty Acid Methyl Analysis (FAME). ARS-2-2024-GL API 20E strip results yielded a profile code 534612, correctly identifying the isolate to the genus level as *Vibrio* spp. Then, the phenotypic profile of ARS-2-2024-GL was generated with the Biolog Gen. III ID microplate system, which classified the sample as *V. parahaemolyticus*. Additionally, FAME analysis of the isolate identified a core group of fatty acids (12:0, 13:0 iso, 14:0, 16:0 iso, 16:0, 17:0 iso, 17:1 ω8c, 17:1 ω6c, 17:0, 18:1 ω9c, 18:0, Summed Feature 3, Summed Feature 5, and Summed Feature 8) yielding a sim index ID of .357 *V. parahaemolyticus*. 16S rRNA sequencing confirmed the isolate identification to be Vp.

Preliminary bacterial challenges were carried out in *L. vannamei* using ARS-2-2024-GL to identify an appropriate challenge method and dosage. Cumulative percent mortality (CPM) from the immersion challenge was 11%, (low dose), 0% (intermediate), 13.5% (medium) and 15 % (high). Reverse gavage injection CPM was 4% (low), 8% (medium) and 39% (high). These findings suggest the outbreak in the RAS was most likely caused by Vp. We are currently investigating additional Vp isolates obtained from the outbreak and establish an effective *L. vannamei* challenge model for these specific isolates.

SWEDISH AQUACULTURE FARMS: A STUDY OF SEASONAL VARIATIONS AND BIOFILM DYNAMICS

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Swedish aquaculture is a growing industry but faces environmental and fish health challenges. Currently, Sweden produces 0.2 million metric tons of fish and fishery products annually, making it a net importer of these products. Aquaculture is often associated with negative environmental impacts like eutrophication and disease outbreaks.

Climate change and seasonal variations pose threats to fish health in aquaculture.
Warmer water temperatures due to climate change create ideal conditions for harmful bacteria to thrive. These bacteria can cause diseases in fish, leading to increased mortality and reduced growth. Additionally, warmer water can accelerate fish metabolism, increasing their oxygen demand and potentially leading to stress and weakened immune systems.

Seasonal changes in water temperature, quality, nutrient levels, and flow can also impact biofilm composition and structure. Biofilms can experience blooms of harmful bacteria during periods of high nutrient input, such as after rainfall or agricultural runoff. These bacteria can release toxins that are detrimental to fish health. Conversely, during periods of low nutrient availability, biofilms may become dominated by less diverse microbial communities, increasing the risk of disease outbreaks.

Understanding the role of microbes in maintaining ecological balance in fish farms is crucial.
To address these challenges and ensure the long-term sustainability of Swedish aquaculture, the study is using advanced techniques such as long-read PacBio sequencing and functional predictive analysis to study the role of microbes in maintaining ecological balance in fish farms. By analyzing biofilms from inlets and outlets, the study aims to gain insights into the complex interplay between seasonal variations and biofilm dynamics.

Proactive measures are needed to mitigate the negative impacts of climate change and seasonal variations on fish health and productivity.
By understanding these complex relationships, researchers and aquaculture farmers can implement strategies to mitigate the negative impacts of climate change and seasonal variations on fish health and productivity. This will help safeguard both the environment and the global food supply.

STRAIN-SPECIFIC RESPONSE TO STRATEGIC REDUCTION OF SOY PROTEIN CONCENTRATE BY INCREASING SOYBEAN MEAL IN THE DIET OF RAINBOW TROUT, *Oncorhynchus mykiss*

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Soy-based ingredients are essential in salmonid diets, providing a sustainable, cost-effective alternative to fishmeal. The primary soy-based products used are soybean meal (SBM) and soy protein concentrate (SPC). Optimizing the SBM-to-SPC ratio is crucial for reducing feed costs and improving performance and gut health resistance in trout. In this study, we conducted a feeding trial to optimize the SBM-to-SPC ratio across two commercial strains of rainbow trout to assess strain-specific responses in growth, feed utilization, gut health, and gene expression.

In 8 weeks feeding trial, 1500 fish (4.2 g) were distributed into 10 treatments in triplicates (50 fish/tank) following a 2 × 5 factorial design, where two types of rainbow trout strains, i.e., commercial strain 1 (CT1) vs. commercial strain 2 (CT2) were fed with 5 different isonitrogenous (44% crude protein) experimental diets with varying ratios of SBM to SPC for 8 weeks. Experimental diets were Diet 1 (control), 0% SBM/SPC + 30% fishmeal; Diet 2, 11% SBM + 18% SPC; Diet 3, 22% SBM + 13% SPC; Diet 4, 33% SBM + 6% SPC; and Diet 5, 44% SBM + 0% SPC. Fish were fed twice daily at apparent satiation. All the data were subjected to two-way ANOVA to determine the main and interaction effects of strains and diets and Tukey's HSD for pairwise comparison using R programming.

Results showed that all the growth-related parameters exhibited significant interaction of 'Strain × Diet'. Based on the growth performance, increasing the SBM up to 33% with reduced SPC of 6% (Diet 4) showed no significant ($p > 0.05$) difference with control (Diet 1). Although CT2 strain showed higher specific growth rate (SGR) (Figure 1) than CT1, final body weight (FW) and weight gain (WG) showed no significant ($p > 0.05$) difference between the strains. Daily feed intake (DFI) was significantly ($p < 0.05$) higher in CT1 than CT2 strain, but feed conversion ratio (FCR) and protein efficiency ratio (PER) showed the opposite trend. Interestingly, CT1 showed a significantly ($p < 0.05$) lower viscerosomatic index (VSI) (11.30) than the CT2 strain (15.94). Gut histology and gene expression parameters are being analyzed.

Overall, although CT2 strain performed better than CT1 strain in terms of SGR, TGC, FCR, and PER, opposite trend is true in terms of DFI and VSI. Among the experimental diets, reduction of SPC from 18 to 6% with SBM increment from 11 to 33% showed similar performance like control fishmeal-based diet.

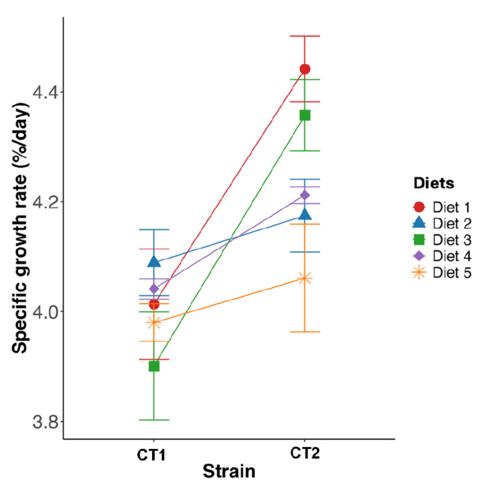

Figure 1: Interaction plot for 'Strain × Diet' on the SGR of trout

FISH SOLUBLE AS A FUNCTIONAL FEED INGREDIENT TO ENHANCE SOYBEAN MEAL UTILIZATION IN TWO STRAINS OF RAINBOW TROUT, *Oncorhynchus mykiss*

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Fish solubles (FS), a nutrient-dense functional feed ingredient derived from fish processing byproducts, can complement soybean meal (SBM)-based diets by improving growth performance, gut health, and nutrient absorption, ultimately supporting sustainable and cost-effective aquaculture practices. This current study aimed to study strain-specific (commercial strain 1, CT1 vs. CT 2) response of rainbow trout to high SBM diets supplemented with FS on growth, feed utilization, gut health, gene expression, and fillet quality.

In a 66 days feeding trial, 600 fish (22.1 g) were distributed into 10 treatments in triplicates (50 fish/tank) following a 2 x 5 factorial design, where two types of rainbow trout strains, i.e., CT1 vs. CT2 were fed with 5 different isonitrogenous (45% crude protein) experimental diets: Diet 1, FM-based diet; Diet 2, 20% SBM; Diet 3, 35% SBM; Diet 4, 20% SBM with 5% FS; Diet 5, 35% SBM with 5% FS. All the data were subjected to two-way ANOVA to determine the main and interaction effects of strains and diets (three-way ANOVA when analyzing the fillet quality for two different storage days) and Tukey's HSD for pairwise comparison using R programming.

Results showed that fish fed Diet 5 exhibited significantly ($p < 0.05$) higher growth performances than other diets. The CT2 strain showed better growth performance than the CT1 strain, and a similar observation was followed for feed utilization. However, no difference between these strains was observed for daily feed intake. The body indices like viscerosomatic index and hepatosomatic index were significantly ($p < 0.05$) higher in the CT2 strain than the CT1 strain. Fillet yield was significantly ($p < 0.05$) higher in fish fed Diet 5 and in CT2 strain. Significant effects of Diet, Strain, and Storage days were observed on fillet quality parameters. Significantly ($p < 0.05$) lower liquid loss and highest pH were recorded for Diet 5, CT1, and Day 8 than their counterparts. A better color profile was observed for the CT1 and Diet 3. The texture profile analysis showed that the CT1 strain had higher fillet hardness than the CT2 strain, and the opposite trend was observed for cohesiveness. Gut histology and gene expression parameters are being analyzed.

Overall, Diet 5, with 35% SBM and 5% FS, showed better growth performance and feed utilization. The CT2 strain showed better growth and feed utilization, but fillet quality was found to be better in the CT1.

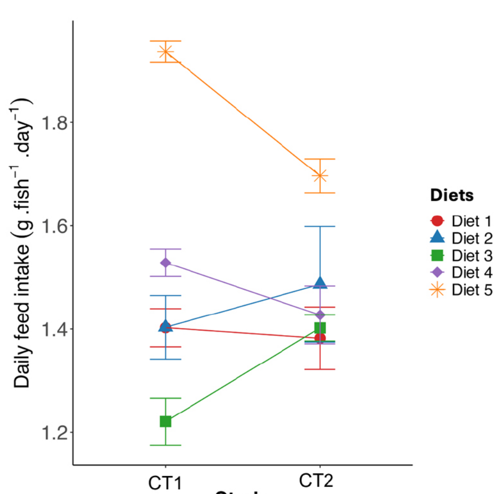

Figure 1: Interaction plot for 'Strain × Diet' on daily feed intake

GOLDEN ALGAE MANAGEMENT: PROPHYLACTIC AND REACTIVE USE OF HUMIC ACID

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Golden algae (*Prymnesium parvum*) blooms present significant challenges to aquatic ecosystems and aquaculture operations, producing toxins that impair respiratory functions in gill-breathing organisms and leading to considerable economic and ecological losses. Current management strategies often rely on algaecides, chemical treatments, dilution, or adjustments to water chemistry, but these methods may be impractical in certain systems. Humic acid, an organic compound derived from natural decomposition, provides a carbon source for beneficial bacteria and has been used commercially in recirculating aquaculture systems and agriculture for diverse purposes.

This study explored the potential of humic acid as an alternative approach to managing golden algae blooms by assessing its ability to reduce nutrient concentrations and control algal proliferation. A controlled experiment was conducted using various dosages of humic acid (prophylactic, low, and high) applied to outdoor systems mimicking pond environments, with and without soil presence. Key water quality metrics, including total phosphorus and nitrogen, were monitored before and after treatment. Results showed significant phosphorus binding within six hours across all treatments and the most notable nitrogen reductions observed in soil-present conditions. By week 5, prophylactic treatments significantly reduced golden algae cell counts, while low and high doses achieved similar results by week 6, keeping cell concentrations below bloom thresholds (<10,000 cells/mL).

These findings highlight the efficacy of humic acid, particularly as a prophylactic treatment, in preventing and mitigating golden algae blooms while reducing nutrient availability. This research supports the use of humic acid as a cost-effective, environmentally sustainable management tool for golden algae and potentially other harmful algal blooms, with promising applications in aquaculture and natural aquatic systems.

TRANSCRIPTOMICS UNCOVERS EARLY MOLECULAR SIGNATURES OF MALPIGMENTATION IN SOUTHERN FLOUNDER *Paralichthys lethostigma* LARVAE

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Hatchery stock replenishment efforts for flatfish populations often result in high rates of malpigmentation. Efforts to identify the molecular processes leading to malpigmentation in flatfish have implicated some pathways, but the exact mechanisms leading to malpigmentation in hatcheries remain poorly understood. Furthermore, identification of predictive gene expression profiles that could indicate future high levels of malpigmentation may allow hatcheries to test tanks early and not spend resources on predicted high-malpigmentation batches.

To investigate the molecular pathways leading to malpigmentation and determine early gene expression predictors of malpigmentation, we collected southern flounder larvae at 27, 34, and 41 days post-hatching from larvae in our Fisheries and Mariculture Laboratory (FAML) and a Texas Parks and Wildlife Department (TPWD) hatchery, where malpigmentation rates average 30%.

We determined the rate of malpigmentation for each tank at day 55 and conducted 3' tag-sequencing on the collected larvae to profile gene expression. Principal component analysis demonstrated mRNA expression signatures clustering by age (Fig 1A), rearing site (Fig 1B), and tank malpigmentation rate (Fig 1C).

On Days 27 and 34, PC2 separated out 9 samples from tanks with a high malpigmentation rate (Fig 1C), indicating expression profiles that may predict future malpigmentation. Genes previously implicated in pigmentation pathways and that had strong PC2 loadings included, prostaglandin E receptor 2b (*ptger2b*) and retinol binding protein 4 (*Rbp4*) which contributed to negative PC2 eigenvalues and therefore may be predictive of future malpigmentation at 27 days post-hatching or earlier. Genes for melanin production and thyroid hormone signaling strongly contributed to positive PC2 values. Taken together, our results provide novel insights into the molecular underpinnings of malpigmentation and identify potential marker genes for facilitating early diagnosis of malpigmentation.

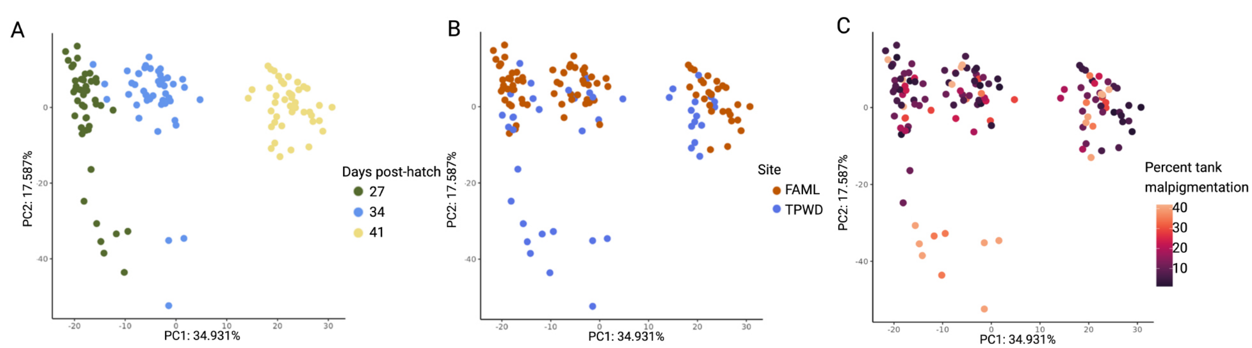

Figure 1. Principal component analysis of gene expression. Samples are labeled by A) age B) site, and C) tank. Malpigmentation rates were assessed at 55 days post-hatching.

RESEARCH TO REDUCE MALPIGMENTATION IN PRODUCTION OF SOUTHERN FLOUNDER *Paralichthys lethostigma* FOR STOCK ENHANCEMENT IN TEXAS

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As a result of decades of declining populations of Southern Flounder (*Paralichthys lethostigma*), some fishery-management agencies have begun producing flounder for stock-enhancement. One problem is that normal pigmentation may not develop in hatchery fish. The most prominent form of malpigmentation is pseudoalbinism, in which the eyed side of the fish is almost entirely white, which makes them exceptionally vulnerable to predators. At one hatchery, we determined that 30% of production were pseudoalbinos. In contrast, malpigmentation rates in our laboratory averaged 5%. We compared hatchery and laboratory rearing protocols to design experiments to identify ways to improve hatchery methods so that malpigmentation is minimized or eliminated. Among several differences, larval diet was identified as the top candidate for experimentation.

Experiments were conducted at the University of Texas Marine Science Institute's Fisheries and Mariculture Laboratory to compare the two diets: non-enriched *Artemia* (hatchery) vs. *Artemia* enriched with Algamac DHA10 (laboratory). Five female flounder were strip-spawned, and 10,000 eggs from each spawn were divided equally between two rearing tanks (10 tanks in all), with one tank for each female assigned to each diet treatment. The larvae were reared to 55 days posthatching (dph), when the tank-wise malpigmentation rate was determined based on 100 individuals. In addition, malpigmentation rate was assessed for five tanks of flounder reared in the production hatchery through 55 dph for comparison with the laboratory results.

Malpigmentation rates from the laboratory experiment were 18.4% ± 14.8% (mean ± s.d.) from the non-enriched diet and 4.4% ± 2.4% from the enriched diet. Hatchery-produced fish had a malpigmentation rate of 26.7% ± 4.3%). Therefore, *Artemia* enrichment significantly improved the malpigmentation rate in the laboratory. For the subsequent production season (now underway), the hatchery changed the larval diet to enriched *Artemia*, and malpigmentation rate will be measured for 10 tanks to determine how well the laboratory results transfer to the hatchery. Some of the results may be available by the time of this presentation.

But, malpigmentation rates in the laboratory using non-enriched *Artemia* (18.8%) were lower than the rates observed in the hatchery (26.7%), and the 14% decrease due to the enriched diet would not bring the hatchery malpigmentation rate in line with the laboratory. This indicates that additional factors contribute to malpigmentation in the hatchery. Differences in light intensity and spectrum used in the hatchery vs. laboratory were examined. The hatchery used LED illumination at higher intensities (405 ± 66 lux) than the fluorescent lighting used in the hatchery (274 ± 22 lux) and the two light sources have very different spectral characteristics. Malpigmentation rates for the non-enriched diet (both locations included) showed a positive linear relationship with light intensity, suggesting that higher light intensities may increase malpigmentation rates. The effect of light intensity on malpigmentation rate is being tested in experiments that are currently in progress.

THE OYSTER AQUACULTURE TRAINING PROGRAM (OAT) AT THE VIRGINIA INSTITUTE OF MARINE SCIENCE

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The Oyster Aquaculture Training (OAT) program is offered by the Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science (VIMS) in Gloucester Point, Virginia. It is a 4-5 month, paid, hands-on internship, focusing on various principles of oyster aquaculture from the hatchery to the field. Participants are selected from a pool of applicants based somewhat on past relevant education and work experience, but moreso based on a clearly demonstrated desire to have a career in shellfish aquaculture. OATs learn and work alongside researchers during ABCs oyster hatchery and spring/summer field season from roughly March to July. To ensure a one-on-one experience, we accept a maximum of two trainees each year.

During the OAT program, trainees rotate through various stages of oyster aquaculture, including hatchery, nursery, field grow-out operations and laboratory. Participants learn the process of conditioning, spawning, larval husbandry and setting. They are exposed to algae culture techniques and gain an understanding of broodstock and larval algae requirements. They learn how to monitor water quality and various processes of seawater filtration and pumping. OATs are exposed to upwelling systems, including bottle systems, and they learn to build and maintain numerous types of field gear deployed across five farm sites. In the ABC laboratory, participants learn how ploidy analysis is performed as well as some basic disease diagnostic procedures.

In addition to hands-on experience, regular lectures are conducted by VIMS staff and faculty to provide participants with background information pertinent to shellfish aquaculture. These lectures include oyster biology, shellfish diseases, principles of genetics and breeding and site selection considerations, as examples. Field trips are taken to multiple research and commercial hatcheries, along with visits to commercial farms in order to expose participants to different farming methods and aspects of oyster aquaculture, particularly aquaculture operations at a commercial scale. There is an opportunity for participants to go on 2-3 day externships to industry facilities, based on their particular interests. By the end of the program, participants have a clear understanding of various areas of oyster aquaculture and should be confident in their ability to perform tasks in oyster hatchery, nursery and field operations.

OVERVIEW OF RESEARCH FARM SITES OPERATED BY THE AQUACULTURE GENETICS AND BREEDING TECHNOLOGY CENTER

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The Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science (VIMS) in Gloucester Point, Virginia performs research, training and broodstock grow-out across five locations in Chesapeake Bay.

VIMS operates a lease along the shoreline of the Gloucester Point campus where gear types include rack and bag, bottom cages and an adjustable long line system. The salinity at this site ranges generally from 14-23ppt and two endemic diseases can be found here including *Perkinsus marinus* and *Haplosporidium nelsoni*. Portions of the lease are used to grow and select ABC broodstock, whereas bottom cages are used to grow line or family seed or sometimes deploy smaller collaborative projects. The adjustable long line system, capable of holding roughly 600 baskets, is primarily used for testing ABC family material. There are also a limited number of rebar racks used on that lease to host projects or grow out ABC material. VIMS also has access to a pier in Sarahs Creek, a creek off the York River a few miles from VIMS. ABC uses a system of 40+ Taylor floats there for a variety of purposes including, but not limited to overwintering experiments, temporary storage and for naturally conditioning broodstock.

ABC has an agreement with Rappahannock River Oyster company to use part of their leased grounds near the mouth of Locklies Creek. Gear utilized on site includes bottom cages and specially designed set of tall rebar racks (24" depth at mean low water), allowable as part of a special permit through the Virginia Marine Resources Commission (VMRC). Custom wire trays or cages have been affixed to the racks; at the designated height, oysters are reared high in the water column and get regular air exposure. This site is primarily used for broodstock grow out for industry and the salinity generally ranges from 10-15ppt.

ABC has an agreement with KCB Oyster Holdings, LLC in the Coan River to use part of their leased grounds. Gear utilized on site includes bottom cages, rebar racks and a specially designed set of tall rebar racks (23" depth at mean low water), allowable as part of a special permit through VMRC. Custom wire trays hold adjustable long line baskets. This site is used to select low salinity ABC broodstock, to test ABC family material and for hosting collaborative research projects. The salinity is typically 8-13ppt and the disease pressure is low.

ABC co-owns and operates an adjustable long line system with UMCES Horn Point Laboratory in the Choptank River, MD. The salinity can be as low as 5-13ppt. ABC typically uses 2/3 of the farm (~500 baskets) for testing ABC family material.

IMPLEMENTATION OF GENOMIC SELECTION IN THE EASTERN OYSTER, CRASSOSTREA VIRGINICA, AT THE AQUACULTURE GENETICS AND BREEDING TECHNOLOGY CENTER, IN THE MID-ATLANTIC USA

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Advances made through family breeding at the Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science have yielded substantial gains in economically-important traits for the eastern oyster, *Crassostrea virginica,* including improvements in survival, growth rate and meat yield. Shape characteristics, such as fan shape and cup depth, are monitored and included in a multi-trait selection index. Genetic gains from family breeding are transferred to the commercial oyster industry through yearly production of two licensed family-based broodstock lines. The lines are derived from top families for improved performance in low salinity, low disease-pressure environments and moderate salinity, high disease pressure environments.

Through combined efforts of the East Coast Oyster Breeding Consortium members, a 66K SNP genotyping array has been developed specifically for east coast oyster populations. ABC has utilized this tool to genotype 10,570 individuals (2,277 parents, 8,293 progeny) from 10 years of family production and testing (2013-2023).

In spring 2023 and again in 2024, genomic selection was used to select broodstock candidates through the calculation of genomic estimated breeding values (GEBV). The spread of GEBV within families indicated a high degree of genetic gain was possible using genomic selection to select the best individuals with families over pedigree-based approaches in operation. In 2023, the first GEBV-based spawning designs were executed to create 57 high salinity, 57 low salinity and 5 low-ranked GEBV families. Families were replicated across 3 field locations in spring 2024 covering a range of environments with variable salinity (6-24ppt) and disease pressure. Local endemic pathogens include *Perkinsus marinus* and *Haplosporidian nelsoni*. Progeny tests for the 2023 families were completed in November 2024, at which time survival was assessed for each family replicate and randomly-selected samples of oysters from each replicate were analyzed to collect data on traits including total weight, length, height index, width index and meat yield. This data has been used to compare the improved accuracy for selection using genomic information and rate of genetic gains forecasted by GEBV as the first validation of genomic selection in this breeding program.

SCARED STRONG: USING PREDATOR CUES TO BOLSTER OYSTER RESTORATION AND AQUACULTURE

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Using predator cues to enhance prey defenses may improve survival of cultivated species and enhance species restoration efforts, but assessment of such benefits across relevant scales and identifying the chemical cues responsible for these morphological changes is needed. Oysters, *Crassostrea virginica,* develop heavier, stronger shells in response to chemical cues from predators and injured conspecifics. We investigated if oyster morphology could be manipulated with predator cues to improve survival and bolster restoration. Through a series of collaborative experiments with state agencies, NGOs, and industry, we tested the costs and benefits of this approach in the field. Although diploid and triploid seed oysters exposed to predator cues were initially smaller after one month of exposure to blue crab *Callinectes sapidus* predators (Figure 1), after one year, they had similar sizes and tissue masses to controls (Figure 2). We restored an oyster reef using spat-on-shell raised with blue crabs or raised in controls without predators and found that oysters raised with blue crabs had 68% higher survivorship than controls after one year (Figure 3). Concurrently, we ran bioassays to identify the chemical cues predators release that stimulate oysters to grow stronger shells. We exposed oysters to eight different concentrations of urine extracted from blue crabs as well as candidate molecules for inducing defenses. Oyster shell strength increased by 20 – 100% when exposed to predator cues and followed a standard dose response curve with crab urine concentration causing shell strength to peak at ~0.19 mL urine/L seawater. Five molecules in blue crab urine that trigger shell hardening in oysters have been identified. Thus, remote setting can benefit from manipulating oyster shells with predator cues to improve survival for oyster restoration and on-bottom aquaculture. Our findings demonstrate the utility of using predator cues to enhance the survival of target species and highlight an opportunity to employ nontoxic methods to control pest-based mortality.

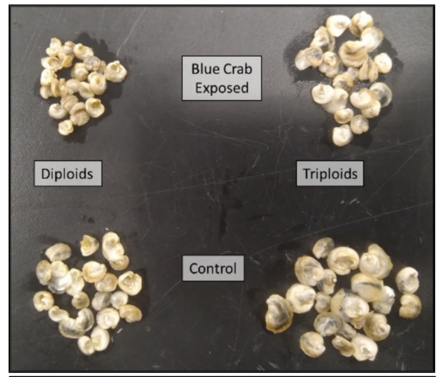

Figure 1. Diploid and triploid seed oysters after one month of growth in a nursery and exposed to blue crab exudates or grown in controls without predators.

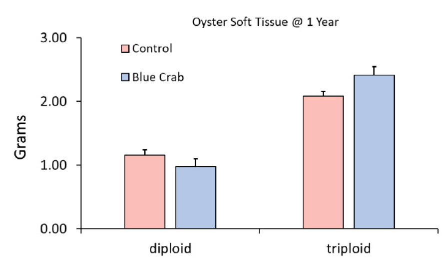

Figure 2. Oyster soft tissue weight after 1 year of growth in an off-bottom oyster farm. Significant different in tissue weight were not found among predator exposed oysters and controls in diploids or triploids.

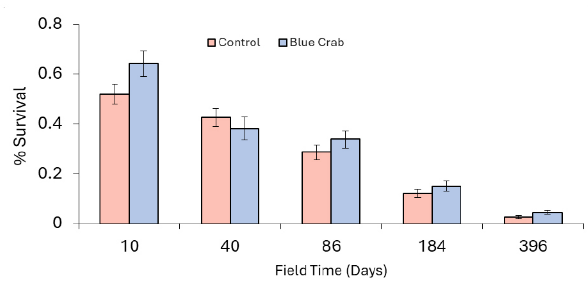

Figure 3. % oyster survival on a restored reef when oyster spat were exposed to blue crabs or controls for 1 month prior to field placement. Survival was 68% higher in oysters initially exposed to predators, which was significant $p < 0.01$.

WOMEN IN VIRGINIA'S SHELLFISH AQUACULTURE SECTOR: A GENDERED PERSPECTIVE OF INDUSTRY INVOLVEMENT AND CHANGE

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Aquaculture in the United States is an important piece of many local economies, yet limited data exist that represent the number of women working in aquaculture, nor their perspectives on the sector. This research sought to respond to that lack of data by collecting demographic information and first-hand perspectives from women working in the sub-sector of Virginia shellfish aquaculture. Semi-structured interviews with 23 women working in Virginia's shellfish aquaculture industry targeted topics of job satisfaction, gender-specific experiences, and industry challenges along with opportunities. Many participants identified gender-specific challenges, however, more than half were confident that they would continue in the industry. Additionally, participants observed that more women were entering the workforce over the course of their careers. Other themes that emerged included challenges related to climate change (e.g., effects of rainfall-related salinity changes on their operation) and potential opposition toward floating aquaculture gear. Participants also discussed the value of feeling connected to other women in the field. This project provides a look into an underrepresented yet increasingly important population within Virginia's shellfish aquaculture industry and sets the foundation for additional research to understand identity-based challenges and opportunities within U.S. aquaculture.

VALUES, RISKS, AND TRUST: UNDERSTANDING DETERMINANTS OF STAGE PROGRESSION WITHIN THE SOCIAL LICENSE TO OPERATE FRAMEWORK IN THE CONTEXT OF RECIRCULATING AQUACULTURE SYSTEM DEVELOPMENT

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Environmental threats and rising consumer demand pose critical challenges for the seafood industry. Recirculating aquaculture systems (RAS), an efficient, land-based technology to cultivate aquatic organisms, is poised to address these challenges. Stakeholder opposition, however, has deterred progress on many proposed RAS developments. This relationship between industrial actors and stakeholders is characterized by social license to operate (SLO), which entails the informal, often tacit judgment of an industrial actor's right to do business as intended. Research concerning novel or contentious technologies suggests that SLO is informed by stakeholders' values, risk perceptions related to the industry, and trust in industry actors, among other factors. While SLO is widely referenced in the aquaculture industry – and often depicted as a developmental stage model – limited work has tested such a model with empirical data, and none has used the RAS context in particular.

This research builds on past qualitative and quantitative studies completed by the authors that have sought to understand the drivers of SLO by focusing on contemporary RAS development in several U.S. communities. Using an online survey with an embedded experiment – a series of vignettes featuring news media depictions of a hypothetical RAS facility development – the present research empirically tested the drivers of SLO stage progression, and how these factors may change over time as the hypothetical RAS facility advances through critical stages in community engagement and permitting. Rural and urban U.S. residents ($n = 2200$) were surveyed to determine whether sentiments about RAS development differ across these two categories. Understanding the influence of environmental and cultural values, trust, and risk perceptions on SLO at varying stages of facility development is a novel approach to assess SLO progression. Analysis for this survey is ongoing and focuses on the how these key factors drive RAS development support among the sample. Using a structural equation modeling approach, we will determine the influence of values and risk perceptions on interpretation of the RAS development vignettes, and determine the stage and time ordered judgement making process that defines the SLO model.

Findings can provide decision-makers such as RAS professionals, municipal governments, extension agents, and regulators with evidence-based guidance on how to communicate with stakeholders and facilitate relationships with community partners, increasing the likelihood that the U.S. seafood industry can continue to explore domestic RAS development in a just and equitable manner. Ultimately, the project will contribute to guiding deliberative processes between communities and RAS developers – thus, helping to bridge the gap between researchers, communities, and practitioners.

ADVANCEMENTS IN AQUACULTURE: INNOVATIONS BY THE FOOD PROCESSING AND SENSORY QUALITY RESEARCH UNIT

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The USDA-ARS's Food Processing and Sensory Quality (FPSQ) research unit, based in New Orleans, LA, traditionally concentrated on catfish. It has now broadened its aquaculture projects to encompass salmonids. FPSQ recently completed design and construction of a recirculating aquaculture system designed to support diverse experimental designs and accommodate climate-related research. The program's longstanding emphasis on off-flavors continues, supported by a team of experts in fish biology, food technology, sensory analysis, and chemistry. Recent advancements from FPSQ include a novel method of geosmin and MIB quantification using isotope dilution, significantly reducing variability in analyte recovery and enhancing sample throughput. This method will help addresses the economic loss due to off-flavor catfish. This research has further revealed that off-flavors in fish can be absorbed orally, demonstrating that geosmin can accumulate through ingestion. This discovery opens new pathways for strategies to mitigate off-flavors, playing a vital role in enhancing sensory quality in aquaculture. Ongoing and future research includes determination of consumers' rejection thresholds for off-flavors in fish, sources of off-flavors, and pre- and post-harvest mitigation strategies. Future plans for novel feeds ingredients and alternative protein sources are also in place.

MICROBIAL DYSBIOSIS AND FUNCTIONAL SHIFTS IN VIBRIO CLADES AS DRIVERS OF EASTERN OYSTER MORTALITY

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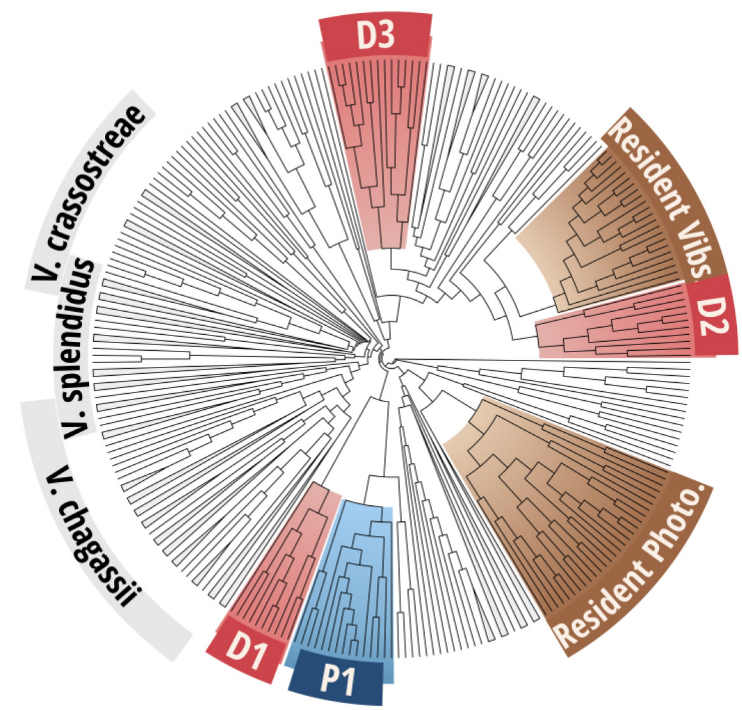
Shifts in Vibrio abundance and community structure have been implicated as potential precursors to mass mortality events in oysters. This study investigates the role of Vibrios in these mortality events by comparing the genomes of 110 Vibrio strains isolated from the viscera of diploid and triploid Eastern oysters (*Crassostrea virginica*) in 2022 and 2023. Isolates were collected either pre-mortality or during intense mortality events across two consecutive years.

Phylogenomic analyses revealed the presence of distinct Vibrio cladesthat dominated pre-mortality (P clade), or during-mortality (D clades). P clade Vibrios were identified as *Vibrio mediterranei*, and D clade is comprised of three distinct Vibrio groups – *V. astrianarerae, V. harveyi,* and *V. alginolyticus*.

Our analysis revealed a striking absence of P clade Vibrios during mortality events, and co- isolation of P and D clade Vibrios occurred in only a single oyster. We applied pangenomic analyses to compare the metabolic capacities of these clades and identified 16 metabolic pathways enriched in either the P or D clades. Pathways enriched in P clades suggest symbiotic interactions, including the utilization of host-derived sugars and amino acids, detoxification, and waste removal. In contrast, D clade Vibrios are enriched in pathways indicative of pathogenicity and antagonistic interactions with the oyster host, including type III secretion systems and effectors.



Persistent diverticula pathology in oysters leading up to and during mortality events suggests that nutritional deprivation, compounded by microbial interactions, plays a critical role in oyster mortality. Based on our findings, we propose a mortality model where P clade Vibrios function symbiotically pre-mortality, contributing to oyster health through metabolic cooperation. However, shifts in the surrounding environment create anaerobic, nutrient-poor conditions within oyster tissue micro-niches that favor the invasion and persistence of D clade Vibrios. The displacement of P clade symbionts and opportunistic colonization by D clades likely triggers a positive feedback loop of environmental stress and microbial antagonism, culminating in oyster mortality.

Our findings emphasize the need for longitudinal studies that actively monitor microbial metabolic dynamics to determine (1) whether P clade Vibrios indeed provide essential functions to oyster health that are lost during dysbiosis and (2) how opportunistic D clade pathogens exploit these shifts to drive mortality. This work underscores the importance of microbiome interactions in oyster health and provides insights into the microbial contributions to aquaculture-related mortality events.

THE DEMISE OF THE CHEVRON DOCTRINE AND THE LEGAL IMPLICATIONS FOR THE AQUACULTURE INDUSTRY

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The Supreme Court decided two landmark cases in its last term, *Loper Bright Enterprises v. Raimondo* and *Corner Post, Inc. v. Board of Governors*, that fundamentally alter how courts will consider regulatory rulemaking and challenges to administrative rules and regulations. This presentation will consider the legal and practical implications of these decisions and how the aquaculture industry should be prepared to respond accordingly.

COMMERCIALIZATION OF FUNCTIONAL FEEDS FOR RAINBOW TROUT *Oncorhynchus mykiss* PRODUCTION IN THE USA. A REPORT ON THE EFFICACY OF MANAGING ON-FARM MORTALITY DUE TO ELEVATED WATER TEMPERATURES AND PREVALENCE OF OPPORTUNISTIC PATHOGENS

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In many areas of the USA, rainbow trout (*Oncorhynchus mykiss)* aquaculture is characterized by having summer season reduced water flows and elevated water temperatures (up to 20º C). Feeding rates and growth rates have been managed using liquid oxygen additions to the rearing units; however, disease outbreaks due to the prevalence of opportunistic pathogens are common during the summer season. These disease outbreaks are commonly treated with chemical therapeutants, including antibiotics, but have had limited efficacy due to the mixed pathogen etiology of the disease events.

There have been many scientific studies describing the efficacy of feeding natural, immune-stimulating compounds as part of a daily ration under laboratory conditions. To that effect, there are numerous feed additive suppliers offering commercial versions of these immune-stimulating compounds. This report outlines the development and commercialization processes utilized to construct an efficacious feed additive blend that resulted in successful commercial application of an improved integrated pathogen management program on one commercial farm in North Carolina, USA.

PASSIVE ACOUSTIC MONITORING OF *Litopenaeus vannamei* BEHAVIOR UNDER DIFFERENT FEEDING FREQUENCIES

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Acoustic technology is emerging as a valuable tool for ethological studies, leveraging the click sounds emitted by shrimp mandibles during feeding. Passive acoustic monitoring (PAM) was employed to investigate the feeding behavior of *Litopenaeus vannamei* subjected to varying feeding frequencies. Shrimp groups (average weight 3.31 g) were placed in 12 tanks, with three treatment replicates for feeding frequencies of one, two, four, and eight times daily. The same quantity of pelleted feed was provided either as a single feeding or divided into two, four, or eight portions throughout the day. Feeding activity was monitored over an eight-hour period (8 am to 4 pm) using hydrophones connected to a digital recorder. Uneaten feed was collected at the end of each recording session to estimate feed consumption. Audio recordings were analyzed using Raven® Pro 1.5 software.The feeding behavior of *L. vannamei* varied with the feeding frequencies, showing increased click emissions and feed consumption in groups fed more frequently. Across all frequencies, an initial peak in click activity was observed during the first feeding of the day. Additional peaks in acoustic activity occurred only after subsequent feedings in groups subjected to two, four, and eight daily feedings. These findings highlight the advantages of implementing multiple feeding frequencies up to eight times daily to enhance feeding activity and feed consumption in *L. vannamei*.

INVESTIGATING NITROGEN CYCLE AND WATER QUALITY IN ALTERNATIVE POND-BASED PRODUCTION SYSTEMS FOR THE REARING OF LARGEMOUTH BASS *Micropterus nigricans*

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Aquaculture's rapid expansion emphasizes the critical importance of water quality for fish health and productivity. Innovative systems like split-ponds (SP) and pond-side tanks (TK) have been used for cultivating Largemouth Bass (LMB) while addressing challenges such as cannibalism and feed fluctuations. These systems also enhance water treatment through the nitrogen cycle, a fundamental process in aquatic ecosystems. Nitrification plays a vital role in this cycle by converting harmful unionized ammonia (NH_3), which can disrupt fish cell membranes, then into the relatively less toxic nitrite and nitrate. This study investigates the impact of water circulation in enabling the nitrogen cycle, improving nitrification to effectively transform toxic ammonia into nitrate and alleviating ammonia-related toxicity in the water, thereby promoting the health and productivity of fish.

Three ponds were selected for SPs, three ponds for TKs and three ponds for full ponds (FPs). Each pond had an area of 322.5 m^2 (25.8m x 12.5m), and an optimal attainable depth of 1.5 m. SPs were horizontally divided into two unequal sections (25% and 75%) by concrete wall with a perpendicular wall in the larger section extending to facilitate U-shape circulation. Water circulation was further governed by paddlewheel, gate, submersible pump, and piping. Tanks were adjacent to fishless full ponds; water was circulated by pumping water from the nearest part of the full pond into a tank, then drained to the farthest part of the full pond. Each system contained 1250 fingerling LMB, with an average size of 58.02 mm (± 2.71). The experiment ran from June to Sept. 2024. Water samples were collected in 18 different locations for weekly measurements of physiochemical variables: low & high range ammonia, nitrite, nitrate, total organic carbon, total nitrogen. Temperature and pH were measured daily.

Data processing is ongoing, but preliminary analysis of unionized ammonia (NH_3) concentrations across the treatment ponds has provided initial insights. Early results suggest that NH_3 levels follow the trend TK < SP < FP (Figure 1), with a statistically significant difference noted between FP and TK ($P = 0.0061$). Given the equal stocking density of fish, the presence of water circulation in TK and SP may partially explain these observations. Final conclusions will be discussed in the oral presentation.

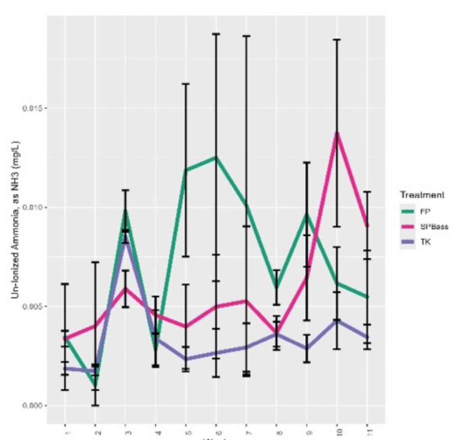

Figure 1. Weekly NH_3 concentrations in LMB rearing systems.

IMPACT OF NUTRITIONAL STATUS ON THE PATHOGENESIS AND SEVERITY OF MOTILE *Aeromonas* SEPTICEMIA IN CHANNEL CATFISH *Ictalurus punctatus*

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Motile Aeromonas Septicemia (MAS) is a significant disease affecting channel catfish (*Ictalurus punctatus*) in aquaculture settings. The objective of this study was to investigate the impact of nutritional status on the pathogenesis and severity of MAS by comparing pathological findings.

In this study, we used a bath immersion model to simulate the natural route of infection and examined gross and histopathological changes in the stomach, spleen, intestines, and hepatopancreas over time. Channel catfish were divided into four treatment groups: fin-clipped and fed (FCF), not fin-clipped but fed (NCF), fin-clipped and unfed (FCN), not fin-clipped and unfed (NCN). A bacterial challenge using vAh (ALG-15-097) was administered, and fish (n=90) were sampled at 2-, 4-, and 8-hours post-challenge (HPC) for gross and histopathological examination. Key organs including the stomach, spleen, intestines, and hepatopancreas were analyzed for pathological changes. A semiquantitative grading system was used to assess lesion severity, and results were compared between treatment groups. Gross pathological findings revealed that fed groups exhibited more severe external lesions, such as bilateral exophthalmia and hemorrhages around the gills, mouth, and fins, compared to unfed groups. Internally, spleen enlargement and engorged gastric arteries were prominent in fed fish but not in unfed fish. Histopathological examination showed early onset of edema, lymphoplasmacytic infiltration, and epithelial erosions in the stomach and intestines at 2 HPC, progressing to severe hemorrhage and necrosis by 8 HPC. The spleen exhibited severe congestion, edema, and melanosis, particularly in unfed fish, which supports the spleen's role as a primary immune organ during vAh infection. This study demonstrates that nutritional status significantly influences the distribution and severity of MAS-induced lesions in channel catfish. Fed fish showed more pronounced gastrointestinal lesions, suggesting that postprandial increases in blood flow to the stomach and intestines may facilitate bacterial proliferation. In contrast, unfed fish exhibited more severe splenic damage, indicating that nutrient deprivation may shift the immune response towards splenic sequestration of bacteria. These findings highlight the importance of nutritional status as a modulating factor in the pathogenesis of MAS and underscore the need for further research on the interactions between feeding, immune response, and disease severity in aquaculture settings.

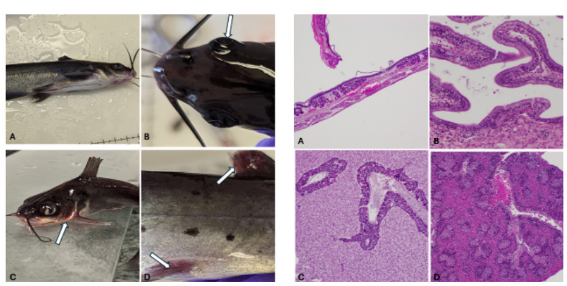

Fig: External lesions and histology controls

"GENDER REVEAL" FOR OYSTERS? AN INVESTIGATION OF EARLY SEX DETERMINATION MECHANISMS IN PACIFIC OYSTERS, *Magallana gigas*

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Pacific oysters, the most widely cultivated oyster species globally, contribute to over $89 million in sales in the United States alone. Oysters are intriguing sequential hermaphrodites, undergoing sex changes at various points during their lifespan. Nevertheless, the absence of secondary sex characteristics and the lack of sex chromosomes in this species remain a challenge for studying basic mechanisms of sex determination and also for the non-lethal sexing of animals. We set out to answer two questions to address cryptic sex states among oysters: 1. Are there specific early and/or later gene expression patterns that reliably predict final sex state of oysters at spawning time? and 2. Can these gene expression signals be detected from sampling hemolymph, which serves as the invertebrate equivalent of blood?

Hemolymph samples were repeatedly and non-lethally collected four times from the same group of marked individual oysters during the period prior to conditioning and throughout gonad maturation (Figure 1). Sex of each oyster were determined at the end of gonad maturation by gonadal biopsy. RNA-seq was used as a comprehensive discovery method to detect temporal differential expression patterns in male and female hemolymph samples. Genes involved in muscle growth, such as paramyosin (Ensembl Gene ID: G24796), were upregulated in females compared to males at the first sampled time point (26 days) (Figure 2). Genes involved in nutrient transport were upregulated in females compared to males at 36, 51 and 79 days, consistent with the observation of enlargement of eggs and vitellogenesis. Resolving markers and mechanisms of oyster sex determination will enhance the efficiency of spawning and crossing practices, potentially enabling controlled production of specific sex ratios and/or sterile oysters.

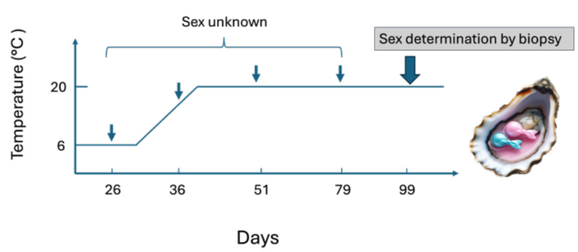

Figure 1. Experimental timeline. small arrows indicate sampling points.

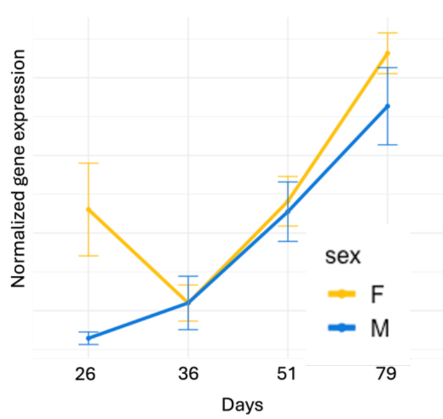

Figure 2. Paramyosin, a key component in the adductor muscle, shows sex-biased expression at day 26.

GENERATION AND EFFICACY OF A LIVE ATTENUATED VACCINES AGAINST COLUMNARIS DISEASES IN A SALMONID MODEL OF INFECTION

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Columnaris disease is a pervasive threat to a wide range of wild and cultured freshwater fish worldwide. There are currently no efficacious preventative methods available and most commercially valuable species cultured in the United States are susceptible, including salmonids, catfish (*Ictalurus* spp.), and tilapia (*Oreochromis* spp.). *Flavobacterium columnare,* the historical etiologic agent, has been recently reclassified into four related species: *F. columnare, F. covae, F. davisii* and *F. oreochromis.* In salmonids, *F. columnare* and *F. davisii* are the main agents associated with outbreaks in both wild and captive populations. We hypothesized that accumulation of mutations in *F. columnare* and *F. davisii* by serial passage in the presence of rifampin can generate immunogenic live attenuated vaccines (LAVs) that confer protection to vaccinated fish challenged with heterologous Columnaris causing-agents. Four lineages of rifampin-resistant strains were generated from two genetically distinct parent strains (n=4) by multiple passages in increasing increments of Rifamycin SV sodium salt. Attenuation was initially evaluated quantifying cytotoxicity in an CHSE cell line comparing the passaged and parental strains. Chinook salmon and rainbow trout challenges were then used to assess strain virulence and the protection conferred by LAV candidates against virulent *F. columnare*. Multiple passaged strains demonstrated significant reductions in cytotoxicity in CHSE ($p < 0.05$). Selected strains showed attenuated virulence in the salmonid challenge model. Immunization by immersion of some candidates proffered protection against a virulent WT strain of *F. columnare*; however, standardization of vaccination protocols are still needed. In conclusion, passaging of *F. davisii* in increasing concentrations of antibiotic is a viable method for generating LAV candidates with the potential to protect against heterologous WT strains of Columnaris causing-agents in salmonids.

MRNA VACCINE: A PROMISING THERAPEUTIC APPROACH FOR ISAV IN ATLANTIC SALMON

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Messenger RNA (mRNA) vaccines have emerged as a cutting-edge platform for combating infectious diseases. These vaccines consist of a delivery vehicle and one or more nucleic acids encoding single or multiple antigen candidates. Once taken up by host cells, these cells become factories for antigen production. Recently, particularly due to the SARS-CoV-2 pandemic, the production of mRNA vaccines has accelerated, though this progress has not yet been paralleled in cultured fish. Infectious salmon anemia (ISA) is a WOAH-notifiable disease caused by the ISA virus, a single-stranded RNA virus belonging to the Orthomyxoviridae family. Its genome comprises eight single-stranded RNA segments that encode at least ten proteins. Among these, the P3 or fusion [F] protein and the hemagglutinin-esterase [HE] protein have shown promising results in a recent pilot study of ISAv mRNA vaccines. Consequently, the aim of this study was to further explore the effects of these mRNA vaccines on the survival and immune response of Atlantic salmon during a cohabitation experiment. Fish were intramuscularly (i.m.) injected with a 200 µL dose of either a high (1 µg/g), 1:10 (0.1 µg/g), or 1:100 (0.01 µg/g) dilution of the respective vaccine, with PBS serving as a sham control. Half of the fish in each group received a booster after approximately 1000-degree days. In a parallel module, a group of unvaccinated fish was intraperitoneally (i.p.) infected with ISAv (HPR4 isolate at TCID50 1x 10^4). The viral donor fish were kept in a quarantined module for six days post-injection before being introduced to each experimental cohabitation tank, achieving a 5:1 ratio of cohabitant to donor fish. Fish were monitored three times daily, and mortalities were recorded. Serum, head kidney, and spleen samples were collected from all groups (n=5) one day after the onset of mortality. Our findings indicate that, out of the 28 vaccine treatments evaluated, two Fusion protein targets resulted in significantly higher survival rates compared to sham vaccinates, consistent with the pilot study. Current analyses are focused on assessing the presence of specific ISAv-antibodies using ELISA. Moreover, the modulation of genes related to vaccination and antiviral responses in Atlantic salmon has been assesed by RT-qPCR.

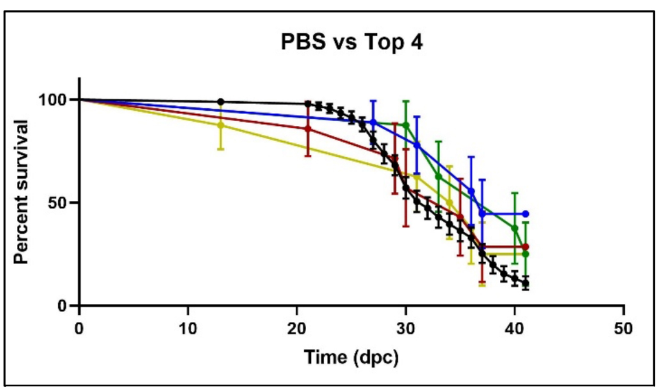

Figure 1. Survival of Atlantic salmon i.m. injected with a sham control (black line), 1 µg/g single dose (blue line), 0.1 µg/g boost (red line), 0.01 µg/g single dose (green line), and 0.01 µg/g boost (yellow line) of the candidate mRNA vaccine.

POTENTIAL OF SUB-UNIT AND LIVE-ATTENUATED VACCINES AGAINST PISCINE LACTOCOCCOSIS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Lactococcus petauri is an emerging pathogen in rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*) aquaculture in the Americas that has resulted in significant economic loss. Piscicine lactococcosis often presents as an acute septicemia with clinical signs and gross changes including: hyperpigmentation, lethargy, exophthalmia, hemorrhage of the skin, eyes, fins, and/or gills and death. Vaccination is key to preventing and controlling lactococcosis outbreaks.

In California, a two-step vaccination protocol using immersion followed by injection months later of autogenous whole-cell killed vaccines is being used to prevent the disease. To create alternative vaccines, the potential of sub-unit vaccines and live-attenuated vaccines (LAVs) was recently investigated. To generate the LAVs candidates, a recently isolated *L. petauri* was passaged on nutrient media with increasing increments of rifampin to generate random mutations. After 21 passages, nine strains were generated. Significant *in vitro* attenuation was observed in four strains. However, only one strain resulted in >90% survival during *in vivo* trials.

Additionally, we characterized immunodominant *L. petauri* antigens with potential applications for recombinant antigen subunit vaccine development. Rainbow trout sub-adults, were inoculated with formalin-killed whole-bacteria preparations in Montanide ISA 763 A adjuvant to generate anti- *L. petauri* IgM. Enzyme-linked immunoassays identified that vaccinated fish produced a significantly higher anti-*L. petauri* antibody response compared to control fish. Serum from the rainbow trout was used in a shotgun immunoproteomic approach for discovery of immunogenic *L. petauri* peptides via LC/MS-MS. This analysis identified two main immunogenic proteins using the trout serum. Further ELISA analyses revealed that serum from rainbow trout exposed to formalin-killed *L. petauri, L. formosensis* and *L. garvieae* preparations were strongly reactive to the selected proteins, suggesting that these proteins are highly conserved across the different piscine lactococcosis causing agents.

Further oral, immersion and injectable challenges suggests mucosal route provided immunostimulation against *L. petauri*, but further research is needed before these potential vaccines can be used in the field. Although LAV and sub-unit vaccines appear to be safe to trout fingerlings and protective against piscine lactococcosis, intra-coelomically immunized fish with LAV or whole-cell killed vaccines develop a significantly stronger protective response.

ENHANCING VETERINARY TRAINING IN AQUATIC MEDICINE: COLLABORATIVE MINI-SYMPOSIUMS TO ADDRESS THE GROWING NEEDS OF LATIN-AMERICAN AQUACULTURE

Esteban Soto*, Paola Barato, Henrique Figueiredo, Guilherme Campos Tavares, Fernando O. Mardones, Benjamin R. LaFrentz, Melanie Ammersbach, Fernando Y. Yamamoto, Sara Patricia Bonilla, Roberto Olivares, Ezekiel Salas, Rubén López, Maria de la Luz Chavacán Ávila, Ariadne Hernández Pérez

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With the growth in Latin-American aquaculture, the emergence and global dissemination of numerous pathogens, and the lack of formal training on aquaculture medicine at many Latin-American Veterinary Schools, it is imperative that easily accessible workshops and continuing education opportunities are presented to enhance the training of veterinarians in aquatic medicine for the growing aquaculture industry. Well-trained fish veterinarians are needed in many Latin-American countries to ensure health program standards are successfully implemented to benefit aquatic animal health and farm operations.

In the past decade for example, Latin-American aquaculture has been challenged with numerous emerging pathogens. Emerging viral and bacterial pathogens have caused significant economic losses in salmon, trout, and tilapia aquaculture. Still, at this point, few Latin-American veterinary schools offer courses focused on fish medicine. As an inevitable outcome, there is a demand for well-trained, knowledgeable aquaculture veterinarians in Latin-America and the Caribbean.

During 2024, the University of California-Davis (USA), CORPAVET/MolecularVet (Colombia), the Universidad San Francisco de Asis Veritas (Costa Rica), Universidade Federal of Minas Gerais (Brazil), Universidad Nacional Autónoma de Mexico (Mexico), Corhuila and the Universidad SurColombiana (Colombia), the Latin Comparative Pathology Group from Davis Thompson Foundation, the World Aquatic Veterinary Medical Association, and the World Aquaculture Society, partnered to offer four mini-symposiums on fish medicine to veterinarians in Latin-America in Costa Rica, Brazil, Colombia and Mexico. The symposiums were provided in different languages (Spanish, Portuguese, and English), and live-translated when needed. The main format was oral presentations, but also fostered discussion sessions and a one-day hands-on laboratories where anesthesia, euthanasia, necropsy, and sample collection of fish were demonstrated or performed by students.

The mini-symposiums provided supplementary training to practicing veterinarians, and easily accessible, valuable, and up-to-date information to veterinary trainees. Additionally, the mini-symposiums served as a forum to exchange up-to-date information on infectious and non-infectious diseases of cultured fish, including disease prevention, diagnosis, and treatment, particularly those reportable at international levels. Finally, the workshop fostered national and international cooperation to support improvements in the health of aquaculture animals.

In total, over 400 attendees/students participated in the mini-symposiums. The support of WAVMA also allowed one of the mini-symposiums to be available for other interested participants on-line. An overwhelming positive response was received, and although more educational opportunities are needed, these type of programs are a step-forward into the implementation of better aquaculture practices in Latin-America.

CHARACTERIZING THE EFFECTS OF TRIPLOIDIZATION ON GUT BARRIER INTEGRITY AND IMMUNE RESPONSE IN CHINOOK SALMON: A HISTOLOGICAL AND MOLECULAR ANALYSIS

Manuel Soto-Dávila*, Abigail G. Dixon, Megan Dyck, Valentina Wong-Benito, Maricruz Guevara, Tania Rodríguez-Ramos, Paola Avaria-Vasquez, Rochelle Supinski, Brian Dixon

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Ploidy is a fundamental genetic concept that refers to the number of sets of chromosomes in an organism's cells. Most salmonids are naturally diploid, possessing two sets of chromosomes, but triploid salmon are increasingly produced through breeding techniques to enhance specific traits. Triploid salmonids, which have three sets of chromosomes, demonstrate accelerated growth rates and improved disease resistance, making them particularly valuable in aquaculture. While the triploidization of salmonids offers several advantages, it also presents challenges and concerns. A significant issue is the potential reduction in genetic diversity, reduced adaptability and resilience to environmental changes, and diseases. Studies have shown that triploid Chinook salmon exhibit 10-30% higher mortality rates and increased susceptibility to diseases compared to their diploid counterparts under aquaculture conditions. Chinook salmon, the largest and most valued species of Pacific salmon in North America, are prized for their size, resilience, flavor, and nutritional composition, making them an attractive candidate for diversifying Canadian aquaculture. Consequently, we investigated the impact of triploidization on the gut of Chinook salmon, an organ vital for their overall health and survival. We also evaluated how molecular and structural markers are modulated in each ploidy during an infection challenge using *Vibrio anguillarum*. To achieve this, we assessed the expression of genes associated with the innate and adaptive immune responses, as well as gut barrier integrity, in the pyloric gut, midgut, and hindgut of both diploid and triploid Chinook salmon. Additionally, we measured protein levels of key immune markers through Western blot and ELISA. Histological analysis was conducted to determine the structural effects of triploidization on the gut. Our results indicate that triploidization influences the modulation of genes across different gut sections, as well as the response of each section to *V. anguillarum* challenge. Notable differences were observed in the levels of four proteins—β2m, IL-1β, IgM, and IgT—between the ploidies, with particularly interesting findings of consistently higher β2m levels in infected triploids compared to diploids. We anticipate that the histological findings will help clarify whether these molecular variations between ploidies correspond to structural changes in the Chinook salmon gut or are confined to molecular alterations. This study aims to establish a reliable protocol for future molecular research on Chinook salmon and provide a standardized comparison of how the three gut sections respond to pathogens in diploid versus triploid salmon.

Figure 1. Pyloric gut of diploid Chinook salmon.

PREVALENCE OF PATHOGENIC BACTERIA IN NILE TILAPIA *Oreochromis niloticus* FARMED IN SOUTHWEST MEXICO: A REAL-TIME PCR ASSAY

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In southwest Mexico, mortality of farmed tilapia *O. niloticus* is recurrent. In 2018, 2019, 2020, and 2022, samples of internal organs and lesions of farmed tilapia were taken to detect the most crucial fish pathogens. First, real-time PCR protocols were standardized to obtain a reliable qualitative detection of the selected bacteria using fixed tissues. 599 farmed tilapias in rural ponds and cages from Guerrero, Oaxaca, and Chiapas Pacific belonging to southwest Mexico were analyzed. This Mexican region is characterized by tropical weather, where, in general, the water temperature of the cultured tilapia was optimal for the growth of Nile tilapia. Still, sometimes extreme temperature values were registered. Most of the sampled tilapia showed a normal appearance, but some displayed hemorrhage areas, desquamation, bulging at the base of the caudal fin, exophthalmia, etc. No *Francisella sp.* was detected in any sample, *Staphylococcus sp.* was the bacterial genus more prevalent over time (from 0 to 64%) (figure 1), the low prevalence was found in Aeromonas sp. (4.3%), and *Streptococcus iniae* had 1.4% prevalence in Chiapas (2019).

Streptococcus agalactiae, the most dangerous bacterial pathogen for tilapia, was detected at high prevalence (0 to 59 %) in the three Mexican states. This is the first report of bacterial pathogens in farmed tilapia in Mexico using the real-time PCR assay. Detection of *S. agalactiae* in high prevalence constitutes a significant health risk for tilapia aquaculture in Mexico and a potential spread of these pathogens to other aquaculture areas.

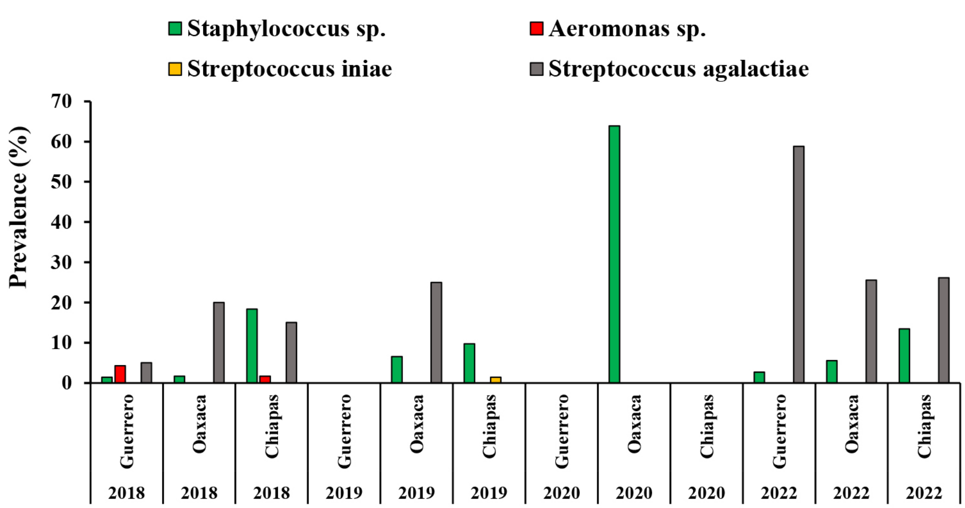

Fig. 1 Bacterial prevalence in farmed tilapia from the southwest Mexico (Guerrero, Oaxaca, and Chiapas). ns, no sample.

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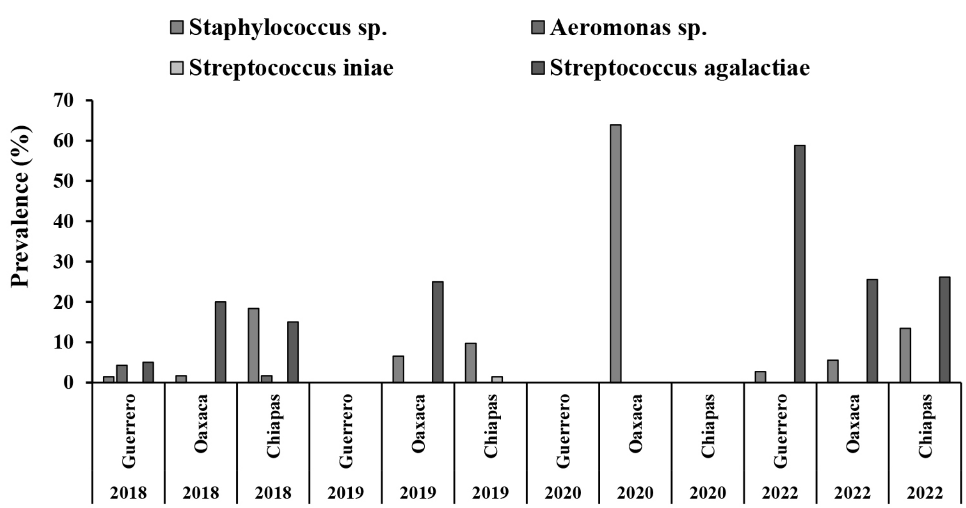

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APPLICATION OF *Faecalibacterium* GENOMICS FOR HUMAN AND SHRIMP GUT HEALTH

Eduarda G. Sousa*, Ana Lua O. Vinhal, Thaís C. V. Rodrigues, Jean-Marc Chatel, Vasco Azevedo and Marcus V. C. Viana

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Faecalibacterium is a bacterial genus in which *F. prausnitizii* is considered a next-generation probiotic for human gut health. Since 2021, six other species from the genus have been described, and genomic data suggests dozens of undescribed species have associations with different hosts, diets, and geographical locations. As the probiotic effect is strain-dependent, this diversity represents an unexplored potential for strain screening and the development of new probiotic products.

Recently, *Faecalibacterium* has been associated with a healthy gut of the Pacific white shrimp (*Penaeus vannamei*), and *F. praunitzii* was shown to protect it from the pathogen *Vibrio parahemolyticus*, a major seafood-borne zoonotic pathogen that causes gastroenteritis in humans and acute hepatopancreatic necrosis disease (AHPND) in shrimp. *P. vannamei* is the most cultivated shrimp species, with a global production value of U$ 29 billion in 2022. Its production has faced economic losses due to outbreaks of devastating bacterial diseases, such as white feces syndrome, related to dysbiosis in the shrimp gut microbiota. In this context, probiotic *Faecalibacterium* strains could be incorporated into the shrimp diet. Genomic analyses can help identify new species and variants that can be used for diagnosis methods, probiotics development, and culture media design for previously unculturable bacteria. This study aimed to identify marker genes for *Faecalibacterium* species in the shrimp gut and to develop selective culture media to isolate strains of undescribed species, with applications for shrimp aquaculture.

The reference genomes of 70 *Faecalibacterium* species identified by the Genome Taxonomy Database (r220) were retrieved and annotated using Prokka v. 1.14.6. Orthologous genes were identified using OrthoFinder v. 2.5.5, and the exclusive genes of each genome were identified using an R script, with their functional annotation predicted using eggNOG-mapper v. 2.1.12. The 70 reference genomes had between 8 and 183 candidate marker genes. Among the 63 genomes from undescribed species, 34 had between 1 and 12 of these genes annotated as enzymes and from 1 to 17 as proteins that participate in metabolic pathways. The marker genes will be validated through a co-abundance analysis of those genes in DNA sequencing reads from public P. vannamei gut microbiomes. The validated genes will be investigated for their potential application in developing selective culture media. Additionally, metagenome-assembled genomes will be obtained from the public DNA sequencing reads to evaluate the functional diversity of *Faecalibacterium* strains from the *P. vannamei* gut.

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DECLINE AND RECOVERY OF THE CHESAPEAKE OYSTER POPULATION: UPDATING AN OLD METRIC

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The decline of the Chesapeake Bay oyster, *Crassostrea virginica,* resource in the post-Colonial period through the combined impacts of overfishing, environmental degradation, and disease epizootics has been well described in the literature. Newell (1988) estimated a two order of magnitude reduction in Bay oyster biomass (188 x 10^6 kg to 1.9 x 10^6 kg). Rothschild et al. (1994, https://www.jstor.org/stable/24847607) noted that the Maryland oyster population had "declined more than 50-fold since the early part of the century." Wilberg et al. (2011, https://www.jstor.org/stable/10.2307/24875491) reported that "oyster abundance declined 99.7% (90% credibility interval [CI], 98.3 to 99.9%) since the early 1800s and 92% (90% CI, 84.6 to 94.7%) since 1980" in the upper Chesapeake Bay. These depressing statistics remain widely quoted, yet they are outdated and in need of revision. The extant bay oyster populations are exhibiting increased tolerance of endemic diseases, and with careful management, including significant investment in the private fishery in Virginia, the resource has rebounded in the past two decades to support a sustainable annual harvest in the region of 1,000,000 bushels for Maryland and Virginia combined. We review survey and commercial landings data during the post epizootic recovery period, discuss future stability of the resource, and suggest a somewhat less depressing metric for comparisons with pre-Colonial oyster populations.

UNDERSTANDING INTERACTIONS WITH ENDANGERED MARINE MAMMALS AND TROPICAL AQUACULTURE

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Marine aquaculture is a rapidly growing field both in technology and protected species monitoring and mitigation. In the U.S. Pacific Islands Region, the Hawaiian monk seal (*Neomonachus schauinslandi*) is an endangered endemic species whose range overlaps with an aquaculture facility using open-water finfish net pens. Aquaculture facilities can attract wildlife, including protected species, resulting in unwanted interactions. NOAA Fisheries has partnered with Blue Ocean Mariculture to investigate and adaptively manage monk seal interactions at its aquaculture facility following a 2017 mortality event and subsequent Endangered Species Act section 7 consultation. This is a coordinated effort between managers of the regulatory branch at NOAA's Pacific Islands Regional Office, scientists at the Pacific Islands Fishery Science Center, and Blue Ocean Mariculture. To date, over 160 hours of camera footage and three years of human observations have been reviewed. Through the use of aquaculture personnel observations, deployed underwater HD cameras, and artificial intelligence, we can identify and analyze monk seal behaviors associated with the aquaculture net pens, which allows us to test mitigation strategies for minimizing the targeted behaviors. We have identified 71 distinct monk seal interactions and behaviors associated with the net pens that we aim to minimize. Our results show that when used together, human observation and cameras are an effective way to identify and monitor Hawaiian monk seal interactions at an offshore aquaculture facility. This data is being used to implement protocols and mitigation measures to deter Hawaiian monk seals from predating the pens and to reduce the probability of protected species injury or death. Outcomes of this ongoing project will provide a more detailed understanding of protected species behaviors around aquaculture net pens, support adaptive management, and the regulatory processes necessary to reduce these risks.

WHAT'S NEXT FOR OPEN OCEAN AQUACULTURE MANAGEMENT IN THE PACIFIC ISLANDS

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The National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) Pacific Islands Regional Office and the Western Pacific Fishery Management Council (Council) are working to establish an aquaculture management program in the Pacific Islands Region (PIR). We will present an overview of the current and potential future system for permitting aquaculture in Federal waters of the PIR.

Aquaculture in Federal waters in the PIR is not currently subjected to management oversight, with limited exceptions. This current situation increases the potential for the development and proliferation of unmanaged aquaculture operations in federal waters in the PIR. NMFS national and regional priorities seek to increase opportunities for sustainable aquaculture to promote safe, sustainable, seafood production. Aquaculture operations could supplement wild-caught fish, increase food security, reduce reliance on seafood imports, and provide economic opportunity and job creation.

The first steps in developing a program included preparing a Programmatic Environmental Impact Statement (PEIS) to support early planning for a future management program and to evaluate the potential effects of alternative management provisions under consideration. The final PEIS was published in September 2022 and can be viewed here: https://www.regulations.gov/document/NOAA-NMFS-2021-0044-0003/.

NMFS and the Council are now preparing a draft management plan for publication in 2025 and will be seeking public comments once it is published. If approved, aquaculture in the U.S. Exclusive Economic Zone (EEZ or Federal waters) around the U.S. Pacific Islands of American Samoa, Guam, Hawaii and the Northern Mariana Islands would be managed under the Council's Fishery Ecosystem Plans (FEPs) and their implementing regulations.

The proposed aquaculture management program is being designed to regulate, manage, and promote the development of an environmentally sound and economically sustainable aquaculture industry in Federal waters of the PIR. The program would enable NMFS and the Council to provide enhanced planning, coordination, and oversight of aquaculture in PIR Federal waters, and is intended to help provide operational stability and maintain Council and NMFS commitments to sustainable and environmentally sound fisheries management.

EVALUATING THE INTEGRITY OF A STRATIFIED RANDOM STOCK SURVEY DESIGN IN LIGHT OF PROJECTED CLIMATE-INDUCED CHANGES IN SPECIES DISTRIBUTIONS: AN ATLANTIC SURFCLAM CASE STUDY

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Instances of range expansion and poleward shifts in species continue to occur on the Northeast Continental Shelf due to climate-induced warming. Changes in the distribution of commercial species has severe implications for fisheries stock assessments, as changes in species' distributions outpace revisions in stock survey designs, therefore increasing uncertainty in stock biomass estimates. The distribution of the commercially important Atlantic surfclam, *Spisula solidissima*, a biomass dominant on the Mid-Atlantic Bight shelf, has been projected to expand offshore and north as shelf warming continues, landing this species into what has historically been a cold-water habitat for the commercially important ocean quahog, *Arctica islandica*. This anticipated range shift prompts an investigation into the statistical strength of the Atlantic surfclam federal survey design over the remainder of the 21st century. The survey is organized into 4 parts: the inshore and offshore regions of the MAB west of the Great South Channel, and the periphery and interior of Georges Bank. Anticipated outcomes of this distributional shift among these four units include a degradation in the integrity of the stratified random sampling regime as the stock redistributes itself, leading to decreased confidence in status of stock estimates. This is particularly true for the two offshore regions for which stratum design and sample density were originally developed to optimize biomass estimates of the colder-water ocean quahog, a species being displaced over time by the Atlantic surfclam. Results of this investigation show that the Atlantic surfclam survey in all four regions provides reliable estimates of biomass over a 79-year period, with bias generally decreasing over time. This perhaps unexpected improvement in survey performance accrues from the influence of warming temperatures on geographic distribution, where a reduction in large-scale patchiness is observed as clams disperse more evenly across the shelf over the coming decades. However, the survey becomes increasingly incapable of capturing the entirety of the Atlantic surfclam biological habitat over time, as an increasing number of clams occupy areas outside of the survey boundaries, with the worst mismatch occurring in the early 2080s when approximately 12% of total surfclam biomass will be missing from the survey.

INTRODUCTION TO OZONATION AND ITS BENEFITS FOR RECIRCULATING AQUACULTURE SYSTEMS

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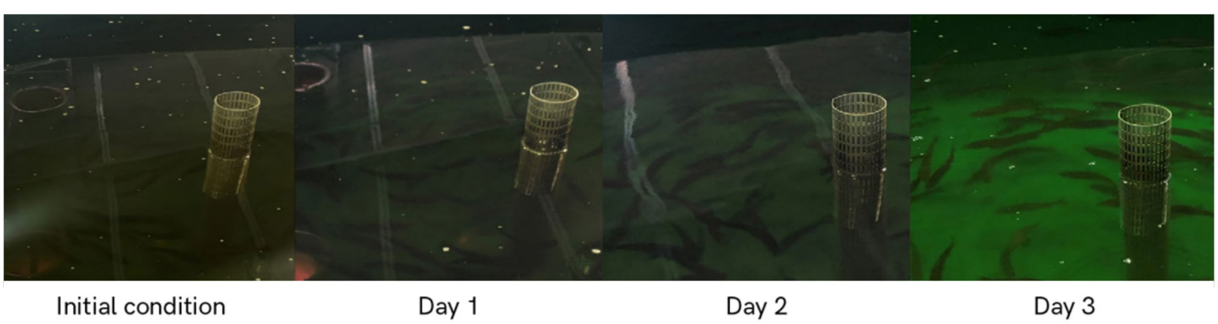
Recirculating aquaculture systems (RAS) are closed-containment systems where fish are farmed in reused water. RAS has become increasingly important as the available water is better utilized, achieving production continuity. However, such systems that are characterized by prolonged hydraulic retention time, high production intensity and feed loading, the water is heavily loaded by organic and inorganic compounds, where proteins, ammonia, urea, heavy metals and dissolved organic matter (DOM) are the most pronounced. In presence of high DOM, the overall water quality is deteriorating, creating a favourable environment for microorganisms' abundances to thrive resulting in disease outbreaks or being responsible for a muddy taste of fish flesh making them not suitable for consumption. Disinfection and vaccination have been applied as part of the management of RAS to control or eliminate pathogens. To address the need for environmentally friendly disinfectants, drifting away from chemotherapeutants, ozone has been introduced as an alternative in RAS.

Ozone is a strong oxidizing agent, reacting rapidly with the non-biodegradable DOM. When ozone is added into water, protein degradation is enhanced, while the water clarity, UV transparency and the processes of filtration and nitrification are improved. Ozone as disinfectant is able to kill bacteria, parasites and viruses. In a non-meticulously designed system, ozone can reach the tanks affecting adversely the fish. Depending on the water matrix, additional issues emerge, e.g. formation of brominated by-products in salt water. The risk of losing fish from overdose, the failure to monitor the ozone concentration and the high investment and running costs are limiting parameters and lead to a reluctance by the aquaculture industry to use ozone.

Over the past years we have participated in several projects aiming to resolve a number of issues in RAS water but also issues related to ozonation itself. Several examples, based on our industrial experience, will be given, where ozone technology has been integrated in different fish farms to improve the overall water quality, enhance fish welfare, remove off-flavour in fish meat, optimize different water treatment technologies to strengthen green and blue effects and to regulate the ozone dose avoiding over or under dosing and without forming any brominated by-products in salt water facilities.

Figure 1: The ozonation effect of RAS water.



RECONSTRUCTING BOTTOM WATER TEMPERATURE DISTRIBUTIONS ON THE MID-ATLANTIC CONTINENTAL SHELF

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The bottom water temperature (BWT) distribution on the Mid-Atlantic continental shelf is characterized by a region of cold water, the Cold Pool, that occupies the inner and central shelf during summer and provides a habitat for the boreal ocean quahog (*Arctica islandica*), a long-lived (about 500 years) bivalve species. Time series of BWTs the ocean quahog experienced can be recovered from growth increments recorded in the shell using a growth-temperature relationship. This study combines spatial patterns of BWT variability with shell-derived temperature time series to reconstruct BWT on the Mid-Atlantic continental shelf. Spatial patterns of variability were obtained from an empirical orthogonal function (EOF) analysis of BWT distribution climatologies constructed using the 1993 to 2020 Global Ocean Reanalysis. The EOF analysis for the May to June climatology, when the Cold Pool is present, showed that most of the spatial variability was explained by the first three modes, which represented across-shelf variability in BWT, the Cold Pool, and along-shelf variability, respectively. The spatial patterns were combined with prescribed temperature time series of BWT at discrete locations on the Mid-Atlantic shelf to reconstruct BWT distributions. These reconstructions provide guidance for identifying the number and quantity of growth-temperature relationships needed to obtain temperature from the ocean quahog growth rates. The temperature time series derived from the shell growth rates, combined with the BWT spatial patterns and temperatures estimated from fossil shells, allow extending reconstructions to earlier times to assess decadal variability in BWT distributions on the Mid-Atlantic continental shelf.

GREAT LAKE STATE REQUIREMENTS FOR LIVE FISH MOVEMENTS AND VIRAL HEMORRHAGIC SEPTICEMIA MANAGEMENT: CHARACTERIZING DIFFERENCES AMONG STATE REGULATIONS

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A virus with a complex history in the Great Lakes region, Viral Hemorrhagic Septicemia virus (VHSV) IVb was first detected in the region in 2005 in wild fish, and a cascade of regulatory action was quickly enacted at the federal- and then state-levels. The United States Department of Agriculture (USDA) quickly instituted a stop movement order of live fish, including those cultured by private, public, and tribal fish producers, across Great Lakes state lines. This stop movement order immediately impacted and/or raised concerns among fish producers from all sectors in the Great Lakes region, with private farmers worried about business continuity and public and tribal hatcheries concerned about meeting seasonal stocking quotas. After push back from fish producing stakeholders against the agency's decision, the USDA amended the order and gave state natural resource and/or agriculture agencies jurisdiction over managing live fish movements.

As of 2024, state agencies take varied approaches to managing fish movements with specific requirements in place to potentially curb the spread or introduction of VHSV IVb within or into their state. Such requirements include VHSV testing of as part of fish health certificate requirements as well as requiring specific testing for fish coming from "VHS-affected" areas or for fish that the individual states deem as susceptible to VHSV IVb. As part of a USDA National Animal Disease Preparedness and Response Program funded project that focuses on the risk of VHSV IVb spread in the Great Lakes region, we sought to summarize and characterize the state-specific requirements for inter- and intra-state fish movements and for VHSV management and response. Collaborating with Minnesota Sea Grant and the National Sea Grant Law Center, we summarized publicly available state regulations and/or regulations provided by the representatives from the appropriate agency in each Great Lake state. Additionally, representatives from Tribal Nations located within the Great Lakes region were consulted regarding any VHS management and/or fish movement regulations that are put in place in their own nations. Regulations surrounding the following areas were summarized: 1) state specific definitions related to VHS; 2) areas related to VHS management and fish movements where external guidance is followed; 3) multi-agency roles and responsibilities in VHS management and fish movements; 4) fish health certificate requirements for movement; 5) VHS testing requirements for movement; and 6) additional movement requirements for fish. Upon completion, the summaries were validated by representatives from state agencies or tribal nations and any corrections were made.

Here, we discuss the consistencies and differences observed among Great Lakes states and tribal nations in how VHSV and fish movements are managed. Additionally, we discuss how these findings looked at in tandem with findings from a regional VHS risk assessment can assist state and tribal agencies in making fish movement and VHS management decisions.

TRANSLATING RESULTS FROM A VIRAL HEMORRHAGIC SEPTICEMIA RISK ASSESSMENT INTO RISK-BASED TOOLS FOR COMMERCIAL AQUACULTURE PRODUCERS IN THE GREAT LAKES REGION

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In 2021, the United States Department of Agriculture's (USDA) National Animal Disease Preparedness and Response Program funded the first proposal to focus on disease risk in the aquaculture sector. Specifically, the funded project aimed to assess the risks of spreading Viral Hemorrhagic Septicemia virus (VHSV) IVb in Great Lakes region associated with moving live fish from a fish farm or hatchery. A virus with a complex history in the Great Lakes basin, VHSV IVb was first detected in 2005 in wild fish, and a cascade of regulatory action was quickly enacted at the federal- and then state-level. The USDA quickly instituted a stop movement order of live fish, including those cultured by private, public, and tribal fish producers, across state lines. After push back from fish producing stakeholders against the agency's decision, the USDA amended the order and gave state natural resource and/or agriculture agencies jurisdiction over managing live fish movements.

As of 2024, state agencies take some varied approaches to managing fish movements with specific requirements in place to potentially curb the spread of VHSV IVb. Additionally, detections of VHSV IVb still occur annually in wild fish in isolated areas in the Great Lakes Basin and detection of the virus has occurred in wild broodstock of salmonid species at spawning weirs; however, there has never been a detection of the pathogen on a fish farm or hatchery. Because of the absence of detections in propagated fish populations, continued sporadic detections of the virus in wild populations, and the consequences of the detecting the virus on a farm or hatchery, perceptions of disease introduction risk vary among stakeholders.

To this end, a regional VHSV risk assessment was conducted, which included the evaluation of the likelihood of VHSV entry onto Great Lakes fish hatcheries. Likelihood of VHSV entry was evaluated using a framework adapted from the World Organisation of Animal Health (WOAH) Import Risk Analysis methods as well as from Secure Food Systems Proactive Risk Assessment methodology. Specific disease entry pathways onto fish hatcheries were identified and systematically evaluated through the qualitative synthesis of input provided by a Great Lakes Public and Tribal Fish Hatchery workgroup as well as available scientific literature related to known host-pathogen-environment interaction mechanisms. Throughout this process biosecurity gaps on fish hatcheries were identified based on workgroup input and additional pathogen-specific mitigations were determined. Upon completion of the final step, the likelihoods of VHSV entry were assigned ratings using a likelihood rating scale utilized by the World Organization for Animal Health for risk analysis.

While results of the risk assessment are specific to public and tribal fish hatcheries, here we discuss how results have been adapted to inform risk-based biosecurity that commercial fish farms can implement if they are in a VHS-high risk scenario. Additionally, findings from the risk assessment have been translated into a tool that can help fish producers determine what constitutes a VHS-high risk scenario.

RESOLVING SPECIES-SPECIFIC FUNCTION IN MICROBIOME ANALYSIS IS KEY TO MANAGEMENT DECISIONS IN AQUACULTURE

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The functional dynamics within bacterial communities are increasingly recognized as crucial to cultured fish and shellfish health. Interest in microbiome analysis has soared in response and a number of studies on environmental, gut, and skin bacteria have been conducted within aquaculture contexts. The majority of investigations use NGS platforms to sequence the hypervariable V3/V4 region of the bacterial 16s rRNA gene, providing identification and relative abundance data for all genera. While the V3/V4 region can also provide species-level differentiation in some cases, the necessary resolution for accurately defining closely related species or detecting uncommon or recently identified genera is lacking. Bacterial metabolism, growth strategies, pathogenicity, and capacity for beneficial or antagonistic microbial interactions are broadly species dependent. The work presented here represents a compilation of data from our custom full-length (FL) 16S rRNA gene sequencing platform and database. We describe species-specific bacterial functions related to nitrite and salinity tolerances in nitrifying biofilms of recirculating aquaculture systems (RAS), along with sulfur and iron-metabolizing bacteria profiles that inform on biofilter performance. We find that full-length 16S rRNA identification of important bacterial groups such as Vibrio is key to avoid overrepresenting health risks from benign species, while identifying those that are truly threatening. Further overrepresentation of production risks due to some secondary metabolites (toxins, taste and odour chemicals) produced specifically by cyanobacteria and myxobacteria have been detected, which can blur data interpretation and management decisions. Lastly, probiotic retention within treated animals and environments can be misrepresented due to failures in differentiating common non-therapeutic members of the probiotic genus. These examples and others will display the scientific value of full-length 16S rRNA sequencing in RAS biofilter function, water quality surveillance for indoor and outdoor aquaculture, pathogen profile identification, and investigations of probiotic efficacy, all of which are relevant to critical management decisions for aquaculture producers.

IDENTIFYING ACUTE STRESSFUL CONDITIONS IN FOUR LOUIAIANA OYSTER HABITATS FROM HIGH FREQUENCY DATA

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Estuarine habitats are experiencing increasing change due to the effects of climate change and other anthropogenic stressors. Increasing temperatures, continued agricultural runoff, hypoxic zones, and changes in salinity are all factors that can affect the growth, success, and mortality of eastern oysters (*Crassostrea virginica*). As both oyster fisheries and aquaculture face these dynamic conditions, understanding and identifying the frequency and duration of stressful conditions and how they interface with eastern oyster tolerance ranges is key to better prediction of habitat suitability for oysters in key growing areas. Many habitat suitability indices for oysters rely on monthly averages of environmental data; however, it is likely that acute stressful conditions are still occurring within the months represented by those mean conditions, even in sites considered suitable. To test this hypothesis, continuous monitoring data collected every 15 minutes from four oyster-growing regions in Louisiana were analyzed to determine exposure to stressful conditions from temperature, salinity, dissolved oxygen, or phytoplankton biomass (as chlorophyll-*a*). Stressful events were assessed as either lethal and nonlethal based on published tolerance ranges (or optima). Preliminary data shows a general absence of lethal synergistic stressors in the four systems and 18 months of data analyzed thus far. Nonlethal stressors occurred at frequencies in excess of 17 events in the study period, lasting upwards of 31 days, with West Cove having the highest number events. Overall, dissolved oxygen as a nonlethal stressor ($x < 4$ mg/L) was observed most frequently with durations ranging from 2-31 days. Additionally, temporal trends of nonlethal stressor events showed higher frequencies in summer months (May-August). Additional results including frequency, duration, and overall site scoring, as well as recommendations for incorporating such analyses into oyster aquaculture site selection, will be further discussed.

TRACKING HARMFUL CYANOBACTERIA AND THEIR EFFECTS ON LOUISIANA OYSTERS USING MOLECULAR TOOLS

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The emergence of toxigenic cyanobacteria in Louisiana estuarine systems raises concerns regarding the impacts of fitness and public health risks of exposure to valuable shellfish species like the eastern oyster, *Crassostrea virginica*. The freshwater cyanobacterium, *Microcystis aeruginosa*, is of particular concern due to its salt tolerance, production of the cyanotoxin microcystin, ability to outcompete other phytoplankton species when pulses of nutrient-rich freshwater are delivered to receiving basins, and documented presence in oligo- and mesohaline Louisiana estuaries. This spatiotemporal overlap of eastern oysters and *M. aeruginosa* makes monitoring efforts and research resolving feeding of the eastern oyster on this, and similar, species of pressing importance. However, individual cells of *M. aeruginosa* are small (2-4 μm) but can also form large colonies (100 μm) comprised of hundreds to thousands of cells, both of which are difficult to enumerate accurately using traditional microscopy. Furthermore, morphological identification of *M. aeruginosa*, or other cyanobacteria species that produce toxins non-constitutively, does not tell us if a given cell is producing harmful cyanotoxins.

Through the application of molecular techniques (real-time quantitative PCR; RT-qPCR) we are detecting the presence of low concentrations (250 cells ml^{-1}) of *M. aeruginosa* in local oyster habitats and determining which portion of those cells possess the genes to produce microcystin (approx. 65%). These data are providing important data that help explain microcystin concentrations in oyster tissues ranging from 0.194 to 0.941 ng MC g^{-1} oyster tissue wet weight (MCY-DM ELISA). We also used RT-qPCR to quantify possible rejection of a non-toxic strain of *M. aeruginosa* in lab-based oyster feeding experiments. These results showed that rejection of *M. aeruginosa* was consistent even with alternative prey available (Fig. 1), although total pseudofeces production rates increased with a diet rich in the cyanobacterium. Molecular methods are proving critical tools in enhancing our understanding of toxigenic cyanobacteria as components of the natural estuarine phytoplankton communities, relating species abundance to toxin accumulation in oyster tissue, and understanding oyster feeding behavior as these cells become more abundant in prey communities.

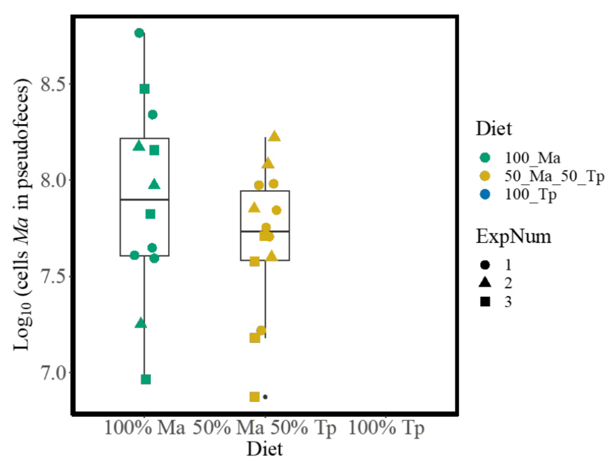

Figure 1: Abundance of *M. aeruginosa* in oyster pseudofeces in diets consisting of 100% *M. aeruginosa* (green) and 50% *M. aeruginosa*/50% *Thalassiosira pseudonana* (yellow). From Sweet et al., in prep.

EXPERIMENTAL OFFSHORE AQUACULTRUE OF THE ATLANTIC SURFCLAM *Spisula solidissima*

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The Atlantic surfclam (*Spisula solidissima*) is an economically important fisheries species in the mid-Atlantic region of the United States. Although not widely commercially practiced, there is potential to diversify surfclam products brought to market through aquaculture production. Although aquaculture farms are often established in protected coastal areas, farming in the open ocean presents an opportunity to farm shellfish where space is less competitive, water quality is often higher, and where species naturally occur. Here, we have collaborated with fishing industry partners to provide information about the potential for the surfclam to be cultivated at commercial scales in the open ocean. We conducted two deployments of over 300,000 hatchery reared seed-sized (15mm shell length) surfclams in fall 2023 and spring 2024 in federal waters off the coast of Atlantic City, New Jersey. Surfclams were deployed in large steel cages (1.2 x 1.2 meters). Within the cages, surfclams were contained within plastic mesh Hexcyls, often used on oyster longline farms. To determine the effects of stocking density on surfclam growth, surfclams were stocked at five different stocking densities ranging from 266 to 1,995 clams per hexyl (average shell length 14.68 +/- 2.70 mm) in the fall, and from 118 to 880 clams per hexly (average shell length: 21.74 +/- 5.3 mm) in the spring. Surfclam cages from both deployments were retrieved in August 2024. Surfclam growth rates were comparable what would be expected in natural populations, indicating the potential for offshore aquaculture to be used for the commercial production of a steamer (55mm) sized surfclam product. Additionally, preliminary results suggest that lower stocking densities result in faster growth rates of surfclams. This collaborative research effort will provide information about the potential to produce surfclams in offshore aquaculture farms, making use of new growout areas that have environmental conditions that reflect the natural habitats for this species.

MULTISTRESSOR LABORATORY EXPERIMENTS WITH THE ATLANTIC SURFCLAM (*Spisula solidissima*): OCEAN WARMING AND ACIDIFICATION

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The Atlantic surfclam (*Spisula solidissima*) is a widely distributed bivalve along the Atlantic coast of the United States and Canada where this species supports a large commercial fishery. Shifts in the distribution of surfclam populations indicates that the Atlantic surfclam is responding to changes in ocean conditions. To examine how changing ocean conditions may impact surfclam growth and survival, laboratory experiments were used to observe surfclam performance at ambient and manipulated levels of temperature and carbonate chemistry (reflective of ocean warming and ocean acidification, respectively). In a flow-through laboratory experiment using natural seawater, surfclams were exposed to one of nine treatments, with three levels of pH and temperature in a fully crossed design. Temperature and pH regimes fluctuated with ambient conditions, while maintaining consistent offsets between each treatment. After six weeks of acclimation, growth, feeding, digestion, respiration rates, and transcriptomics were measured in surfclams from each treatment. Results from this experiment indicate resilience in the Atlantic surfclam to moderate ocean acidification (low pH average = 7.59) across non-stressful thermal regimes (high temperature average = 19.0°C). Surfclams in low pH treatments grew similarly to those in high pH treatments (condition index and shell length), and no differences in metabolic rates were observed across all treatments. The design of this experiment highlights the importance of integrating natural environmental variability into laboratory studies and studying multiple environmental stressors concurrently. These results will help to inform models of surfclam growth and distribution under different climate change scenarios.

ASSESSMENT OF ZOOPLANKTON IN MANAGED NURSERY PONDS AND FLOATING RACEWAYS FOR PRODUCTION OF PHASE ONE LARGEMOUTH BASS, *Micropterus nigricans*

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Largemouth bass (LMB) are an important recreational fish and are increasingly grown as a food fish. There is need for methods to consistently produce quantities of LMB fingerlings. Current practice to produce phase one LMB relies on dedicated nursery ponds filled on demand and harvested after several weeks. Managed nursery ponds are fertilized to promote natural food, primarily zooplankton and insect larvae. Fingerlings produced are then feed trained and graded to manage the risk of cannibalism.

Floating raceways represent a novel approach for phase one production of phase I LMB. Mesh size may be chosen to retain fish and possibly components of the bloom. The volume of water pumped through the raceway is directly related to the quantity of natural food presented to the fish. It offers convenient monitoring and ease of harvest. Multiple raceways with diverse age groups can be placed in a single managed pond.

Previous studies conducted at the Kentucky State University Aquaculture Research Center have demonstrated proof of concept. The system also produced smaller sized fingerlings in contrast to conventional pond rearing method. Thus, questions regarding whether sufficient food (density and diversity) are being delivered and how they differ between the pond and floating raceway were explored in this study.

Investigators used eight 0.04 ha ponds fertilized to enhance the spring zooplankton bloom and a fertilization protocol based on methods used at the A.E. Wood Hatchery in San Marcos TX. Fish were not confined in the control (pond) treatment. Treatment fish were confined in a 2.6 m^3 growing area inside the raceway with water pumped from the pond. Each treatment was stocked with 6000 LMB swim up fry. Fish and zooplankton samples were collected on a weekly basis and fish were harvested after 35 days. Zooplankton samples were obtained from three sources (Raceway, Raceway pump, and Pond) and were analyzed for quantity and diversity to assess zooplankton community structure. Water quality parameters were monitored on a weekly basis.

Fish were recovered from both treatments. Average weight was 3.57±0.54 grams in the control treatment and 0.86±0.18 grams in the raceway treatment. This represents a respective specific growth rate of 20 and 16 % body weight/day. Mean weight was significantly higher in control treatment than in the raceway treatment (*P-value 0.003*). Mean recovery was 785±352 fish in the control treatment and 2234±886 fish in the raceway treatment. Recovery rate was highly variable in both treatments such that there were no significant differences between treatments (*P-value 0.179*). Zooplankton data will be presented at the meeting.

STRATEGIES FOR SUSTAINABLE IMTA PRODUCTION AND POST-HARVEST PROCESSING OF TROPICAL SEAWEED SPECIES IN THE SOUTHEAST U.S. AND CARIBBEAN

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The production of macroalgae ("seaweeds") within integrated multi-trophic aquaculture (IMTA) systems has many benefits for improving the environmental sustainability of marine finfish production ventures while offering opportunities for improved economic viability of such operations. Although IMTA seaweed production is well established globally in operations centered around production of temperate species of marine finfish, the techniques, technologies, and overall production strategies for seaweed species native to the Southeast U.S. and Caribbean within IMTA production systems is at a rather incipient stage in the U.S. In order for U.S. producers of seaweeds to be able to compete in an import dominated marketplace, there is a critical need for identifying strategies that allow for economically viable domestic seaweed production. Some of the keys to viability in the Southeast U.S. and Caribbean include identifying native seaweed species that have: 1) the greatest ability to utilize nutrients in marine finfish production effluent water: 2) the highest market value; 3) the post-harvest techniques, including value-add strategies, that will allow for highest economic returns for producers in the region. Results of research and development activities centered around IMTA production of native seaweed species of the Southeast U.S. and Caribbean region will be presented and discussed. Aspects of this work have been supported by the Gulf States Marine Fisheries Commission (GSMFC) and the National Oceanic and Atmospheric Administration (NOAA).

MARINE FINFISH SPECIES SELECTION FOR A VIABLE U.S. AQUACULTURE INDUSTRY: CRITICAL CONSIDERATIONS AND STRATEGIES FOR DEVELOPMENT

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Despite the burgeoning U.S. consumer demand for marine finfish, the overwhelming majority of seafood consumed in this country comes from overseas. The story has remained seemingly unchanged for decades with the vast potential for U.S. aquaculture production of marine finfish facing the reality of failed operations far outweighing the success stories. While every story is unique, upon closer examination the similarities begin to emerge in terms of the key factors associated with the failure of domestic marine finfish operations. In many cases, such factors include species selection and associated issues with systems and/or site selection. Every species of marine fish has a its own unique physiological, biological, behavioral, and market considerations that must be accounted for in the development and operation of commercial marine finfish operations. Operations that thoroughly understand and capitalize on such factors are typically those which end up achieving success in the industry. Factors associated with the selection of marine finfish species for the U.S. aquaculture industry will be presented and discussed, including many considerations that are all too often neglected in both the project development and operational phases of the business. The U.S. has incredible potential for increasing domestic production of marine finfish species across a variety of different types of aquaculture production systems, and the key is to have more commercial success stories in the industry to help unlock this vast potential.

RESTORATIVE AQUACULTURE IN SOUTH FLORIDA: USING OYSTERS IN INNOVATIVE NATURE-BASED SOLUTIONS TO RESTORE BISCAYNE BAY AND IMPROVE COASTAL RESILIENCE

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There is an increased need for development and implementation of nature-based solutions for improving coastal resilience in regions on the front lines of climate change impacts. South Florida has been identified as one of the most at-risk communities in the nation in the face of climate change, and Biscayne Bay is widely considered ground zero in its exposure to many of the most common coastal resilience challenges. While historical populations of native oysters (*Crassostrea virginica*) in Biscayne Bay have been decimated over the past decades, remnant populations remain in various portions of the Bay. Current restorative aquaculture activities centered at the University of Miami – Rosenstiel School aim to connect community partners, scientists, habitat restoration specialists, and conservation practitioners to build capacity in utilization of oyster restoration in Biscayne Bay for living shoreline initiatives. Additionally, research is underway in exploring the utilization of native Biscayne Bay oysters in land-based integrated multitrophic aquaculture (IMTA) systems to develop strategies for advancing marine aquaculture in the region through effective product diversification that may allow for improved environmental sustainability and economic viability. Leveraging expertise in restorative marine aquaculture, ecological genomics, outreach, and education, this project has the potential to play a critical role in coastal resilience initiatives throughout the region while providing valuable ecosystem services associated with native oyster restoration. Research activities on oyster aquaculture at the University of Miami – Rosenstiel School will be presented and discussed along with future directions of the project.

ELECTRIFYING SEA FARMING: SUSTAINABLE EQUIPMENT INNOVATION THROUGH THE BOAT YARD, LLC'S USDA NE SARE-FUNDED PROJECT

Chad Strater

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The Boat Yard, LLC is pioneering the development of electrified sea farming equipment, offering sustainable solutions that help shellfish and seaweed farmers reduce their reliance on fossil fuels while enhancing productivity. Supported by a USDA Northeast Sustainable Agriculture Research and Education (SARE) grant, The Boat Yard's work focuses on the design, deployment, and testing of solar and battery-powered farming tools essential to aquaculture operations.

Key innovations include solar- and battery-powered oyster tumblers, water pumps, refrigeration systems, and hauling equipment—designed to meet the energy-intensive demands of sea farms without contributing to carbon emissions. By integrating renewable energy sources like onboard solar panels with advanced battery storage and cloud monitoring, these systems offer consistent, off-grid operation tailored to the needs of small- and mid-scale aquaculture businesses. The solar-powered tumblers ensure efficient oyster cleaning and sorting, while battery-powered water pumps and haulers streamline critical tasks in a quiet and sustainable manner.

Refrigeration systems powered by solar and battery arrays play a crucial role in preserving shellfish quality, allowing farmers to store harvested products directly on-site without the need for diesel generators. These innovations are designed to be modular and easily deployable, offering flexibility and scalability for various farm sizes and configurations.

The Boat Yard's hands-on collaboration with farmers has enabled continuous design refinement and testing, ensuring that the equipment is practical, efficient, and built to withstand harsh marine conditions. This presentation will showcase the progress made, highlighting the performance of these renewable-powered systems and their potential to reduce costs and emissions while supporting resilient, sustainable aquaculture practices.

OUTWARD FDI IN NORWEGIAN SALMON EXPORT

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A large and growing literature on international trade focuses on the effect from outward foreign direct investment on trade dynamics in exports. However, this is an issue that has received limited attention in relation to the seafood trade despite the large quantities of seafood being traded. Using Norwegian firm-level customs data this paper investigates the effects of outward FDI on the export of fresh farmed salmon.

There are several explanations to why exporters of fresh salmon may choose to engage in outward FDI. First, outward FDI may be beneficial to firms that seek to transfer more labor-intensive processing of the raw product abroad to lower costs. Second, outward FDI makes it possible for exporters to penetrate deeper into new markets to better access important market information. Third, some firms may use horizontal outward FDI to expand their distribution network.

Our results suggest significant effects from outward FDI on export value, as well as on relevant trade margins, in Norwegian export of fresh whole salmon. The results indicate a positive association between outward FDI and trade value in general, as well as on prices to a given destination market for the largest exporters. The degree of export diversification, i.e. export of new products to new markets, has heterogeneous effects on different trade margins. We also control for traditional gravity variables and document that exporters charge higher prices to the most distant markets after controlling for outward FDI and export sophistication. Our results add to a growing literature exploring the micro fundaments of the aquaculture supply chain.

TOXICITY OF BIFENTHRIN TO BAITFISH

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Transportation of unintended organisms (hitchhikers) in a load of baitfish can be a problem when they cross State lines; this could be a violation of the Lacey Act. The Lacey Act prohibits the importation or shipment of injurious species into or within the U.S. Efforts are being made to find compounds to eliminate hitchhikers without harming the baitfish or their shelf-life. The primary hitchhikers are crawfish, but bullfrog tadpoles are also a problem. Baitfish in this study include: golden shiner, fathead minnow, black salty, goldfish and bluegill. This study determined the 24-hour acute toxicity of bifenthrin to these baitfish in well water.

Median lethal concentration (LC50) values at 24 h were 0.19 mg/L for golden shiner, 0.14 mg/L for fathead minnow, 0.88 mg/L for goldfish, 2.85 mg/L for black salty, and 0.26 mg/L for bluegill. Timing last summer only allowed minimal preliminary studies on the toxicity of bifenthrin to crawfish, but studies next summer should define these toxicities.

HIGH-SPEED IMAGING IN AQUACULTURE: APPLICATIONS OF FLOW IMAGING MICROSCOPY IN SHELLFISH HATCHERIES

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Microscopy is a daily ritual in most shellfish aquaculture facilities: counting and measuring organisms and investigating quality are critical to success. However, this can take hours each day, and results may vary dramatically between operators. Other techniques have since been developed for counting phytoplankton and are now being utilized by hatcheries to speed up and tighten up their daily microscopy tasks. One such technique, flow imaging microscopy, utilizes high-speed imaging to automatically image, count, and measure organisms as they flow past a camera. In this presentation, we will demonstrate how flow imaging microscopy is used for a variety of organisms (e.g. microalgae, oysters, mussels, clams) at multiple points in their development (e.g. fertilization and larval development) in research-forward shellfish hatcheries. This presentation will cover methodologies used for counting, measuring & classifying organisms of interest using flow imaging microscopy and associated data used for hatchery operations.

OBJECTIVE EVALUATION OF OFF-FLAVOR MITIGATION TECHNOLOGIES: A MASS BALANCE APPROACH FOR INFORMED DECISION-MAKING

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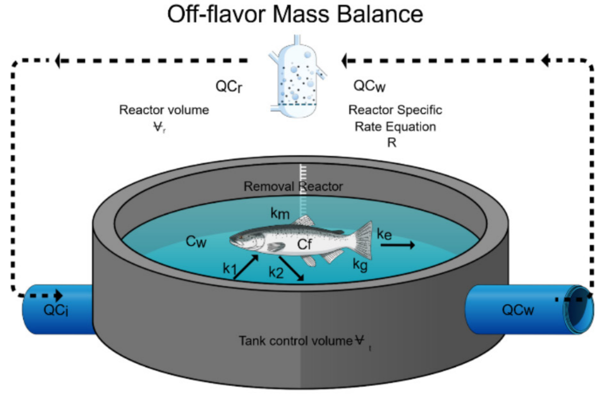
To keep up with ever increasing seafood demand, aquaculture in the US and abroad must expand beyond traditional coastal culture methods and dramatically increase production. A major bottleneck experienced when bringing aquaculture onshore is the accumulation of the off-flavor compounds geosmin (trans-1, 10-dimethyl-trans-9-decalol) and 2-methylisoborneol (MIB)—particularly in recirculating aquaculture systems (RAS). These terpenoids give a "muddy" flavor to farmed fish, especially high value and popular fish like Atlantic salmon (*Salmo salar)*. Knowledge regarding the rates at which these compounds are taken up by fish muscle, eliminated, and biologically transformed remains unestablished. Various technologies have been developed for commercial scale removal of off-flavor compounds and other applications. No comprehensive models exist to predict the bioaccumulation or elimination of off-flavor compounds in RAS with these technologies in mind, or to assess the efficacy of the technologies themselves. This research attempts to fill that gap with a model which not only quantifies the mass transport of off-flavor in RAS, but allows for the objective comparison of different remediation and removal approaches.

1. $\frac{dC_f}{dt} = k_1C_w - (k_2 + k_e + k_m)C_f$

2. $V_t\frac{dC_w}{dt} = Q(C_{w_i} - C_w) +$
$(k_2)C_fm_f - k_1C_wm_f$

3. $V_r\frac{dC_{w_r}}{dt} = QC_w - QC_{w_r} - R$

Off-flavor Mass Balance



Total System perspective

EVALUATION OF AN OPEN FORUMULA REFERENCE DIET FOR CALIFORNIA YELLOWTAIL *(Seriola dorsalis)* BROODSTOCK

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The reliable production of marine finfish is contingent on the quantity and quality of the eggs available from the broodstock. Proper broodstock nutrition is critical for maintaining a supply of good quality eggs. Here we compared an open formula reference diet (OFD; 18 mm pellet) to fresh fishery products (FFP; sardines and squid) and a commercial broodstock diet (Europa, Skretting; 12 mm pellet), when fed to California yellowtail (CYT*; Seriola dorsalis*). The aim was to determine how egg production, and egg and larval quality would be affected by these diets.

A total of sixteen F2 generation CYT broodstock (2 females; 2 males per tank) were held in four 10 m^3 tanks under ambient temperature and photoperiod. In Year 1 we compared the OFD to FFP and in Year 2 we compared the OFD to Europa. During each year we alternated each diet twice over four 6-week periods through the spawning season. In Year 1 the OFD treatment yielded 10 spawn events totaling 5.4 million eggs, with an average viability of 59.9%, compared to the FFP treatment which yielded 15 spawn events for a total of 7.0 million eggs, with an average viability of 45.1% (Table 1). Egg and larval quality measurements (egg diameter, oil diameter, percent oil volume, notochord length at hatch, hatch rates, and survival to first feeding) were not statistically different between treatments. Results from Year 1 demonstrated that the OFD treatment performed similarly to the FFP treatment and lead us to compare the OFD treatment to a commercially available broodstock diet (Europa). In Year 2 the OFD fed fish spawned 20 times and produced 16.9 million eggs with an average viability of 53.6%, while the Europa fed fish spawned 21 times and produced 18.1 million eggs with an average viability of 53.8%. Both treatments showed no significant differences in egg or larval quality. Based on the results from these trials, the OFD can be used in future experimental work as a tool to determine dietary needs for CYT broodstock.

Table 1. Egg production and quality results for California yellowtail (*Seriola dorsalis*) broodstock fed an open formula reference diet (OFD), Fresh Fishery Products (FFD), and the commercial diet (Europa).

	Year 1		**Year 2**	
Diet	OFD	FFP	OFD	Europa
Number of Spawns	10	15	20	21
Total Eggs	5,498,331	7,020,265	16,932,570	18,179,420
Eggs/Spawn	549,833	468,018	846,629	865,687
Viability (% ± SD)	59.9 ± 30.8	45.1 ± 36.9	53.6 ± 27.2	53.8 ± 22.3
Hatch Rates (% ± SD)	19.1 ± 31.5	21.8 ± 26.2	55.9 ± 27.2	51.0 ± 25.3
SFF (% ± SD)	44.9 ± 40.6	39.9 ± 39.6	72.3 ± 23.1	70.9 ± 29.8
Egg Diameter (mm ± SD)	1.38 ± 0.03	1.35 ± 0.03	1.35 ± 0.04	1.38 ± 0.03
Oil Diameter (mm ± SD)	0.32 ± 0.01	0.31 ± 0.01	0.33 ± 0.03	0.33 ± 0.01
POV (% ± SD)	1.29 ± 0.13	1.23 ± 0.10	1.55 ± 0.27	1.46 ± 0.17
NL Length (mm ± SD)	4.01 ± 0.33	4.13 ± 0.23	4.27 ± .022	4.20 ± 0.20
YSV (m3 ± SD)	0.32 ± 0.24	0.30 ± 0.14	0.23 ± 0.11	0.27 ± 0.07

REEVALUATING THE CO-CULTURE OF EASTERN OYSTERS WITH SEA URCHINS *Lytechinus variegatus* ON FLORIDA'S GULF OF MEXICO COAST

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The potential of culturing wild-collected sea urchins *Lytechinus variegatus* with eastern oysters *Crassostrea virginica* was demonstrated at farms on Florida's Gulf of Mexico coast in a previous pilot study. Preliminary results showed urchins reduced fouling on oysters and culture bags at one site, resulting in interest by growers as biofouling control is a major expense and effort in off-bottom culture. To move this proof of concept forward, research was needed to reevaluate the co-culture of hatchery-produced urchins and oysters over a crop cycle at commercial densities.

Field trials were conducted at commercial farms over a nine-month period to assess oyster performance and biofouling on oysters and gear cultured with and without urchins. Due to hatchery problems, urchins were collected from natural populations. In the nursery trial, 12 subadult urchins (30 mm test diameter) were stocked with triploid oyster seed (1200/bag, 16 mm shell height) and reared in 4 mm mesh floating bags. Urchins did not survive at farms where salinities were less than 25 psu. At a higher salinity farm site, urchin survival after 2.5 months ranged from 8.3 to 66.6%. In the intermediate growout trial at the latter farm site, oysters were restocked at 400 per 9 mm mesh bag. Urchin density (0, 10, 15, 20/bag) and placement of floats varied. After 3.5 months, urchin survival was highest in bags with floats placed on top (87-98%) versus sides (53%). Growth and survival of oysters in all treatments were similar, suggesting urchins did not influence oyster performance. There was a 64% reduction of barnacles on oysters in the treatment with 15 urchins. The biggest difference observed in the biofouling assessment was the matrix of tube-building amphipods and accumulated sediment and detritus on oysters without urchins compared to those with urchins (Figure 1).

For the final growout trial, oysters were reduced to 150 per 14 mm mesh bag and stocked with subadult and adult (49 mm test) urchins. After 3.5 months, growth rates were higher for subadult urchins versus adults and survival (96%) was higher for urchins in bags with floats placed on top. As in the intermediate trial, oyster growth and survival did not differ. Results of biofouling on oysters were also similar; however, fouling weights on bags were lowest in the treatment with 20 adult urchins. Overall, results did not clearly demonstrate the potential of using urchins for biofouling control of oysters and limit the potential for oyster and urchin co-culture in Florida.

Figure 1. Oysters stocked without urchins (top) and with urchins (bottom) after 7 months in growout trials.

THE SIGNIFICANCE OF WOMEN IN THE DEVELOPMENT OF A CLAM FARMING INDUSTRY: THE CEDAR KEY STORY

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The hard clam aquaculture industry centered in Cedar Key, Florida, is a dramatic success story. In just 30 years, over 180 growers produce 90% of the state's crop supporting some 500 jobs with an economic impact estimated at $30.6 million. But its beginnings followed that of a failure. During 1989-1991, a federally funded job retraining program introduced oyster harvesters in Apalachicola Bay to oyster farming as an alternative to fishing natural stocks. Participants were to receive aquaculture leases to start their new businesses, but the local government vetoed privatizing bay bottom. The project was also mired with sociopolitical and technology transfer problems.

Further down the Gulf coast, a 4-H school enrichment project initiated in 1990 by women in the town of Suwannee taught youth how to raise oysters and clams. And the tide of anti-leasing sentiment began to turn. The following year, oystering in the Suwannee Sound was closed due to the presence of *Salmonella*. Women community leaders lobbied their legislators to acquire funding for a similar retraining program and provided support during several projects conducted in Cedar Key over the next six years. Women were involved in administering and managing the projects and chairing an advisory committee of representatives of local, county and state governments and agencies to address leasing, permitting, and other issues that arose. As a result, 850 acres of shellfish aquaculture leases were developed allowing over 200 project graduates the opportunity to begin a new way of life by farming clams and oysters.

This presentation is a tribute to the women who initiated and supported the clam culture industry over the years and also to those who represent the future of clam farming in Cedar Key.

CLIMATE CHANGE IMPACTS TO A CLAM AQUACULTURE INDUSTRY IN FLORIDA AND POTENTIAL ADAPTATION STRATEGIES

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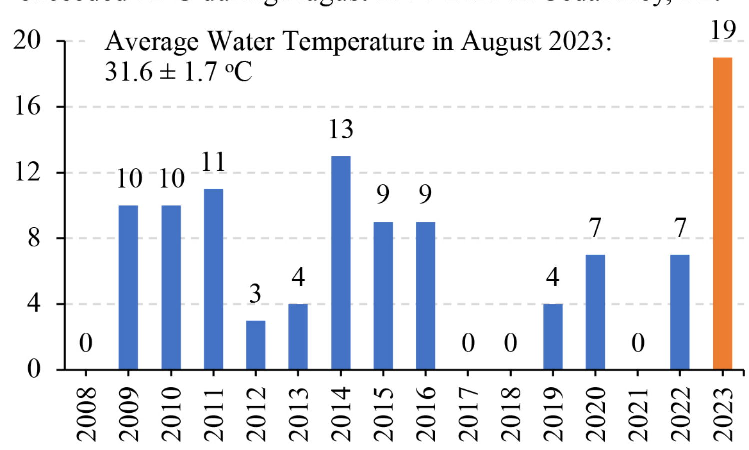
Hard clam *Mercenaria mercenaria* aquaculture centered in Cedar Key, Florida, is a 30-year economic success story by any measure. Over 180 growers produce more than 90% of the state's crop, supporting 430 jobs and $29.3 million in industry output. However, the past two years have brought unprecedented challenges from weather and climate-related events, raising growers' concerns about the profitability and even sustainability of their operations in the face of climate change. Florida is particularly vulnerable to climate change due to its low elevation, geographic location, and landscape configuration. Further, shellfish aquaculture is particularly sensitive to multiple drivers including sea level rise (15 cm in 30 years), coastal habitat loss, and increased hurricanes and harmful algal blooms.

In August 2023, Hurricane Idalia made landfall near Cedar Key as a category 3 hurricane with a storm surge of 3.26 meters from MLLW, causing crop losses exceeding 75% with projected damages of $25.9 million. Even before the hurricane, growers reported substantial mortalities due to extreme temperatures. According to the NOAA National Weather Service, August 2023 was the warmest on record in the area; water temperatures at leases exceeded 32°C on 19 days in that month (Figure 1). Another heat wave in June 2024 affected new crops which were also vulnerable to lower salinities. Variability in rainfall has led to inconsistent river discharges resulting in additional water quality stressors during summer months. As recovery efforts from Hurricane Idalia were underway, the industry suffered a new setback in September 2024 when Hurricane Helene, a category 4 storm, made landfall in the same location with a surge of 3.99 meters.

Weather and climate adaptation strategies being considered include reducing clam densities and changing cultivation methods from bags to bottom plants, which would allow clams to bury deeper in the substrate. In the longer term, selective breeding efforts of clams for greater tolerance to higher temperatures are ongoing and may strengthen adaptive capacity. Growers may also adapt by shifting to other species, such as the eastern oyster, or new strains, such as a hybrid with the native clam *Mercenaria campechiensis*. Relocation of leases is unlikely. But climate change may lead to a shorter growing season and development of markets for smaller clam sizes. These strategies and future concerns will be discussed.

Figure 1. Number of days in which water temperatures exceeded 32ºC during August 2008-2023 in Cedar Key, FL.



EVALUATING CARVACROL AS A PLANT-DERIVED DIETARY SUPPLEMENT FOR CHANNEL CATFISH *Ictalurus punctatus* FINGERLINGS

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Carvacrol is a monoterpene derived from plants, such as thyme and oregano, and has been shown to promote growth, improve health, and mitigate bacterial infections in various animal models. Previous results from this research group have demonstrated minimum inhibitory (MIC: 98 µg/mL) and bactericidal concentrations (MIB: 195 µg/mL) against *Aeromonas hydrophila, Edwardsiella ictaluri,* and *E. piscicida,* which are important bacterial pathogens in US catfish aquaculture. Interestingly, concentrations lower than the MIC and MIB presented the potential to inhibit biofilm formation and hemolytic activity of the aforementioned bacteria and pathogenic strains of *Streptococcus iniae*. Herein, the impact of dietary carvacrol on the growth and blood parameters, oxidative status, immune responses, and intestinal microbiota of channel catfish was evaluated.

Carvacrol was supplemented in experimental diets at concentrations of 0.0 (control), 0.5, 1.0, 2.0, and 4.0 g/kg of feed, and fish were fed twice daily at a rate of 4% body weight. Catfish juveniles were distributed to 25 aquaria, each containing 30 fish, and each dietary treatment had five experimental units. The aquaria operated as a recirculating aquaculture system maintained under a 12:12 light-dark cycle, and the feeding trial lasted for 84 days. At the end of the study, production performance parameters were evaluated. Three fish per tank were sampled for hematology and plasma analysis, as well as condition indices, Fulton's condition factor, histology, and activity of antioxidant enzymes. A three fish from each tank were sampled for whole-body proximate composition (dry matter, protein, fat, and ash). Digesta samples were collected to assess prospective differences in the intestinal microbiota using 16S rRNA targeted amplicon sequencing. Significant differences were observed for weight gain, where fish offered diets supplemented with 4.0 g/kg had decreased production performance, feed intake, and increased viscerosomatic and hepatosomatic indices. No significant differences were observed in the intraperitoneal fat index, condition factor, and survival. No differences were observed in hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, or mean corpuscular hemoglobin concentration. However, red blood cell production increased significantly with 0.5 g/kg and 4 g/kg carvacrol treatments. Results suggest low concentrations of carvacrol did not negatively impact the production performance of channel catfish fingerlings.

CULTURE CONDITIONS TO MAINTAIN MITOTIC ACTIVITY OF SPERMATOGONIA STEM CELL IN YELLOWTAIL AMBERJACK *Seriola lalandi*

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In Mexico, the northwest region concentrates the largest fishing and aquaculture production in the country. However, several of the species used in fishery production are overexploited. Most fish species in this region exhibit late sexual reproduction, approximately four to six years to mature in a natural environment. Therefore, maintenance and management in captivity makes it difficult to obtain reproduction protocols. In view of this problem, biotechnological strategies such as *in vitro* culture and transplantation of stem germ cells or "surrogacy reproduction" are an alternative for the conservation and reconstitution of species. The stem germ cells (spermatogonia or oogonia) in addition to having the capacity to reconstitute species, they also present characteristics such as sexual plasticity and the ability to transfer genetic information to the following generations. For this reason, a protocol for cryopreservation of spermatogonia stem cell was initially established in the yellowtail amberjack (*Seriola lalandi*). Therefore, the objective of this work was to establish the conditions for the *in vitro* culture of spermatogonia stem cell that promote cell proliferation, from previously cryopreserved tissue.

Previously cryopreserved testicular tissue was subjected to the enzymatic disaggregation process at 0.3% trypsin. For *in vitro* culture, the protocol established in rainbow trout (*Oncorhynchus mykiss*) was used. A cell concentration of 0.6×10^4 cells/cm^2 was seeded in 6-well plates coated with 0.1% gelatin with 2 mL of medium. Half the volume of the medium will be changed every 3 days. The cells will be incubated in an oven at a temperature of 28°C with a 5% CO^2 atmosphere. The growth factors that will be added to the basal medium are Leibovitz's L-15 medium Hepes (25 mM), Penicillin (50 U/mL), Ampicillin (50 µg/mL), Streptomycin (50 µg/mL), FBS (1%), Bovine serum albumin (0.5%), L-aspartic acid (20 µg/mL), L-cystine (20 µg/mL), L-proline (20 µg/mL), L-glutamic acid (20 µg/mL), Adenosine (200 µM), fish serum (0.25%), 2-mercaptoetanol (50 µM), Ascorbic acid (50 µM), bovine insulin (25 µg/mL), bFGF human (1 ng/mL), fish embryo extract (1 µg/mL). The percentage of stem spermatogonia will be validated after 6, 12, 21 and 27 days of culture. In each collection, cell concentration, mitotic activity and cell viability will be validated through flow cytometry.

The results of the present work are being analyzed, but from them it is expected to optimize the in vitro culture conditions for stem spermatogonia in the yellowtail amberjack (*S. lalandi*). This study would represent the first step towards the establishment of stem spermatogonia cell lines, aiming in the future at germ cell transplantation for sterile recipients with intermittent reproduction.

COMPARATIVE EFFECTS ON INTESTINAL INTEGRITY OF A PLANTMEAL- VS. FISHMEAL-BASED DIET BETWEEN TWO STRAINS OF RAINBOW TROUT

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The current trend in aquafeed development is moving away from dependence on traditional protein sources (i.e.: fishmeal) to lower-cost, sustainable alternatives (i.e.: plant protein). However, the incorporation of high levels of plant sourced protein to replace fishmeal in carnivorous salmonids can deleteriously affect the intestine. The intestine is important for the regulation of nutrient absorption and barrier function contributing to overall growth and health in fish, and the paucity of studies in this area encourages the need for further understanding and research.

Here, we report the effects of a plantmeal- vs. fishmeal-based diet on intestinal integrity on two strains of rainbow trout, a commercial strain (CS) and a selected strain reared to grow on an all-plant protein (soy-based) diet (ARS-*Sel*). A feeding trial of 7 months was conducted to assess overall fish performance. Further electrophysiological characterization was performed using *ex vivo* intestinal segments (anterior proximal, posterior proximal, mid, and distal intestine) mounted in Ussing chambers to measure transepithelial electrical resistance (TEER) and conductance. TEER and conductance both measure overall intestinal barrier function. For both parameters, significant differences were observed between intestinal segments within strains, as well as differences between strain and diet. Specifically, fishmeal resulted in significant TEER differences between CS and ARS-*Sel* within anterior proximal (Figure 1) and distal intestine, whereas the plant-based diet resulted in significant effects between strains in the mid intestine. Interestingly, these results illustrate not only differences in regional intestinal permeability within a strain, but there are also major differences from feeding a plantmeal- vs. fishmeal-based diet on intestinal integrity between strains. These results plus histological analyses of distal intestinal segments, as well as expression of genes involved in intestinal permeability (i.e.: claudins) and inflammation will be presented.

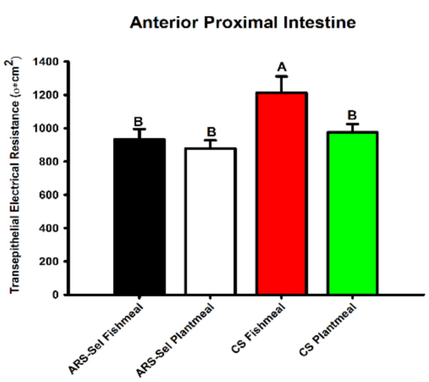

Figure 1. Transepithelial electrical resistance (TEER) from anterior proximal intestine (N = 9 fish). Significant differences indicated by different letters (two-way ANOVA performed on log-transformed data, $p < 0.05$).

ASSESSING THE IMPACTS OF CLIMATE CHANGE ON SEA URCHIN PRODUCTION

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Anthropogenic changes are significantly impacting animal physiology, altering the ways in which we produce seafood—particularly when, where, and how it is done. Despite extensive research efforts, most studies have focused on short-term climate change experiments, leaving critical knowledge gaps about the medium- and long-term effects on production practices. We will overview several ongoing projects that aim to address these gaps, with a particular focus on commercial sea urchin production. As wild fisheries decline, aquaculture has emerged as a promising alternative for sustaining the sector. We will present work on multigenerational breeding and high-frequency field sampling to explore how ocean acidification and warming affect sea urchin physiology and reproduction, key factors influencing production output and the timing of practices. Additionally, we will share the latest findings from a high-resolution species distribution model (SDM), used to map historical species distributions and forecast future shifts under ongoing anthropogenic changes which can inform future aquaculture siting. While this research focusses on sea urchin species produced in the Atlantic, these insights have broad implications for other commercially important species and production practices in both aquaculture and fisheries.

INTEGRATING GRAZING SEA URCHINS WITH SHELLFISH CAN REDUCE BIOFOULING

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Nuisance biofouling in shellfish aquaculture production requires large intervention efforts, farmer costs, and potentially lost opportunities for further shellfish growth. In some areas, these farm activities have received opposition from sectors of the public concerned about the impacts on coastal aesthetics or property values which can limit aquaculture's social carrying capacity. One potential solution that could help address these concerns is to integrate sea urchins with shellfish. Sea urchins can actively feed by grazing upon the biofouling that develops on aquaculture gear and/or the external shell surface of shellfish. In some areas of the US this integration could help expand the emerging aquaculture sector of sea urchin production as well as creating further economic opportunity for shellfish growers. Here we will overview two case studies using differing species and gear types. In one study we integrated two emerging aquaculture species together, Atlantic Sea Scallops (*Placopecten magellanicus*) with Green Sea Urchins (*Strongylocentrotus droebachiensis*) grown in lantern nets in a Maine based sea farm. In another study we integrated Eastern oysters (*Crassostrea virginica*) with the commercially unexploited Atlantic Purple Sea Urchin (*Arbacia punctulata*) grown in on-bottom oyster bags and cages in a Rhode Island coastal pond farm. Here we will overview the results of these case studies highlighting the potential benefits towards reducing biofouling and farm related activities and grower perspectives for uptake.

EVALUATING THE EFFECTS OF GLUTAMINE SUPPLEMENTATION IN HYBRID STRIPED BASS (*Morone chrysops x M. saxatilis*) FED A SOYBEAN-MEAL-BASED DIET

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As marine feedstuffs have transitioned to strategic ingredients in aquafeeds, plant protein feedstuffs now provide a majority of crude protein in most aquaculture diets. Although this shift often reduces the overall price of diets through incorporation of less expensive raw materials, specific attention must be given to the nutritional profile of diets high in plant protein feedstuffs, especially the amino acid (AA) ratios. In addition to dietary essential AA, traditionally classified nonessential AA have been recognized as potentially limiting as standard levels of fishmeal have decreased in carnivorous fish diets. Therefore, a 6-week comparative feeding trial was conducted to assess the effects of glutamine supplementation as a functional AA in soybean-meal (SBM)-based diets fed to hybrid striped bass (HSB). The basal diet (38% crude protein (CP), and 10% crude lipid) was composed of practical ingredients with SBM contributing 75% of total CP. Along with the basal diet, experimental diets were supplemented stepwise with L-glutamine (Gln) at levels 0.0, 0.5, 1.5, 2.0, and 4.0% of dry diet weight. Responses of HSB were statistically assessed via growth parameters, condition indices, whole-body proximate composition, histological condition of the distal intestine and various innate immune parameters.

Groups of 10 juvenile HSB (~8.18 g/ fish initial weight) were stocked into 24, 38-L aquaria fashioned as a recirculating aquaculture system with quadruplicate aquaria randomly assigned to each dietary treatment. Fish were fed to apparent satiation twice daily for 6 weeks. HSB fed the diets supplemented with glutamine exhibited a significant ($P<0.05$) positive quadratic response of weight gain (Figure 1). The optimal supplementation level was estimated at 1.64% of diet. Although not significant, a similar quadratic trend was also observed for feed efficiency. Body condition indices and nonspecific immune responses including neutrophil oxidative radical production in the whole blood were not significantly influenced by diet.

Analysis of AA fluxes in plasma and various other tissues of HSB is currently ongoing, along with determination of whole-body proximate composition. Histological analysis of HSB distal intestines is also currently ongoing to assess potential intestinal enteritis mitigation given this condition has been observed in some carnivorous species fed high levels of SBM. Nonetheless, preliminary results indicate beneficial effects of Gln supplementation in juvenile HSB.

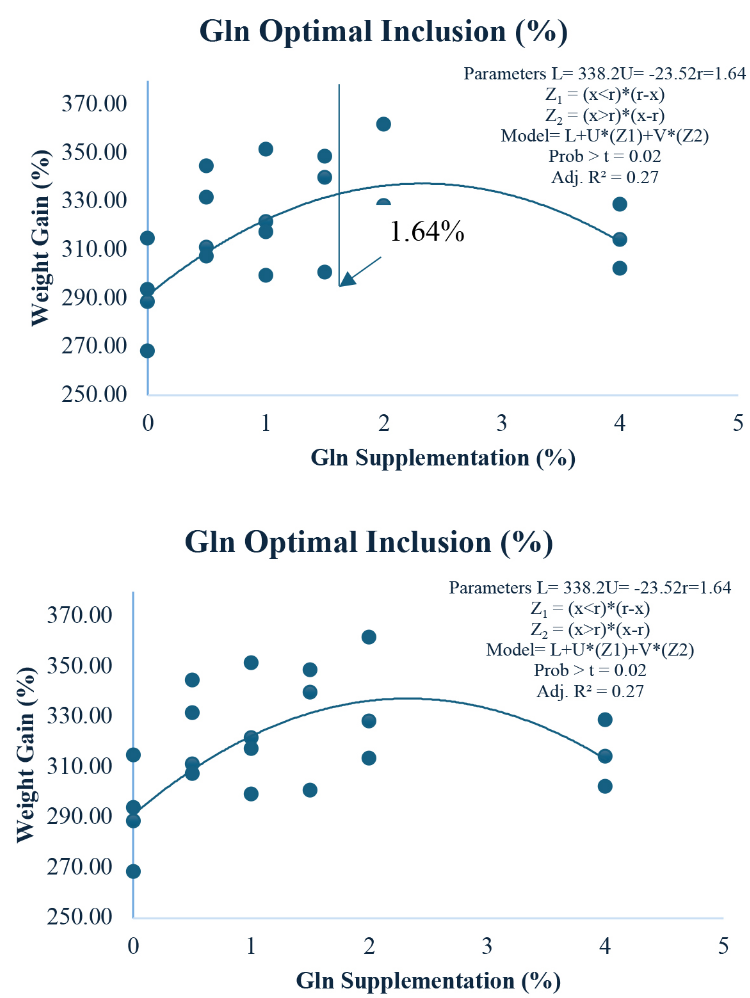

Figure 1. Optimal inclusion of dietary Gln in SBM-based diets of HSB.

PERFORMANCE OF JUVENILE RED DRUM (*Sciaenops ocellatus*) FED BLACK SOLDIER FLY LARVAE MEALS PRODUCED ON SUBSTRATES WITH VARIOUS SEAFOOD PROCESSING BYPRODUCTS

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As the demand for marine ingredients outpaces the supply of these commodities, alternative protein ingredients are necessary to support continued expansion of the aquaculture industry. In addition, there is an increasing consumer demand for the inclusion of low-impact raw materials that can access underutilized waste streams from other value chains. Black soldier fly larvae (BSFL) meal is one such ingredient with the potential to satisfy both the high-quality nutrient requirements to replace fishmeal in aquafeeds and satisfy the modern consumer sustainability demands. In the current trial, BSFL meals were produced by growing BSFL on either a commercial house fly substrate or one in which 75% of the commercial substrate was replaced with either processing byproducts (filleted carcasses) from red drum or tuna on an equal-weight basis. The BSFL raised on the various substrates were dried at 60C, processed into meals and then supplemented in the diets of juvenile red drum to replace Special Select™ menhaden fishmeal (MFM) at rates of 0, 50, 75, and 100% on a dry-matter basis. The reference (Ref) diet was composed of practical ingredients including MFM at 15% of dry weight to provide digestible protein at 36% of dry diet. The experimental diets included those in which BSFL reared on the Gainesville substrate replaced MFM at either 50% (G50) or 75% (G75), BSFL reared on red drum processing byproduct replaced MFM at 50% (RD50) or 75% (RD75), and BSFL reared on tuna processing byproduct replaced MFM at 50% (T50), 75% (T75), or 100% (T100).

Quadruplicate tanks of 17 juvenile red drum (~5.0 g initial weight) were cultured in 24, 38-L aquaria fashioned as a recirculating aquaculture system. Fish were fed to apparent satiation twice daily based on a percentage of body weight, which was adjusted weekly for the duration of the 6-week comparative feeding trial. At the end of the trial, no statistical differences in weight gain were observed for any dietary treatment; however, higher dietary inclusions of BSFL reared on either seafood processing byproduct in place of MFM presented numerically decreasing percentage weight gain (Figure 1) and feed efficiency. Similarly, body condition indices and muscle yield expressed as a percentage of body weight were not statistically different; however, a numerical increase in hepatosomatic index was observed in fish fed diets in which BSFL reared on seafood processing byproducts replaced 75 and 100% of dietary fishmeal. The current study suggests that 50% replacement of fishmeal using BSFL reared on unique seafood processing byproducts is feasible while higher replacement (75 and 100%) is suboptimal based on reduced growth and overall health indices.

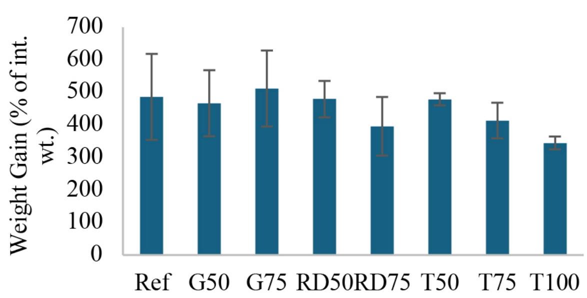

Figure 1. Effects of substituting fish meal with various BSFL products on the weight gain (%) of juvenile red drum over a 6-week feeding trial.

MANILA CLAM (*Ruditapes philippinarum*) POPULATION DECLINE IN WILLAPA BAY, WASHINGTON

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Since 2016, clam farmers in Willapa Bay Washington have been reporting declines in commercial Manila clam (*Ruditapes philippinarum*) harvests. They have also noted an absence of juvenile recruitments and very few young clams during harvest. An internal poll of commercial clam farmers in Willapa Bay reported that since 2013 average annual harvests are down 500,000 pounds. In response, several farmers have ceased operations, laid off clam harvesting crews, and subleased tidelands to other growers due to the inability to meet market demands. The causes for declining harvest were not immediately apparent. Potential causes for the decline included widespread mortality events, HAB's, extreme heat or cold weather events and, more recently, the dramatic expanded range and increased abundance of the invasive European green crab (*Carcinus maenas*), which preys on clams. The dinoflagellate (*Akashiwo sanquinea*) which has been implicated as a producer of yessotoxin, deadly to clams, has been observed in nearby Washington Department of Fish and Wildlife (WDFW) monitoring sites. Predation pits have been observed in graveled clam ground along western Willapa Bay in previous studies by the Pacific Shellfish Institute (PSI) and the Willapa Grays Harbor Oyster Growers Association (WGHOGA), but video had not identified the source.

Funded by WDFW, in the spring of 2023, PSI and WGHOGA scaled up the effort to determine the factors causing the decline in commercial clam harvests in Willapa Bay. Research included: grower surveys, video surveys for potential predators, installation of predator nets at 4 locations followed by population surveys under and adjacent to the nets every 6 months, and summarization and correlation of nearby HAB and water quality data. Initial video surveys point to European green crab (EGC) excavation pits, where manila clams are dug up and exposed, as an explanation of part of the decline (Figure 1). Increased EGC trapping has occurred in these areas after exposure and possible predation was documented. The potentially harmful algae, *Phaeocystis*, has been noted in 2023-24 WDFW plankton surveys of nearby marine waters.

Figure 1. European crab (*Carcinus maenas*) predation pit and uncovered manila clams.

ESTABLISHING *IN VITRO* OYSTER HEART MODELS TO INVESTIGATE *Bonamia ostreae* INFECTION AND HOST-PARASITE INTERACTIONS

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Infections by the parasitic Haplosporidian *Bonamia ostreae* have devastated European flat oyster, *Ostrea edulis*, populations, posing an ongoing threat to aquaculture and restoration efforts today. Understanding the mechanisms of host-parasite interactions is therefore critical for developing effective management strategies that support oyster restoration, promote sustainable aquaculture, and inform marine policy. This study aimed to establish an i*n vitro* model to investigate the effects of *B. ostreae* on oyster physiology, with a particular focus on heart function.

A novel co-culture system was developed using heart tissue from previously infected oysters to preserve both the integrity and infection status of whole hearts *in vitro* (Fig 1A). Uninfected hearts remained viable and continued contracting *in vitro* for up to nine months, while hearts infected with *B. ostreae* maintained the parasite in co-culture for over three months (Fig 1B). This system allowed for the assessment of the impact of *B. ostreae* on heart function, revealing a significant increase in resting heart rate in infected cultures. Furthermore, early histological analysis with Hemacolor indicated that Bonamia is likely replicating in culture, evidenced by the presence of double-nucleated parasites (Fig 1B). Moreover, preliminary metabolomic, and proteomic analyses offer insights into the broader physiological changes triggered by the infection, suggesting complex interactions between the parasite and host *in vitro*.

To our knowledge, this is the first study to successfully maintain *B. ostreae in vitro* for an extended period, offering a reliable platform to investigate the host-pathogen interactions in *O. edulis* at both cellular and organ levels. Future work will focus on further characterizing the molecular changes and exploring potential interventions to mitigate the impact of *B. ostreae* on oyster populations.

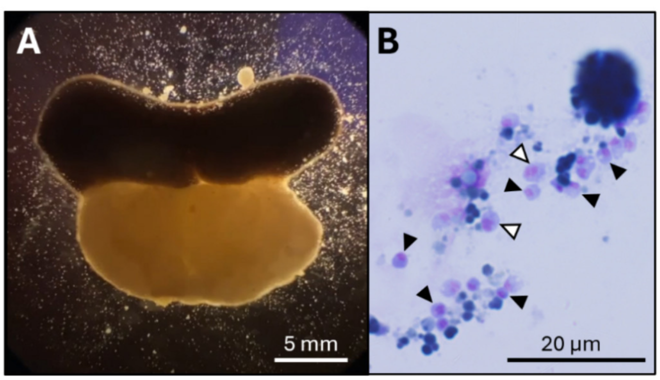

Fig. 1: ***In vitro*** **maintenance of** ***Ostrea edulis*** **heart explant infected with** ***Bonamia ostreae***. A. Oyster heart explant maintained *in vitro*. B. *Bonamia ostreae* stained with Hemacolor. Black arrowheads indicate single-nucleus parasites, while open arrowheads highlight double-nucleus parasites, suggesting replication within the *in vitro* co-culture.

NOAA SEA GRANT'S 10-YEAR AQUACULTURE ROADMAP

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In 2016, the Sea Grant Association published the 10-Year NOAA Sea Grant Aquaculture Vision. The Vision served as a foundational document guiding strategic investments to support the sustainable growth of the U.S. aquaculture industry. In January 2024, Sea Grant initiated a new planning program to create a 10-Year Sea Grant Aquaculture Roadmap (2025-2035). There are two goals of the Roadmap: 1) to guide Sea Grant's aquaculture investments and contributions to address key farming and other aquaculture community needs, and 2) the Roadmap will inform Congress, the Sea Grant Network, the aquaculture community, NOAA, and other state and federal agencies about the role Sea Grant has in fostering sustainable U.S. aquaculture. Sea Grant contracted with the Eastern Research Group, Inc. (ERG) to create the Roadmap. ERG coordinated a project team consisting of aquaculture staff members in the National Sea Grant Office and the Sea Grant Aquaculture Liaison. A steering committee composed of members of national or regional aquaculture associations, NOAA's Office of Aquaculture, the Sea Grant Advisory Board and Sea Grant extension and education staff from across the Sea Grant Network was established and met regularly.

The one-year project consisted of three key engagement phases designed to gather input and diverse perspectives from the U.S. aquaculture community. Phase I was a reflection phase to gather feedback on successes, lessons learned and opportunities for change. Phase II was a forward- looking phase to identify actions, opportunities, and priority investments to further advance sustainable US aquaculture with Sea Grant's support. During Phase III, draft content for the Roadmap was vetted and revised for the final draft Roadmap. Input from 308 people was obtained during the listening sessions and input from another 65 people (as of September 2024) using an online survey. Participants included farmers, equipment suppliers, regulators, educators, and researchers, as well as representatives from Tribal and Indigenous groups, aquaculture associations, non-governmental organizations, federal and state agencies, and the Sea Grant Network.

Preliminary results identified the following four service areas for Sea Grant to address:

1. Expansion of applied and basic research
2. Engagement and outreach services, best practices, and knowledge exchange
3. Public awareness and consumer education
4. Workforce and career development

Within each service area are 4-6 proposed actions. During the presentation details of the proposed actions and outcomes will be shared.

THE AQUACULTRUE NETWORK INFORMATION CENTER (AQUANIC) AS AN EARLY COMMUNITY OF PRACTICE

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The Aquaculture Network Information Center (AquaNIC), established in 1994, was a federal and state partnership between the National Sea Grant College Program, USDA CSREES, and the National Agricultural Library. AquaNIC was coordinated through the Mississippi-Alabama Sea Grant Consortium, Auburn University's Department of Fisheries and Allied Aquacultures, and the Illinois-Indiana Sea Grant College Program. Land Grant institutions, Sea Grant Colleges, the USDA Regional Aquaculture Center Program, and others with an expertise in aquaculture provided significant oversight and contributed to the resource base.

The mission of AquaNIC was to be the gateway to the world's electronic resources in aquaculture. AquaNIC housed or provided links to thousands of state, national, and international aquaculture publications and newsletters. A media section contained photographs, Microsoft PowerPoint slide sets, digital video, and aquaculture software. Several directories were maintained including an international database of people involved in aquaculture and a database of more than six hundred aquaculture Web sites. A news section provided users access to a calendar of events, classified advertisements, and weekly news flashes. One of the most frequently used sections was the job services that contained job announcements and resumes. A beginner's section provided general information and threaded discussion groups for a variety of commonly cultured aquaculture species and the production systems in which they are grown. An assortment of key links beneficial to outreach educators was included in an educator page and included a list of frequently asked questions and a Java driven aquaculture chat room for educator updates and specialized discussion sessions. AquaNIC hosted web sites for several organizations in the early days of the World Wide Web. These sites included the World Aquaculture Society, The National Shellfisheries Association and the Hybrid Striped Bass Association.

AquaNIC was one of the first, if not the first, virtual aquaculture communities of practice and was widely used. For example, In 2002 AquaNIC was accessed 11.5 million times by 1.2 million visitors from more than 140 countries. AquNIC ceased to exist around 2004 when new eXtension sites were created for freshwater aquaculture and marine aquaculture. The eXension concept for aquaculture lasted only a few years.

FROM CERTIFICATION TO TRANSFORMATION: ASC'S ROLE IN EVOLVING AQUACULTURE

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As the fastest growing food sector, aquaculture has the potential to further contribute to food security, economic progress and the improvement of livelihoods globally. However, seafood farming can have environmental and social impact.

The Aquaculture Stewardship Council (ASC) was founded in 2010 to establish a mechanism to transform aquaculture production towards environmental sustainability and social responsibility through incentivizing market demand. Progress has been made by developing the most robust standards, based on best practices and sound science, and the highest levels of assurance.

Today, over 2M tonnes of seafood is produced annually against the ASC standards, with ASC certified farms producing seafood across 58 countries and ASC labelled products available in 116 countries. This represents upwards of one-third of global farmed salmon production, to as little as 1.5% of global tilapia production.

However, a 'one size fits all' approach is not suitable for the industry. barriers such as constraints in scope, limited sustainability demand and farm scale result in a realization that only 2% of globally farmed seafood (excluding aquatic plants) has achieved ASC certification.

With a clear mission to transform aquaculture, ASC is dedicated to address the impacts resulting from aqua feed and deliver mechanisms for measurable improvements. ASC formally launched the ASC Feed Certification Programme and the Improver Programme by ASC– both intended to further scale improvements across the industry. Though to deliver more responsibly farmed seafood globally and the assurances that sustainability issues are addresses, will require multiple coordinated approaches.

This presentation will review progress, challenges and barriers to improvements, and ASC's approaches to driving larger-scale improvements and delivering meaningful assurances.

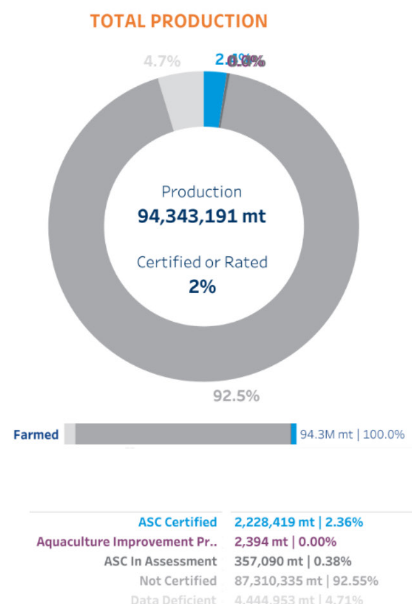

Figure 1: Global aquaculture production (CRC 2024).

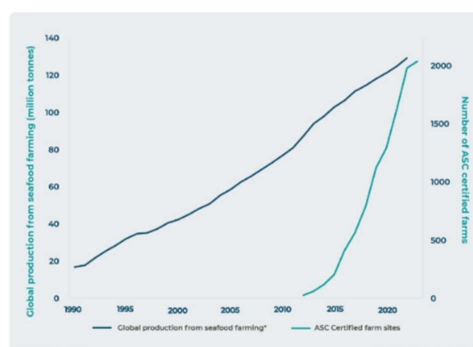

Figure 2: Growth of global aquaculture and ASC certified farms.

REVIVING MAINE'S REEFS: RESTORING AMERICAN OYSTERS *Crassostrea virginica* NEAR THE NORTHERN LIMIT

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The American oyster (*Crassostrea virginica*) was once prevalent in many rivers of Southern Maine, as evidenced by oyster shell middens in the Damariscotta and documented accounts of abundant oysters by early American colonists. In the 18th through 20th centuries, overharvesting combined with declining water quality from industrialization nearly eliminated *Crassostrea virginica* from Maine. Scattered populations continue to exist in several estuaries. Today, the growth of the oyster industry throughout the state may be contributing to an increase in local populations, however, they remain at a fraction of their historical abundance.

The Basin Oyster Project has been working to restore oysters in the Basin off the New Meadows River since 2020 as a collaborative effort between multiple stakeholder groups throughout coastal Maine, including academic institutions, conservation NGOs, town commissions, and industry members. In this case study, we describe the Project's efforts to restore oysters and the results of monitoring oyster settlement, growth, and survival and the effects of oyster restoration on biodiversity and substrate to date.

Live oysters for restoration have been acquired through a purchase program with local aquaculturists and from raising oyster seed through aquaculture on Limited Purpose Aquaculture permitted sites. Oysters of various shell lengths have been bottom planted at 2 locations for the past 4 years. In 2024, oyster spat recruitment and settlement was monitored by deploying mesh bags filled with 2L of cured oyster shells and checking the shells for the presence of oyster spat or scar marks every 2 weeks from mid-June through mid-October. Starting in 2023, the naturally settling adult population has been monitored annually through shoreline surveys in the low and mid-intertidal zone. In 2023, oyster growth and survival was quantified by measuring and checking for mortality and settling oysters in a designated group of oysters contained in trap-wire plots. A likely predator of American oysters, the European green crab (*Carcinus maenas*), has also been monitored through consistent intertidal surveys to determine abundance and size class distribution. Annual environmental monitoring has included tracking water quality parameters and changes in substrate type and marine biodiversity over time.

Our preliminary analysis shows promising signs for the Basin oyster population. Oysters in Maine are among the few populations that could potentially benefit from climate change. Oyster populations in Maine are frequently recruitment-limited due to water temperatures rarely breaching the minimum spawning threshold. However, the water temperature in the basin has been increasing since 1995, revealing a positive sign for future oyster spawning. Species richness in both restoration sites has also significantly increased, showing that oyster restoration can indeed help ecosystem health as whole and return community benefits ($p=0.0.002642$)

TRACKING HARMFUL CYANOBACTERIA AND THEIR EFFECTS ON LOUISIANA OYSTERS USING MOLECULAR TOOLS

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The emergence of toxigenic cyanobacteria in Louisiana estuarine systems raises concerns regarding the impacts of fitness and public health risks of exposure to valuable shellfish species like the eastern oyster, *Crassostrea virginica*. The freshwater cyanobacterium, *Microcystis aeruginosa*, is of particular concern due to its salt tolerance, production of the cyanotoxin microcystin, ability to outcompete other phytoplankton species when pulses of nutrient-rich freshwater are delivered to receiving basins, and documented presence in oligo- and mesohaline Louisiana estuaries. This spatiotemporal overlap of eastern oysters and *M. aeruginosa* makes monitoring efforts and research resolving feeding of the eastern oyster on this, and similar, species of pressing importance. However, individual cells of *M. aeruginosa* are small (2-4 μm) but can also form large colonies (100 μm) comprised of hundreds to thousands of cells, both of which are difficult to enumerate accurately using traditional microscopy. Furthermore, morphological identification of *M. aeruginosa*, or other cyanobacteria species that produce toxins non-constitutively, does not tell us if a given cell is producing harmful cyanotoxins.

Through the application of molecular techniques (real-time quantitative PCR; RT-qPCR) we are detecting the presence of low concentrations (250 cells ml^{-1}) of *M. aeruginosa* in local oyster habitats and determining which portion of those cells possess the genes to produce microcystin (approx. 65%). These data are providing important data that help explain microcystin concentrations in oyster tissues ranging from 0.194 to 0.941 ng MC g^{-1} oyster tissue wet weight (MCY-DM ELISA). We also used RT-qPCR to quantify possible rejection of a non-toxic strain of *M. aeruginosa* in lab-based oyster feeding experiments. These results showed that rejection of *M. aeruginosa* was consistent even with alternative prey available (Fig. 1), although total pseudofeces production rates increased with a diet rich in the cyanobacterium. Molecular methods are proving critical tools in enhancing our understanding of toxigenic cyanobacteria as components of the natural estuarine phytoplankton communities, relating species abundance to toxin accumulation in oyster tissue, and understanding oyster feeding behavior as these cells become more abundant in prey communities.

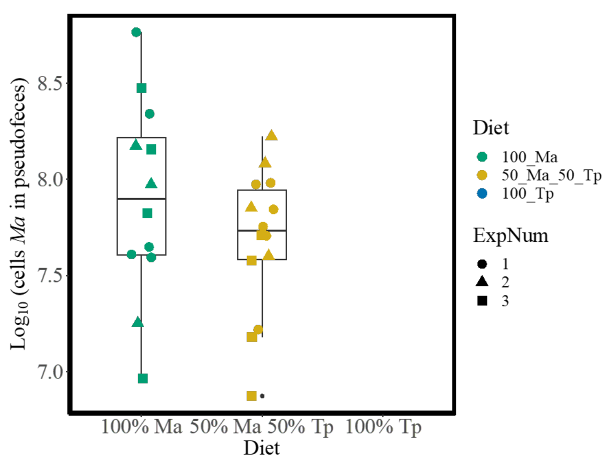

Figure 1: Abundance of M. aeruginosa in oyster pseudofeces in diets consisting of 100% M. aeruginosa (green) and 50% M. aeruginosa/50% Thalassiosira pseudonana (yellow). From Sweet et al., in prep.

ANTIMICROBIAL ACTIVITY OF *Centella asiatica* EXTRACTS AND THEIR POWDERED DIETARY SUPPLEMENT EFFECTS ON GROWTH, SURVIVAL, AND DISEASE RESISTANCE IN *Macrobrachium rosenbergii* LARVAE

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The giant freshwater prawn, *Macrobrachium rosenbergii*, is a species of significant economic importance in global aquaculture. However, its cultivation faces challenges, including disease outbreaks caused by pathogens such as *Vibrio alginolyticus* and *V. parahaemolyticus*, prolonged larval development, and high production costs. This study explored the potential of *Centella asiatica* as a natural feed additive to enhance larval performance, resilience, and production outcomes. The antimicrobial activity of ethanolic and aqueous extracts of *C. asiatica* was assessed using *in vitro* tests. Results indicated a minimum inhibitory concentration (MIC) of 500 μg/mL for ethanolic extracts against *V. alginolyticus* and *V. parahaemolyticus*, while aqueous extracts showed limited efficacy.

Dietary feeding trials were conducted using diets supplemented with varying levels of *C. asiatica* powder (0%–0.4%). The 0.2% supplementation yielded the best results, significantly improving survival rates (61.3%), production, and growth metrics, while reducing the rearing period to 21.0 ± 0.3 days compared to 25.3 ± 0.6 days in the control group. The challenge with *Vibrio alginolyticus* was conducted by exposing *M. rosenbergii* post-larvae to a bacterial concentration of 6.8×10^6 CFU/mL under laboratory conditions. Cumulative mortality was monitored every 3 hours over 72 hours. Larvae fed *C. asiatica*-supplemented diets demonstrated improved resilience and reduced mortality compared to the control group.

Histopathological analysis of hepatopancreatic tissue revealed that larvae fed *C. asiatica*-supplemented diets exhibited enhanced tissue integrity, reduced hemocyte infiltration, and lower histopathological damage compared to the control. These findings suggest that *C. asiatica* supplementation mitigates the adverse effects of *V. alginolyticus* infection and supports larval health. This research highlights *C. asiatica* as a cost-effective and sustainable feed additive for freshwater prawn aquaculture. By reducing reliance on synthetic antimicrobials and enhancing larval growth and resilience, the study demonstrates the potential of plant-based additives to advance sustainable aquaculture practices. The application of *C. asiatica* can contribute to improved production efficiency, lower mortality rates, and more economically viable prawn farming systems.

NURTURING FUTURE AQUACULTURE PROFESSIONALS: MENTORSHIP OF GRADUATE STUDENTS ALONGSIDE HIGH SCHOOL STUDENTS

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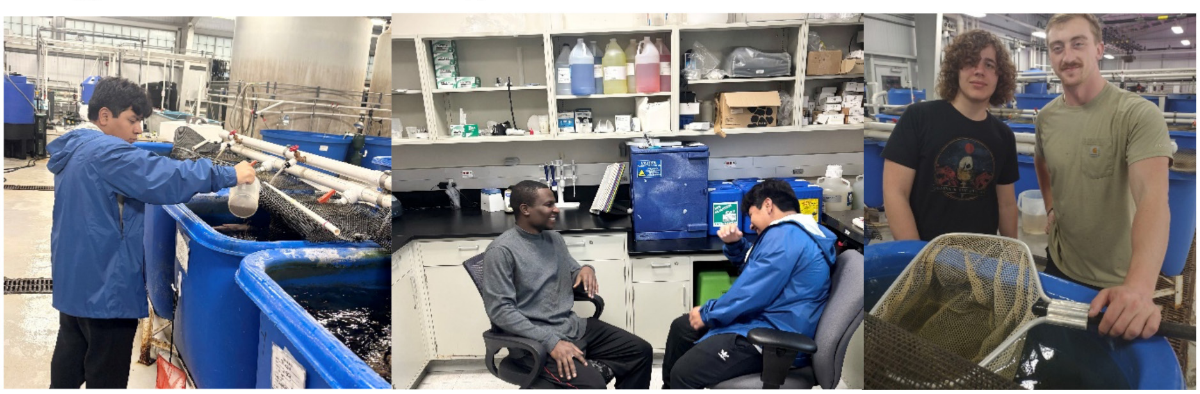
In envisioning a pressing need for self-sufficiency in local aquaculture food production, initiatives that enhance production and provide hands-on training for future professionals are needed. The tilapia research and Extension work at Kentucky State University (KSU) Aquaculture Research Center has provided an invaluable platform for early exposure to aquaculture by integrating mentorship of graduate students paired with Frankfort High School (FHS) students. This mentorship has strengthened capacity and enriched the quality of research, teaching, education and student experience in the fields of genetic improvement, reproduction, human nutrition, and farming of Nile Tilapia. This initiative was designed not only to fulfill high school requirements for graduation, but also to provide role-models, guidance, and responsible professional behavior. This has resulted in hands-on, practical skills and training in the following areas: (i) responsible and professional timeliness and work ethics, (ii) fish handling and spawning procedures, (iii) management of recirculating aquaculture systems, (iv) fish processing and preparation for cooking, (v) fish feeding and sampling, and (vi) water quality measurements. These activities are pivotal in enhancing students' understanding of aquaculture, life-skills, and fulfilling our research and Extension responsibilities (Figure 1). KSU's partnership with FHS has provided an opportunity for early exposure to higher education in aquaculture since 2016. This endeavor bolsters Kentucky's food security by training Kentucky residents, and establishes a sustainable example for mentorship in aquaculture, arming younger generations with the knowledge and abilities required to contribute to a more resilient food system.

Figure 1. Jonathan Barnes, FHS student, feeding fish (A), and conversing with graduate student mentor, Ayomide Taiwo (B) in 2024. FHS student, Jacy Langley (left) alongside graduate student, Cole Daleiden (right) in 2023 (C).

A **B** **C**



DEVELOPMENT OF A DISEASE STRATEGY MANUAL FOR SHRIMP HEPATOPANCREATIC MICROSPORIDIOSIS (HPM)

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Hepatopancreatic microsporidiosis (HPM) is an emerging shrimp disease caused by a microsporidian parasite *Enterocytozoon hepatopenaei* (EHP). The EHP is an intracellular parasite of penaeid shrimp that has caused substantial economic losses to shrimp producers throughout Asia. The disease was first seen in farmed *Penaeus monodon* in Thailand and later appeared in farmed *P. vannamei* in Thailand and elsewhere in Asia. Infected populations exhibit severe growth retardation and low-level mortality. The variability in the size of affected shrimp results in reduced market value. In addition, farmers often fail to notice HPM-related signs and continue to provide feed and other inputs as usual, leading to further economic losses.

This HPM-specific strategy manual provides information relevant to its prevention and control, is a component for the development of national contingency plan. It includes information on: (i) the nature of HPM: its etiology, and susceptible species; (ii) diagnosis: a description of gross clinical signs, field and laboratory methods; (iii) treatment and prevention measures: the use of EHP-free shrimp, RNAi technology, immunostimulants, metabolic modulator, and farm management practices; (iv) epidemiology: EHP's geographic distribution, persistence in the environment, reservoir hosts, mode of transmission, and risk factors; (v) principles of control and eradication: methods for control, containment and eradication, trade and industry considerations; and (vi) policy development and implementation: specific objectives, options and strategies for eradication and control, education, funding and compensation.

SELENOMETHIONINE ADDITION IMPROVE LARGEMOUTH BASS (MICROPTERUS SALMOIDES) GROWTH PERFORMANCE AND SURVIVAL UNDER 7 STRESS CONDITIONS

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1600 Largemouth bass with initial body weight of 19.27g were fed two diets of control diet or Selenomethionie addition diet for 8 weeks in pond cages for growth performance test. The Selenomethionie addition dosage is 0.48mg/Kg. The Se content in control diet was 1.387mg/Kg, and 1.780mg/mg in Selenomethionine addition diet. After growth trial, 420 Largemouth bass from each treatment were randomly selected for stress tests in tanks. Each stess standed for 30h and 60 fish for each stress. 7 stress conditions include 32°C high temperature stress, 8°C low temperature stress, 4mg/L ammonia nitrogen stress, 2mg/L nitrite stress, transport stress, 1% salinity stress and 1-2mg/L low oxygen stress. After 30h of stress, fishes were return to pond cages for 14d and go on fed with control diet or Selenomethionie addition diet according to their initial treatment.

After 8 weeks of growth, Selenomethionie addition significantly improved Largemouth bass growth performance. The body weight gain rate was improved for 4.68% ($P \leq 0.05$), FCR was decreased 4.30% ($P \leq 0.05$). Selenothionine addition had significantly improved Se content in fish flesh.

Selenomethionine addition had improved fish survival in 30h of stresses. There had 22 fishes died of 60 largemouth bass fed control diet after 30h of high temperature stress. 8 fishes died of 60 largemouth bass fed control diet after 30h of ammonia nitrogen stress. And 2 fishes died of 60 largemouth bass fed control diet after 30h of cold temperature stress. There was no fish died in Selenomethionine addition diet in any stress conditions.

In followed 14d of feeding trial after 30h of stress, the death of fish of both treatments were seriously. However, Selenomethionine treated fish have higher survival. For total 420 fish, the control diet have 38 survival, and Selenomethionine treated fish have 69 survival.

In conclusion, addition Selenomethionine in Largemouth bass diet improved fish growth performance and improved fish stress resistance in hot temperature, ammonia nitrogen and low oxygen conditions and improved fish bacteria resistance after stress with higher survival after stress and go on infection conditions.

APPLIED SHELLFISH RESEARCH AT THE AUBURN UNIVERSITY SHELLFISH LABORATORY, DAUPHIN ISLAND, ALABAMA

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The Auburn University Shellfish Laboratory (AUSL) opened in 2003 on Dauphin Island, Alabama. The lab's mission is to better understand the value of natural environments and promote best practices that lead to sufficient and sustainable shellfish production which in turn supports harvesters, growers, producers, distributors, and consumers of shellfish. Over the past two decades, the laboratory has graduated 18 masters and 4 PhD students. The research group currently consists of 9 full-time employees and 5 graduate students. Facilities include laboratories for microbiology and flow cytometry, sample processing, and digital imaging, an indoor wet lab, temperature controlled broodstock and spawning rooms, an open-air hatchery and nursery, and two off-bottom oyster aquaculture research farm sites, located in Grand Bay, AL and Bayou Sullivan, AL.

AUSL leads applied research projects involving all life stages of the Eastern oyster, *Crassostrea virginica*, throughout the production cycle. Research projects include: broodstock conditioning, biomarkers of gamete quality, increasing resilience through genomic selection, assessing performance of lines and ploidies in variable environments, use of aquaculture for restoration, hardening or priming to increase tolerance to stressors, oyster behavior and physiology in response to environmental stressors, phytoplankton community monitoring, seafood safety, bird interactions with floating oyster gear, sudden unexplained mortality events, and probiotics. AUSL also leads a workforce development program for Mississippi and Alabama.

Future plans for AUSL include collaborative establishment of an oyster broodstock breeding program for the Gulf of Mexico. AUSL also aims to expand research on oyster health and disease, including oyster immunology and oyster-microbial interactions. The lab will continue to collaborate with groups throughout the United States to produce oysters for research, extension, and outreach, and provide support for the commercial oyster aquaculture industry.

Figure 1. Aerial photo of AUSL research farm site in Grand Bay, Alabama.

THE COMMERCIAL OYSTER AQUACULTURE SECTOR TRAINING (COAST) PROGRAM: PHASE I

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As the off-bottom oyster aquaculture industry expands, there is an increasing demand for skilled employees. Desired proficiencies include oyster production and husbandry, but also record keeping, boating, and handling of product to ensure consumer safety. Although there are free online courses available for some of these skills, hands-on training in the industry is the best option. Therefore, the commercial oyster aquaculture industry needs workforce development programs that offer funding for on-farm training of apprentices.

In 2023, the Commercial Oyster Aquaculture Sector Training (COAST) program was initiated in the northern Gulf of Mexico. This program is a workforce development initiative aimed to recruit workers to the oyster aquaculture industry and provide them with training to meet industry demand. The current two-year funding cycle allows for participation of 10 apprentices in various aspects of oyster farming, including production and rearing, business management, food safety, and serving. Participating businesses from Alabama and Mississippi receive a portion of the apprentice's wages to support training efforts.

One benefit of this program is its flexibility, allowing the farm to select their own apprentice, determine the number of hours worked each week, and identify areas of training needed for their business. Assessments to date indicate all apprentices increased their knowledge in oyster aquaculture competencies. In addition, all farms and apprentices indicated that they would be interested in continued participation in COAST. Suggestions for improvement of the program include providing more funding to allow apprentices to dedicate themselves to the industry full time, as well as some formal training outside of the on-farm duties.

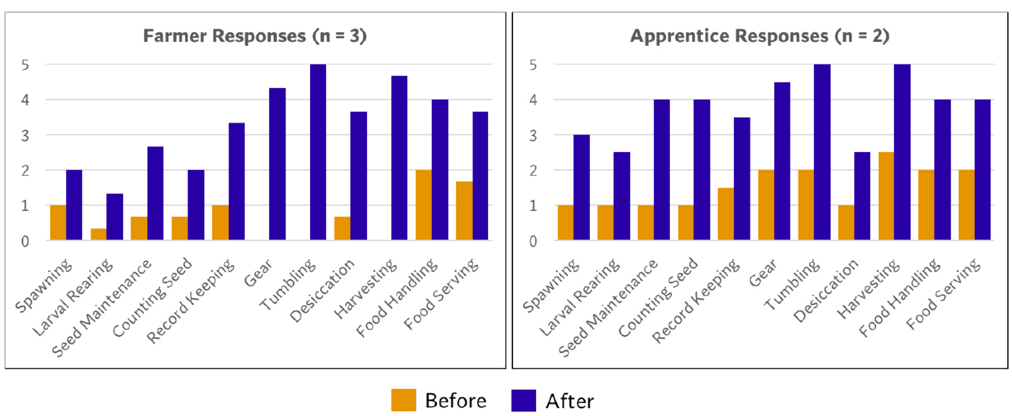

Figure 1. Questionnaire results from farmers rating their apprentice (left) and apprentices rating themselves (right), indicating an increase in knowledge after participation in the COAST program.

AN AUTOMATED OPEN-SOURCE SOLUTION FOR CRYOPRESERVATION TO ESTABLISH AQUATIC GERMPLASM REPOSITORIES

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The development of reliable genetic repositories is challenging. Effective cryopreservation calls for the use of industrial-scale programmable freezers that are costly and essentially unobtainable for institutions with limited resources. The Aquatic Germplasm and Genetic Resources Center here at Louisiana State University has addressed these challenges in the past with various versions of their open hardware 3D printed apparatus to make cryopreservation cheaper and more accessible. Among these include the Positional Cooling Platform Device (CryoKit), designed to float on liquid nitrogen and achieve various cooling rates (Figure 1). The issue being faced now is that devices such as the CryoKit can have very unpredictable cooling rates. The current solution to this is using a range of thicknesses of Styrofoam on the underside of the kit that helps the genetic samples achieve different cooling rates, but there is a considerable margin of error that can be observed in these rates because of the proximity the kit needs to be to the liquid nitrogen.

The goal now is to broaden the cooling rates that the CryoKit can achieve and make these cooling rates more predictable and consistent. To achieve this, a sort of lift system could be constructed that allows for a gradual descent into the nitrogen container. Additionally, the system could be entirely autonomous, utilizing microcontrollers, precision servos, and temperature sensors to control the rate of descent as well as ascend in the case the apparatus is cooling too quickly. Finally, a low-power aquatic air pump designed for home fish tanks with pneumatic tubing and an air stone could be used to percolate the liquid nitrogen in the container (Figure 2), raising vapors and increasing the temperature gradient within the enclosure.

The AGGRC has already created and tested a precision time-temperature sensor (coined as the Turtle) that could handle the immediate temperature data necessary for the lift. The aquatic motor and air stone used for percolating the nitrogen has also already been tested and shown to increase the temperature gradient drastically. These materials as well as the additional motors and microcontrollers are all easily accessible and cost-effective, increasing the price of the CryoKit's implementation slightly, but still being orders of magnitude more achievable than the costly freezers previously discussed. Additionally, all the code and wiring schematics for this project would be published, allowing anyone to recreate it. The aim here is to create a system that can intelligently and automatically handle the cooling necessary for cryopreservation similarly to an industrial freezer, taking the strain away from the institutions using this setup as a preservation alternative. Of course, this setup would be an add-on to the existing solutions that have been published, entirely compatible and dynamic. This project is currently referred to as the Autonomous Positional Cooling Platform Device (CyroLift).

Figure 1. CryoKit v1 suspended in container of liquid nitrogen.

Figure 2. Commercial aquatic air pump connected to air stone.

LIGHT INTENSITY MODULATES THE EFFECT OF THERMAL STRESS ON THE GIANT CLAM *Tridacna maxima* AND ITS SYMBIOTIC ZOOXANTHELLAE

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Gradual climate change and extreme marine heatwaves (MHWs) pose a serious threat to tropical marine ecosystems, endangering the fisheries and aquaculture resources they support. Effective adaptive management and the development of mitigation strategies depend on accurate predictions of the potential impacts of frequent and intense elevated temperature events on the productivity of these vital resources. A deeper understanding of species physiological responses to climate change-related stressors can help anticipate the risks. In this study, we explore mechanisms driving the sensitivity of the giant clam *T. maxima* to elevated temperature, providing insights that can aid in mitigating the effects of MHW on giant clam fisheries and aquaculture.

To investigate the effects of temperature and light intensity on the physiological condition of *T. maxima*, we conducted laboratory experiments at the Pacific Centre of Ifremer in Tahiti. Giant clams (n = 192; mean shell length= 6.5 ± 0.5 cm) from Reao lagoon (Tuamotu Archipelago) were exposed to eight experimental treatments combining four temperature levels (24°C, 27°C, 29°C, and 31°C) and two light intensities (150 and 1000 µmol s-1 m-2). After 18 days of exposure we used PAM fluorometry, respirometry, and measurements of zooxanthellae density (Chl-a and cell count) to characterize the physiological responses of both the symbiotic microalgae and animal hosts.

Our key findings emphasize the crucial role of light in determining the sensitivity of giant clam to warming. The effects of light and its interaction with temperature were stronger than temperature alone for several measured responses, including the photosynthetic yield of zooxanthellae (Figure 1A) and the respiration rate of giant clams (Figure 1B). These findings open up new opportunities for aquaculture and fishery management to minimize the impact of future MHWs.

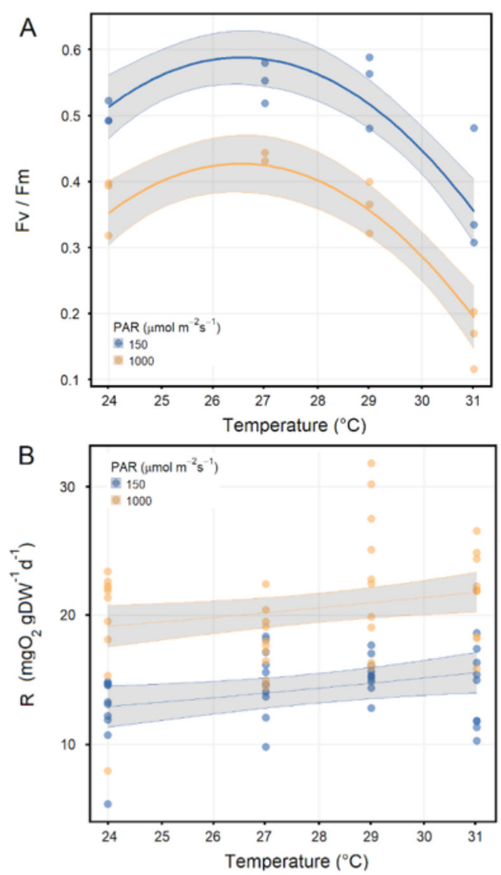

FIGURE 1. Physiological traits as a function of temperature and light. (A) Maximum quantum yield (Fv/Fm) of zooxanthellae; (B) daily respiration rate of giant clams.

ENHANCING MARINE ECOSYSTEMS AND PROMOTING SUSTAINABLE AQUACULTURE

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Agriculture today faces unprecedented challenges—climate change, soil degradation, biodiversity loss, and a growing population. Traditional monoculture systems deplete soil nutrients, exacerbate pest outbreaks, and increase greenhouse gas emissions. To address these issues, integrating agriculture with land-based aquaculture offers a transformative solution that enhances productivity, restores ecosystems, and reduces pollution.

The Case for Integrated Agriculture and Aquaculture Systems

Integrated systems like **Recirculating Aquaculture Systems (RAS)** minimize water usage and nutrient discharge while increasing food production. Mixing agricultural products with aquaculture effluent creates immediate crops, such as leafy greens, herbs, and vegetables, while simultaneously purifying water.

Key Benefits:

- **Pollution Reduction:** Research shows that crops integrated with RAS systems absorb **80-90%** of nitrogen and phosphorus from aquaculture effluent, preventing harmful nutrient runoff.
- **Resource Efficiency:** RAS reduces water use by **up to 99%** compared to traditional systems, ensuring sustainable water management.
- **Dual Outputs:** Systems combining aquaculture with saline-tolerant crops or halophytes yield food and animal feed while recovering **40-50%** of nutrients.

Practical Strategies for Implementation

1. **Education and Training:** Equip farmers with skills in regenerative practices, sustainable aquaculture, and integrated nutrient management.
2. **Demonstration Sites:** Establish training centers showcasing land-based RAS paired with agricultural crops, such as tomatoes, basil, and lettuce.
3. **Infrastructure Investment:** Develop systems that connect aquaculture effluent directly to crop production, optimizing resource cycling.

Conclusion

Integrated agriculture and aquaculture systems offer a practical, scalable approach to sustainable food production. These systems reduce pollution, enhance biodiversity, and build resilience against climate change. By adopting this innovative paradigm, we can protect waterways, improve ecosystem health, and secure sustainable food systems for future generations.

This transformation is not optional—it is a necessity. Let us lead the charge toward a future where agriculture and aquaculture harmonize to nourish both people and the planet.

DESIGN CONSIDERATIONS FOR COOL-WATER AQUACULTURE OF WALLEYE AND SAUGER USING RECIRCULATING AQUACULTURE SYSTEMS

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Cool-water fish culture for percid species like Walleye and Sauger comes with a unique set of challenges for each fish developmental stage. These challenges necessitate design solutions that are unique to the rearing of these species. One approach to addressing these challenges is to rear these species in Recirculating Aquaculture Systems (RAS). Increased survival (60-80%) and overall improvements in health have been achieved by rearing these species in RAS. Water quality management and tempering of the water supply are critical for all stages of life, from incubation to grow-out. Incubation and early rearing are complex due to fish behavior and a tendency towards cannibalism that requires a narrow range of environmental conditions to be maintained in RAS. These conditions include darkened rearing environments, controlled water turbidity, and use of spray bars at the tank water surface. In addition, treatment is needed for recirculated water, including the following unit processes: filtration for removing settleable and suspended solids, carbon dioxide stripping, oxygen addition, disinfection, and biofiltration if the system is recycling more than 75% of its water. Means for system reliability are critical, requiring equipment redundancy and monitoring and alarms for both the individual tanks and the systems. Additional design considerations for the culture of these fish species include evaluation of vertical vs. horizontal treatment equipment, use of modular systems, biosecurity measures, and incorporation of training and startup assistance. By taking into consideration the needs specific to percid species, RAS facilities can be designed for successful rearing of cool-water species for stock enhancement. Two current project examples with be reviewed for the design of new hatchery facilities for these fish species.

SHELLFISH AND CLIMATE CHANGE: ACTIONS TO UNDERSTAND AND REDUCE IMPACTS

Wyllys Chip Terry

CEO, BlueTrace

As a technology company working with over 500 different shellfish producers and distributors, BlueTrace has a unique perspective on the shellfish industry.

We all know shellfish is a triple bottom line win: Good for our health, good for our local communities and good for our environment--plus they taste great. However, running a shellfish operation is tough. Things can and do go wrong all the time. The only way these companies can deliver benefits over the long haul is if they are profitable. The BlueTrace presentation will delve into some of the things we see successful shellfish businesses overcoming.

My curiosity and love of new challenges has led me on an unlikely journey from a Ph.D. in American History to leading businesses at the cutting edge of analytics and software. I have had leadership roles at companies ranging from natural language processing (Zoominfo) and information (Forrester Research) to social media (BzzAgent) and retail analytics (dunnhumby). My most immediate previous experience includes five years building, selling, and integrating a social media startup into a large retail analytics company.

I started my career in traditional product and marketing roles and have grown into general management roles that include engineering and other functions.

My current role co-founding and running Shellfish Solutions is the most gratifying of my career. Being able to serve an industry that is helping rural waterfront communities (like where I grew up) build businesses that are good for the environment and great for the community is really rewarding.

Professional Preparation

Tufts University, BA American History, 1991
Boston University, Ph.D. American History, 1995
Full merit scholarship, graduate advisor 2-time Pulitzer Prize winning historian, Alan Taylor

Relevant Experience

SHELLFISH SOLUTIONS (OYSTER TRACKER), Maine — Co-founder, CEO 2017- PRESENT
We have grown from an idea to a company serving over 400 clients in the seafood industry. Key to our success has been recruiting a team of talented engineers, product developers, sales and marketing folks. We participated in both the Mass Challenge and the Clean Tech Open Accelerators and were in the top five of both in the final pitch contest. We raised Venture Capital from leading firms and successfully completed an SBIR Phase I and II grant from NOAA.

DUNNHUMBY, Boston and London — SVP Engineering/Global Product Director 2013 - 2016
After the acquisition of BzzAgent (see below) by dunnhumby, a global leader in retail analytics, I managed the US and UK engineering and product teams for the global media business. Among many new products and improvements, we used shopper transaction data (aka Big Data) to personalize media targeting and improve results for millions of campaigns for major retailers like Kroger, Tesco, and others in Europe, South America and Asia. Redemption rates rose to an unprecedented 50%+ for some campaigns.

(Continued on next page)

BZZAGENT, Boston and London — SVP & Integration Manager 2010 - 2016
Led the engineering and product teams for a venture backed startup—dramatically increasing on-time delivery and throughput by instituting an agile process and improving requirements gathering. New innovations led to the signing of major clients like P&G and growth that led to our acquisition. During the acquisition I led the integration between BzzAgent and dunnhumby.

Zoominfo, Waltham, MA — GM Business Solutions 2007 - 2010
Building on a natural language processing solution, pivoted the business from its roots in recruiting to the world of sales and marketing. The "Sales Intelligence" business grew from less than $100k to over $10 million in annual revenue. Company sold to a private equity firm.

Forrester Research, Cambridge, MA — VP Strategy and Marketing 2003 - 2007
Led a strategic review that resulted in a reorganization of the entire company—and an accelerated growth rate. Increased the efficiency of the sales force by automating lead scoring, nurturing, and opportunity identification. Marketing improvements led to a reduction in the cost of sales.

Princeton Review, Cahners, Homeportfolio.com — various Product and Marketing roles 1993-2003
I taught SAT and GMAT prep courses for Princeton Review through graduate school. That led to a role as product manager for their first digital product and a career in technology. I learned a tremendous amount from talented leaders as I transitioned from the academic world to the business world.
Leadership

Roles:
- Board Member and former Board Chair of Courageous Sailing—an innovative youth development non-profit that serves 1,000+ underprivileged children every year.
- Judge for Fish 2.0 business competition.
- Champion sailor, avid mountain biker & skier, and mediocre poker player, husband and father.

NON-LETHAL TISSUE SAMPLING AND SPECIES IDENTIFICATION FOR *Crassostrea virginica* IN BISCAYNE BAY, FLORIDA: IMPLICATIONS FOR HABITAT RESTORATION EFFORTS AND SPECIES CONSERVATION

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Oyster reefs provide critical ecosystem services, including water filtration, shoreline protection, and habitat for marine organisms. However, oyster populations have declined globally, with some species, such as *Crassostrea virginica*, considered functionally extinct in certain regions. In Biscayne Bay, Florida, *C. virginica* populations were once abundant historically but have since experienced dramatic declines. Recent findings have identified a remnant *C. virginica* population spread across various sites in the bay. Oyster aquaculture research at the University of Miami has recently been centered around collecting native *C. virginica* broodstock in Biscayne Bay to identify areas where this species naturally occurs while simultaneously establishing a broodstock population of native oysters at the University of Miami Experimental Hatchery (UMEH) facility. This work aligns with ongoing efforts to assess the potential of native oysters as a nature-based solution for habitat restoration in Biscayne Bay. In this project, native *C. virginica* broodstock were collected from sites along the western shoreline of Biscayne Bay, quarantined, transferred to broodstock holding tanks, and finally genotyped using non-lethal sampling methods. Tissue samples were collected after relaxing the oysters in a 5% Epsom salt solution. Given the high variability in shell morphology, molecular analysis was employed for accurate species identification. Genomic DNA was extracted, and mitochondrial cytochrome c oxidase I (COI) gene fragments were amplified using PCR for species identification. DNA sequences were analyzed and compared to reference sequences from other regions. The results confirmed that all oysters sampled (n=43) were *C. virginica*, with the highest genetic similarity to *C. virginica* populations from the Gulf of Mexico. The presented results provide a robust protocol for anesthetizing oysters for non-lethal tissue sampling, a molecular approach for assessing oyster species identification in restoration projects, and overall contribute to ongoing efforts to identify and restore oyster habitats in Biscayne Bay, Florida.

DETERMINING EFFECTIVE SALT (NACL) TREATMENT CONCENTRATIONS FOR COLUMNARIS INFECTIONS IN LARGEMOUTH BASS *Micropterus nigricans*

Vincent Teye*, John Kelso, Robert Durborow, Tifani McKay, Gunnar Psurny, Lucien Blakemore, and Adetola Ogundipe

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Feed trained largemouth bass fingerlings will be transferred into aerated 10-gallon aquaria and experimentally infected with columnaris causing bacteria (CCB). Infected fish will be exposed to salt concentrations of 6, 9, and 12 parts per thousand and will be monitored for signs of columnaris causing bacteria infection, such as skin ulcers, fin rot, and lethargy. The salt treatment levels will be monitored and maintained throughout the experiment. Each tank will be observed for mortalities and dead fish will be removed and recorded daily. To confirm columnaris causing bacteria in the experimental fish, gill, skin, brain and kidney samples will be collected from dead fish and cultured on Tryptone Yeast Extract Salts (TYES) media.

Data collected from the experiment will first be tested for normality and analyzed using ANOVA and Tukey's post-hoc test. The optimum salt treatment level that can control columnaris causing bacteria infections in largemouth bass will be determined based on survival percentage rate (number of fish survived x 100 / initial number of fish) and the absence of signs of columnaris causing bacteria in the fish.

Results will be disseminated to our stakeholders who raise largemouth bass and have to deal with preventing and treating columnaris infections. It is estimated that the initial results of the study will be ready by the April 2024 conference date. (This research is supported by the intramural research program of USDA NIFA, Evans-Allen project # KYX-80-23-34A, Accession Number 7004685).

THE DEVELOPMENT OF UNI IN PURPLE SEA URCHINS COLLECTED FROM CENTRAL CALIFORNIA COAST BARRENS

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An overabundance of the pacific purple sea urchin (*Strongylocentrotus purpuratus*) along the central coast of California has led to an ecosystem phase shift from kelp forests to urchin barrens. Human intervention in the form of collecting purple urchins from barrens offers some relief to kelp forests, but urchins collected from barrens often do not contain a high yield of high-quality uni. These empty urchins do not have a high market value, and divers do not have an economic incentive to collect them. The collection of empty urchins from barrens and their cultivation in an aquaculture setting increases uni production in empty urchins and incentivizes people to help restore kelp forests by collecting purple sea urchins from barrens.

This study aims to determine an effective, sustainable, and accessible diet for the uni development of purple urchins collected from barrens. Trial 1 highlights the effects of varying diet crude protein (CP) and starch levels on the yield and quality of uni. The composition of the six diets includes 20% CP and 30% starch, 20% CP and 20% starch, 20% CP and 10% starch, 40% CP and 30% starch, 40% CP and 20% starch, and 40% CP and 10% starch. After 8 weeks of feeding the diets, the yield and quality of the uni was assessed. Uni yield was determined by measuring the gonad somatic index. Uni quality was measured in terms of color, firmness, and texture. The L*A*B* values of the uni color were measured using a HunterLab MiniScan EZ 4500L portable spectrophotometer. Additional L*A*B* values were determined using ImageJ software. Firmness of the uni was measured using a Food Tech Corp TMS-Pro Texture Analyzer. Subjective measurements of color, firmness, and texture were collected using a 1-4 scale, 1 being exceptional and 4 being unacceptable. Trial 2 focuses on the influence of different carotenoid sources in a purple urchin diet on the color of the uni. The carotenoids provided in each of the eight diets were beta-carotene, spirulina, dunaliella, spinach powder, astaxanthin, Panaferd, krill meal, and a control diet with no carotenoid source. These diets were fed for 8 weeks, and the yield and quality of the uni was analyzed with similar methods as Trial 1. Carotenoids in the diets were analyzed and compared to the carotenoids in the uni.

The results from this study will help to establish a feeding plan using diets that are economically and environmentally sustainable. By determining a diet for the uni development of purple urchins, this study will encourage commercial divers and aquaculturists to continue collecting urchins from barrens with the confidence that they can raise them to a marketable standard.

OYSTERS, THEIR HATCHERY AND CLIMATE CHANGE IN CHINA AND SOUTHEAST ASIA: CHALLENGES, OPPORTUNITIES AND THE WAY FORWARD

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[10]Haskin Shellfish Research Laboratory, Rutgers University, USA
[8]Ocean University of China, Qingdao, Chian

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China accounts >85% of global edible oyster (shellfishes in general) production, which is and expected to produce even more in coming years to meet global demand for seafood protein by using this carbon-neutral coastal delicacy – the blue food. This massive coastal industry is, however, facing severe challenges to simultaneously increase meat quality and quantity using indigenously developed oyster seed collection, hatchery and cultivation technologies – especially, when oysters in south China are encountering unprecedented mass mortality at the time of harvest in winter – the so called "winter mass mortality", human dominance on coastal habitats and climate change. Though the reason for such mass mortality in variable with environment and time, seasonal shift in temperature, salinity and pH due to climate change appears to be involved in weakening oyster immune response and thus make them vulnerable to opportunistic pathogens. This mass mortality is not only directly affecting quality of oysters produced but also seriously affecting wild seed production and collection. The collected few wild seeds also experience mortality and their performance often unpredictable. The future of oyster farming is dependent on the development of hatcheries and advanced breeding technologies such as genomic selection. In this talk, we will discuss the current status of edible oyster aquaculture and the challenges this sector is facing due to climate change and multiple stressors. End the talk by showing how our "university, industry and grower's network and cooperation" is developing and transferring the grower-centered oyster hatchery technology that are recently getting popular in China and found to be more effective and sustainable, especially in South China and southeast Asia.

GLUTAMATE NEURONS SERVE AS EXCITATORY INTERNEURONS INNERVATING SEROTONIN NEURONS IN GANGLIA OF THE BIVALVE *Crassostrea virginica*

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Mammals and various invertebrates have excitatory glutamate neurons in their nervous systems. Human neurodegenerative diseases associated with glutamate dysfunctions include Parkinson's, Alzheimer's, Huntington's, autism, depression and schizophrenia. Recently, the glutamate NMDA receptor was found in and reported to be involved in regulating bivalve metamorphosis in *C. gigas, Mercenaria mercenaria* and *Mya arenaria*. In addition, our lab found glutamate neurons present in visceral ganglia of *Crassostrea virginica* and showed they have a neurophysiological function as ganglionic neurons causing gill lateral cell cilia to increase their beating rates. Serotonin neurons from the visceral and cerebral ganglia of *C. virginica* innervate the gill lateral cells and increase the cilia beating rates. Based upon this and the presence of glutamate neurons in *C. virginica*, we hypothesize that glutamate neurons are present in the cerebral ganglia of *C. virginica*, and that they serve as ganglionic interneurons in the cerebral ganglia, as well as the visceral ganglia, innervating serotonin neurons. To test this, we first used immunohistofluorescence (IHF) microscopy to determine the presence of glutamate neurons in the cerebral ganglia. Briefly, the cerebral ganglia were excised, snap frozen, cryostat sectioned, fixed with EDAC (N-Ethyl-N'-(3-dimethyl-aminopropyl) carbodiimide hydrochloride), treated with blockers, and incubated with glutamate primary antibodies and FITC conjugated secondary antibodies. Sections were viewed on a Zeiss epilume fluorescence microscope with a ProgRes C3 Peltier cooled camera, 100 watt mercury lamps and FITC excitation/emission filters. Results showed the presence of glutamate neurons in the cortex of the cerebral ganglia. The next set of experiments were to determine if glutamate GluR-1 receptors in the cerebral and/or visceral ganglia innervated the serotonin neurons. Cerebral and visceral ganglia were excised and prepared for IHF as above. To view if GluR-1 receptors were co-localized on serotonin neurons in the cerebral and visceral ganglia, the same procedure was employed using glutamate GluR-1 receptors primary antibodies with Texas Red secondary antibodies and serotonin primary antibodies with FITC secondary antibodies. Sections were then viewed using FITC and Texas Red excitation/emission filters. The IHF results showed the presence of GluR-1 receptors co-localized on the serotonin neurons in the cortex of the cerebral and visceral ganglia. This study complements other work of our lab which showed a neurophysiology function of glutamate as an excitatory neurotransmitter in the cerebral and visceral ganglia of *C. virginica*. Together they demonstrate that *C. virginica* is a useful model to study neurophysiology as well as the pharmacology of drugs affecting nervous systems.

This work was supported in part by grants 2R25GM06003 of the Bridge Program of NIGMS, 0537231071 of the CSTEP Program of NYSED, P120A210054 of the MSEIP Program of the DoEd, and NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University.

PRESENCE OF THE LIGHT SENSITIVE PHOTORECEPTOR RHODOPSIN IN MANTLE RIM SENSORY TENTACLES OF THE BIVALVE *Crassostrea virginica*

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Gill lateral cells (GLC) of *Crassostrea virginica* are innervated by serotonin and dopamine nerves from their ganglia. Most bivalves have GLC cilia that respond to serotonin and dopamine, with serotonin being the neurotransmitter that increases GLC cilia beating rates and dopamine being the neurotransmitter that decreases beating rates. The motor aspects of GLC innervation have been well studied, but there is limited information about sensory inputs. Our previous work found *C. virginica* senses light and other cues, such as food and crab extract when applied to the mantle rim and adjusts GLC cilia beating rates accordingly. Shining light on the mantle rim decreased GLC cilia beating rates, but when shined directly on the gill had no effects on cilia beating rates. This light-sensitive reaction was duplicated by applying histamine to the mantle rim and blocked by applying the histamine antagonist, famotidine, prior to shining light. Recently, published articles have found the presence of a rhodopsin-like gene in adult *Crassostrea gigas.* We hypothesize that in *C. virginica* the photoreceptor, rhodopsin, is present in mantle rim tentacles. To test this, we used immunohistofluorescence (IHF) microscopy and Western Blotting (WB) on mantle rim tentacles to view and detect the presence of rhodopsin. Mantle rims were excised from adult *C. virginica* and prepared for IHF or for PAGE followed by WB. Briefly, for IHF, mantle rims sections were snap frozen, cryostat sectioned, fixed with EDAC (N-Ethyl-N'-(3-dimethyl-aminopropyl) carbodiimide hydrochloride), treated with blockers, and incubated with rhodopsin primary antibodies and FITC secondary antibodies. Sections were viewed on a Leica epilume fluorescence microscope with a Leica DFC400 camera, 50 watt mercury lamps and FITC excitation/emission filters. For WB, mantle rims sections were homogenized in detergent and centrifuged. Protein concentrations of the supernatant were determined by detergent compatible Bradford. Aliquots were treated with Laemmli followed by PAGE (20-40 μg protein/well). After electrophoresis gels were sandwiched for transfer onto nitrocellulose membranes. After WB membranes were blocked, then incubated with rhodopsin primary antibodies and HRP secondary antibodies in TBST and 2% blocker at 4°C. Blots were resolved by KPL TrueBlue Peroxidase Substrate and viewed. IHF showed the presence of rhodopsin in mantle rim tentacles. WB detected discrete bands of rhodopsin protein present in mantle rim tentacles as well. The study shows the presence of the light sensitive receptor rhodopsin in mantle rim tentacles of adult *C. virginica* and complements other work of our lab that showed a neurophysiological response to light when shined onto the mantle rim. It also continues to demonstrate that *C. virginica* is a useful model to study neurophysiology as well as the pharmacology of drugs affecting the nervous systems.

This work was supported in part by grants 2R25GM06003 of the Bridge Program of NIGMS, 0537231071 of the CSTEP Program of NYSED, P120A210054 of the MSEIP Program of the DoEd, and NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University.

USING A CLASSROOM AQUAPONICS PROJECT TO IMPROVE URBAN (CITY) STUDENTS' PERCEPTION OF STEM DISCIPLINES AND CAREER PATHWAYS

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There is a need for secondary schools to provide more hands-on experiences in science, technology, engineering, and mathematics (STEM), and specifically, more contextualized project-based investigation (PBI) environments in the classroom that manifest the next generation science standards. This study investigated how, and to what extent, a 10-week contextualized aquaponics project-based investigation (APBI) affected urban (city) high school students' attitudes toward STEM, aquaculture and aquaponics, and interest in future STEM-related disciplines and/or STEM career pathways. Currently, there is little research published in technical literature on how APBI may engage students in increasing attitudes and interest in aquaculture/aquaponics fields as a career choice and, more generally, STEM disciplines.

Using a quantitative quasi-experimental research design, two different student groups participated in a hands-on APBI project and were given pre- and post-attitude/interest surveys (n=22). The 12 survey items were rated by a 5-point Likert-type scale that measured changes in student interest and attitudes toward STEM as discipline and as an area of interest. In addition, the survey included a profile of the respondents with the demographic items.

The results revealed that the intervention contributed to the treatment group students' positive attitudes toward STEM in general, and aquaculture and aquaponics specifically, and to students' developing an interest in the disciplines of STEM and/or as career pursuits. Results suggest that APBI models may be effective in attracting urban (city) students to STEM-related disciplines and careers.

DEVELOPMENT AND EARLY RESEARCH WITH A MICROHAPLOTYPE GENOTYPING-BY-SEQUENCING PANEL IN PACIFIC OYSTER (*Magallana gigas*)

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Genotying tools for Pacific oyster (*Magallana gigas*) have not advanced as rapidly as other aquaculture and agricultural species. To facilitate genome-enabled selection strategies a genotyping panel that is inexpensive, scalable and effective for confidently assigning kinship among samples is needed. In this study we describe the development of a microhaplotype panel from oyster populations used by the USDA ARS Pacific Oyster Genomic Selection (POGS) project.

Initially a haphazard sample from three populations of adult Pacific oysters were taken and sequenced using Whole Genome Resequencing methods to obtain a robust library of variation within and among breeding populations. Using the WGS data a greedy-algorithm was implemented to choose 288 loci that maximized heterozygosity and even distribution along the 10 chromosomes of *M. gigas*.

To develop and refine the panel a data set of 48 full-sibling families was used with 4 offspring per family. The increased number of offspring was used to identify loci with significant null allele frequencies. DNA was extracted, quantified and then standardized for library preparation. A GT-seq laboratory protocol was used to produce individual libraries that were then pooled by plate and submitted for Illumina sequencing.

Reads were demultiplexed, mapped to the amplicon sequences and processed using Microtyper. Run statistics were evaluated to quantify evenness in reads per locus, per sample and per plate. The microhaplotype panel was refined using multiple rounds of library preparation and sequencing where primer concentrations were primarily modified to achieve even coverage among loci.

The panel will be used to assign parentage to samples from the POGS project that have been part of Ostreid-Herpes Virus 1 experiments using a microvariant from San Diego Bay, California. Results from these studies will be presented to demonstrate the utility of the microhaplotype panel for enabling Pacific oyster breeding for increasing survival to exposure of a highly pathogenic virus that has the potential to significantly impact the Pacific Coast shellfish industry if the virus expands its range beyond San Diego Bay.

PROBIOTICS ENHANCE EARLY IMMUNE DEVELOPMENT, GROWTH, AND RESISTANCE TO VIBRIOSIS IN PACIFIC OYSTER (*Crassostrea gigas*) LARVAE

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The bacterial species *Vibrio coralliilyticus* has been linked to mass mortality events of *Crassostrea gigas* larvae in hatcheries on the U.S. Pacific Coast. These mortality events reduce supplies of seed to farmers. In aquaculture, multiple studies report the benefits of rearing shellfish larvae with putative probiotics for protection against pathogen-induced mortalities.

In our probiotic studies, we found that additions of three marine bacteria isolates were highly beneficial to oyster larvae cultured under standard hatchery conditions. A single dose of the probiotic combination to one-day-old larvae increased subsequent larval metamorphosis success for both the Miyagi and Midori stocks of *C. gigas,* compared to controls with no additions of probiotics.

In axenic larval culture assays, *V. coralliilyticus* was found to suppress the larval immune response while also prompting cell death. Probiotic treatment prior to *V. coralliilyticus* infection stimulated expression of larval genes involved in immune signaling proteins and effectors. This immunomodulation in response to probiotic treatment corresponded to an increase in the survival of *C. gigas* larvae infected with *V. coralliilyticus* (Figure 1).

Further development of the axenic larval culture protocol has shown that a very small dose of *V. coralliilyticus* (20 CFU mL^{-1}) results in 94% larval mortality compared with 5% mortality of the non-pathogen exposure control. Application of only 207 total CFU mL^{-1} of the probiotic mix reduced larval mortality to 8% when challenged with 2×10^3 CFU mL^{-1} *V. coralliilyticus*, further highlighting the effectiveness of probiotics in protecting oyster larvae against this bacterial pathogen under defined assay conditions.

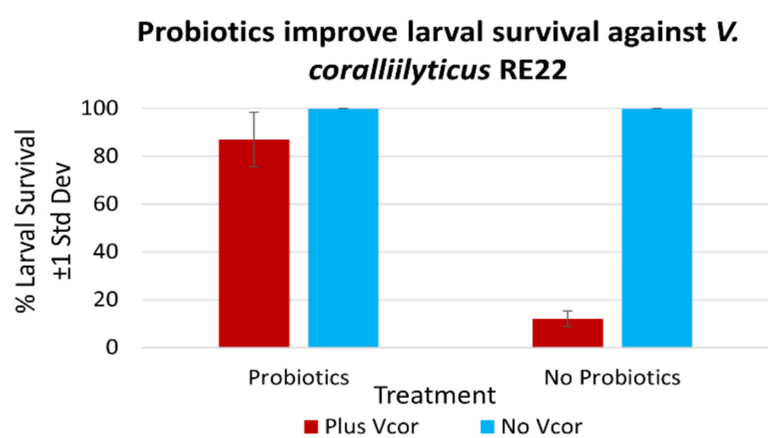

Figure 1. Percent larval survival of C. gigas larvae treated with probiotics prior to V. coralliilyticus RE22 infection. Axenic larvae were incubated with and without probiotics in sterile seawater for 46 hours prior to being challenged with a lethal dose of *V. coralliilyticus* RE22 (red) or a sterile seawater control (blue). Larvae were preserved with 0.1% formalin after 48 hours post-Vcor addition and the %larval survival was averaged across six replicates per treatment.

COMMUNITY-LED MANGROVE RESTORATION FOR ECOSYSTEM RESILIENCE AND SUSTAINABLE LIVELIHOODS IN COASTAL KENYA

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Mangrove forests are vital ecosystems that provide critical services, including coastal protection, carbon sequestration, and support for fisheries. However, extensive degradation due to unsustainable practices and climate change has left many mangrove areas in urgent need of restoration. In Kilifi County, Kenya, community-driven initiatives are addressing these challenges by integrating ecological restoration with sustainable development goals.

The aim of this project is to restore degraded mangrove ecosystems through participatory approaches that enhance biodiversity, sequester carbon, and support local livelihoods. Specific goals include: 1. Rehabilitating 600 hectares of degraded mangrove forest using best practices, 2. Empowering local women and youth through Capacity-Building workshops and economic opportunities in mangrove-based aquaculture, and 3. Promoting community awareness about the ecological and economic importance of mangroves.

We have successfully replanted 5 hectares of mangrove using native species selected for site-specific conditions, trained 200 community members in sustainable aquaculture and mangrove restoration techniques, and established partnerships with local schools to introduce environmental education programs, fostering a conservation ethos among youth.

Future work includes expanding the mangrove restoration efforts to achieve the 600-hectares target, scaling up mangrove-based aquaculture projects, and developing community-driven monitoring systems to ensure long-term sustainability. We plan to follow the "Best Practice Guidelines for Mangrove Restoration" recently published by the Mangrove Alliance[1] and educate local communities using the 4-part animation video series on mangrove restoration prepared by Wetlands International.[2]

References

[1] Best Practice Guidelines for Mangrove Restoration. 2024. The Blue Carbon Initiative. Global Mangrove Alliance. https://www.mangrovealliance.org/best-practice-guidelines-for-mangrove-restoration/.

[2] Mangrove Restoration, a 4-part animation video prepared by Wetlands International, a member of the Global Mangrove Alliance.

Acknowledgments

We extend our gratitude to FUCOBI Foundation of Ecuador, local stakeholders, and international partners (Grow with US) for their invaluable support in making this initiative possible.

PROMOTING MANGROVE CONSERVATION THROUGH CIRCULAR ECONOMY INITIATIVES: A SUSTAINABLE LIVELIHOOD APPROACH

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Mangrove forests are invaluable ecosystems providing critical environmental services such as carbon sequestration, shoreline protection, and biodiversity conservation. Despite their importance, mangroves face threats from deforestation, climate change, and unsustainable land use. This project highlights a dual approach to mangrove conservation by integrating ecological restoration with circular economy initiatives that empower local communities in coastal regions.

Central to this initiative is the establishment of small-scale, mangrove-friendly businesses such as honey production from beehives powered by solar technology, through a solar project supported by ESRAG – Environmental Sustainability Rotary Action Group.[1] By situating beehives in and around restored mangrove areas, the project creates incentives for communities to protect and sustain these vital ecosystems. The resulting mangrove honey, marketed locally, generates income, reduces reliance on exploitative practices like logging, and raises awareness about the ecological and economic value of mangroves.

This approach embodies the principles of a circular economy by promoting resource efficiency and waste reduction while enhancing biodiversity. The integration of renewable energy in powering beekeeping operations further aligns with global sustainability goals. Educational programs and training workshops ensure that local participants gain the necessary skills to manage their enterprises effectively, fostering a sense of ownership and long-term commitment to mangrove conservation.

The project demonstrates the transformative potential of coupling conservation with community-driven economic activities. By addressing both environmental and socio-economic challenges, this model offers a scalable and replicable framework for achieving sustainable development in coastal regions worldwide. We will follow the “Best Practice Guidelines for Mangrove Restoration” published by the Mangrove Alliance[2] and educate local communities using the animation video series on mangrove restoration.[3] At the Aquaculture 2025 meeting, we aim to share our insights and engage with stakeholders to inspire broader adoption of such integrated strategies for ecosystem restoration and community resilience.

References

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[2] Best Practice Guidelines for Mangrove Restoration. 2024. The Blue Carbon Initiative. Global Mangrove Alliance (GMA). https://www.mangrovealliance.org/best-practice-guidelines-for-mangrove-restoration/.
[3] Mangrove Restoration, a 4-part animation video prepared by Wetlands International, GMA member.

Acknowledgments

We extend our gratitude to FUCOBI Foundation of Ecuador, local stakeholders, and international partners (Grow with US) for their invaluable support in making this initiative possible.

iMCR: A NEW PARADIGM IN RAS DESIGN A NEW PARADIGM IN RAS DESIGN

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Growing fish using recirculating aquaculture systems (RAS) has been well-established. Economics of such systems still remains a challenge with systems often being characterized as too capital intensive, too high costs of operation, too labor intensive, and too complicated. The basic engineering principles of employing RAS is well established: round tank diameter-depth ratios, biofilter design, and water quality unit processes for oxygen and carbon dioxide control. The mixed cell raceway (MCR) was first introduced in 2000 and is basically a rectangular fish tank but hydraulically functions as a series of round tanks each with its own center drain. There are numerous MCR's around the world but just as RAS has not seen wide scale adaptation due economic viability, neither has the MCR design.

We have developed a completely different design that we refer to as the iMCR (provisional patent has been filed) where all processes are in the same tank structure with interior walls defining unit processes. The iMCR has several new features making the MCR simpler, less costly, reduced operating costs, and reduced risk for operational failure. The iMCR design results in the same water quality for the entire length of the raceway, whereas current MCR designs see a linear decrease in water quality from one end to the other end of the raceway. The iMCR design allows multiple stages of fish growth to be conducted in the 'same' tank by placing divider gates between stages (perpendicular to the long axis of the rectangular tank). In essence, fingerlings are never touched from placement till harvest at market size. Purging can be performed in the same iMCR.

These features all contribute to reduced fish stress, which is the key to fish performing near their genetic potential. The whole system operates on very low hydraulic head (less than 3 ft) and oxygen and CO2 control result from the design of the biological filter providing gas control at no additional cost (pure oxygen is made available as a backup only or for very high biomass loading). Since the fish are only 'touched' by humans at the beginning and end of the production cycle, labor costs are also reduced significantly.

RAS DESIGN: MASS BALANCES

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Water flow is the mechanism by which oxygen is transported into a fish culture vessel and the waste products being generated within are removed. The design of a recirculating aquaculture system (RAS) should ensure that the important parameters affecting water quality and fish productivity, e.g., oxygen, ammonia, carbon dioxide, and suspended solids are properly balanced. This requires calculating the value of each of these parameters independently to determine the thresholds for each. Then, having done the necessary calculations, the system must be operated at the lowest flow rate possible while still maintaining a particular parameter at its design value, e.g., ammonia. The simple word equation is:

Transport ***in*** *of "x"* + ***production*** *of "x" = transport* ***out*** *of "x"* (3.1)

The production or **P term** can be the production of oxygen, ammonia, suspended solids, or CO_2. Design examples will be given.

A mass balance is depicted in Figure 1 for the general case where part of the flow is recirculated and part of the flow is flow-through.

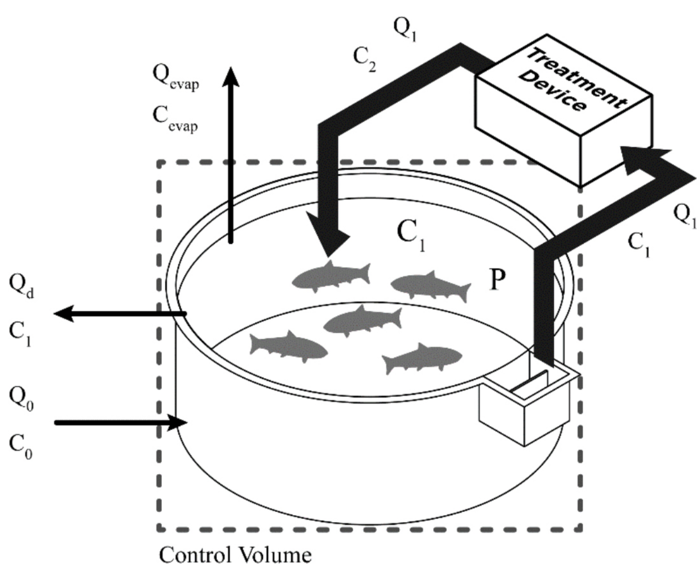

Figure 1. General mass balance on a fish culture tank. Treatment occurs exterior to the tank. (Symbols are defined in full paper)

A QUARTER CENTURY OF CERTIFICATION AND RANKINGS FOR IMPROVEMENT: ARE WE WHERE WE WANTED TO BE AND WHERE SHOULD WE GO IN THE NEXT 25 YEARS?

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Aquaculture production has had a meteoric rise in production, and many statistics of volume point to this success. Certification and rankings have been integral, especially early on, to help ensure that products reaching markets are produced with little environmental impact. But after a quarter century, have certification and ranking programs delivered on the promise of ensuring aquaculture products are produced with lower environmental impacts? Furthermore, to what degree should certification and ranking programs engage with a broader suite of impacts including but not limited to societal and animal welfare issues.

And with all their success of certification programs, why is such a low percent of product covered by these schemes? Additionally, companies are being sued listing sustainability claims on packaging that may not align with the ideals of those that are suing them. What is a path forward? What other programs exist that can ensure that aquaculture continues its path of continual improvement? This talk will introduce a session on how we can ensure the future of aquaculture is less impactful than it is now. Talks will highlight new programs as well as a reimaging of ideas that have been around for a while. It is only through an honest assessment of where we are, and what levers do we have to improve aquaculture that we can address the current challenges and will help us focus energy on solutions for the next quarter century. Through continual improvement, we can all help create a best future to ensure aquaculture maximizes its ability to provide food security and nutritionally superior products that maximize the equity and environmental justice for communities and societies across the world.

WILL YOU EAT SEAFOOD THAT IS CULTURED IN A LABORATORY? TAKE OUR SURVEY!

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Cultivated or cultured meat, is genuine animal meat (including seafood and organ meats) that is produced by growing animal cells directly in fermenters or bioreactors. There is significant interest in developing the ability to increase meat production through this method. We are interested to see how the aquaculture industry perceives this new entry into the food space. Please take our brief survey (QR code below), and there is an opportunity at the end to provide an email address if you would like to be mailed the survey results.

Link to the survey

SURVIVABILITY OF NEOPLASTIC HEMOCYTES OF *Mercenaria mercenaria* AT VARYING WATER TEMPERATURE AND SALINITY

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Disseminated neoplasia, or hemocytic neoplasia, has been documented in several marine invertebrate bivalves, including *Mercenaria mercenaria.* Tumor cells are thought to spread among naive populations through the water column. The disease is characterized by an expansion of atypical, anaplastic neoplastic cells that fill the vasculature. The infiltrating neoplastic cells decrease hemolymph flow and may use vital nutrients leading to organ dysfunction and eventually death of the bivalve. Due to the high rate of infection and dissemination, mortality occurs quickly across dense aquacultured populations, providing high demand for identifying environmental parameters which increase neoplastic hemocyte survivability in order to help farmers maximize their growth and earnings.

Neoplastic cells from surface *M. mercenaria* clams collected in Wellfleet, Massachusetts, were evaluated for mortality and survival at varying salinities and water temperatures. Highly neoplastic clams were identified as >90% neoplastic via hemolymph (blood) smear. A mixture of hemolymph (containing neoplastic hemocytes) and filtered artificial seawater was incubated for 1 hour. After 1 hour, the mixture was stained with 0.1% Erythrosin B and evaluated for live and dead cell counts using a hemocytometer. Hemocytes were exposed to the following salinities: 0, 10, 20, 25, 30, 35, and 40 ppt at room temperature (22-23°C). For the temperature exposures, neoplastic hemocytes were exposed to 0, 10, 15, 20, 25, 30, 35, or 40 °C while salinity remained constant at 25 ppt.

Survivability was calculated as a percent (surviving/total cells) for each target parameter. Preliminary findings show neoplastic cells had the highest survival rates at mid-range values (30 ppt, 25°C). Lowest survivability was documented at low extremes (0 ppt, 0°C). In contrast, higher extremes saw a slight decrease in survivability from the mid-range water parameters but remained higher than the zeros (ppt, °C). Neoplastic hemocytes of *M. mercenaria* appear to tolerate a wide range of salinities, suggesting they can survive and spread within a wide range of seawater environments.

A BROAD APPROACH TO ADVANCING RIBBED MUSSEL (*Geukensia demissa*) AQUACULTURE PRACTICES TO ACCELERATE COMMERCIALIZATION AND MEET GROWING DEMAND

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Demand for cultured ribbed mussels (*Geukensia demissa*) has grown exponentially in recent years due to their use in a wide range of ecological engineering projects, such as bacterial remediation, bioextraction, ecosystem stabilization, living shoreline development, and coastal resiliency. However, consistent ribbed mussel seed production has remained challenging and unreliable. Unlike the well-established husbandry techniques for the Eastern oyster (*Crassostrea virginica)* and the hard clam (*Mercenaria mercenaria*), culture techniques for ribbed mussels remain severely underdeveloped. To meet the growing demand for cultured ribbed mussels, present studies will build upon the work of the Ribbed Mussel Aquaculture Collaborative (RMAC) and seek to address significant gaps in the ribbed mussel aquaculture literature. All major aspects of ribbed mussel hatchery production, including broodstock conditioning, spawning, larviculture, settlement/recruitment, and juvenile culture are being studied. Since the project's primary goal was to accelerate the commercialization of ribbed mussel aquaculture, deliverables and outputs were anticipated to include practical tools, techniques, and resources that could be easily adopted by private and public shellfish hatcheries, alike. The chief anticipated outcome of this project was a well-laid foundation for a reliable ribbed mussel seed supply. While this ongoing study has helped begin to develop repeatable methodology in ribbed mussel hatchery and nursery techniques, large protocol gaps continue to exist perpetuating inconsistencies in meeting seed demand.

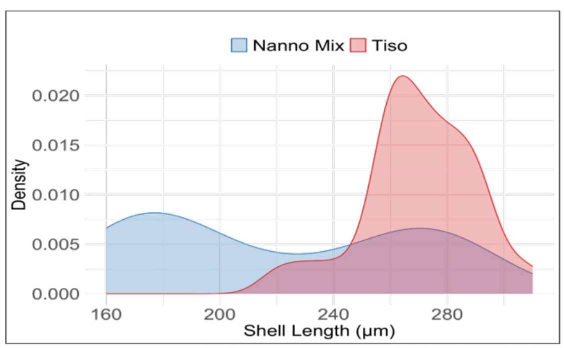

Fig. 1 – Larvae fed Nanno mix (blue) exhibit a bimodal distribution in shell length. Larvae fed Tiso (red) exhibit a negatively skewed shell length distribution.

INVESTIGATING IDEAL DIET AND TEMPERATURE OF MONKEYFACE PRICKLEBACKS *Cebidichthys violaceus* FOR COMMERCIAL FINFISH AQUACULTURE

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Freshwater eel populations are threatened due to overfishing and habitat degradation. Attempts to use aquaculture to sustain the fishery have often failed, owing to the complex lifecycle and the carnivorous nature of eel species. Monkeyface pricklebacks (*Cebidichthys violaceus*) have potential as an alternative cultivated finfish, possessing several characteristics warranting an assessment of the species that includes herbivory, potential high stocking density, tolerant of fluctuating environmental conditions, and no fishery competition. Though not technically an eel, *C. violaceus* is eel-like in appearance and is reported to taste like unagi. Their herbivorous, intertidal ecology has the potential to facilitate the use of fish free feeds and grow in warmer-than-ambient conditions. However, the ideal formulated diet and temperature to efficiently rear *C. violaceus* is unknown. To test this, we measured growth, feed intake, condition, metabolic rates, fillet composition, and critical thermal maximum (CTM) of juvenile monkeyface pricklebacks that were reared in separate experiments: (1) on four different diets (fresh seaweed, seaweed pellet, mixed pellet, and soy pellet) and (2) across five different temperatures (14-26°C) when fed the ideal diet.

Monkeyface pricklebacks exhibited the highest growth rates and body condition on the mixed and soy pellet diets and in the 20-23°C treatment. Metabolic costs did not differ between diets, but they were impacted by temperature. FCR was lower on pellets and feed intake increased with temperature. Fillet composition was generally influenced by diet but not temperature. Fish could also increase their CTM by 2°C after acclimating to warmer temperatures. If raised on a mixed and soy-based pellet at 20°C, monkeyface pricklebacks have the potential to increase their growth, which could reduce pressure on freshwater eel populations as farming increases the supply.

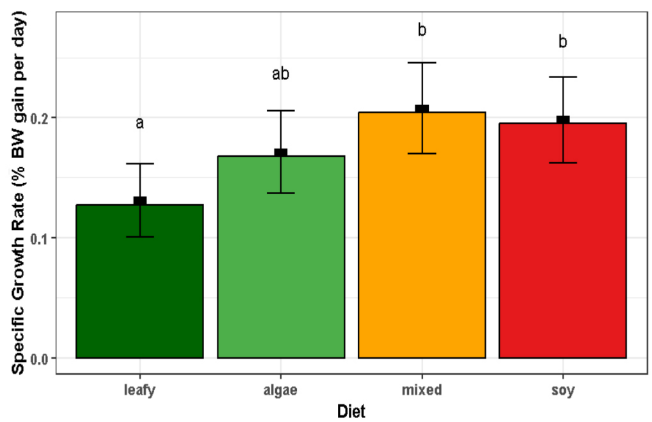

Figure 1. Specific growth rate of juvenile monkeyface pricklebacks consuming different feeds.

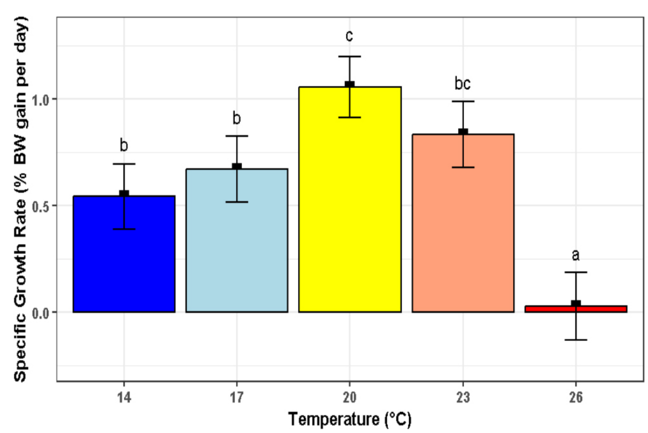

Figure 2. Specific growth rate of juvenile monkeyface pricklebacks at different temperatures.

BIODIVERSITY AND OYSTER HEALTH ON AQUACULTURE LEASES AND RESTORED SANCTUARY REEFS IN CHESAPEAKE BAY

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Biodiversity on oyster reefs includes organisms that affect oysters as their food, predators, parasites, and competitors. The organisms present and their interactions with oysters may differ on aquaculture leases and restored sanctuary reefs. This study takes an enhanced perspective on biodiversity and oyster health by comparing the species detected on aquaculture leases and restored sanctuary reefs at three timepoints that are critical for the oyster life cycle: the spring phytoplankton bloom, the summer reproductive window, and the fall spike in *Perkinsus* parasitic infections. The aim is to identify species linked to oyster health and improve the understanding of ecosystems supported by oyster aquaculture and restoration.

We sampled 11 reefs across two rivers in spring, summer, and fall of 2024 and used salinity, temperature, dissolved oxygen, turbidity and chlorophyll to characterize environmental conditions. We leveraged a variety of biodiversity metrics to capture diverse taxa. First, environmental DNA ("eDNA") was extracted from water and sediment to identify animals through the DNA they shed into the environment and is effective for rare or microscopic organisms. Second, we deployed settlement tiles for approximately 30 days at each timepoint to track small fouling organisms that compete with oysters for space and food. Finally, GoPro videos and audio recordings target mobile organisms, such as fish, jellyfish, and crustaceans. Oysters were diagnosed for *Perkinsus marinus* infection and scored for body condition to assess how oyster health is linked to biodiversity.

Aquaculture leases and restoration reefs had distinct biodiversity as estimated by audio profiles based on eight unique sounds (**Figure 1**). Audio from oyster toadfish was only consistently present on restoration reefs, while non-reef control sites had the lowest sound diversity. Sediment tiles showed differences in small fouling organisms across rivers and timepoints. Restoration reefs had more oysters and more complex habitat than aquaculture leases, but also greater disease impacts. Sequencing of eDNA from water and sediment will yield further insights into biodiversity profiles and linkages with oyster health, indicating species that merit increased attention in managing oyster aquaculture and restoration.

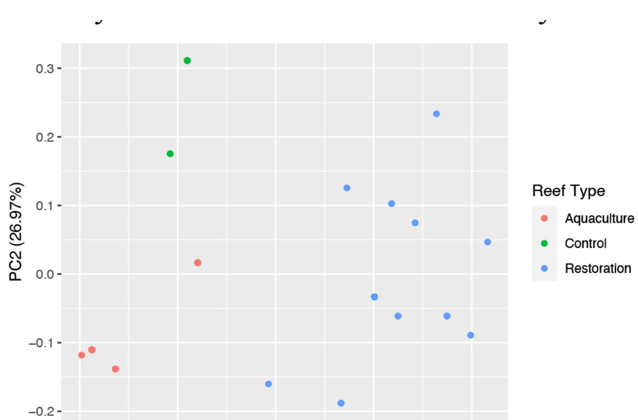

Figure 1. *Biodiversity sound profiles by reef type*

SHRIMPVET FARMING TECHNOLOGIES AIM TO REVOLUTIONIZE VIETNAM'S SHRIMP SECTOR

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Vietnam's shrimp industry faces challenges from low prices, high production costs, and environmental concerns. This is exacerbated by competition from major producers like Ecuador and India, leading to a price crisis. ShrimpVet introduces a new farming model aiming to revolutionize the sector. This model emphasizes efficiency, sustainability, and technology transfer. ShrimpVet's 30-hectare farm utilizes a modular design, advanced technology, and mangrove integration to minimize environmental impact and maximize productivity with a lower carbon footprint. By optimizing economies of scale and reducing labor costs, ShrimpVet aims to achieve profitability even with low shrimp prices. ShrimpVet's vision includes expanding to 50 modules by 2030, covering only 1% of Vietnam's shrimp farming area while matching current national production. This model also addresses the generational shift in farming by offering a technologically advanced and profitable approach, potentially revitalizing Vietnam's shrimp sector and ensuring its future sustainability.

THE INAUGURAL SESSION OF THE S.C. COMMERCIAL SEAFOOD APPRENTICESHIP PROGRAM

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This abstract is being submitted for the session entitled “Apprenticeships, Internships and Other Workforce Programs for the Aquaculture Community.” Created through a partnership between the S.C. Sea Grant Consortium, Clemson University, the Town of McClellanville, and the local commercial seafood industry, The S.C. Commercial Seafood Apprenticeship Program is a one-month, full-time program that was created in order to develop a skilled workforce for the commercial fishing and mariculture industry in South Carolina and the southeast region, while simultaneously preserving and transferring the knowledge and expertise of local commercial fishers and farmers. Our goal is to create a recruitment pipeline both for those seeking work and for those seeking workers in the commercial fishing and mariculture industry.

The program teaches participants the skills they need to get started in the commercial fishing or mariculture industry and is best suited for those seeking entry-level employment in the industry. Apprentices receive immersive marine safety training, classroom based training, and hands-on training with local industry experts. Upon completion of the program, participants will have their U.S. Drill Conductor certification and be certified in First-Aid and CPR, a completion stipend, gear and course materials, and networking assistance to help apprentices find work upon completion of the program.

Our first session ran from April 15 - May 10, 2024. We had fifty-nine applicants. Six people began the program and five apprentices graduated. We had three men and three women participate. Students learned about the biology of fish and shellfish, habitat requirements, fisheries management, farming practices and techniques in the Lowcountry, production and the grow out process, the permitting process, equipment and gear types. They were able to observe and take part in the entire mariculture process from bagging seed to planting, harvesting, and preparing for sale.

MANAGING SMOLTIFICATION FOR IMPROVED OUTCOMES FOR ANADROMOUS FISH

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Any aquatic organism transitioning between life in freshwater to saltwater must address divergent osmoregulatory challenges in these environments to maintain or quickly reinstate homeostasis. Survival following the transition from freshwater to saltwater necessitates a reversal in the functioning of osmoregulatory organs/tissues, such as the gills, gut, and kidney. Among salmonids, these physiological changes are accompanied by morphological and behavioral changes that collectively define a transformation known as "smoltification".

Different strategies can be used to manage parr-smolt transformation in production aquaculture, including photoperiod manipulation (i.e., 'winter signal' followed by continuous light) and the use of smoltification feed. SuperSmolt™ Feed Only (SSFO) is a smoltification feed containing a proprietary blend of sodium chloride, free tryptophan, and magnesium and calcium salts that induces smoltification via stimulation of calcium-sensing receptors in the fish's body. SSFO induces smoltification in obligate anadromes, like Atlantic Salmon and Coho Salmon, without the need for photoperiod manipulation. Additionally, SSFO can induce smoltification in facultative anadromes, like Rainbow Trout/steelhead, that are largely unresponsive to changes in photoperiod.

SSFO has been used primarily to smoltify commercially reared juvenile salmon before seawater transfer, but it is now increasingly being used as a tool to prepare commercially reared trout for grow-out in sea cages and in public hatcheries raising various anadromous salmonids for conservation purposes. This presentation will briefly review the physiology of smoltification—what happens, when, and how—and provide insights from recent research involving managed smoltification using SSFO.

COLLABORATIVE SCIENCE IN THE COMMERCIAL AQUACULTURE SPACE: LEVERAGING RIVERENCE'S SENTINEL PROGRAM FOR DATA GENERATION AND DISCOVERY

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Artificial selection is a powerful tool that can be used to make dramatic changes to observable traits in just a few generations. However, when breeders select for one trait, they inadvertently select for or against many other traits that may be important. Artificial breeding programs focused exclusively on production performance metrics commonly fail to identify the associated vulnerabilities, giving rise to stocks that are fast-growing or high-yield, but prone to infectious disease or other hereditary disorders. Consequently, animal breeders have begun to rethink their approach to genetic improvement and are looking to the principles of natural selection, vigor, and biological fitness as guides.

Riverence Brood's Rainbow Trout breeding strategy includes an iterative research program to screen candidate indicators of biological fitness so that these traits can be targeted for selection. The so-called "Sentinel Program" entails an annual 'common garden' experiment in which family groups representing all of the genetic variation within the broodstock (i.e., "sentinels") are raised to market-size according to routine commercial practices. At harvest, individual fish are evaluated in terms of various candidate fitness indicators and superior performance is then traced back to families who genetics are emphasized in future breeding schemes.

Over the last 5 years, the Sentinel Program has identified fitness-based breeding targets and enabled Riverence Brood to continuously improve upon our genetics. Additionally, the R&D group has leveraged the Sentinel Program to create additional opportunities for scientific inquiry. Each year, roughly 1000 fish are genotyped to determine familial relationships and individually characterized in terms of various morphological, physiological, and performance-related metrics. For each fish, a wealth of metadata is available and virtually any tissue can be sampled for histology, gene expression, or other analysis. Collectively, these data can be used to contextualize or interpret other results or probe other questions related to fish health, nutrition, reproduction, or other topics. Recently, samples collected from sentinel fish and the associated meta-data have been used to collaboratively explore the basis of variation in the prevalence and abundance of external parasites, expression of antimicrobial peptides, and viral pathogen susceptibility and transmission.

First and foremost, the Sentinel Program informs Riverence's selective breeding decisions for Rainbow Trout. However, it is also an unmatched opportunity for collaborating researchers to gather valuable samples and data to further their own scientific endeavors involving this species. This presentation will provide an overview of the Sentinel Program and outline opportunities for collaborative science and the development of robust, long-term datasets.

INVASIVENESS OF ORNAMENTAL FISH IN THE CONTERMINOUS UNITED STATES

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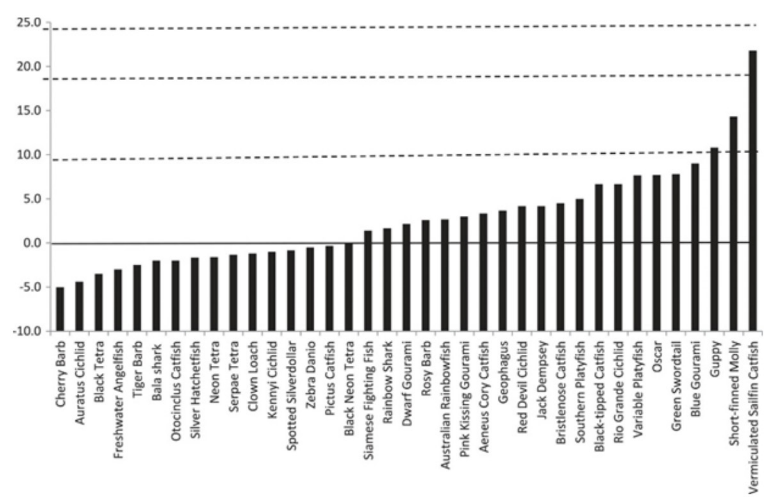
The trade in ornamental fish is large and global, with much of the trade volume being produced in captivity, representing an important aquaculture industry. Because ornamental fish often originate from tropical climates, aquaculture production is in warm regions such as Florida, where hundreds of species and varieties are reared. The introduction of ornamental fish, whether through aquarium release or escape from aquaculture, affects industry development and sustainability, and is one of the chief environmental concerns for ornamental aquaculture. Our broad goal is to examine the invasiveness of ornamental fish. Our specific objectives were to 1) evaluate the aquaculture escape pathway for the introduction of ornamental fish, 2) identify risk of ornamental fish to the conterminous U.S., and 3) evaluate the causes of successful and failed invasions. We used risk screening of popular species, thermal suitability, and known invasion history to examine invasiveness.

The aquarium release pathway was often found to be conflated with ornamental aquaculture, although they are separate introduction pathways. The ornamental aquaculture pathway was only important for regions with a combination of suitable habitat (warmer water temperature) and significant ornamental aquaculture production (Florida). Ornamental fish are predominantly low risk (non-invasive), with a few exceptions. Even for established non-natives, impacts are rarely known or found. Successful invasions were due to unique microhabitats (hot springs), availability of subtropical and tropical climates, or tolerance to cold temperatures. The causes of failed ornamental fish invasions are often difficult to identify. However, the lack of cold tolerance was a dominant and often obvious cause of failed invasions, likely due to the relative paucity of temperate ornamental fishes, although there has been some development of native North American ornamental species. In other cases, careful experimentation was required, as with the case of the Guppy (*Poecilia reticulata*), which failed to establish due to native species biotic resistance.

Figure 1. Risk scores for ornamental fish. Scores ≤ 10 indicate non-invasive (Hill et al. 2017).



EXPLORING PLASMID-BASED HORMONAL TREATMENT TO INDUCE PUBERTY IN LATE-MATURING FEMALE FISH

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A significant challenge in aquaculture is the delayed sexual maturation of commercially valuable marine fish, challenging the production of a reliable seed supply. Previous research in various fish species has demonstrated that prolonged treatment with either Follicle-Stimulating Hormone (FSH), Insulin-like Growth Factor 1 (IGF-1), and Estradiol (E2) can induce gonadal development in immature fish. However, current available hormonal treatments, using recombinant proteins, require frequent administration, leading to high costs and labor, and cause stress and mortality to the fish.

This study aimed to test a multi-hormonal treatment with plasmids expressing *Seriola* FSH and IGF-1 and implants releasing low levels of Estradiol (E2) to induce gametogenesis in the late-maturing female *Seriola rivoliana*. Successful outcome will ultimately promote sustainable marine aquaculture, especially in species that exhibit late maturation or failure to breed.

One year old female *Seriola rivoliana* were used for this study. The fish received 0.5 mg/kg BW intramuscular injections of serFSH and/or serIGF-1 expressing plasmids at time 0, 1.5 and 4.5 months. 5 mg E2 implants were administered every 3 months. Gonadal development and circulating FSH and E2 levels were analyzed.

Four months into the experiment, plasma FSH levels were significantly elevated only in the plasmid treatment groups compared to controls (Fig. 1), with no significant difference observed between the double FSH and FSH-IGF-1 plasmid treatments, both administered with an E2 implant. In addition, mid-vitellogenic oocytes were observed across all treatment groups, including the E2 implant group, but were more prevalent in the FSH/IGF-1 treatment group (Fig. 2). These findings suggest that prolonged administration of plasmids expressing FSH and IGF-1, along with E2 implants, can effectively induce gonadal development and trigger precocious puberty.

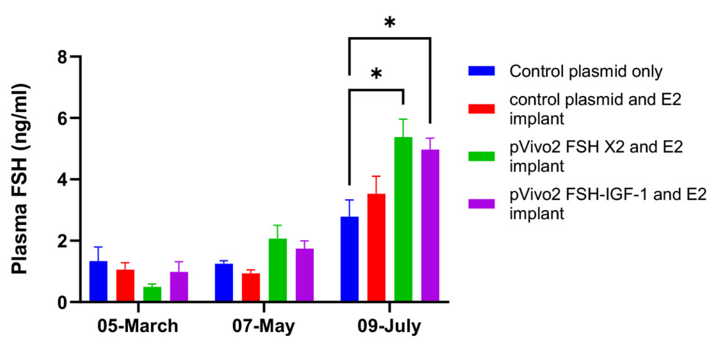

Fig. 1- circulating plasma FSH levels at time 0, 2 months, and 4 months. Results are presented as mean ± SEM.

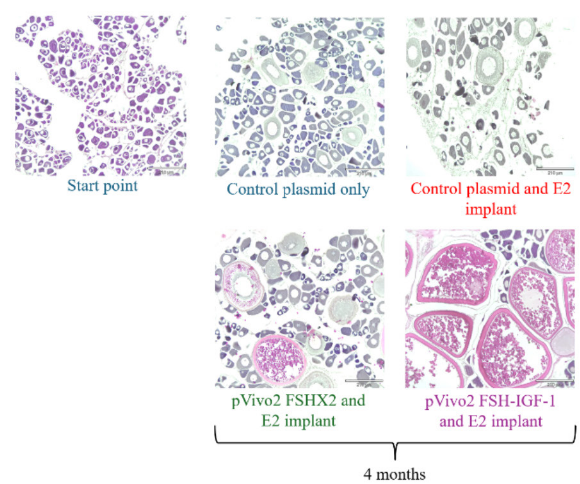

Fig. 2- representative gonad H&E staining samples at time 0 and 4 months.

EFFICACY OF A COMMERCIAL WATER CONDITIONER ON TRANSPORTATION-, TEMPERATURE-, AND BACTERIAL CHALLENGE-STRESS RESPONSES OF *Ictalurus punctatus* FINGERLINGS

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Catfish [channel (*Ictalurus punctatus*) and hybrid catfish (♀ *I. punctatus* × ♂ *I. furcatus*)] are the most produced domestic aquaculture finfish species. While grow-out occurs nationwide, Mississippi hatcheries produce most food-fish fingerlings. This necessitates transporting large numbers of fingerlings long distances. Additionally, due to inefficiencies and complications in pond-stock turnover, producers receive new fingerlings at inopportune times and suboptimal pond conditions. The objectives of this study were to determine the physiological, immune, and stress responses of *I. punctatus* fingerlings to transportation, temperature, and bacterial challenge stressors when individuals were transported in conditioner-treated water compared to untreated water. First, 720 Marion strain *I. punctatus* fingerlings (8.45±2.72g) were split evenly and transported approximately 255 km in 18°C oxygenated water from Greensboro, AL to Auburn, AL at a density of 0.45 kg/L. One group was exposed to a commercial water conditioner (*V…*) and the second group was not (*NV…*). After 2 hours and 48 minutes of travel, the two containers were each split into 4 different groups. One group was stocked at 18°C (-.*CFN*), second was stocked at 27°C (-.*HFN*), third was exposed to a lethal concentration (1.27x10^7 CFU/mL) of *Flavobacterium covae* (ALG-00-530) at 18°C (-.*CFC*), and fourth was exposed to a lethal dose of the same *F. covae* isolate at 27°C (-.*HFC*). These 8 groups consisted of 6 tanks (2 for sampling, 4 for survival monitoring) randomly distributed among 4 sets of 12 flow-through tanks housed in a secure system. Blood, mucus, gills, kidney, and spleen sampling from 3 individuals from each treatment occurred before and after transport, as well as 2, 6, 24, and 48 hours post stocking. Survival (Figure 1), blood cortisol, glucose, and lactose concentrations, mucus lysozyme activity, gill histology, and kidney, spleen, and gill immune gene expression were analyzed. Survival probability of all treatments exposed to *F. covae* was different from treatments naïve to the pathogen (P < 0.001), and the VCFC treatment was different from all other treatments, except NVHFC (P = 0.179). No distinguishable trends over time occurred in any treatment regarding blood cortisol, glucose, lactose concentrations, or mucus lysozyme activity. These findings provide valuable insight into fingerling transportation and stocking challenges, as well as progress towards a viable stress management strategy in the commercial catfish industry.

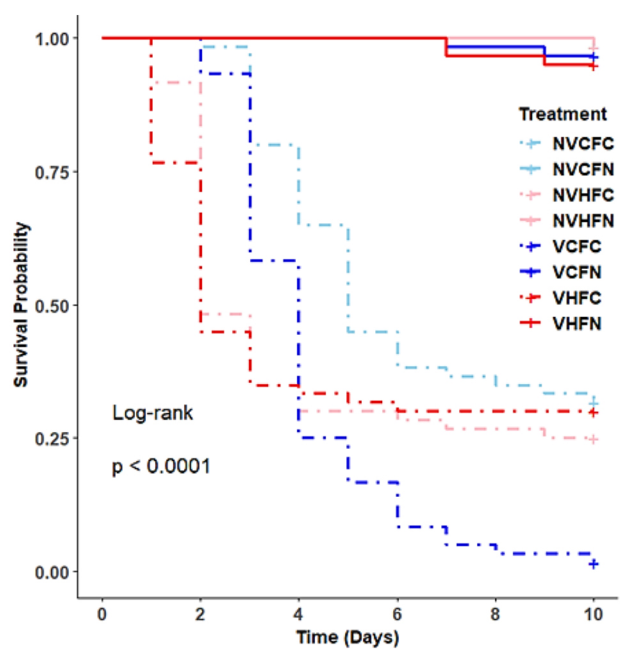

Figure 1. Survival probability of *I. punctatus* fingerlings subjected to variable stressors. Results visualized via Kaplan Meier plot. p < 0.05 considered statistically significant

EVALUATION OF ORANGE-SPOTTED GROUPER *Epinephelus coloides* MYOBLAST ADHESION ON SCAFFOLD MATERIALS USING QCM-D

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Transitioning from a linear to a circular bioeconomy represents a pivotal shift in resource utilization and waste management. A circular bioeconomy emphasizes reuse, recycling and repurposing of materials, as opposed to a linear bioeconomy, where resources are extracted, and waste is disposed.

This shift from a linear to circular use of resources leads to a significant reduction of waste and environmental impact. While it is more difficult to convert well established food supply chains into a circular bioeconomy, it is easier to develop solutions in newer products like cell-cultured seafood. To encourage a circular use of resources in cell-cultured seafood, scaffold materials may be made from a variety of seafood processing byproducts. Figure 1 shows Overview of waste/byproducts of seafood processing industry.

In this study, Orange-spotted Grouper (*Epinephelus coloides*) myoblast cells were grown on multiple upcycled scaffold materials and the cell adhesion was evaluated using quartz crystal microbalance with dissipation (QCM-D). This method is a very accurate and precise method, which provides real-time measurements on cellular adhesion and biocompatibility of scaffold materials.

QCM-D provides real-time kinetic data, which is not possible with other methods. While adhesion to scaffolds has been measured using other methods, there are no current studies using QCM-D to measure the adhesion of cell lines to scaffold materials in cellular agriculture/aquaculture. The results of this study will identify varying levels of adhesion for three different hydrogels. This information can be used for designing an optimum scaffold for cell-cultured seafood and fish meal production.

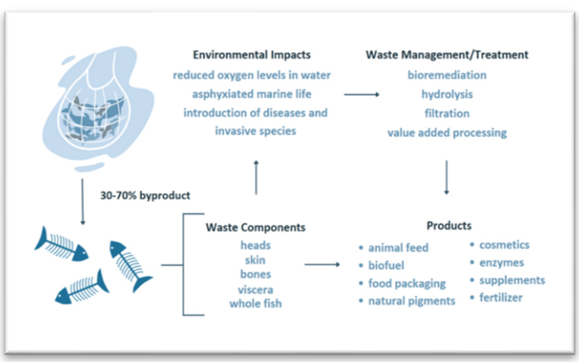

Figure 1. Overview of waste/byproducts of seafood processing industry. (Credit; Julia Tvedt, Boce Zhang, and Razieh Farzad, UF/IFAS Extension)

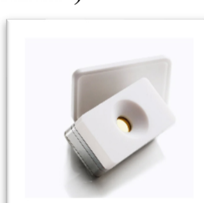

Figure 2. QSense® open-module QCM-D instrument. (Credit; Biolin Scientific)

SOME DON'T LIKE IT NOISY: SHIPPING NOISE ALTERS THE BEHAVIOUR AND HABITAT USAGE OF WAVED WHELK *Buccinum undatum* (*Gastropoda*)

Thomas Uboldi*, Nathalia Byrro-Gauthier, Youenn Jézéquel, Rejean Tremblay, Fréderic Olivier, and Laurent Chauvaud

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Besides marine mammals and fishes, there is great confidence today that anthropophony also negatively affects different classes of marine invertebrates. Since laboratory experiments can only provide partial answers to the effect of noise on animal behavior and habitat usage, we investigated the effect of shipping noise on the gastropod species *Buccinum undatum* from large to small scale.

Deploying an underwater loudspeaker from the coast in the isolated Miquelon Bay, we exposed two distinct acoustic telemetry arrays to contrasting shipping noise pressure levels (126 dB re 1 µPa2 Hz^{-1} and ambient; site A and B, respectively) over a 10-days period to evaluate the effect of noise on mobility, habitat usage and distribution of wild specimens. Following such results, we then exposed individuals to different noise pressure levels (control, 122.5, 154.1 and 174.8 dB) in a large basin over 2 h period to assess finer behavioral changes though accelerometry.

Over the long term, acoustic telemetry revealed that individuals exposed to shipping noise had lower daily total/net covered distances and speed, exhibiting a lower potential for recovery after release (Fig. 1). Whelks also exhibited a smaller habitat usage potential (HUP), although they did not displayed any area avoidance from the polluted site.

According to laboratory findings, individuals exposed to 174 dB were less active compared to other treatments, with lower Overall Dynamic Body Acceleration (ODBA) and shorter moving time.

These results demonstrate for the first time that over different intensity and time scales, shipping noise negatively affects the locomotor capacity of marine gastropods, constraining their already reduced potential for dispersal with major consequences for the fisheries of several species.

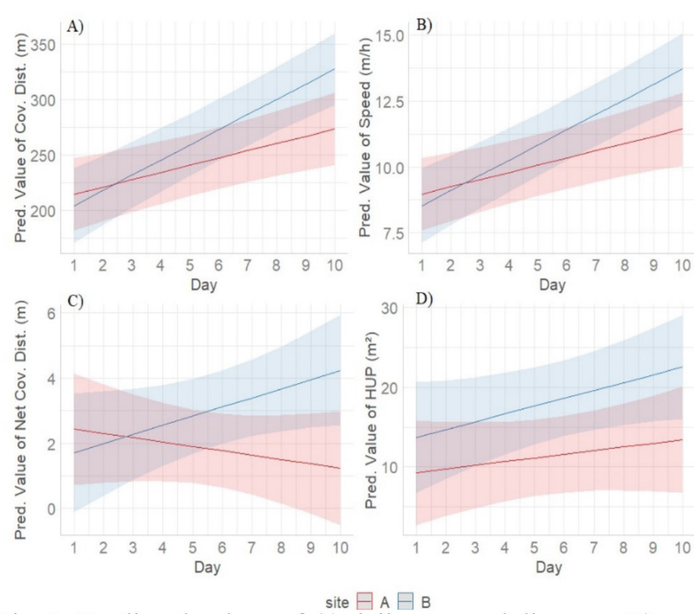

Fig. 1: Predicted values of A) daily covered distance, B) speed, C) daily net covered distance and D) daily HUP according to the applied model concerning the interaction between site and days.

SOME DON'T LIKE IT NOISY: SHIPPING NOISE ALTERS THE BEHAVIOUR AND HABITAT USAGE OF WAVED WHELK *Buccinum undatum (Gastropoda)*

Thomas Uboldi*, Nathalia Byrro-Gauthier, Youenn Jézéquel, Rejean Tremblay, Fréderic Olivier, and Laurent Chauvaud

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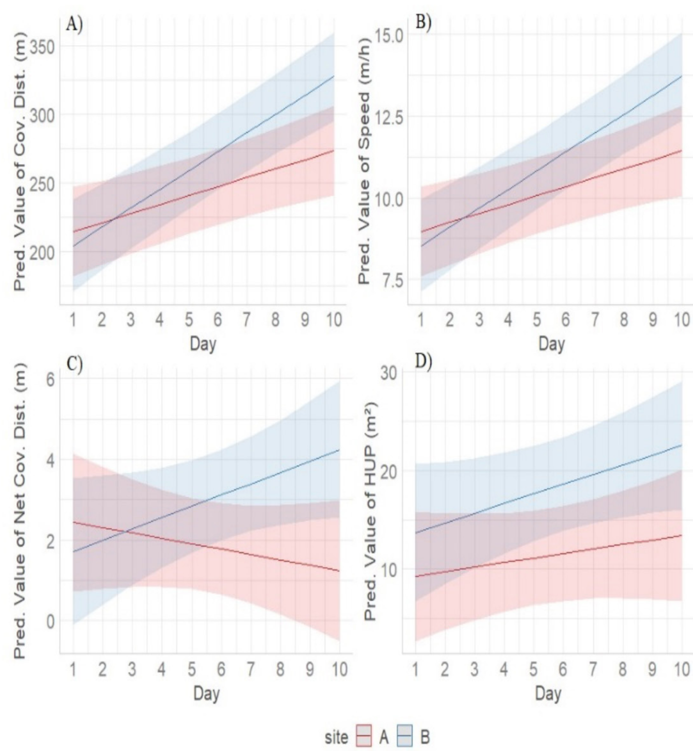

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DIETARY INCLUSION EFFECTS OF FERMENTED MAJOR OIL SEED BYPRODUCTS ON GROWTH AND HEALTH PERFORMANCE OF CLIMBING PERCH (*Anabas testudineus*)

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This study investigated the replacement of fish meal (FM) with fermented soybean meal (SM) and mustard oil cake (MOC) in the diet of Climbing Perch. Given their availability, rich amino acid profiles, low cost, and sustainable nature, plant protein sources are valuable in aquafeed. However, anti-nutritional factors (ANFs), toxic compounds, and high fiber content can reduce their nutritional value and digestibility, negatively affecting fish performance. Fermentation by beneficial microorganisms such as bacteria, yeast, and fungi has been reported to enhance the nutritional quality of feed ingredients and provide probiotic health benefits to fish.

A 60-day feeding trial was conducted using five iso-proteinous (38%) and iso-lipidic diets, where equal proportions of fermented Mustard Oil Cake (FMOC) and Fermented Soyabean Meal (FSM) were incorporated at 15%, 20%, 25%, and 30% to replace FM protein. A control diet was prepared with 15% non-fermented SM and MOC (Table 1). A total of 300 fish (4 g) were randomly distributed (20 fish/tank) into 15, 100-L aquariums as triplicates and fed twice daily at satiation. After the growth trial, fish growth performances, feed utilization, whole body nutrient compositions and hematological parameters were assessed. Results showed significantly higher growth in fish fed the D2 diet, followed by D3 and D1; D4 and D5 diet groups showed significantly lower growth performance (Table 2). Feed utilization performance parameters were not significantly different among diet groups D1, D2 and D3; D4 and D5 groups showed significantly lower performance. Total cholesterol, triglyceride and total protein content was significantly higher in fish fed D2 diet compared to other treatments. Significantly lower whole-body protein, lipid and ash content were observed in D5 diet compared to other treatments. Fish fed D1 to D4 diets showed significantly lower whole body moisture content compared to D5. Most of whole-body amino acid content was significantly higher in fish fed D1 to D4 diets compared to D5 diet. The findings indicate that 20% of fermented SM and MOC can be included in the dietary formulation of climbing perch, which combined 35.2% of FM in the formulation without negatively impacting the growth and health performance of this species.

Table 1: Feed formulation of experimental diets.

Ingredients	Diets				
	D1	D2	D3	D4	D5
FM	25	22.6	16.2	10	4.2
SM	15	0.0	0.0	0.0	0.0
FSM	0.0	15	20	25	30
MOC	15	0.0	0.0	0.0	0.0
FMOC	0.0	15	20	25	30
Others	45	47.4	43.8	40	35.8

Table 2: FBW: Final Body Weight (G); WG: Weight Gain Percentage (%); SGR: Specific Growth Rate; FCR: Feed Conversion Ratio of Climbing Perch. Means in a column with different letters were significantly different (P < 0.05).

Variables	Diets				
	D1	D2	D3	D4	D5
FBW	15.0^{b}	16.9^{c}	15.4^{b}	10.0^{a}	8.9^{a}
WG (%)	279^{b}	324^{c}	284^{b}	150^{a}	125^{a}
SGR	2.2^{c}	2.4^{d}	2.2^{cd}	1.5^{b}	1.3^{a}
Survival (%)	86.7	87.1	90.0	85.0	78.3
FCR	1.4^{a}	1.3^{a}	1.3^{a}	2.0^{b}	2.4^{b}

DEVELOPING A UNIFIED AQUACULTURE INDUSTRY IN NEW YORK

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The aquaculture industry in New York spans the entire state. Land-based fish farms and aquaponic operations are primarily distributed across the upper region of the state while shellfish and seaweed operations are found in the marine waters around Long Island. Despite being one of the largest states in the Great Lakes and Northeast regions, with an extensive coastline, New York ranks 19th in aquaculture production according to the 2018 Census of Aquaculture. There is not a statewide aquaculture association that unifies or supports the industry, making it challenging for it to expand without additional support. In 2020, New York Sea Grant created the Aquaculture Specialist position to work with the industry and increase awareness about its value and benefits. An industry needs assessment was conducted in 2021which has guided the efforts of this position. It has led to developing workgroups with industry stakeholders, facilitating meetings with industry members to improve networking, and developing resources for the industry and the public to increase awareness about New York's aquaculture industry.

EVALUATION OF OYSTER SHELL AS TREATMENT MATERIAL TO IMPROVE WATER QUALITY FOR FISH SEED PROPAGATION

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Reviewed literatures revealed Oyster shell as a potential adsorbent material that can be used to control pollution in groundwater. The study examined the impact of exposure and duration of exposure of water (tagged C-0) that was reported incapable of supporting fish egg hatching to quantities of crushed Oyster shell (COS) for curative to use the water for fish seed propagation. The water was studied alongside proven convenient water for fish egg hatching (F-0) in the study area. The water (C-0) in 5 separate containers was exposed to COS at 1.08g/l for 13, 9, 5 and 3 days and 0.80g/l for 3days prior to use for seed propagation. The five experimental treatments were studied alongside C-0 and F-0 with zero shells. Water quality conditions of each treatment were monitored during the study. Fertilized *Clarias gariepinus* eggs were incubated in each treatment in triplicates. Eggs were monitored for fertilization, hatchability and larval growth.

The pH and hardness in F-0 (6.63, 63.3mg/l) and C-0 (4.47, 104.0mg/l) were significantly different ($P<0.05$). The pH of C-0 increased from 4.47 to 8.45 while the hardness increased from 104.0mg/l to 153.3mg/l when the water was exposed to crushed Oyster shell at 1.08g/l for 9 days. When the exposure duration was extended to 13 days, the pH did not change; however the hardness increased to 169.3mg/l (Table 1). Egg hatching and larval development in C-0 were improved with COS. Fastest growth was recorded when it was exposed to COS at 1.08mg/l for 3 days (Table 2). The improvement could result from the ability of COS to induce pH increment of the acidic water due to its high calcium content.

Table 2: Water parameters in each treatment prior to egg incubation

Treatments	pH	Temperature(ºC)	Dissolved Oxygen(mg/l)	Hardness(mg/l)
F-0	6.67c	25.1	7.06b	64.48e
C-0	4.70d	25.1	7.94a	106d
C-1.08^{13}	8.45a	25.1	5.59d	169.3a
C-1.08^{9}	8.45a	25.2	6.41c	153.3c
C-1.08^{5}	8.01a	25.2	6.40c	168.0a
C-1.08^{3}	7.21b	25.1	6.31c	160.6b
C-0.80^{3}	7.09b	25.2	5.99d	159.3b
Statistical test	*	ns	*	*

Table 3: Propagation values realized under different experimental treatments.

Variables	F-0	C-0	C-1.08^{13}	C-1.08^{9}	C-1.08^{5}	C-1.08^{3}	C-0.80^{3}	Statistics
% F	82.5	84.2	83.3	85.0	84.2	85.4	84.6	nss
%H	47.5a	21.3c	42.5	44.2b	43.3b	42.1b	41.3b	*
%S	39.5a	7.8c	34.7b	36.8a	33.7b	35.6a	33.3b	*
SGR	8.4c	8.3c	8.7b	7.6e	8.2c	9.1a	8.0d	*

THE EFFECT OF *mir202* KO ON STERILITY IN NILE TILAPIA AND THE METHOD FOR MASS PRODUCTION OF KO FISH

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Sterilization of aquaculture species has been recognized as beneficial for various reasons. First, it can promote growth by conserving energy that would otherwise be used for gonadal development. Second, it can prevent genetic disturbance to wild populations caused by escaped farmed individuals reproducing in natural environments. Third, it helps farmers protect their aquaculture strains from being illegally produced outside their premises. Furthermore, it could serve as a means to address regulatory challenges associated with implementing genome editing technologies for the aquaculture industry in some countries.

Recent studies using zebrafish and medaka have revealed that certain microRNAs influence fertility by regulating gene expression in their reproduction. This study investigates the effect of microRNA202 (*mir202*) knockout(KO) on sterility in Nile tilapia (*Oreochromis niloticus*), a globally important aquaculture species. We also study the method of large-scale production of *mir202* KO fish.

Using the CRISPR/Cas9 genome editing tool, the *mir202* gene was knocked out. Homozygous KO individuals were produced in the F2 generation, and the gonad development of each sex in KO individuals was physiologically and genetically analyzed. In addition to *mir202*, to create a monosex population, genes essential for male differentiation (*gsdf* and *dmrt1*) and female differentiation (*cyp17* and *cyp19a1a*) were simultaneously knocked out. As a result of the *mir202*KO, female individuals became sterile. To apply this technology in an aquaculture setting, we studied the germ cell transplantation (GCT) method into *dnd*KO hosts to evaluate the possibility of the sterility phenotype in the next generation.

In *mir202*KO females, arrested ovarian development was observed. The ovaries from 8-month-old were tiny, string-like structures, observed with only stage I-III oocytes inside. In males, while testes developed, a significant delay in development and reduction in sperm count were noted, resulting in an extremely low natural fertilization rate of 1.27 ± 2.61%. Based on these findings, we produced an all-female sterile population with a double KO with the masculinization-related gene (*mir202* and *gsdf*). Furthermore, by transplanting oogonia obtained from these sterile females into *dnd*KO hosts, we achieved the large-scale production of sterile individuals in the next generation.

These results demonstrate that the integration of sterilization through *mir202* knockout and germ cell transplantation offers an effective method for large-scale production of all-female sterile populations of Nile tilapia.

Table 1: Fert Rates of ♂*mir202*$^{-/-}$x♀WT

	# of Cross	# of eggs	# of Fert	% of fert
male_1	2	1800	0	0.00%
male_2	2	2000	0	0.00%
male_3	2	1500	0	0.00%
male_4	2	3500	0	0.00%
male_5	2	2000	0	0.00%
male_6	2	1855	**55**	**2.96%**
male_7	1	487	**35**	**7.19%**
male_8	1	1058	0	0.00%
Total Summary		**14200**	**90**	**0.63%**

COMPARATIVE METABOLOMICS ANALYSIS OF *Holothuria cinerascens* AND *Pseudocnella sykion* FROM KWAZULU-NATAL, SOUTH AFRICA

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Sea cucumbers are soft-bodied marine invertebrates revered for their potent health benefits derived from biologically active molecules. However, research has primarily focused on commercially valuable species, leading to overexploitation and neglect of other species, which may also hold potential for beneficial metabolites and nutritional composition.

This study explores the metabolic composition between two sea cucumber species, *Holothuria cinerascens* and *Pseudocnella sykion*, from Southern Africa, using untargeted ^{1}H-NMR and UPLC-QTOF-MS. Our findings reveal distinctive metabolic profiles, with *H. cinerascens* exhibiting elevated levels of compounds associated with osmoregulation, energy and amino acid metabolism, and defence mechanisms compared to *P. sykion.* These disparities may stem from genetic, ecological, and reproductive variations between the species. While providing insights into sea cucumber metabolism, this study also underscores the challenges in holothurian metabolomics research that warrant further exploration. Expanding sea cucumber research to understand their metabolic pathways and composition offers prospects for discovering novel bioactive compounds, improving aquaculture practices, fostering economic opportunities, and advancing sustainable resource management and conservation efforts.

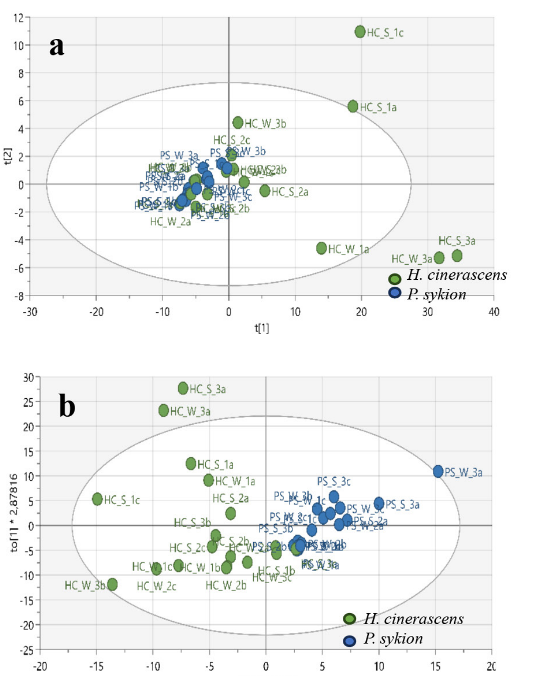

Figure 1: PCA-X results (a) and OPLS-DA score plot (b) showing the metabolic separation between H. cinerascens (green) and P. sykion (blue).

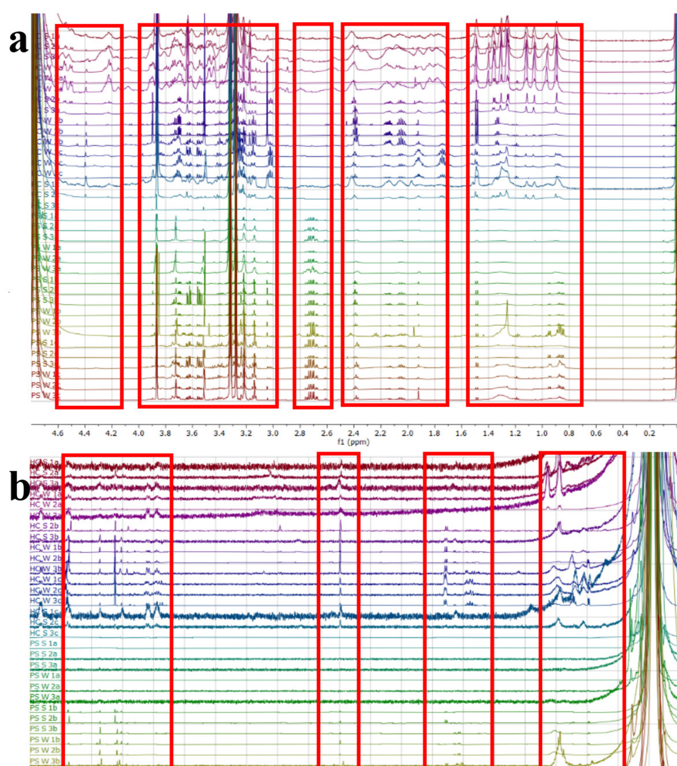

Figure 2: NMR spectral comparison showing the differential chemical shift regions within the sugar-aliphatic (a) and aromatic (b) zones between H. cinerascens ("HC") and P. sykion ("PS").

PRELIMINARY TESTING OF A LIFE CYCLE ASSESSMENT (LCA) USER-FRIENDLY TOOL TO INTEGRATE ECOSYSTEM SERVICES INTO BUSINESS AND ECONOMIC PLANNING FOR SEAWEED AQUACULTURE

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As a major component of the NOAA Sea Grant project "Business and Economic Planning for Seaweed Aquaculture Systems in the U.S.", a fully-interactive, user-friendly tool was designed which allows stakeholders (farmers, extension agents, etc.) to develop their own Life Cycle Assessments (LCAs) for integrated kelp nursery-growout operations. The tool has two major components: 1) an Excel spreadsheet aimed at collecting basic production parameters and material input requirements for the model farm; and 2) a customized LCA model built within the open-source platform openLCA, which accesses data from the spreadsheet to generate a comprehensive assessment of the ecosystem services generated by the aquaculture operation, including nutrient removal and the potential for negative CO_2 emissions. The LCA model relies on open-source databases (USLCI and US Environmental Protection Agency/USEEIO) compiled by the U.S. government and made accessible through the Federal LCA Commons website. As a result, users are able to develop comprehensive LCAs that fully obviate the need for expensive proprietary software.

Because the LCA tool is fully integrated with an Excel-based financial model, users can incorporate the economic value of ecosystem services to the tables and forms produced by the financial component of the integrated model. Ultimately, users could generate estimates of potential revenue flows from Payment for Ecosystem Services (PES) schemes, if they become available to farmers. A practical demonstration of the tool is included using real-word data from cooperating kelp farming operations in Alaska.

SEA LICE INFECTED FROM DIFFERENT ATLANTIC SALMON PHENOTYPES SHOWED DIFFERENT MICROBIOME AND TRANSCRIPTOME SIGNATURES

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Salmon aquaculture is constantly threatened by pathogens that impact fish health, welfare, and productivity. One significant problem is the sea louse, which is controlled using pesticide treatments; however, the emergence of delousing drug resistance has been reported. Strategies based on salmon breeding selection represent a sustainable alternative for producing fish resistant to sea lice infestation. The effects of resistant and susceptible salmon phenotypes on the sea louse population must be better evaluated.

This study reported the sea lice microbiome and transcriptome signatures infecting different salmon phenotypes. Sea lice were collected from salmon families designated as resistant (R) and susceptible (S) after three successive infestations. RNA-Seq analysis and microbiome assessments were performed on sea lice using NovaSeq (Illumina) and MinION (Nanopore) platforms, respectively.

Differences in transcriptome expression patterns were observed between sea lice obtained from different salmon phenotypes. Sea lice from susceptible salmon showed a high number of genes up-regulated associated with metabolic processes; however, sea lice obtained from the resistant phenotype exhibited up-regulation of genes associated with stimulus response and biological regulation. Furthermore, sea lice obtained from the susceptible salmon showed high bacterial diversity. Interestingly, the bacterial genera Tenacibaculum and Pseudomonas were identified in the sea lice coming from the susceptible salmon family. Our results enhance the understanding of the sea lice infestation mechanism by incorporating the microbiome and transcriptome modulation study, which could be used to improve sea lice control strategies.

THE ON FARM UTILISATION OF A MOLECULAR QPCR TOOL FOR THE PREDICTIVE MANAGEMENT OF PARALYTIC SHELLFISH TOXIN-PRODUCING HARMFUL ALGAL BLOOMS FOR THE AQUACULTURE INDUSTRY

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Paralytic shellfish toxins (PSTs) produced by marine dinoflagellates significantly impact shellfish industries worldwide. Early detection on-farm would allow additional time for management decisions to minimize economic losses. The development and validation of the Phytoxigene DinoDTec qPCR assay allows for the utilisation of a standardized workflow to quickly detect and quantify the presence of *sxtA4*. The sxtA4 gene is a critical gene in the biosynthesis of PSTs and is upstream from any tailoring enzymes that are responsible for the many PST variants. The workflow is simple and inexpensive and does not require a specialized laboratory. The workflow consists

1. water collection and filtration using a custom gravity sampler,
2. Cell lysis for DNA, and
3. Rehydrating the lyophilized reagents from the DinoDtec kit to prepare the master mix for the the quantitative polymerase chain reaction (qPCR) assay.
4. Add the lysed sample with the master mix and place in instrument, Results are generated in 60 minutes

Water samples spiked with *Alexandrium catenella* showed a cell recovery of >90% when compared to light microscopy counts. The performance of the lysis method (90.3% efficient), Longmire's buffer, and the DinoDtec qPCR assay (tested across a range of *Alexandrium* species (90.7–106.9% efficiency; $r^2 > 0.99$)) was found to be specific, sensitive, and efficient. We tested the application of this workflow weekly from May 2016 to 30th October 2017 to compare the relationship between *sxtA4* copies L^{-1} in seawater and PSTs in mussel tissue (*Mytilus galloprovincialis)* on-farm and spatially (across multiple sites), effectively demonstrating an ~2 week early warning of two *A. catenella* HABs ($r = 0.95$). Our tool provides an early, accurate, and efficient method for the identification of PST risk in shellfish aquaculture.

RESEARCH NEEDED TO ADVANCE MARINE FINFISH AQUACULTURE IN THE U.S.: WHAT DO ECONOMIC ANALYSES SHOW?

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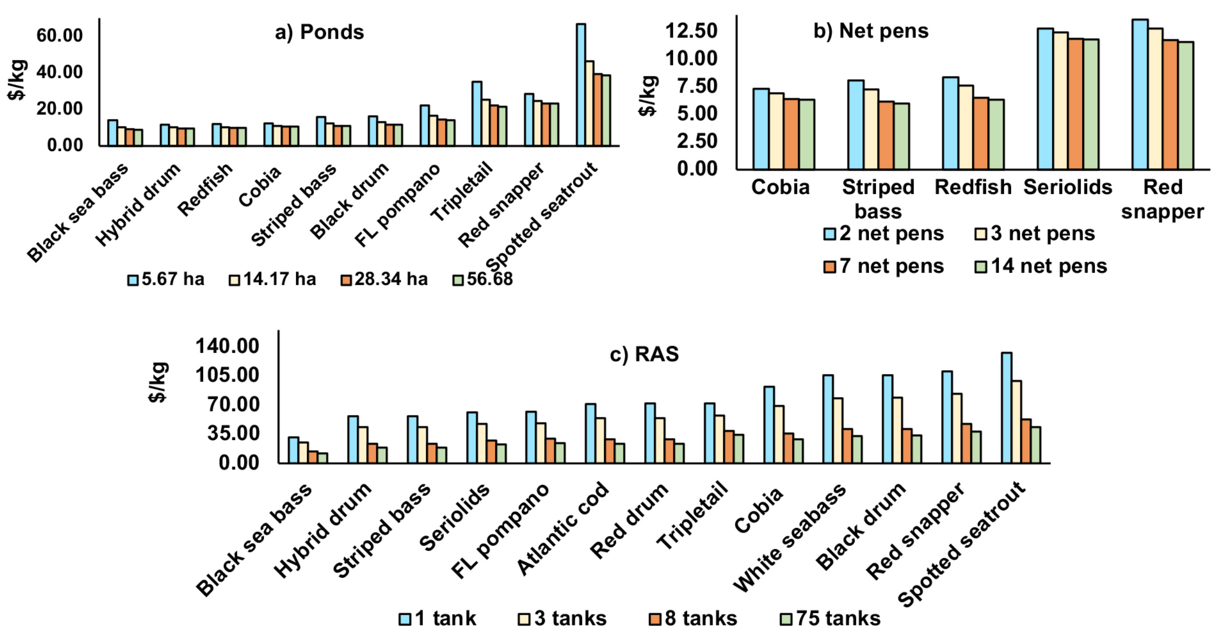
The U.S. has potential for substantial expansion of marine finfish aquaculture. Research investment has led to identification of candidate species for commercialization. Growout production costs were estimated for four scales of production in ponds, recirculating aquaculture system tanks (RAS), and net pens. The five species evaluated for net pen production (redfish, *Sciaenops ocellatus*, striped bass, *Morone saxatilis*, cobia, *Rachycentron canadum*, red snapper, *Lutjanus campechanus*, and seriolids (generic analysis for almaco jack, *Seriola rivoliana,* California yellowtail, *S. lalandi*, and greater amberjack, *S. dumerili*) were profitable. In ponds, four of the 10 species evaluated were profitable, including redfish, hybrid drum, ♀*Pogonias cromis* x ♂*Sciaenops ocellatus*, black sea bass, *Centropristis striata*, and cobia. None of the 13 species analyzed for RAS production were profitable. The lower per-kg costs in net pens, followed by pond production explained the differences in profitability. RAS production costs were two to five times greater per kg than in ponds or net pens. Capital assets were used more efficiently in ponds and net pens, resulting in lower percentages of fixed costs than in RAS. Research production trials conducted under conditions that simulate commercial production with an endpoint of market-sized fish are needed to provide databases from which to optimize management, increase efficiencies, and reduce breakeven prices. Until yields (kg/cubic meter) in RAS are much greater than at present, ponds and net pens are the likeliest production systems for profitable marine finfish production.

Figure 1. Breakeven prices (per-kg cost of production) in a) ponds; b) net pens; and c) RAS.



SEAFOOD ECONOMIC ANALYSIS AND MARKETING RESEARCH PROGRAM: APPLIED RESEARCH AND EXTENSION IN SUPPORT OF U.S. AQUACULTURE

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The Seafood Economic Analysis and Marketing Research (SEAMaR) program has strived to address economics and marketing questions affecting the U.S. aquaculture industry. Working collaboratively with other researchers and Extension specialists around the nation, the program has performed work on several areas including the costs of compliance for U.S. aquaculture farms, the impacts of the COVID-19 pandemic on the U.S. aquaculture sector, economic impact of aquaculture and seafood, assessing feasibility of emerging species and new technologies, social license for aquaculture, financial benchmarking, and consumer preferences studies for aquaculture and seafood products. The program is stakeholder driven with the express intention of integrating research and Extension activities to maximize impacts.

DEVELOPING THE NEXT GENERATION OF ULTRA-LOW PHOSPHORUS FEEDS FOR FRESHWATER AQUACULTURE IN QUEBEC: A MULTI-FACETED PROJECT

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Phosphorus (P) is a limiting element for algae growth in freshwater and causes eutrophication when present in excess. In fish farms and particularly in the province of Quebec, P emissions through wastewater are controlled by a strict regulation (4.2 kg P/T/year) which is holding back industry expansion.

P releases can be limited in several ways, notably through the composition of aquafeeds. Indeed, previous studies conducted by the Department of Animal Sciences at Laval University have shown that it is possible to reduce P emissions by reducing dietary P levels without decreasing production performance [1–4]. Nevertheless, the use of low-P diets in salmonids is a cause for concern as it can affect the mineralization of bone tissues and lead to vertebral deformities and malformations. Thus, further progress and better characterization of low-P feed effects can still be made to reduce P releases and prevent P deficiency in fish.

In this context, a multi-faceted project was launched to develop new-generation feeds with very low phosphorus content for three salmonid species of high economic value in Quebec: rainbow trout *Oncorhynchus mykiss*, brook trout *Salvelinus fontinalis* and Arctic char *Salvelinus alpinus*. Supported by major players in the aquaculture sector, this 3 phases project will enable nearly 15 low-P feeds to be tested in laboratory and on-farm trials.

During the initial phase of the project nutrient digestibility was determined for 20 feed formulations. At the end of the 2-week collection period, precocious indicators of phosphorus status, including 12-h inorganic phosphorus (Pi) accumulation in the tank water, as well as the determination of scale mineralization and enzyme activity associated with mineralization/demineralization dynamics in scales. These results will be presented in the context of phosphorus digestibility and Pi accumulation.

Through this project, a new generation of very low P feeds could result in significantly lower P emissions without compromising fish growth performance or animal welfare.

FROM BISPHENOL A (BPA) TO PHTHALATES TO PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS): HOW ENVIRONMENTAL CHEMICALS ARE HARMING FISH, SHELLFISH AND HUMAN HEALTH AND WHAT WE CAN DO ABOUT IT

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Endocrine disrupting chemicals (EDCs) are chemicals that alter the actions of hormones. In recent years, experts from medical, scientific, and environmental activist groups have demanded action from regulatory agencies to protect humans and wildlife alike from the harm induced by EDC exposures. These demands are based on strong evidence from epidemiology, wildlife, and controlled laboratory studies. Some environmental chemicals have received a lot of attention in recent years including BPA, phthalates, PFAS (forever chemicals), and others.

This talk will describe the conclusions drawn by experts from different scientific and medical disciplines and discuss recent findings that have changed the landscape of EDC work.

There is a need to identify better "sentinel" species to characterize both exposures to EDCs and their effects, and fish and shellfish may provide valuable insights in these areas.

There is also a need to expand our understanding of vulnerable periods of life, and the increasing concern that traditional methods used to evaluate toxicity of environmental chemicals are insufficient for EDCs.

Finally, there are reasons why current regulatory approaches have failed to protect human and wildlife health, but collaborative science could help to address these gaps.

TRANSCRIPTOMICS AND FUNCTIONAL ASSAYS INFORM HEMOCYTE IMMUNE RESPONSE IN PACIFIC OYSTER

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Innate immunity is an ancient defense mechanism that operates in multicellular organisms to detect and eliminate pathogens and distinguish self from non-self. Animal immune cells deploy diverse behaviors during pathogen elimination, including phagocytosis, secretion of inflammatory cytokines, and expulsion of nuclear material - the casting of extracellular DNA "traps" (e.g. ETosis). Hemocytes are circulatory cells in bivalve mollusks and are reported to have roles in immunity, biomineralization, and nutrient transport. A thorough understanding of hemocyte cell types and their discrete or overlapping functions is critical for assessing bivalve responses to pathogen challenge and other physiological processes. However, current resources describing hemocyte types and their associated function are often conflicting or vague, making interpretation of specific cellular immune responses to infection challenging. We have applied flow cytometry, microscopy, and biochemical approaches to explore immune cell behaviors and hemocyte transcriptional signatures in the Pacific oyster (*Crassostrea gigas*). Our findings suggest a variety of cell types may be competent for a range of anti-microbial responses, including ETosis. We also find that some immune cell type behaviors may be activated by non-canonical signaling pathways. These data provide an opportunity to explore both conserved and novel aspects of pathogen defense mechanisms associated with shellfish innate immunity.

ASSESSING NURSERY GEAR EFFECTIVENESS FOR BAY SCALLOP GROWTH AND SURVIVAL IN OUTDOOR FLOW- THROUGH NURSERY SYSTEMS

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Bay scallops (*Argopecten irradians*) are economically and ecologically important in aquaculture. During the early nursery phase, they are vulnerable to environmental stressors and predation. This project evaluates four nursery gear systems—upweller silos, bottom boxed bags, floating plastic baskets, and free-stocked scallops—to determine which optimizes scallop survival and growth in a controlled outdoor flow-through setting.

This study is conducted at Roger Williams University's outdoor nursery system, where scallops are housed for three months in four fiberglass tanks with water from Mount Hope Bay. Each tank holds 600 scallops, with 150 scallops assigned to each gear type. Growth performance is assessed biweekly by measuring shell height and length, while meat and shell weights are recorded monthly. Weekly monitoring includes survival, mortality rates, and biofouling impact on water flow and scallop health.

Objectives include comparing growth rates, mortality, and biofouling across gear types, as well as evaluating the scallop Condition Index (CI) and Specific Growth Rate (SGR). Statistical analyses, including analysis of variance (ANOVA) and analysis of covariance (ANCOVA), will determine significant differences in scallop development across gear types. We hypothesize that scallops in upweller silos and floating plastic baskets will show superior growth and survival due to enhanced water flow and nutrient access, while boxed bags and free-stocked scallops may have lower growth and survival rates. We anticipate biofouling levels will differ among gear types, potentially restricting water flow and impacting scallop growth in floating baskets.

This study aims to inform best practices for scallop nursery management by identifying gear that optimizes growth and reduces labor-intensive maintenance, supporting sustainability. A detailed comparison of scallop growth, survival, and health across different nursery gear systems will help scallop farmers make informed gear selections, enhancing efficiency and sustainability. This study could guide farmers in selecting gear systems that promote healthier, faster-growing scallops, reduce maintenance issues, and support sustainable aquaculture practices. The outcomes may improve both economic viability and environmental sustainability of scallop farming operations, contributing to long-term industry growth.

EFFECTS OF TETRATHIONATE IN THE GROWTH AND TRANSCRIPTOME OF *Vibrio parahaemolyticus* CAIM1400 CULTIVATED UNDER ANAEROBIC CONDITIONS

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Tetrathionate ($H_2S_4O_6$) is a sulphurated compound abundant in the intestine of aquatic and terrestrial animals during inflammatory processes. Some pathogenic bacterial species have a complex of reductase that lets them use the compound as an alternative acceptor electron in anaerobic conditions, enhancing its growth in anoxic conditions even in carbon source starvation. A genomic survey recently revealed that the marine bacterium *Vibrio parahaemolyticus* has a genetic locus putatively related to anaerobic reductases of tetrathionate (Fig. 1), however there is no information about its functionality in the species. Thus, this work focuses on testing the effect of tetrathionate on the growth of *V. parahaemolyticus* and exploring the molecular changes that the species experience by transcriptomic tools.

V. parahaemolyticus CAIM1400 serotype O3:K6 strain was cultured in M9 + 0.3% glucose medium with and with our tetrathionate of potassium ($K_2S_4O_6$) at 3, 5, 10 and 20 mM in anaerobic conditions. The bacteria growth was monitored during 24 hours by optical density. The cells of the cultures without and with 5 mM $K_2S_4O_6$ were harvested when they reached approximately 0.6 ODs**.** The RNA extraction was done with RNeasy Plus Micro kit (Qiagen)**.** The RNAseq library synthesis was done with Stranded Total RNA (Illumina), and sequencing was carried out in a miniseq illumina platform. The processing and analysis of transcriptomic sequences was done with Trimommatic (v0.32), Bowtie software (v.2.3.5.1), FADU (v1.8.3), DESeq2 (v1.40.2) and fgsea (version 1.26.0) of Bioconductor package in R studio.

Tetrathionate improves the growth of *V. parahaemolyticus* in anaerobic environments with low carbon source availability (Fig. 2). A total of 854 differential expressed genes between the cultures of *V. parahaemolyticus* without and with tetrathionate 5 Mm, 456 upregulated and 398 down-regulated (Fig. 3). The main molecular pathways altered are related to nutrients transport, ATP hydrolysis activity, oxidoreductases, transcriptional regulators and flagellum components (Fig. 4).

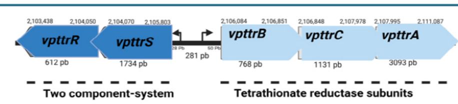

Figure 1. Tetrathionate reductase locus of *Vibrio parahaemolyticus* CAIM1400. The locus containing the tetrathionate reductase operon, vpttrBCA (2,106,087-2,101,87) with its promoter region 28 bp upstrean, neighborin tetrathionate-sensing TCS, vptttR/S (2,103,442-2,105,802).

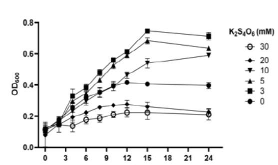

Figure 2. Effects of tetrathionate in anaerobic cultures of *V. parahaemolyticus* CAIM1400. Values represent mean ± SD from three biological replicates

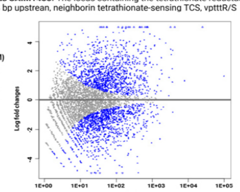

Figure 3.Diferentially expressed genes of *V. parahaemolyticus* CAIM1400 cultured under anaerobic conditions with out and with tetrathionate 5mM. The Y-axis represents the fold change agains the mean normalized counts. Blue dots indicate the differentially expressed genes with at least 1.0 change and statistical significance adjusted P < 0.05. Upper dots represents Up-regulated genes and down dots down-regulated.

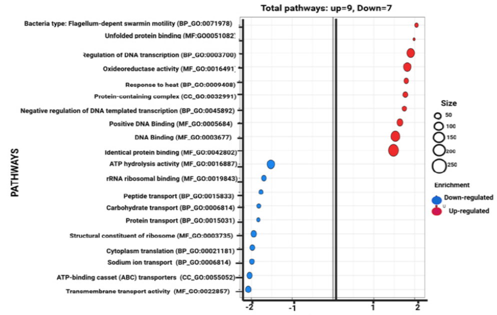

Figure 4. Over represented pathways of genes differentially expressed between *V. parahaemolyticus* CAIM1400 cultured in anaerobic conditios with out and with tetrathionate 5mM. X- axis represents enrichment score calculated by gene set enrrichment analysis vs Y- axis respresents the molecular pathways acording to Gene ontology data base . The groups are integrated by at least 50 and maximun 250 genes

IS HOOKING HERITABLE? EVALUATION OF GENETIC PARAMETERS FOR HOOK SHELL TRAIT IN THE EASTERN OYSTER *Crassostrea virginica*

Robin Varney* and Jessica Small

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Genetically improved lines of the eastern oyster (*Crassostrea virginica*) have been produced through selective breeding to expand the commercial oyster aquaculture industry. The Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science has been engaged in family breeding of the eastern oyster for over two decades; resulting in considerable gains in economically important traits including survival, growth and meat yield. Shell shape characteristics, such as fan shape and cup depth, are routinely monitored and incorporated into a multi-trait selection index. The shell trait of "hook" at the hinge has been observed in some cultured lines of eastern oysters and can make oysters more difficult to shuck. Previous analyses indicate that the hook shell trait is heritable and can be modified through selective breeding.

In Fall 2023, evaluation of the hook shell trait was incorporated into routine ABC monitoring of shell shape characteristics to estimate heritability and genetic correlation with other shell characteristics. Initial analysis of the hook trait was evaluated on a scale of –2 to +2, with 0 indicating no hook present and easy to shuck, –2 indicating the hinge was very curved up toward the flat side of the oyster and +2 indicating the hinge was very curved down toward the cup. The hook trait was evaluated in 8,027 18-month-old oysters from 78 families grown at three field locations which vary in salinity and disease exposure. Restricted maximum likelihood methods were used to estimate multi-variate genetic parameters of the hook shell trait over all samples and for each field location separately. The heritability estimate for the hook shell trait over all samples was 0.22 ± 0.07, and ranged from 0.19 to 0.39 at each of the three field locations. Over all samples, positive genetic correlations were observed between the hook shell trait and total weight (rg = 0.37) and meat yield (rg = 0.18), but negative genetic correlations were observed between hook and width index (rg = -0.51) and height index (rg = -0.22). Although these results are only from one year of evaluation with an imprecise scale, they suggest that the hook shell trait has moderate heritability and can potentially be modified through selective breeding.

EFFECTS OF FLUID VELOCITY ON CO_2 UPTAKE RATE BY THE RED SEAWEED *Agarophyton vermiculophyllum*

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Macroalgae are responsible for a significant fraction of CO_2 uptake by the ocean. Consequently, the potential of macroalgae for ocean-based carbon removal (Ocean CDR) in engineered systems needs to be assessed. This study provides a fundamental understanding of the significance of bulk fluid velocity on specific uptake rate of dissolved inorganic carbon during cultivation of the red seaweed *Agarophyton vermiculophyllum*.

A clonal culture of *A. vermiculophyllum* was immobilized onto mesh panels and cultivated in a raceway recirculation tank. The thalli tissue proliferated to form a branched, highly-porous mat aligned in the direction of bulk flow (Fig. 1). Fluid velocity around and through the seaweed biomass was measured by a pitot tube. The tissue mat was subjected to bulk velocities ranging from 10 to 56 cm/s. The carbon uptake rate at each velocity was estimated from the difference of CO_2 partial pressure in the aeration gas entering and leaving the tank.

Fig 1: Biomass panel with pitot tube.

The specific carbon uptake rate of 3.6 ± 0.32 mmol C/ g AFDW-day did not change when the bulk velocity was increased (Fig 2). Velocity distribution measurements across the width of the fluid channel showed increased flow around the tissue and decreased flow through the tissue. Bulk fluid velocities were attenuated within the tissue to a narrow range of flow spanning 12 cm/s to 32 cm/s, and so the internal sections of the tissue mat were not experiencing the same fluid velocity as the exterior of the biomass. Therefore, increasing bulk velocity beyond 10 cm/s had no significant effect on the CO_2 uptake rate. This study has shown that in order to better predict the effects of current flow on ocean CDR by seaweeds, hydrodynamic conditions around and within the seaweed tissue mat need to be thoughtfully considered.

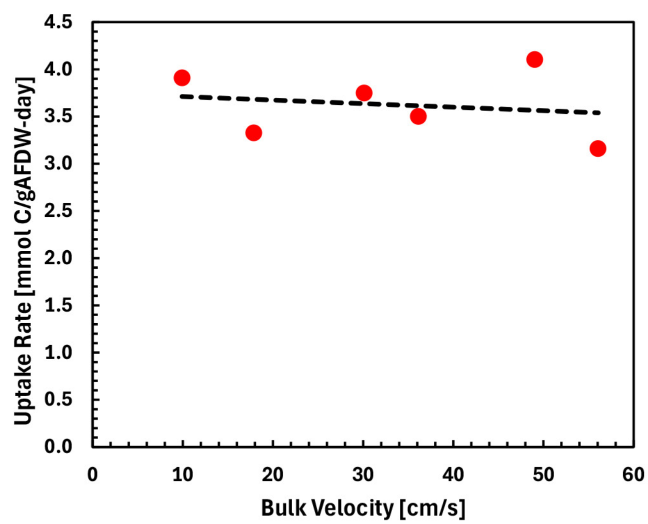

Fig 2: Effect of bulk velocity on carbon uptake rate.

CARBON DIOXIDE CONTROL IN RAS

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Effective control of dissolved carbon dioxide is essential for maintaining optimal water quality in recirculating aquaculture systems (RAS). In low-intensity aquaculture systems that rely on air-based aeration, carbon dioxide removal occurs alongside the addition of dissolved oxygen. However, high-intensity systems that use pure oxygenation and operate with low water exchange rates face the challenge of carbon dioxide accumulation, which can reach toxic levels. The safe threshold for dissolved carbon dioxide varies depending on species, developmental stage, and water quality, but generally, concentrations should remain below 15–20 mg/L to prevent adverse effects.

Unlike oxygen and nitrogen, carbon dioxide concentration in water is governed not only by gas-liquid equilibrium but also by acid-base reactions. Gas-liquid equilibrium influences the transfer of carbon dioxide between air and water, and the acid-base reactions determine the chemical form in which dissolved inorganic carbon is present in water. As a result, dissolved carbon dioxide levels are a function of both total dissolved inorganic carbon and the water's pH.

This presentation will address the fundamentals of carbon dioxide control in intensive recirculating aquaculture systems, focusing on state-of-the-art strategies and real-world applications. Topics will include physical gas transfer mechanisms and the implications of carbonate chemistry on carbon dioxide management. A steady-state mass balance approach will be presented as a framework for future system designs, with case studies of unit processes and control strategies to illustrate key design considerations.

ASSESSMENT OF THE EFFECTS OF PFAS ON BLUE MUSSEL USING GENE EXPRESSION AND BIOCHEMICAL APPROACHES - IMPLICATIONS FOR COASTAL MONITORING AND ENVIRONMENTAL RISK EVALUATION

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Per- and polyfluoroalkyl substances (PFAS) are man- made, highly persistent and harmful chemicals. These substances have various industrial and commercial applications and eventually get released into the environment. PFAS have emerged as a concerning class of contaminants with over 4700 variants found worldwide. Despite growing concerns due to their bio accumulative and toxic properties, there are significant knowledge gap regarding biota exposure to different PFASs.

In this research, the impact of PFAS is assessed by exposing the blue mussels, *Mytilus spp*, to PFAS mixtures in three conditions- control (no exposure), foodborne route via microalgae- *Tisochrysis lutea* and both food and water exposure , mimicking environment in controlled tanks. The enzyme activities were measured in three different organs of mussels (mantle, gills and digestive gland). The analysis compared control mussels with PFAS-exposed ones, evaluating health effects using various enzymes as biomarkers for oxidative stress, detoxification and neurotoxicity. Early molecular responses were investigated by measuring the relative expression of genes, associated with cell proliferation, apoptosis, cellular stress, and energy metabolism through RT-qPCR analysis. The study revealed significant differences in enzyme levels and gene expressions across tissues under various PFAS exposure conditions. Gills exhibited significantly higher GST activity under dietary exposure. At the molecular level, there was a significant downregulation of energy metabolism genes, with ATP synthase and citrate synthase expressions markedly reduced, indicating a substantial impact on metabolic capacity. Additionally, the AKT signalling pathway was significantly altered, with AKT expression downregulated in PFAS-exposed mussels. These findings highlight the complex biological responses of blue mussels to PFAS contamination.

Thus, the results indicate that PFAS at environmental concentrations appears to cause a specific biochemical response in blue mussels. By enhancing our understanding of the risks associated with these contaminants, the findings shed light on the biochemical and molecular effects of distinct routes of PFAS exposures, contributing to the field of ecotoxicology and aiding in the development of more effective management strategies.

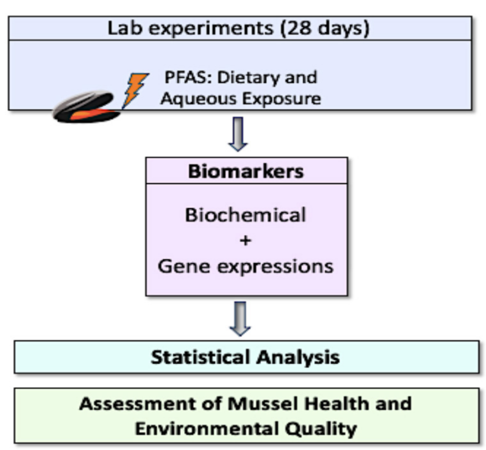

Figure: Overview of thesis

AWARE IN A NUTSHELL - AQUAPONICS FROM WASTEWATER RECLAMATION

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Aquaculture plays a critical role in meeting global food demand and addressing sustainability challenges. In Europe, demand for freshwater fish is rising due to stagnating marine catches, creating opportunities for growth in freshwater aquaculture. However, the sector has seen limited development in recent years due to a lack of innovation, low product diversity, seasonality, and stringent environmental regulations. Additionally, production depends on the availability of natural water resources and habitats, which are increasingly strained by water scarcity and climate change. To advance European freshwater aquaculture, there is a need to:

1. Increase the adoption of innovative, sustainable production methods in urban and peri-urban areas, closer to consumers where land and water are limited;
2. Foster policy and regulatory support for freshwater aquaculture farmers;
3. Build resilience against climate change and water stress.

We propose that these needs can be addressed through local fish farming using reclaimed water. While this approach has been implemented in water-scarce regions globally, Europe lacks the regulatory and policy frameworks to support it, and compelling evidence on the safety, quality, economic viability, and public acceptance of reclaimed water aquaculture is still limited.

With the AWARE project, we aim to break down these barriers and make reclaimed water aquaculture a reality in Europe. Our project will demonstrate a fully integrated aquaponic recirculating aquaculture system (RAS) enhanced with IoT monitoring, blockchain, data security, and automation technologies. Aquaponics, a multitrophic system that grows fish and plants together, requires no soil, recycles water from fish production to support plant growth, and offers substantial sustainability benefits. Both RAS and multitrophic aquaponics have been prioritized for research and innovation by the Strategic Working Group on Fisheries and Aquaculture (SCAR-Fish) of the Standing Committee on Agricultural Research.

Our project will establish the first European case study of reclaimed water aquaponics in Castellana Grotte (Puglia, Italy), building on results from a previous H2020 project on wastewater treatment. Through this pilot, we will:

- Demonstrate effective and sustainable aquaculture using reclaimed wastewater;
- Enhance wastewater circularity, turning it into a freshwater resource at the point of generation;
- Develop a novel farm-to-fork value chain that supports European economic growth and urban agriculture;
- Lay the groundwork for a supportive regulatory and policy framework for European aquaculture.

Total project eligible costs for AWARE are 4.7 million euros, 4.6 million of this is funded by the European Union under grant agreement number 101084245.

INNOAQUA – SUSTAINABLE AQUACULTURE PRACTICES FOR INNOVATIVE SEAFOOD PRODUCTS

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The Farm-to-Fork Strategy of the European Green Deal acknowledges the potential of algae to become an important source of alternative low-carbon footprint protein and contribute to improving the sustainability and competitiveness of the aquaculture sector. Nonetheless, the European algae industry is still in an early phase lagging behind the overall increase seen at a global level, mostly driven by Asia. Within this context, the EU project INNOAQUA aims to pave the path towards the upcoming sustainable and diversified EU in-land aquaculture industry by demonstrating and mainstreaming innovative algae-based foods and solutions, based on sustainability, circularity and digitalization concepts.

The project is centred around the demonstration of the integration of fish and algae cultivation in coupled, land-based RAS/IMTA systems, aiming to minimize energy and nutrient losses and maximise resource efficiency by closing the nutrient loop, at two locations: 1) microalgae and salmon in Norway, and 2) seaweed and sole in Portugal. Next, new biorefining approaches are developed to optimise the extraction yield of targeted valuable compounds from the microalgae, seaweed and fish processing side-products that will be used in the formulation of innovative seafood products.

Earlier results, obtained in the projects iFishIENCi and SLAM-DUNK, showed that microalgae can successfully be grown on effluent water from the production of salmon in RAS (Recirculating Aquaculture Systems) using both biofilm and tubular photobioreactors, and full nutrient uptake can be achieved. In INNOAQUA, we will further optimise these results, through operational and technological enhancements, including tailor-made digital solutions.

The consortium, led by NORCE Norwegian Research Centre AS (NO), consists of 17 partners, from 8 countries: Viking Aqua AS (NO), Marineholmen RASlab AS (NO), Algemy Ingredients SL (ES), A4F Algafuel SA (PT), Safiestela - Sustainable Aquafarming Investments (PT), INESC TEC - Instituto De Engenhariade Sistemas E Computadores, Tecnologia E Ciencia PT), Acondicionamiento Tarrasense Associacion (ES), ERANOVA (FR), PESCANOVA Espana SL (ES), Viva Maris GMBH (DE), Sustainable INNOVATION SL (ES), PEDAL Consulting SRO (SK), European Aquaculture Society (BE), Eco Imagination (FR), and PERSEUS (BE).

Total project eligible costs for INNOAQUA are 7.3 million euros, 6 million of this is funded by the European Union under grant agreement number 101084383.

IGNITION: IMPROVING GREEN INNOVATION FOR THE BLUE REVOLUTION - NEWS TOOLS AND OPPORTUNITIES FOR A MORE SUSTAINABLE ANIMAL FARMING

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IGNITION (Improving Green Innovation for the Blue Revolution), a cutting-edge Horizon Europe project, aims to revolutionize the aquaculture sector by addressing the rising demand for high-quality animal protein while ensuring environmental sustainability and animal welfare in a changing climate. One of the main objectives of IGNITION is the development of effective antigen-based vaccines to combat major diseases, such as tenacibaculosis and infectious salmon anemia virus, which pose significant challenges to intensive aquaculture systems. These innovative vaccines will promote animal health, reduce disease susceptibility, and improve the overall sustainability of the aquaculture industry by relying less on antimicrobials. IGNITION will also focus on enhancing stress and disease resilience through the development of new feeds, formulated to provide essential nutrients and bioactive compounds that boost the immune system and mitigate stress factors in farmed aquatic animals. By bolstering stress and disease resistance, the project aims to improve animal welfare and enhance the sector's overall productivity. To further support animal welfare, IGNITION will focus on the development of non-invasive stress and health biomarkers and biosensors and on defining operational welfare indicators. All these will offer real-time insights into animal welfare and health. Based on the data obtained, IGNITION will employ machine learning techniques and disease prediction software to improve decision-making and facilitate proactive management practices.

Overall, IGNITION's visionary effort aims to transform aquaculture practices, fostering a sustainable, responsible, and thriving future for the industry by providing a wealth of knowledge and innovative solutions for the European aquaculture sector and beyond.

CONSUMPTION OF DIFFERENT BALANCED DIETS IN THE EDIBLE SEA URCHIN *Tripneustes depressus*

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The sea urchin *Tripneustes depressus* is a potential fishing resource on the coasts of Baja California Sur due to the quality of its gonads for human consumption. An alternative to increase gonadal quality in captivity is to control the nutritional balance of the food. The consumption and protein content of different diets formulated based on algae were evaluated in *T. depressus* organisms.

According to the results of the selectivity experiments (evaluating 4 algae) three balanced foods were formulated and produced, two based on the selected algae (*Eisenia arborea*, *Sargassum sinicola*), and one mixed combining both; with a stability greater than 90%. Sixty organisms (8-11 cm diameter) previously acclimatized without feeding (three replicates of 20 organisms for each treatment) and three controls with each type of natural algae were placed in the laboratory. The organisms were fed *ad libitum* for three months. Food consumption was evaluated, as well as the amount of protein consumed weekly; and at the end of the experiment, the gonadal protein efficiency ratio (GPER) was determined, using the gonad wet weight for each treatment.

In general, the consumption of balanced feeds during the whole experiment was higher than 50%, which suggests that all the feeds were well accepted by the organisms. In the case of natural algae, it was between 30-50%, except for *S. sinicola*, which was higher than 60%. The protein contribution per feed consumption was lower for the formulated feeds compared to the natural algae; the former provided approximately 30% of proteins, while in the natural algae it was higher than 50%. The GPER was higher in the natural algae *E. arborea* treatment (90%), compared to the rest of the treatments, due to the higher gonadal weights present in these organisms. Among the formulated feeds, the highest GPER was in the feed based on *E. arborea* (55%).

The use of the natural algae *E. arborea* was favorable in the gain of gonadal weight, as well as in the protein contribution to the organisms. Future studies focused on this could contribute to improving the gonadal quality of *T. depressus* in captivity, and, therefore, to the development of aquaculture in Mexico of this resource.

COMPLEMENTARY EFFECTS OF INSECT MEAL AND SOYBEAN MEAL ON GROWTH PERFORMANCE, GUT HEALTH, AND REGULATION OF INFLAMMATORY, IONREGULATORY HOMEOSTASIS, AND OXIDATIVE STRESS ASSOCIATED GENES IN ATLANTIC SALMON, *Salmo salar*

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Aquaculture is the fastest-growing sector of agriculture and supplies over half of global fish consumption. Alternative aquafeed options have been explored to improve efficiency and lower production and environmental costs. Soybean meal (SBM) is a major ingredient in the aquafeed industry, and the high inclusion of SBM exhibits soybean-induced enteritis in carnivore fish, including Atlantic salmon (*Salmo salar*). The black soldier fly (*Hermetia illucens*) is an efficient, nutritionally sound ingredient. Using black soldier fly meal as a complementary ingredient in SBM-based diets has been shown to improve the performance of rainbow trout in terms of growth performance and mitigate enteritis. Therefore, our goal was to evaluate the effects of whole black soldier fly larval meal (WBLM) as a complementary feed ingredient in soybean meal-based diets on gut health, and regulation of inflammatory, ionregulatory homeostasis, and oxidative stress associated genes in Atlantic salmon.

Seven isonitrogenous (42% crude protein) and isolipidic (20% lipid) experimental diets: fish meal-based diets (FM), low-level SBM-based diets (LS), SBM+5% and 10% WBLM (LS-WB5 and LS-WB10), and high-level SBM-based diets (HS), SBM+5% and 10% WBLM (HS-WB5 and HS-WB10). A total of 630 Atlantic salmon (15 g) were distributed in 21 tanks (triplicates) in a recirculatory aquaculture system and sampled at three time points (days 28, 56, and 84). All the data were subjected to one-way ANOVA using R programming.

At the end of an 84-day feeding trial, the results revealed that feeding dietary SBM at a low (30%) or higher (40%) level did not affect the growth parameters of Atlantic salmon ($p > 0.05$). The histology of the distal intestine showed that there were no differences in the lamina propria thickness ($P = 0.630$), connective tissue thickness beneath the folds ($P = 0.309$), or overall fold size or thickness ($P = 0.356$) among the dietary groups. Gene expression within the liver revealed no differences between diets fed in immune response (*il8, nf-kb,* and *tnfa*: $p > 0.05$), or ionregulatory homeostasis (*claudins 25b* and *occludin*: $p > 0.05$). Similarly, no difference was observed in oxidative-stress-related gene expression within the liver (*gpx1a, gsr, cat,* and *sod1*: $p = 0.845$). Gut microbiome data are being analyzed and will be presented.

Conclusively, the current study has shown that Atlantic salmon could utilize a high level of soybean meal with no detrimental effects on growth, and the addition of whole black soldier larvae meal (WBLM) did help mitigate SBM-induced enteritis, inflammatory response in groups fed a high (40%) SBM diet. Thus, formulating practical diets with FM and high SBM is possible for sustainable Atlantic salmon aquaculture.

APPROACHING 50: PAST CONTRIBUTIONS AND THE BRIGHT FUTURE OF THE COLLEGE OF SOUTHERN IDAHO'S AQUACULTURE PROGRAM

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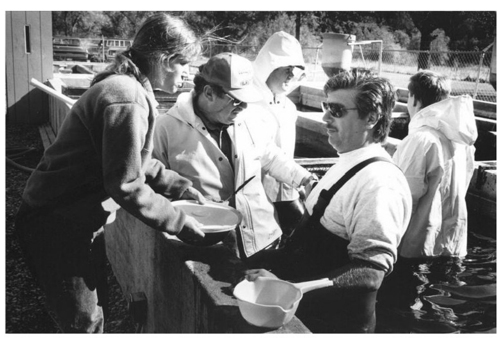
Since its inception in 1977, the College of Southern Idaho (CSI) has played a pivotal role in the advancement of fish culture. Under the leadership of Terry Patterson, Herschel Boydston, and Steve Rivas, students have had the opportunity to engage in one of the most hands-on aquaculture programs in the nation, contributing to the development and growth of the fish culture industry.

In 2022, CSI Aquaculture underwent a significant transformation, including the relocation of hatchery sites and a restructuring of the program. With the introduction of a new degree option, students can now pursue an Associate of Science degree with a Fisheries track specifically designed to transfer to four-year institutions. This expansion of degree offerings provides increased opportunities for individuals pursuing careers in aquaculture, all within the heart of Idaho's thriving aquaculture industry.

The CSI Aquaculture Program is dedicated to equipping students with the skills necessary for superior fish husbandry practices while providing practical experience in applying research within hatchery settings. Graduates will possess a strong proficiency in fish culture techniques, a foundational understanding of study protocols, and insight into the research driving industry innovations. Additionally, CSI students benefit from valuable partnerships through internships, guest lectures, field trips, and collaborative projects at the College's commercial fish hatchery.

As the College of Southern Idaho celebrates its 60th anniversary, CSI Aquaculture reflects on the contributions of those who have shaped its success and looks forward to continuing its legacy of training skilled fish culturists. With over four decades of achievement, the future of the CSI Aquaculture Program remains promising.



VISUALIZING NEAR-REAL-TIME WATER-QUALITY PRODUCT MAPS VIA STREAM

William Wainwright[1,2], Navid Golpayegani[2], Akash Ashapure[1,2], Ryan E. O'Shea[1,2], Arun Saranathan[1,2], Nima Pahlevan[3]

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The Satellite-based analysis Tool for Rapid Evaluation of Aquatic EnvironMents (STREAM) offers globally validated water quality products to facilitate the effective use of NASA/USGS and the Copernicus satellite observations (i.e., Landsat-8/9 and Sentinel-2A/B) for water resource management. Leveraging various NASA-supported R&D efforts, STREAM combines cutting-edge same-day water quality estimation with an easy-to-use, responsive web interface. The processing engine of STREAM is enabled by a rigorously validated machine-learning processing chain (including atmospheric correction and in-water retrievals) to approximate water quality indicators, such as water transparency and concentrations of chlorophyll-*a* and total suspended solids. The web interface allows end-users to visualize water quality maps, identify pixel values, and view time-series plots for a given pixel or a group of pixels (e.g., lake or its sub-basin). STREAM already enables low-latency (< 6 hours) detection of anomalous water-quality conditions for select regions and water bodies and will continue to expand operations as capable. Additionally, a RESTful API allows users to request product maps directly or query for more information. The system is open to the public (https://ladsweb.modaps.eosdis.nasa.gov/stream/) and has conducted daily processing since June 2024. These tools enable rapid insight into emerging water quality changes.

PREPARING LIMITED-RESOURCE KENTUCKY AQUAFARMERS FOR SUCCESS THROUGH COMPREHENSIVE AQUACULTURE BOOTCAMP TRAINING

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The learning curve to enter aquaponics as a business enterprise is steep. Knowledge is a common barrier to successful aquaponic adoption, which is challenging given the timeframe for obtaining the skills needed to succeed, is relatively short. According to the 2018 USDA Census of Aquaculture, Kentucky had only two aquaponics farms; this slow growth can be attributed to limited education and funding resources. High quality, intensive training opportunities can provide beginning aquaponic farmers with the tools necessary for successful business ventures. Hands-on training paired with virtual curricula has potential to enhance the success of new aquaponic operations through practical, application-focused workshops.

Kentucky State University (KSU) Aquaculture extension staff will be conducting an Aquaponic Boot Camp program that will provide immersive training that promotes beginning farmer success and confidence in underserved Appalachian regions. This Aquaponics Boot Camp-3 is a collaboration effort with Ohio State University's Piketon Research and Extension Center, Blue Acre Appalachian Aquaponics in West Virginia and Kentucky State University. Participants will receive science-based information from aquaculture/aquaponics researchers, extension specialists, and industry professionals. Participants will tour established aquaculture/aquaponics farms, network with industry experts, and learn from fellow producers. The training will be offered at three different levels, the "3-I levels": Intensive, Intermediate and Introductory. Intensive training is an in-depth approach that will involve twelve modules, one per month, for a year-long immersive program and will include on-farm practice and classroom courses. Intermediate training will allow participants to choose which modules apply to their unique needs and engage in various educational activities and workshops. Introductory training is focused on entry level participation and will include general information sharing and online material engagement. There will be three focus areas for the training; aquaculture, aquaponics, and business management and marketing.

Intensive training participants will design and conduct pilot-scale aquaponics projects in the production systems at the KSU Aquaculture Research Center where they will utilize the knowledge gained to determine optimal system design, profitable product selection and innovative marketing strategies. At the end of the year, program participants will present their aquaponics production business plan and offer feedback through module/program evaluations. Information will be presented on the program, including recruitment pathways, training topics, previous success with intensive aquaculture training, and information on how to track the Aquaponic Bootcamp Program.

THE COMMERCIAL SHELLFISH AQUACULTURE LAB & TEAM RESEARCH FARM AT THE VIRGINIA INSTITUTE OF MARINE SCIENCE

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To complement both the existing shellfish aquaculture research capacity at the Virginia Institute of Marine Science (VIMS) and collaborative research with the shellfish aquaculture industry, the VIMS Commercial Shellfish Aquaculture Lab & Team (CSALT) permitted, installed (in 2022) and operates a one-acre research shellfish farm in the York River offshore of the VIMS Gloucester Point, VA campus. The farm, situated in 2-4 m water depth with firm bottom with salinities typically ranging from 16-22 PSU, includes three different methods of cultivating shellfish: a line of bottom cages with capacity for 24 cages, a floating line with capacity for 12 floating, flappable cages, and 5 floating lines with capacity for over 1,000 FlipFarm baskets. The farm is managed by the farm manager, with assistance from a 6-month full-time apprentice, lab members and interns.

To date, the farm has provided multiple services. First, the farm has been a platform for a variety of oyster research projects including formal experimental tests of cultivation practices on crop performance, co-culture (e.g., periwinkles, sea lettuce), and effect of cohort diversity on crop performance. The farm also hosts several pilot projects and a long-term monitoring of annual crops of diploid and triploid oysters to gauge patterns of growth and survival. The farm is also a platform for education and training. The farm has hosted class visits and informal tours, and allowed a US Aquaculture Society Student Sub-Unit, the Aquaculture Collective at VIMS (with undergraduates from William & Mary) to get hands-on experience with shellfish aquaculture. Current MA students at VIMS are gaining knowledge and expertise from their work on the farm and we now offer a 2-credit class each semester grounded in experiential learning on the farm (Methods in Shellfish Aquaculture). With a recent donation, a new 6-month full-time apprenticeship was pilot tested in the summer/fall of 2024 Additionally, the farm manager position itself is a potential workforce development mechanism, with the first farm manager recruited into a farm manager position in Texas. Finally, the farm is a platform for outreach and demonstration. The farm has hosted dozens of commercial shellfish farmers as well as regulators and researchers, allowing them to see new gear and techniques, seek advice and compare notes. The farm is highly visible to the public, both on and off campus, and is shared on a live video stream to encourage public awareness of shellfish aquaculture.

EFFECT OF BOTTOM AND FLOATING CONTAINER PRODUCTION ON OYSTER PERFORMANCE: GROWTH, SURVIVAL, SHAPE, SHELF LIFE, AND CONSUMER PERCEPTIONS

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With a variety of methods available to oyster growers to produce oysters (on bottom, bottom cages, suspended containers, floating containers, etc.), commercial shellfish growers are faced with an array of costs and benefits beyond the basic considerations of effect on growth & survival. In Virginia, containerized aquaculture is dominated by two methods: bottom cages and surface floats. To compare these methods, oysters (*Crassostrea virginica*) were deployed in replicate bottom cages (BC) and 6-bag floating cages at Cappahosic Oyster Company in York River (VA, USA) in fall 2021. Oysters were tended with typical commercial practices (e.g., routine density reductions, air drying the floating cages, etc.) with oysters assessed for survival and growth at regular intervals. Product attributes (length, meat weights, shell shape, etc.) were assessed when most of the product was considered 'market ready' at ~18 months (Feb. 2023). The work was supplemented with a restaurant survey of raw oyster consumers in Aug. 2023 with oysters raised with similar methods. In addition, an analysis of shelf life using market ready oysters was performed over nearly 4 weeks to assess shelf life differences between the growing techniques. In this study, BC were compared to two positions in flippable, floating cages: bags in the four outer positions, designated FO, and bags in the two inner positions, designated FI (given prior qualitative observations of differences). While overall survival was ≥87%, survival was lower in the FO treatment than survival in the BC and FI treatments. Growth was highest in BC, followed by FO and FI, respectively. In contrast, oysters raised in the FO treatment had the greatest shell fan ratio (shell height/shell length) and shell cup ratio (shell width/shell length) of all the treatments. Additionally, FO oysters were substantially less biofouled than the oysters from the other treatments. In a test of shelf life during summer (using oysters raised by Cappahosic with identical methods), FO oysters had the longest shelf life while BC oysters had the shortest. However, in a preliminary restaurant survey in summer 2023, BC oysters were requested more frequently by consumers in a blind taste than oysters from FI and FO, respectively. These results suggest that there are tradeoffs between these two methods. This study also does not take into considerations other important factors such as cost, labor, permitting issues, etc. Despite these limitations, these results may help growers make more informed decisions when opting for a particular gear type.

SHELLFISH AQUACULTURE RESEARCH AT THE VIRGINIA INSTITUTE OF MARINE SCIENCE

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The Shellfish Aquaculture Program at the Virginia Institute of Marine Science (VIMS) consists of dynamic team of faculty, staff and students who have identified shellfish aquaculture as a priority, engaging through research, education and advisory services. The program also includes several facilities (e.g. the Acuff Center for Aquaculture, the Eastern Shore Lab, a research farm at the Gloucester Point campus), other resources (e.g., vessels, equipment) and centers of excellence (the Aquaculture Genetics and Breeding Technology Center, the VIMS Shellfish Pathology Lab, the Marine Advisory Program, etc.). The VIMS Shellfish Aquaculture Program is committed to conducting state-of-the-art research and offering world-class education in shellfish aquaculture science. The overarching mission of VIMS' Shellfish Aquaculture Program is to advance and support a thriving sustainable shellfish aquaculture community in Virginia and the United States, through globally relevant shellfish aquaculture science, outreach, and education. Current shellfish aquaculture research projects will be briefly reviewed and include environmental interactions, genetic improvement, environmental challenges, improving aquaculture production, shellfish health & biosecurity, and socioeconomics.

EVALUATION OF AN OYSTER TRAINING PROGRAM FOR SEAFOOD SERVERS

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Despite the recent expansion of oyster aquaculture in the southern US, lack of knowledge about the quality of these farm-raised oysters and even negative perceptions about southern farm-raised oysters continue to persist, especially in regions outside of the southern US. Over the last 2.5 years, we conducted trainings with seafood servers at restaurants in 1) coastal southern US states (at least 54 trainings) as well as 2) 'foodie' cites in potential markets (e.g., Boston, New York, Nashville, Seattle, San Diego, with at least 14 trainings). In these trainings, at least 900 seafood servers participated. In addition, two regional wholesale distributors were provided trainings upon request.

In pre and post surveys, servers self-assessed their comfort answering customers' questions and explaining differences among oyster varieties. Prior to trainings, participants reported a higher average comfort level ($p < 0.01$) answering customer questions (6.2 on a 10-point scale) than explaining differences among oysters (5.6). Overall, participants reported a 27% increase in comfort answering customer questions ($p < 0.01$, paired t-test) and a 43% increase in comfort explaining differences among oyster varieties ($p < 0.01$, paired t-test) with average scores of 7.7 and 7.5, respectively. There was, however, a negative correlation between pre- and post-trainingcomfort, suggesting that knowledgeable servers did not benefit as much from the trainings as less knowledgeable servers.

Participants that volunteered to participate in follow-up interviews (at least 40 conducted) overall spoke favorably of the training. Though few could note significant changes in sales or individual tips, the majority of participants indicated that they (or their staff) were more confident in their knowledge surrounding oysters and many suggested the value of recurring trainings (in some cases to dig deeper on particular topics, in other cases simply as a "refresher").

Data on changes in overall sales were collected from a subset of restaurants in the six weeks before and after the trainings, but were insufficient to conduct an analysisof the trainings' effect on sales. In response, additional approaches to test the effect of trainings were tested and results will be reported.

MINING OMICS DATASETS FOR RESILIENCE BIOMARKERS TO IMPROVE OYSTER BREEDING AND MANAGEMENT STRATEGIES

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Biomarkers associated with environmental and disease resilience traits can be leveraged in breeding and management strategies. However, their discovery has been limited by the complexity of molecular systems and the cost of omics tools used to understand them. Advances in computational approaches including machine learning algorithms, together with the wealth of genomic data that has amassed, enable powerful meta-analyses for improved biomarker discovery in aquaculture species. Omics datasets from a variety of published studies on Pacific oyster with varying thermotolerance were systematically reanalyzed using open-access, reproducible bioinformatics pipelines, and updated references, and annotations. Data integration approaches revealed new and previously identified biomarkers associated with thermotolerance. Meta-analyses of omics datasets from Pacific oyster with different resilience traits and datasets from other shellfish species will reveal additional biomarkers and potential cross-species and/or cross-condition biomarkers. An example of the data analysis workflow and use of a comprehensive database containing these identified resilience biomarkers will be demonstrated during the presentation to promote the use of these products by the aquaculture community. These products will enable molecular tool development for more efficient phenotype selection and health monitoring, selection methods that use a systems biology approach for simultaneous improvement of multiple traits, and ultimately increased animal fitness.

IMPROVEMENT IN DERMO RESISTANCE OF THE EASTERN OYSTER *Crassostrea virginica* AFTER TWO GENERATIONS OF GENOMIC SELECTION

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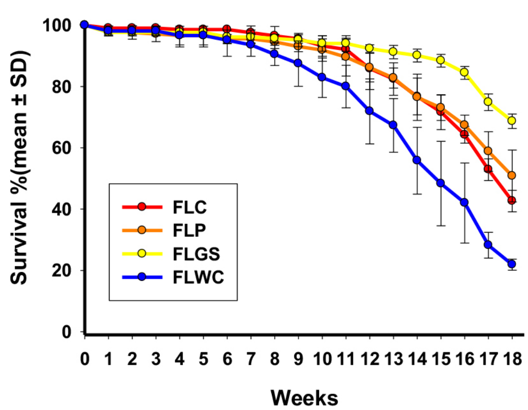
Dermo is an important disease of the eastern oyster *Crassostrea virginica* caused by the protist *Perkinsus marinus* that heavily impacts wild and cultured populations. Selective breeding has improved dermo resistance although progress has been slow, likely because dermo resistance is determined by many genes of small effects. Genomic selection (GS) is a powerful approach to genetic improvement especially for polygenic traits that are difficult to measure. GS was conducted to improve dermo resistance in a wild population from Florida over two generations. In the first generation, GS led to 10.2% increase in dermo resistance as previously reported. For the second generation, F1 oysters were again challenged with *P. marinus,* separated into dead (susceptible) and live (resistant) phenotypes, and used as the training population, with unchallenged oysters from the same F1 population used as the breeding population. Oysters were genotyped with a 66K SNP (single-nucleotide polymorphism) array. A genome-wide association study identified no major quantitative trait loci for dermo resistance, confirming the trait is polygenic with a heritability of 0.26. Five models were evaluated for genomic predictions and the model with the highest prediction accuracy, as determined by cross-validation in the training population, was used to calculated genomic estimated breeding values (GEBVs) for oysters in the breeding population. Oysters with the highest (~10%) or average GEBVs were selected to produce the genomic selected (FLGS) and genomic control (FLC) groups, respectively. Survivors from dermo challenge and unchallenged wild oysters were selected to produce phenotypic selected (FLP) and wild (FLWC) unselected controls, respectively. Progeny from the four groups were challenged with dermo in the lab to assess their resistance. At the end the 18-week challenge, cumulative survival was 69% in FLGS, 51% in FLP, 43% in FLC and 22% in FLWC (Figure). Compared with the genomic control FLC, the genomic selected FLGS showed 60.5% improvement in survival, while improvement in FLP (18.6%) was not significant. FLGS showed 214% increase in survival over the unselected wild control FLWC. Considering improvements made in both F1 and F2, two generations of genomic selection led to 76.9% compounded increase in survival. These results indicate that GS is effective in improving dermo resistance, and significant improvement can be achieved in two generations.



ELUCIDATING THE GENOMIC BASIS OF ENVIRONMENT-DEPENDENT HETEROSIS TO INFORM GENETIC ENHANCEMENT IN CATFISH FOR AQUACULTURE PRODUCTION

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In F1 hybrids, phenotypic values are expected to be near the parental means under additive effects or close to one parent under dominance. However, F1 traits can fall outside the parental range, and outbreeding depression occurs when inferior fitness is observed in hybrids. Another possible outcome is heterosis, a phenomenon in which interspecific hybrid F1s exhibit improved fitness than both parental species/strains. As an application of heterosis, hybrids between channel catfish females (*Ictalurus punctatus*) and blue catfish males (*Ictalurus furcatus*) are superior in feed conversion efficiency, carcass yield, and harvestability, which account for over 50% of the US catfish production. Interspecific hybridization facilitates the combination of genetic material from different species and has proven to be an effective strategy for enhancing phenotypic variability and achieving genetic improvement in catfish. However, deleterious epistatic effects can occur, manifesting as hybrid breakdown in F2 populations. Therefore, to maintain the enhanced production traits in hybrids, it is necessary to rear hybrid F1 fry for each growth cycle. To fully leverage heterosis and develop catfish breeds with stable superior phenotypes, there is a critical need to elucidate the underlying molecular mechanisms of heterosis.

In this study, we performed catfish culture experiments of parental species (blue catfish and channel catfish) and their reciprocal hybrids in both tank/aquarium and earthen pond environments. By longitudinally quantifying fitness parameters such as growth and survival, we confirmed a previously observed phenomenon, which we have formally defined as environment-dependent heterosis. In ponds, hybrids outgrow both parents due to an extra rapid growth phase of (2~4 months) in year 2. This bimodal growth pattern is unique to F1 hybrids in pond culture environment only. In sharp contrast, the same genetic types cultured in tanks display outbreeding depression, where hybrids perform poorly, while channel catfish demonstrates superiority in growth. Throughout this 29-month experiment, we collected 14 tissues samples per fish at the following ages: 3 weeks, 10.8 months, 18.6 months, 21.8 months, 23.5 months, 25 months, and 28.4 months. RNA-seq and DNA methylome sequencing experiments were conducted using selected tissue samples from all four genetic types (blue, channel, reciprocal hybrids) cultured in tank and pond environments. A set of genes in pond-cultured F1 hybrids were identified as upregulated during the critical fast-growing window in the second year, serving as biomarkers and candidate causal genes for environment-dependent heterosis. Additionally, transgressive genes, *cis*- and *trans*-regulatory effects, as well as epigenetic differences, were observed when comparing pond vs. tank culture conditions. Our research has pinpointed key genes that may contribute to the superior phenotypes in F1 hybrids, which serve as targets for genetic enhancement through genome editing. Compared to interspecific hybridization, which combines the entire haploid genomes of two species and results in the indiscriminate mixing of all genes, the precise and stable introduction of beneficial traits via genome editing of specific genes holds significant potential for genetic enhancement to improve catfish production.

TRANSCRIPTOMIC AND PROTEOMIC INSIGHTS INTO THE ROLE OF ANP32A IN RESISTANCE TO TILAPIA LAKE VIRUS

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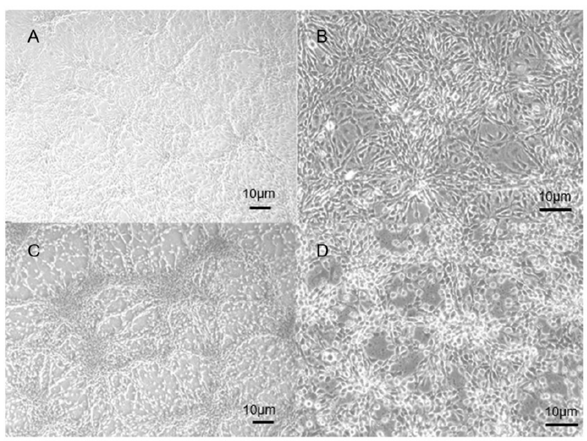
Infectious and parasitic diseases pose significant challenges to tilapia aquaculture, causing notable economic losses and adversely affecting the welfare of these fish species. Utilizing cell lines as experimental models plays a pivotal role in advancing our comprehension of infectious diseases, providing a valuable platform for studying the intricate interactions between pathogens and the host. In combination with genome editing technologies, such as CRISPR/Cas systems, it becomes possible to assess the specific functions of genes within these systems. This approach allows research into potential targets for enhancing resistance to diseases such as Tilapia lake virus (TiLV) in tilapia aquaculture. The ANP32 family, acidic (leucine-rich) nuclear phosphoprotein 32 kDa, play various endogenous roles in regulating gene expression, intracellular transport, and cell death. Interestingly, avian ANP32 proteins are also critical for avian influenza polymerase activity and can influence viral replication, a key factor in viral proliferation. In this current study, we knocked out anp32a and demonstrated a significant increase in levels of resistance to TiLV. We then analysed the transcriptomic and proteomic response of knockout cells to the virus to characterize the mechanism of resistance. This work has the potential to impact strategies for disease control in Nile tilapia.



The difference between WT cells and *anp32a*-KO cells after infected TiLV. (A, B) anp32a-KO cells were infected by TiLV. (C, D) WT cells were infected by TiLV (A, B was in low magnification, C, D was in high magnification).

TAUTOG *Tautoga onitis* RESEARCH INTO EARLY LIFE STAGE STRATEGIES: BROODSTOCK, SPAWNING, LARVAL FEEDS AND JUVENILE CULTURE CONDITIONS

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Tautog (*Tautoga onitis*) is a marine wrasse native to the Northwest Atlantic, which is a highly sought after food fish throughout its range and commands a premium on both the processed and live fish markets. Its life-history characteristics render it vulnerable to overfishing as it exhibits strong site fidelity, low daily movement rates, and seasonal migration patterns and therefore over the last 30 years, commercial landings have significantly decreased. Due to current low availability, high consumer demand, strong price and a proven market for the product, tautog has been identified as a candidate for marine aquaculture. A year-round supply of high-quality, market-size fish would boost regional economies, and could reduce pressure on wild stocks. The current work included objectives to evaluate and optimize broodstock conditioning, spawning, larval and juvenile early life stage culture techniques. There was an evaluation of egg quality and quantity, when broodstock were either naturally ripened or overwintered and conditioned in recirculating systems. Spawn quality was evaluated, as well as larval success and fertilization after spawns under diverse conditioning protocols. Larval tank conditions, larval stocking densities, prey density and prey quality (enrichment) were also assessed as these factors relate to growth and survival. Finally, larval feed quality, density of transitioning larvae, and optimization of juvenile tautog growing conditions were investigated; including optimal thermal range, testing of various substrates and stocking densities of juvenile fish.

Over two years, multiple cohorts of broodstock were collected and held at the NOAA Sandy Hook Laboratory. Spawning success from both naturally and laboratory-conditioned fish led to larval assessments in which it was determined that tank volume and stocking density was positively correlated to survival. In both years at larval culture temperature from 10.6-26.0˚C warmer conditions led to shorter embryonic duration (2 vs 6 days) and faster growth rates (6 vs 10 mm TL at 35 dph), though there was a lack of correlation between temperature and survival success. Continued research and analysis will yield greater insights into optimal culture conditions for tautog, to enable a transition to commercial viability for this important food fish.

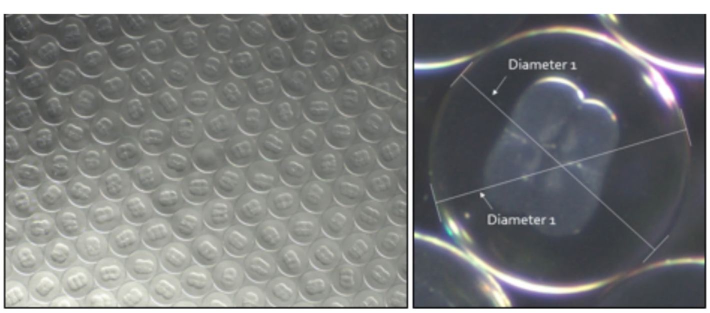

Figure 1. Images of tautog eggs at 2 hr post-fertilization. Left: Group egg image from which ~ 50 viable eggs were measured. Right: Single egg to show examples of two diameters drawn and used to estimate mean egg diameter.

INVESTIGATING SUSPENSION-FEEDING INVERTEBRATES AS BIOINDICATORS OF MICROPLASTICS

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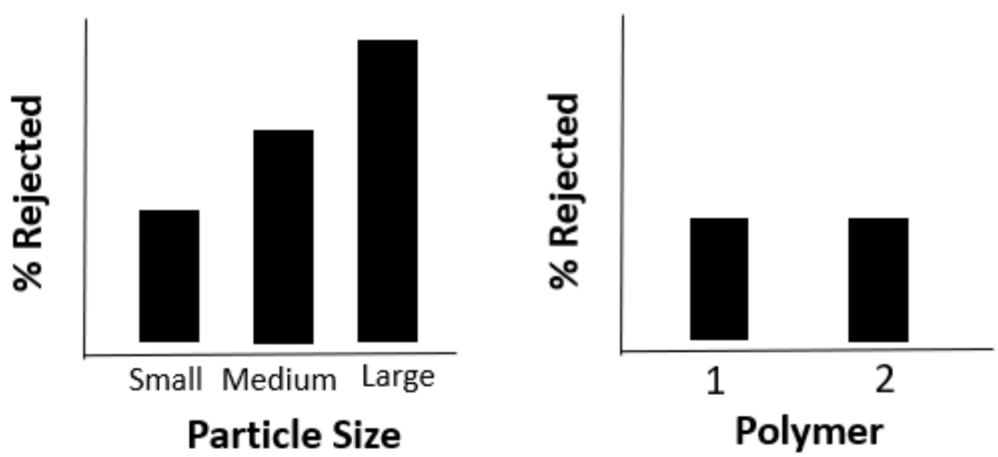
Suspension-feeding animals interact with microplastics of different shapes and sizes suspended in the water column. Bivalve molluscs have been suggested as potential bioindicator species for microplastics as they are known to consume microplastics in the natural environment, are widely distributed, sessile, and easy to collect; however, these molluscs are selective suspension feeders and, thus, do not consume all particles to which they are exposed. This study investigated how three indiscriminate suspension feeders, the Atlantic slippersnail *Crepidula fornicata* (gastropod), the sea grape *Molgula manhattensis* (tunicate), and *Styela clava* (tunicate) interact with microplastics of different sizes, shapes, and polymers to determine their suitability as bioindicator species for microplastics. The data were compared to that of previous experiments with oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*). Animals were offered aged polyester or nylon microfibers of different lengths, nylon, and polyester microfibers of similar lengths, or polyethylene and polystyrene microspheres of similar diameters during a 2-h exposure. Feces and pseudofeces collection during and after the exposures revealed that slippersnails and sea grapes both exhibited size-based rejection of nylon fibers, rejecting longer fibers at higher proportions. Polymer type did not influence ingestion of fibers or spheres. Sea grapes were the most indiscriminate feeders when compared with slippersnails, oysters, and mussels, but were able to egest microplastics just as quickly. Although sea grapes rejected proportionally fewer microplastics than slippersnails, neither species will make an ideal bioindicator because they do not ingest all plastic particles they encounter, they egest the particles quickly, and do not accumulate microplastics in their tissues. Data are currently being analyzed for *S. clava* exposures to include in this comparison.



EVALUATING THE EFFECTS OF WATER TEMPERATURE ON COMPOSITION OF BIOFILM MICRIOBIAL ASSEMBLY IN AQUAPONIC SYSTEMS

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Much of the research on nutrient dynamics in aquaponic (AP) systems has focused on microbial-driven nitrogen transformation and utilization by plants. There is a lack of information on the dynamics of other essential nutrients, such as Phosphorus. This knowledge gap limits the sustainability, productivity, and capacity for crop diversification in AP systems. The microbial community associated with the plant rhizosphere may play a critical role in driving these transformations and utilization pathways in AP systems.

The aim of this study was to determine how temperature influences the microbial community composition of biofilms from AP systems, with special emphasis on organisms that contribute to phosphorus cycling and availability. Three replicated aquaponic systems were utilized, each maintained at a set temperature (22, 25, 28 °C). Each system fed one Center of Disease Control biofilm reactors (BioSurface Technologies, Montana USA) in a flow through design using a peristaltic pump (Cartridge Pump, Fisher Scientific, New Hampshire USA) at a flow rate of (2.75 gal/min). Aquaponic systems were stocked with Nile tilapia (*Oreochromis nilotics*) and bell pepper (*Capsicum annum*) seedlings. Microbial samples from biofilm reactors were collected every seven days for 21 days. DNA was extracted (Zymo Research, *Quick*-DNA/RNA Plus) and 16S rRNA was sequenced (Mk1C, Oxford Nanopore) to determine the differences in community composition over time. A principal component analysis (PCA) was used to visualize the patterns between temperature and developmental stage and a Bray-Curtis diversity test was used to determine significance.

The PCA showed distinct groupings of microbial communities based on temperature (Figure 1). Results from the Bray-Curtis test showed significance (P-value < 0.05) between all treatments. The results show that there is a significant difference between the compositions of the microbial communities of biofilm at different temperatures and plant development stage.

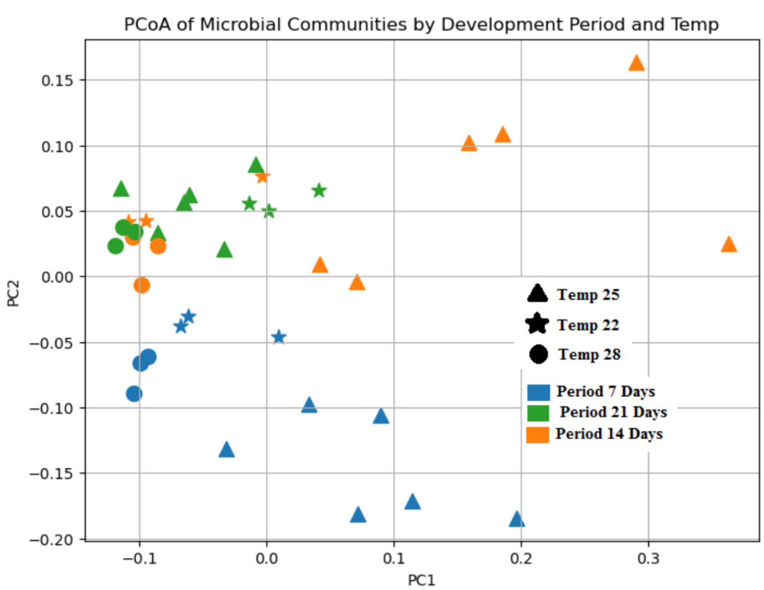

Fig. 1: PCA visualization of temperature and development stage of the microbial community.

BLACK SOLDIER FLY MEAL AS A PARTIAL REPLACEMENT FOR MARINE-DERIVED INGREDIENTS IN MICRODIETS USED TO FEED LARVAL INLAND SILVERSIDES *Menidia beryllina*

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Rapid expansion of aquaculture in recent decades has allowed the industry to meet the growing global seafood demand. However, it is important that future growth in this sector is accomplished sustainably. Because of this, insect meal is rising in popularity as a protein replacement for fishmeal, which is commonly used in formulated finfish diets and is in limited global supply. The objective of this research was to evaluate the effects of microparticulate diets produced with black soldier fly (*Hermetia illucens,*) larvae meal (BSFL; Stratium, Buffalo. NY) on the growth and survival of island silverside (*Menidia beryllina*) larvae.

Two experimental diets were produced and were based on an open-formula reference diet (OFRD) developed for marine fish larvae. The benefits of the OFRD is that the formulation is open-source which allows direct comparison with related studies and can be used by commercial feed manufacturers for product development and formulation. The experimental control diet (OFRD) was based on the standard OFRD formulation whereas in the second diet half (50%) of the marine ingredients, on a crude protein basis, were replaced with BSFL meal. These two experimental diets were compared with one another and with a commercially-available diet (Otohime) in a 10-day feeding trial with larval Inland silversides (*Menidia beryllina)*. Larval growth parameters (lengths, dry weights and condition factors) were not significantly different between those fed the diet containing BSFL meal (50% MPR) and those fed the OFRD. Ultimately, this study suggests that BSFL meal can be used in early life stage diets for some finfish species, in this case inland silversides, without compromising the larval growth.

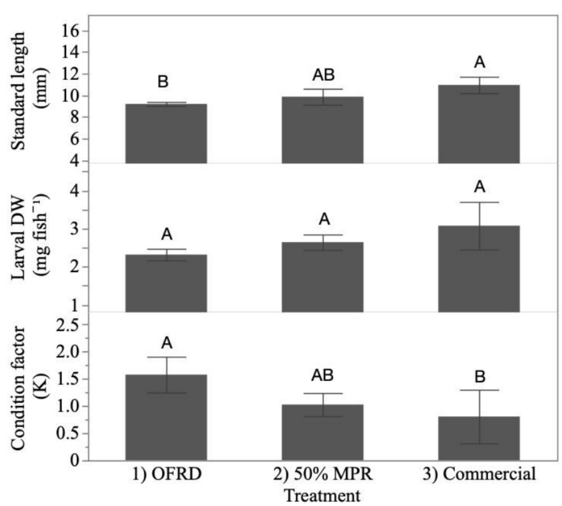

Figure 2. Larval lengths and dry weights measured at the end of the trial. Treatments sharing the same letter were not significantly different (Tukey's HSD, threshold p<0.05)

EFFECTS OF MECHANICAL DISTURBANCES ON BLUE MUSSEL *Mytilus edulis* FEEDING AND RESIDENT GUT MICROBIOME

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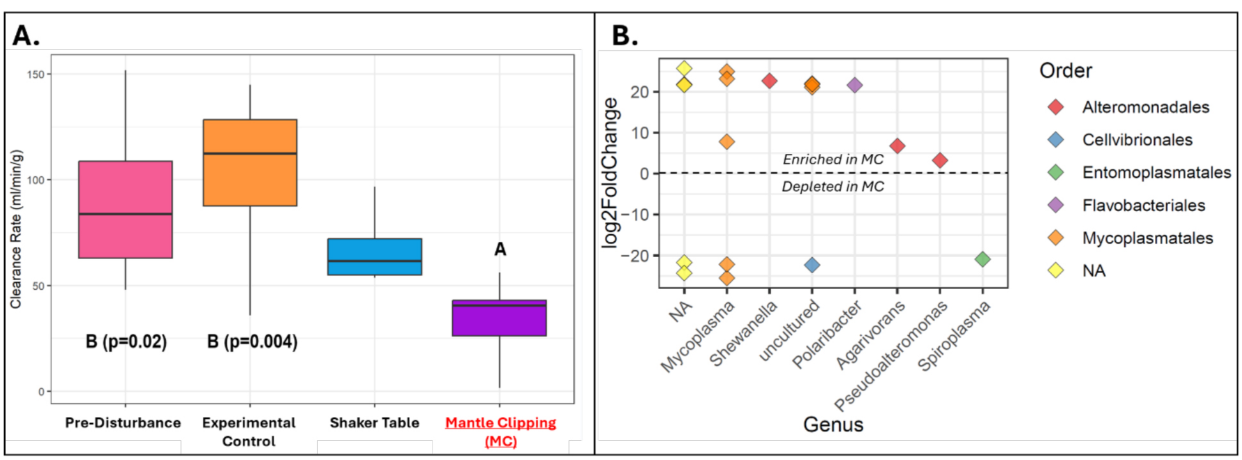
The animal gut microbiome strongly influences host health and ecology. Blue mussels (*Mytilus edulis*) are ecologically significant filter-feeding bivalves whose gut microbiome can support nutrient uptake, immunity, and stress resilience. While disturbances like changes in temperature or diet can directly disrupt the bivalve gut microbiome, it remains unclear whether mechanical disturbances that stress the bivalve host have the potential of indirectly affecting the gut microbiome. We measured the effects of two such disturbances to *M. edulis* from natural populations in Long Island Sound. We aimed to 1) assess the degree of host stress by measuring how mechanical disturbances affect filter feeding, and 2) determine if these disturbances could lead to shifts in gut microbial community composition. Mussels were disturbed via clipping of the mantle organ (simulated crab assault) or shaking (simulated storm event). Disturbed and control mussels were transferred to individual microcosms and rates of clearance of *Tetraselmis* algae were calculated as a measure of host stress. Mantle-clipped mussels remained closed for longer periods and had significantly lower clearance rates compared to controls (Fig. 1A), indicating compromised metabolic state and food capture efficiency. We also analyzed effects of shaking and mantle-clipping on the *M. edulis* resident gut microbiome. Following control or disturbance regimes, mussels were allowed to void gut contents in individual, sterilized microcosms for up to 24 hours, then dissected aseptically to isolate gut tissue. Total gut DNA was extracted, the V4 region of the 16S rRNA gene amplified using Earth Microbiome primers, and libraries sequenced using 300bp paired-end sequencing. The resident gut microbiome of mantle-clipped mussels was significantly different from experimental controls, changing the proportions of several key bacterial taxa (Fig. 1B). These results demonstrate that a mechanical disturbance can result in both host stress and gut microbiome disruption in bivalves, potentially exacerbating the effects of downstream anthropogenic stressors.



<u>Figure 1.</u> (A) *M. edulis* exhibited significantly lower clearance rates immediately after mantle clipping. (B) Differentially abundant bacterial Amplicon Sequencing Variants (ASVs) in control vs. mantle-clipped (MC) mussels. Fold change (y-axis) >1 indicates increased proportion of ASVs (points) in MC mussels, and vice versa.

BRINGING AQUACULTURE TO THE TABLE: EXPANDING KENTUCKIAN PALATES THROUGH RESEARCH AND EXTENSION

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Kentucky State University (KYSU), an 1890 Land-Grant Institution, has provided the citizens of the Commonwealth of Kentucky (KY) with exceptional agriculture extension service since its inception. KYSU has offered a Master of Science in Aquaculture and Aquatic Sciences since 1999. KYSU's Aquaculture Program continues to grow its academic education, innovative research and Cooperative Extension Program.

Extension faculty and staff in the School of Aquaculture introduce aquaculture education and aquatic products to stakeholders throughout Kentucky with a goal of increasing seafood product acceptability, urban food accessibility, and supporting local food production and healthy eating. The Aquaculture Extension team implement aquaponics in local schools as educational tools, provide filleting and cooking demonstrations, and donate fish and produce grown in our research systems to local non-profit organizations. These donations impact local needs-based Community Supported Agriculture programs and soup kitchens. Local aquaculture products have been used to prepare and cook Butter-Garlic Shrimp, Tilapia Ceviche, and Smoked Trout dip featured at KYSU's Annual Legislative Fish Fry, at the 2024 Kentucky Chamber of Commerce Gala, and at non-profit fundraising dinners. Healthy recipe cards and instructional videos have been created in partnership with international graduate students. These extension activities and interactions showcase preparation and cooking of fish and shellfish, and how consumers can introduce them to their dinner tables as a sustainable, and desirable component of a healthy diet.

Perceptions of farmed aquatic animal protein can improve by providing KY consumers with cooking demonstrations, recipes, and research-based information. Future extension activities include taste testing surveys, value-added processing, recipe competitions - all geared to expand Kentuckian palates.

Figure 1. A) Noel Novelo and Oluwafemi Adebayo tasting Hot Pepper Soup; B) Uchechukwu Ohajiudu cooking Hot Pepper Soup; C) Chelsea Walling displaying Aquaponics at HBCU Showcase in Louisville, KY; D) Jeffrey Warner and Chelsea serving Tilapia Ceviche at the 2024 Chamber of Commerce Gala; E) Pan Seared Tilapia and Spinach Recipe.

BUSINESS AND ECONOMIC PLANNING FOR SEAWEED AQUACULTURE SYSTEMS IN THE UNITED STATES

Tammy Warner* and Robert Pomeroy

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This presentation will share business planning and management tools developed as part of a project funded under the NOAA Sea Grant funding opportunity titled, "Addressing Economics and Market Needs of the U.S. Aquaculture Industry" with a particular focus on the integration of a Life Cycle Analysis with the financial planning tool.

Project objectives include: (1) Develop business planning and management tools for kelp aquaculture systems, which improve the economic and financial viability of this industry; (2) Increase access to capital among existing and prospective seaweed farmers via an emphasis on improved industry knowledge for investors/financers/potential market entrants: **(3) Conduct a** comprehensive economic assessment of the ecosystem services provided by seaweed aquaculture; and (4) Develop outreach and education activities through SG extension for industry, regulators and financial institutions to support the development of a seaweed aquaculture industry.

The presentation will focus on objective (3) above and will also demonstrate the use of the financial planning tool (available on NOAA Sea Grant's National Seaweed Hub https://seaweedhub.extension.uconn.edu/). Participants may wish to download the financial planning tool in advance of the session to work along with the presenter.

UNDERSTANDING REPRODUCTIVE TIMING OF AMERICAN OYSTER (*Crassostrea virginica)* POPULATIONS IN MAINE ESTUARIES THROUGH HISTOLOGICAL ANALYSIS

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The Maine (USA) aquaculture industry has grown considerably in recent decades, and with it the farming of the American oyster (*Crassostrea virginica*). Due to a combination of overharvesting, industrial development, and environmental change in the Gulf of Maine, wild populations of American oysters in Maine have been considered functionally extinct since the early 1900s. However, anecdotal evidence indicates that oysters are becoming more prevalent in the intertidal zone outside of culture operations. Still, very little is known about American oyster population dynamics in Maine estuaries. We sought to determine if adult oysters are spawning in two estuaries - the New Meadows and the Sheepscot.

Between May and September of 2024, we conducted sampling of adult oysters in the New Meadows and Sheepscot river estuaries. Fifteen individuals were collected monthly at each site. Sampling was opportunistic in that we obtained oysters from different sources in each estuary, but selective in that we aimed for oysters of a consistent size within an estuary. For each individual we measured wet weight (whole, meat-only, and shell-only) and shell dimensions (height, width, and depth), before preserving and processing tissue for histological analysis of reproductive tissue. In the New Meadows River, oysters (shell height 49-112 mm, average 78.10 mm) were collected from a Limited Purpose Aquaculture permitted site managed by a community-based collaboration working on local oyster restoration. In the Sheepscot River, oysters (shell height 77-146 mm, average 112.3 mm) were collected from a bottom culture oyster lease in June and then held in floating bags nearby. Sondes were deployed for the duration of sampling in the New Meadows and Sheepscot River estuaries to collect continuous data for water temperature, salinity, chlorophyll and turbidity.

Results will focus on characterizing the environmental parameters of the two estuaries, a preliminary analysis summarizing the presence and absence of reproductive tissue and possibly the timing of a spawning event, as well as relating reproduction to environmental parameters. To our knowledge, this study is the first to examine the presence of reproduction in American oysters in Maine estuaries through histology sampling.

SHRIMP SCAMPI: A CITIZEN SCIENCE PROJECT - EDUCATING ABOUT MICROBES AND ENDOCRINE DISRUPTING CHEMICALS LIKE GLYPHOSATE USING FOLDSCOPES

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As part of the OHEEI international initiative, Shrimp Scampi was initiated by Massachusetts (MA) students from Algonquin Regional High School in Northborough, MA to assess the adverse health effects to the environment, wildlife, pets, seafood, and people, caused by the antimicrobial and herbicide Glyphosate, glyphosate-based herbicides (GBH), and other endocrine disrupting chemicals (EDCs) like metals and Bisphenol A (BPA). These EDCs appear associated with obesity, diabetes, non-Hodgkin's lymphoma, non-alcoholic fatty liver disease, antimicrobial resistance, neurodevelopmental syndromes, congenital malformations, and neural tube defects.

Shrimp is the favorite seafood of Americans, most of the shrimp we eat is imported, causing a yearly ~US$4.5 billion trade deficit. Although shrimp viruses and metals have been detected in frozen shrimp sold at MA supermarkets, no official compulsory testing of contaminants in imported seafood is being performed by government agencies. The long-term goal of 'Shrimp Scampi: A Citizen Science Project' is to educate American and Ecuadorian mothers about contaminants in our seafood. In addition to monitoring EDC levels in muscle of frozen shrimp from US supermarkets and wild shrimp from Ecuador, another goal is to perform in-depth reviews of the scientific literature about EDCs like glyphosate, metals, and BPA and their generational epigenetic inheritance.

The Rotary Club of Southborough, MA is helping us to educate by donating foldscopes - paper microscopes (222 foldscopes so far) through their 'Foldscopes: From Southborough to Ecuador' project (https://fucobi-english.weebly.com/). These paper microscopes are making microscopy available to schools in remote areas. Students and teachers have a wonderful time learning about microcosms, and teachers express their gratitude and enthusiasm for being able to have this new classroom tool since they currently do not have a science laboratory.

AMMONIA, NITRITE AND NITRATE PEAKS IN PACIFIC WHITELEG SHRIMP (*Penaeus vannamei*) CULTURE IN BFT SYSTEM: CONCENTRARION, EXPOSURE LIMITS AND RECOVERY TROUGH COMPENSATORY GROWTH

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Peaks and accumulation of nitrogen compounds in biofloc technology (BFT) systems frequently occur during system establishment, causing stress to cultured organisms and compromising their growth and survival. This study investigated the effects of chronic exposure to ammonia, nitrite, and nitrate on *Litopenaeus vannamei*, focusing on both immediate stress responses and compensatory growth during recovery phases.

Three experiments (ammonia, nitrite, and nitrate) were conducted in a 2 x 3 design with two "concentrations" and three "stress" exposure times, divided into two phases: stress and recovery. In the first experiment, shrimp were exposed to high ammonia concentrations (two- and three-fold the recommended safe level, i.e., 7.10 mg·L^{-1} and 10.65 mg·L^{-1}) for periods of 5, 10, and 15 days, followed by 38, 33, and 28 days of "recovery time," respectively. For nitrite (experiment 2), animals were exposed to two concentrations (30.4 and 45.6 mg·L^{-1}), representing two- and three-fold the recommended safe level, for 7, 14, and 21 days, followed by 42, 35, and 28 days of "recovery time," respectively. In the third experiment, chronic exposure was conducted with two nitrate concentrations (0.5- and one-fold the recommended safe level, i.e., 139.45 mg·L^{-1} and 278.91 mg·L^{-1}) over three "stress" exposure times of 10, 20, and 30 days, with "recovery times" of 48, 38, and 28 days, respectively. In all experiments, a control was maintained with ammonia (experiment 1), nitrite (experiment 2), and nitrate (experiment 3) levels below 25% of the safe threshold.

In experiment 1, results indicated that, after the stress phase, survival was impacted by a 10-day exposure to three-fold the safe ammonia level and by 15-day exposures in both high-ammonia treatments. Growth was reduced in all stressed treatments, except for those exposed to two-fold the safe level for 5 days. Results from experiment 2 (nitrite) showed that survival rates were significantly reduced after the stress phase in all groups exposed to three-fold the safe level, as well as in the group exposed to two-fold the safe level for 21 days. Growth was reduced in treatments exposed to high nitrite concentrations for 14 and 21 days. In the third experiment, results indicated that *L. vannamei* displayed higher resistance to nitrate; however, prolonged exposure (more than 20 days) to concentrations above 139.45 mg·L^{-1} negatively affected growth rates. After the recovery phase, shrimp from experiments one (ammonia) and three (nitrate) exhibited total compensatory growth, nullifying significant differences from the control group. In experiment 2, although the animals achieved satisfactory growth rates when nitrogen compound levels were re-established, they did not match the control group, demonstrating partial compensatory growth.

Therefore, if it is necessary to maintain shrimp exposed to high nitrogen compounds, such as during system establishment or when water replacement is limited, the following exposure limits are recommended: (1) for ammonia, exposure should not exceed two times the safe level for a maximum of 10 days; (2) for nitrite, exposure should be limited to two times the safe level for up to 14 days; and (3) for nitrate, exposure should remain within the safe level for a maximum of 20 days.

OPTIMIZATION AND VALIDATION OF A MICROHAPLOTYPE-BASED GENOTYPING PANEL FOR *Crassostrea virginica*

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The eastern oyster (*Crassostrea virginica*) is an ecologically and economically valuable species along the East Coast of the United States and within the Gulf of Mexico. However, the oyster fishery is being threatened by anthropogenic and environmental stressors such as salinity and temperature fluctuations and increased fishing pressure, and an improved understanding of genetic differentiation is vital for future management of *C. virginica*. In addition, the use of low-cost genotyping in oyster breeding programs is increasing. A microhaplotype-based genotyping panel utilizing genotyping-in-thousands by sequencing (GT-seq) technology was previously developed using individuals from Gulf of Mexico and East Coast populations. While this panel successfully captures patterns of genetic variation across the *C. virginica* range, the high degree of genetic diversity and prevalence of null alleles in mollusc genomes pose challenges to effective use of the panel, particularly in achieving accurate genotype calling. Here, we report on the progress of improving genotyping accuracy through additional optimization of the microhaplotype panel using information from pair crosses. Validation of the panel's ability to accurately determine pedigree relationships and differentiate populations will also be presented. Broader applications for aquaculture, restoration, and fisheries management will be discussed, highlighting the potential impact of this genotyping panel on the future of oyster fisheries management and selective breeding.

MATERNAL TRANSCRIPT PROFILES ASSOCIATED WITH EGG VIABILITY IN RAINBOW TROUT *Oncorhynchus mykiss*

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The identification of markers that can serve as predictors of egg quality and for diagnosing problems with embryo development would benefit production efficiency in fish hatcheries. Since mRNA transcription is arrested in the late-stage oocyte the maternal transcriptome stored in the oocyte provides nearly all the mRNA required for oocyte maturation, fertilization, and early cleavage of the embryo. The transcriptome of the unfertilized egg has therefore been targeted to identify informative markers and levels of specific transcripts have been shown to be associated with various measures of egg quality. However, these differentially expressed genes (DEGs) have not been consistent among studies. As a start to determining factors that contribute to disparate results among studies, we compared expression of 65 select transcripts previously reported to be potential markers of egg quality in rainbow trout, between unfertilized eggs that yielded high and low eyeing rates, among three populations using an assay based on the nCounter analysis data system (Nanostrings Technologies; Seattle, WA). The pattern of transcripts differentially expressed with egg quality remain consistent among year classes of the same line supporting value to line specific DEG discovery efforts. On the other hand, less similarity in dysregulated transcripts among lines than within year classes of the same line suggest patterns of transcriptome dysregulation may provide insight into causes of decreased viability within a hatchery population. Although many DEGs were confirmed, there is considerable variability in transcript abundance among eggs of similar quality for each of the genes and low correlations between transcript abundance and eyeing rate. These factors make it difficult to predict the quality of a single batch of eggs based on transcript abundance of just a few genes.

To further characterize the association of maternal transcript profiles with egg quality, we used poly(A) inclusive RNA isoform sequencing (PAIso-seq) to investigate the effects of oocyte ageing on mRNA transcript abundance profiles and poly(A) tail length distributions. Maternal transcripts stored in the egg have short poly(A) tails that must be elongated to allow translation, which takes place for some transcripts before ovulation. We first compared transcript tail-lengths between freshly ovulated oocytes and embryos at 24 hours post fertilization in high quality eggs, and then in unfertilized oocytes in response to in vivo postovulatory ageing up to 14 days.

ELEMENTS OF TODAY'S RAS (INTRO)

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Recirculating aquaculture systems (RAS) will play an important role in the future of the seafood industry because they can be operated more independently of the environment. On the one hand, they reduce environmental impact by recycling water and nutrients. They are also more protected from the environment, making them more resilient to the challenges of climate change. Applications include hatcheries, nurseries and grow-out operations. To date, there is no single standard RAS design. There is also a wide range of filter designs for similar functions using different technologies. However, there is agreement on the basic elements required. These include (1) pumping: to circulate the water between basins and water treatment; (2) solids separation: to remove faecal matter and other particles from the water; (3) biofiltration: to convert and/or remove dissolved nutrients; (4) gas exchange: to oxygenate the water and/or remove gases; and (5) disinfection: to kill or remove pathogens from the water. In simple terms, each sink or source must be balanced to maintain water quality. As the water renewal rate is part of this equation, its level determines the technology required. Closed RAS with a renewal rate of less than 10% of the system volume per day or even less require additional filtration systems such as denitrification, dephosphorisation, backwash water recycling and ion composition control. Care must be taken when designing the hydrodynamics of the system. A distinction must be made between the velocity, hydraulic residence time and flow pattern of the water in the tank. The first has mainly to meet the biological requirements of the farmed species, but is also important for the transport of settled particles. The other two are important for the removal of suspended particles and dissolved substances such as ammonia and carbon dioxide. The design of the tank will affect the flow model to be used. For simplified design calculations, a fully mixed model can be considered as the best approximation for round tanks and a plug flow model for raceways. It is important to use the right model to design the water treatment correctly. A trend is also to consider higher recirculation rates between the basin and the water treatment to achieve better water quality and less daily fluctuations. Technology has developed rapidly in recent decades. In particular, the use of new sophisticated measurement systems at reasonable cost is providing a much broader data base that is improving the understanding of biological and chemical processes in RAS. Examples include screening for fluctuations and shifts within the microbiome or the ionic composition of water. Known problems such as hydrogen sulphide and off-flavour formation are also better understood and can be addressed through improved design and standard operating procedures. However, the complexity of these engineered ecosystems remains, as physical, biological and chemical processes, including their various interactions, must be considered. Standard operating procedures are also improving over time as the knowledge base improves and software solutions assist operators. The next level of operational control is expected to come from the application of AI and computer vision, which will be able to detect key performance indicators such as growth, survival and feed conversion in real time and analyse the complex data to automate predictive modelling.

INDOOR LAND-BASED SHRIMP FARMING: AN INNOVATIVE FARMING APPROACH AS AN ALTERNATIVE TO POND FARMING

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With more than 4 million tons annually Whiteleg shrimp (*Penaeus vannamei*) is the most farmed seafood species in the world. It is dominating also the global shrimp production as it is much easier to farm than other species, which also makes this species suitable for indoor land-based farming. The popularity of shrimp reflects the demand for healthy and convenient food, which is consumed all over the world with versatile preparation methods. Due to the environmental and social challenges of the shrimp industry in Asia and South America and an increasing awareness of stakeholders in importing countries about it there is a rising demand to use new technologies. However, in contrast to most finfish aquaculture several concepts have been developed over the last decades in terms of water treatment (biofloc, aquamimicry, RAS or hybrid systems) and water basin designs (round tanks, raceways, stacked shallow-raceway).

Oceanloop is an innovative RAS, which can be used as a platform technology for the farming of seafood. The concept differs significantly from conventional RAS. It is using raceways to allow a low head design and flexible basin segmentation. Flow-through movable walls can be added, removed, or changed in position easily to react on changing demands at short notice as harvest size, stocking intervals or biomass. Also, the design allows to gentle move the farmed seafood without piping until harvest. This guarantees highest welfare standards for the farmed species. The system operates without piping in the main water recirculation, which is one of the main reasons that there is no need for purging off-flavor. For farming bottom dwelling species as shrimp, which require surface area instead of water volume, Oceanloop has developed special habitats as a basin interior to also allow an efficient volumetric farming of shrimp. Due to the extremely high increase of horizontal surface area and short production cycles of 90 days from post-larvae to harvest size of 30g the annual productivity per area of water surface is 1,600t/ha meaning more than 200 times increase compared to pond farming in South America (7t/ha[a]).

Over the last 8 years the R&D works include the implementation of fast-growing genetics, from partners with most-advanced selective breeding programs; the development of a grow-out feed fitting the requirements of the species and RAS; the use of computer vision to track biomass growth, mortalities and welfare; predictive modelling of growth, feeding and stocking management and digitalized monitored standard operational routines.

[a] Resource use in whiteleg shrimp Litopenaeus vannamei farming in Ecuador - Boyd - 2021 - Journal of the World Aquaculture Society - Wiley Online Library

NOAA SEA GRANT'S CURRENT AQUACULTURE INVESTMENT PORTFOLIO

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This presentation will provide an overview of NOAA Sea Grant's current Aquaculture Investment Portfolio in support of US coastal, Great Lakes, and marine aquaculture research, education, and extension activities. The presentation will focus on the development and implementation of a "Five-Year Aquaculture Investment Plan" for consistent, reliable, and recurring aquaculture funding opportunities. The goal of the Plan, which was initiated in FY2024, is to provide predictable, but flexible funding opportunities to address issues and needs of the US aquaculture community. The various competitions that are included in the Plan will be discussed in detail, along with their anticipated scheduling.

PICKY EATERS, RAPID GROWERS: ASSESSING PHENOTYPIC PLASTICITY OF THE EASTERN OYSTER (*Crassostrea virginica*) IN DIVERSE MAINE ESTUARIES

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Aquaculture of the eastern oyster occurs across a broad range of environmental gradients along the Atlantic and Gulf Coasts of the United States. Multiple breeding programs have been developed in pursuit of developing stocks with improved growth and yield across these variable conditions. However, field trials assessing the genetic lines developed in these programs typically measure size at discrete time points on oysters haphazardly sampled from replicate grow-out cages. Estimates of growth are then derived from the average size measured at each time point for each cage, masking the tremendous variability in the growth of individuals over time. Currently, it's common practice for farmers to grade size classes and carry out large culling events on "slow growing" individuals. A better understanding of the within- and between-individual variation in oysters, and whether it results from genetic variation, environmental differences, or both, will not only reduce time and labor costs associated with grading and culling events, but contribute to the continued improvement of breeding programs.

We monitored individual growth of oysters from a single genetic cohort cultured in two Maine estuaries. Oysters were tagged in the spring (May) and shell height and whole weight was taken bi-weekly throughout one growing season (through October). We combined these measurements with monitoring of water quality parameters including chlorophyll-a concentration, temperature, turbidity, and salinity to assess individual phenotypic response. Present evidence of some growth phenotypes includes "fast" initial growers, and "slow" more consistent growers. By statistically identifying these growth phenotypes, we hope to better understand the variability in their response to changing environments.

Determining the underlying causes, mechanisms, and consequences of the capacity of a genotype to produce different phenotypes in response to environmental variation (phenotypic plasticity) and how this will contribute to the performance of stocks of the eastern oyster is crucial for the continued improvement of breeding programs. Additionally, by facilitating early identification of fast and slow growers, farmers can reduce time and labor costs associated with grading and culling events. Here, we explore several approaches to modeling individual growth to partition out phenotypic variation both within- and between-individuals and define fast and slow growth phenotypes.

POTENTIAL FOR DULSE SEAWEED *Devaleraea mollis* TO MITIGATE EFFECTS OF OCEAN ACIDIFICATION ON LARVAL OYSTERS *Crassostrea gigas* IN CO-CULTURE

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Ocean acidification (OA), driven by elevated CO_2 levels in seawater, negatively impacts economically important marine calcifiers such as Pacific oysters (*Crassostrea/Magallana gigas*) along worldwide coasts. Increased CO_2 interferes with calcification, so oysters are particularly vulnerable when forming their first shell as D-stage larvae. Hatcheries therefore struggle to meet demands for oyster seed due to OA effects on larvae, such as mortality, slow growth, and shell deformities. Hatchery cultivation of seaweed may offer an avenue for seawater remediation: through photosynthesis, macroalgae uptake CO_2 from seawater, while providing a range of important products such as nutritious food, cosmetics, and biofuels. To quantify effects and fill knowledge gaps, this study examines the potential for Pacific dulse (*Devaleraea mollis*) to mitigate OA and the harm it causes on Pacific oyster larvae in land-based co-culture.

The experimental system included three treatments corresponding to ambient (400 µatm CO_2), local upwelling (1000 µatm CO_2), and projected future upwelling (3000 µatm CO_2) OA conditions. Seawater chemistry trials showed that dulse can increase pH by 0.2-0.5 units, depending on OA condition, and can maintain elevated pH under continuous light for at least 20 days (see Fig. 1). Analysis of water samples will show how dulse affects carbonate chemistry across treatments.

Oysters were reared either with or without dulse in each of the three OA conditions. Larvae before and during D-stage at 14, 24, and 48 hours post fertilization (hpf) will be analyzed microscopically for survival, growth, and shell formation. Expression of ion regulation, shell formation, and immune related genes will be analyzed at 48 hpf, demonstrating how relative expression translates to larval condition. Findings will provide useful data for modeling the CO_2 uptake potential of dulse seaweed, applicable to industry and restoration efforts for both shellfish and macroalgae. Outcomes of this study can aid the shellfish industry in adapting to climate change while also providing insight into an avenue for crop diversification.

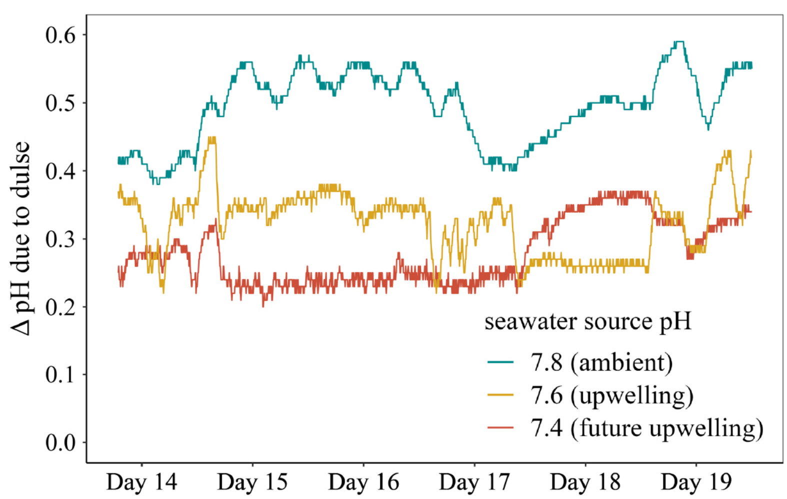

FIGURE 1. Change in pH between paired tanks, one with dulse and one without dulse, that share a water source receiving one of three quantities of CO_2 gas

DEVELOPMENT OF A CRYOPRSERVATION PROTOCOL FOR THE COLLECTOR URCHIN *Tripneustes gratilla*

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The collector urchin, *Tripneustes gratilla*, is an ecologically important member of the grazing community of Hawai'i's coral reefs. Beyond its ability to maintain balance between native seaweeds and corals, *T. gratilla* has also been used as a food source and a biocontrol agent against alien invasive algae species. Due to overexploitation, habitat degradation, and other stressors, their populations face local extirpation. However, artificial reproductive techniques, such as cryopreservation, could provide more consistent seedstock throughout the year to supplement aquaculture efforts. Here, we investigated the urchin embryos' tolerance to various cryoprotectants and cooling rates to develop a cryopreservation protocol for *T. gratilla* (Figure 1). We found that using 1 M Me2SO with a cooling rate of 9.7 °C/min on gastrula stage embryos produced the best results with survival rates of up to 85.5% and up to 50.8% maturation to the 4-arm echinopluteus stage, assessed three days after thawing. Continued research could see cryopreservation added to the repertoire of artificial reproductive techniques for *T. gratilla*, thereby assisting in the preservation of this ecologically important urchin, all while augmenting aquaculture efforts that contribute to coral reef restoration.

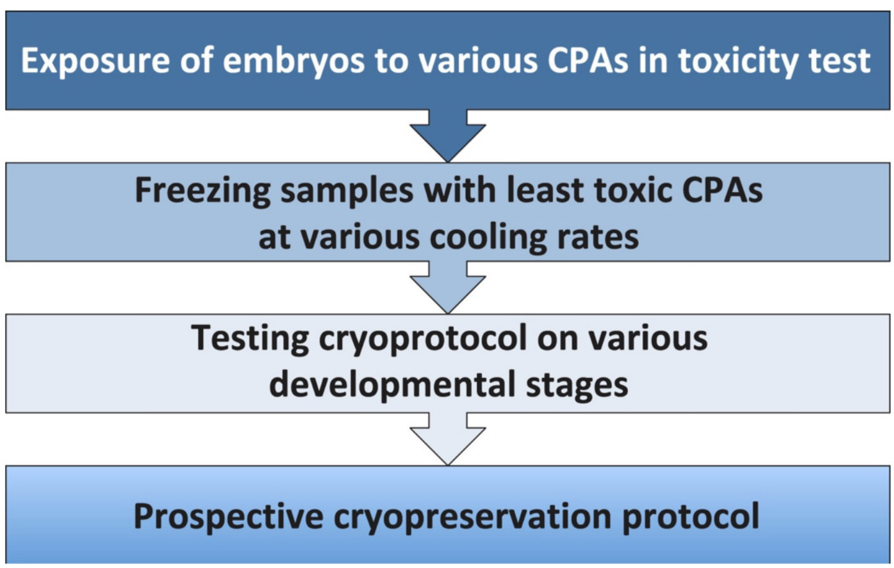

Figure 1: Flowchart illustrating experimental workflow used to identify an optimal cryopreservation protocol for T. gratilla.

ASSESSMENT OF STRATEGIES TO REDUCE THE RISK OF CONTAMINATION OF OYSTER SEED AND PREVENT INTRODUCTION OF HUMAN PATHOGENIC *Vibrio parahaemolyticus* INTO UNAFFECTED AQUACULTURE AREAS

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Oyster aquaculture is a growing industry in New Hampshire (NH) where the introduction of *Vibrio parahaemolyticus,* especially the hypervirulent strain ST36*,* via movement of oyster seed from affected harvest areas is a serious concern. The continued absence of documented illnesses associated with NH product informed policies that restrict seed importation from areas with a history of illnesses. However, this policy remains controversial due to limited knowledge of whether seed is at risk for *V. parahaemolyticus* contamination, and longstanding presumptions that pathogens will eventually arrive from areas near NH, regardless of human efforts. The policy also limits options for growers in attaining seed of greater diversity. If oyster seed treatment prior to transplantation in NH growing areas could purge pathogens this could safely expand seed access. Therefore, we undertook these studies to 1) document the extent that oyster seed is, or is not, at risk for contamination, 2) examine how the oyster microbiome and *V. parahaemolyticus* composition develop upon transplantation in local aquaculture areas to document whether existing *Vibrio* populations persist, and 3) define whether on-shore simulated relay can reduce pathogen load and/or influence susceptibility to contamination as a potential safety measure. These studies revealed that low levels of *V. parahaemolyticus* were rarely detected in hatchery seed, hatchery water treated to reduce microbial contamination, and algal feed, reinforcing that hatchery seed poses little risk when their source water has low resident pathogen populations. When this seed was acclimated to the Great Bay Estuary (GBE) in NH, the patterns of *V. parahaemolyticus* mirrored that of local GBE oysters suggesting they readily reflect local conditions rather than maintain the composition at the time of transplantation. Analysis of *Vibrio* composition following simulated relay in a full factorial design of water source, water temperature, and water salinity indicated that temperature, water source, and their interaction are the primary drivers of abundance, acquisition, and retention of *V. parahaemolyticus*. The combination of natural offshore, low temperature and relatively higher salinity sea water was the best relay method for reducing *V. parahaemolyticus* levels, as well as preventing *V. parahaemolyticus* acquisition and retention in hatchery juveniles once challenged with inoculum after treatment. Combined, these data suggest that if levels of *V. parahaemolyticus* are low, such as is expected from hatchery seed that has not resided in nurseries and untreated water, and with the potential aid of simulated onshore relay to higher salinity, cooler water, with low abundance of *V. parahaemolyticus* as a pre-treatment before transplantation, the risk of contamination, and re-acquisition are low. If our ongoing studies with larger oysters' seed from nurseries and untreated water indicate that onshore relay can assist with purging populations and restricting re-acquisition, this could expand options for seed importation while limiting the risk of introduction of hypervirulent ST36 or other emerging pathogen lineages.

FEDERAL CROP INSURANCE EFFORTS AND IMPROVEMENTS

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The United States Department of Agriculture's (USDA) Risk Management Agency (RMA), created in 1996, serves America's agricultural producers through effective, market-based risk management tools to strengthen the economic stability of agricultural producers and rural communities. RMA is committed to increasing the availability and effectiveness of Federal crop insurance as a risk management tool.

RMA offers a range of crop insurance programs for aquaculture producers. Of the available programs is a program for shellfish growers that was implemented for the 2024 crop year. Since its implementation, RMA has made improvements to it. RMA also has been working closely with oyster growers in Washington state on the research and development of a new insurance product to cover their on-bottom oyster production practices. This session will include a presentation on the available crop insurance programs for aquaculture producers, the improvements to the Shellfish crop insurance program, and the efforts in Washington state.

PUBLIC PERCEPTIONS OF AQUACULTURE IN MAINE

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Public opposition to aquaculture siting has become a significant barrier to growth in Maine, creating delays and unanticipated costs that are especially onerous for beginning farmers. Coupled with this, media coverage of contentious application processes calls into question whether aquaculture has a public perception problem–a problem noted by researchers in studies around the globe. As a result, researchers have put much effort into better understanding public perceptions of aquaculture. Studies have explored what values predict support, what messages are most effective in creating positive perceptions, how people seek out information on aquaculture, and who the most trusted messengers are. Extending this work to the US state of Maine, we conducted a state-wide survey exploring residents' perceptions regarding aquaculture, including concerns and perceived benefits, along with related values. Despite some negative coverage in the press, our survey found that Maine residents overall are relatively positive about aquaculture development–particularly with regard to shellfish and seaweed farming. Interestingly, this sentiment does not vary by demographic characteristics or coastal vs. inland residency. Instead, predictors of positive perceptions are value-related factors including an individual's coastal development priorities, concerns over the growth and size of sea farms, belief that aquaculture provides new opportunities for fishermen and coastal communities, and trust in aquaculture farmers for information on aquaculture.

CLIMATE IMPACTS, OPPORTUNITIES AND ADAPTATIONS FOR THE MAINE AQUACULTURE SECTOR

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Aquaculture is a transformative adaptation strategy that has potential to support sustainability and resilience of coastal regions in the United States. However, the aquaculture sector is also experiencing impacts of climate change such as: fluctuations in crop productivity in response to changing environmental parameters, increased storm frequency and intensity, changes in frequency of pathogen occurrence in aquaculture species, and changes in incidence of public health concerns (e.g. *vibrio* and/or biotoxins). This is true in Maine, where warming waters and increased storm intensity are pushing aquaculture farmers to make changes in their growing practices. To increase industry resilience, the Maine Aquaculture Innovation Center and the University of Maine's Aquaculture Research Institute are working collaboratively on a new project to better understand climate impacts and adaptations experienced by farmers, what they are doing to adapt, and barriers and enabling conditions related to adaptation. This project uses mixed methods, including interviews with farmers, and an industry-wide online survey. The results will inform the Maine Aquaculture Industry Climate Resilience Plan, to be released in 2025.

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A COMMUNITY OF PRACTICE APPROACH TO TACKLING DEI&A IN THE MAINE AQUACULTURE INDUSTRY

Emily Whitmore* and Members of the Maine DEI&A Working Group

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Prioritizing diversity, equity and inclusion is essential for continued growth and sustainability of the aquaculture industry. In Maine, many efforts are underway that have pushed this priority forward, including the 2024 Women in Aquaculture Series, ongoing "Exploring Careers in Aquaculture" courses that are tailored to the interests of students from targeted underrepresented communities, and most recently, a collaborative project with farmers and industry organizations to develop a Women and Non-binary in Aquaculture Network.

These initiatives are the result of an enthusiastic group of individuals and organizations that see the value in a diverse, equitable, and inclusive sector, and who frequently work together to push these initiatives forward. As a result, we have established a DEI&A Working Group in Maine to better coordinate efforts, share challenges and successes, and establish future priorities. The working group meets monthly, sharing updates and reserving time to "deep dive" into topics built into the agenda. Operating under a co-developed working agreement, the meetings have become a valuable space for members. This presentation will provide an overview of the DEI Working Group, and will show how communities of practice can be an important resource for people working in the DEI space.

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SCIENCE AT THE FARM: MEASURING ENVIRONMENTAL DRIVERS AND INTERACTIONS AT SEAWEED AND SHELLFISH FARMS IN ALASKA

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The Mariculture Research and Restoration Consortium project brings together researchers from the University of Alaska Fairbanks, state and federal agencies, tribal and scientific entities with nine shellfish/seaweed farms throughout three regions of coastal Alaska. This large interdisciplinary project is measuring environmental drivers and examining the ecological effects of aquatic farms through various lenses from the physiochemical properties of the water column to the interactions with invertebrate, fish, avian, and marine mammal communities. We are studying farm productivity across this large geographic gradient using standardized growing methods and a research-scale array with a suite of environmental sensors. One of the highlights of the project is how academic and agency scientists are working alongside aquatic farmers to support their information needs. In turn, farmers are collecting and submitting samples and data that contribute to addressing a suite of questions about how farms are influenced by and interact with their environment. This research project will provide valuable insight to inform site selection of future farms and to inform future farming practices, with the ultimate goal to support an ecologically and economically sustainable mariculture industry in coastal Alaska.

HARNESSING THE PYTOCHEMICAL PROPERTIES OF ETHYL CAFFEATE FOR MODULATING THE ANTIMICROBIAL ACTIVITY, THE IMMUNE RESPONSE IN SYSTEMIC AND MUCOSAL ORGAN, AND DISEASE RESISTANCE AGAINST *Vibrio parahaemolyticus* in HYBRID GROUPER (*Epinephelus Fuscoguttatus* ♀× *Epinephelus Lanceolatus* ♂)

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Ethyl caffeate (EC) is a naturally occurring compound found in *Bidens pilosa* with a range of health-promoting effects, such as antibacterial activity and antioxidant properties. Hybrid groupers have rapid growth rates, strong disease resistance, and short production cycles. However, nowadays, the intensification of hybrid grouper farming has led to poor water quality, with the main bacterial disease being *Vibrio* sp. This study evaluated the effect of EC on the antimicrobial activity against *Vibrio* sp., the nonspecific immune responses in vitro and in vivo, as well as the challenge test against *Vibrio parahaemolyticus*. The EC concentration range of 5–50 µg/mL was able to inhibit the growth of *V. parahaemolyticus*, *V. alginolyticus*, and *V. harveyi* with values of 19.7 ± 0.56, 19.3 ± 1.53, and 20.6 ± 1.53 mm. Moreover, The EC can potentially increase the metabolite seconder activity of beneficial bacteria; for instance, when combined with LAB and *P. acidilactici*, it can completely kill the *Vibrio* sp. at 6 hours incubation time. The EC has been proven to have no toxic effect based on MTT assay in head kidney leukocytes at a concentration of 0.5-10 µg/mL and has antioxidant activity that is able to enhance ROS production and phagocytic activity. At the transcriptional level, EC supplementation can upregulate the activity of TOR, NF-kB, and Nrf2 signaling pathway-related genes. The EC supplementation of the diet increased the immune activity on the systemic and mucosal organs after post-challenge with *V. parahaemolyticus*, resulting in a higher survival rate. Further research is needed to determine whether EC supplementation can support growth performance.

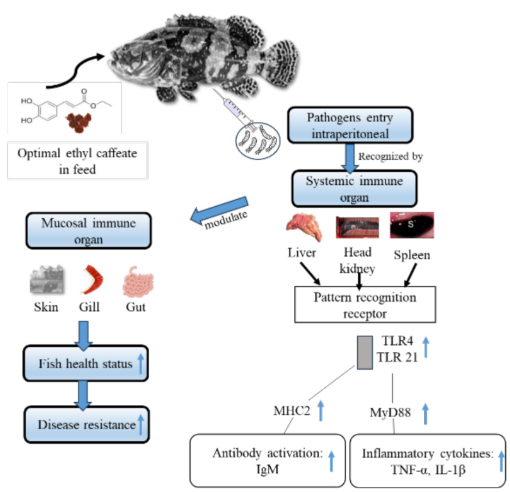

Figure 1. The potential action pathways of supplemental ethyl caffeate regulated systemic and mucosal of hybrid grouper post challenge against *V. parahaemolyticus*.

STRATEGIC PRIORITIZATION FOR RESEARCH AND DEVELOPMENT OF CANDIDATE MARINE FINFISH FOR AQUACULTURE DEVELOPMENT

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Expanding aquaculture in the United States can help meet the increasing demand for seafood alongside traditional fisheries while minimizing environmental impact. Currently, over 85% of seafood consumed in the U.S. is imported, much of it from foreign aquaculture, highlighting a missed opportunity for local growth. By fostering domestic marine aquaculture, the U.S. can reduce its $24.2 billion seafood trade deficit and create opportunities for economic growth. In 2021, a special issue of the *Journal of the World Aquaculture Society* presented review articles that provided an in-depth look at marine fish candidate species and developed a roadmap to help researchers, industry members, funding agencies, investors, and other stakeholders prioritize commercialization opportunities. The contributing authors assessed the scientific, technological, and commercial readiness of these candidate species. Since then, NOAA Fisheries has led an initiative to strategically identify top marine finfish species for commercial aquaculture development in the U.S., narrowing a list of 21 species to three to five priority species, which will become focal points for the NOAA Aquaculture Program. Through collaboration with USDA, Harbor Branch Oceanographic Institution, and partners in academia and industry, NOAA has evaluated species based on technology readiness, agency capabilities, knowledge gaps, and economic potential. Species currently under consideration include (alphabetical): Almaco Jack (*Seriola rivoliana*), Black Sea Bass (*Centropristis striata*), California Yellowtail (*Seriola dorsalis*), Cobia (*Rachycentron canadum*), Florida Pompano (*Trachinotus carolinus*), Red Drum (*Sciaenops ocellatus*), Sablefish (*Anoplopoma fimbria*), Striped Bass (*Morone saxatilis*), and Tripletail (*Lobotes surinamensis*). This presentation will highlight NOAA's species prioritization initiative, which aims to establish a foundation for sustainable marine finfish aquaculture in the U.S., addressing both short- and long-term goals for industry growth and sustainability.

PARTNERING WITH NOAA: COOPERATIVE RESEARCH, INNOVATION, AND TECHNOLOGY TRANSFER

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NOAA's Office of Aquaculture actively engages with industry, universities, and other research organizations to drive sustainable aquaculture through innovation and strategic research partnerships. In coordination with NOAA's Technology Partnerships Office, the Technology Transfer Program manages a portfolio of patents, licensing agreements, and Cooperative Research and Development Agreements (CRADA) that enable rapid and effective technology transfer, bringing NOAA's innovations directly to market. These partnerships allow NOAA to contribute expertise, specialized facilities, and intellectual property, fostering collaborative development of impactful aquaculture technologies. This talk will provide an overview of partnerships with NOAA, including how to identify research partners, select the appropriate CRADA structure, assess funding requirements, and how to collaborate with NOAA's Office of Aquaculture to accelerate innovation, develop impactful technologies, and address the evolving needs of the U.S. aquaculture industry.

CREATURES OF HABITAT: ANALYSIS OF FISH RECRUITMENT IN NEARSHORE AREAS OF ALASKA'S INSIDE PASSAGE

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Researchers use a variety of methods to analyze fish population recruitment in nearshore habitats around the globe, each with their own benefits and weaknesses. Originally developed by Amman (2004), the Standard Monitoring Unit for the Recruitment of Fishes (SMURF) is a device that allows for sampling of fish recruits in nearshore habitats in a way that is cost-effective and passive. Considering the recent progression of Alaska's aquaculture industry, we deployed SMURF devices on mooring lines and used nets for SMURF collection at kelp aquaculture sites, setting the groundwork for a longer study to understand how kelp farms influence fish species diversity and abundance throughout the kelp harvest cycle. We deployed SMURFs near the surface and at depth in a kelp farm site and similar control site. After three days, we retrieved the SMURFs and identified and measured the fish species inside, while also conducting underwater visual surveys of fish and invertebrate species to collect data on biotic communities in the area before kelp is planted. This process will be repeated after kelp is planted, while kelp is growing, and again after the kelp has been harvested. In the pre-kelp phase of this study, SMURFs found small invertebrates but no fish, while visual surveys found a variety of benthic fish and invertebrate species. These early results offer a foundation from which to view the seasonal habitat provisioning impacts of kelp aquaculture, especially on species of commercial and regulatory importance, in Alaska's Inside Passage.

MONITORING SUSPENDED SEDIMENT PLUMES INDUCED BY REMOTE OPERATED VEHICLE SURVEYS WITHIN AN ON-BOTTOM OYSTER LEASE

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On-bottom oyster aquaculture in Maryland, USA has witnessed record production and revenue within recent years. With the oyster industry's growth and continued use of antiquated equipment from the early 1800s, there is an increasing need for advancement of culturing technologies that improve yield while reducing working hours and environmental impact. Remote operated vehicles (ROV) are increasingly being used to aid a variety of agricultural operations. Similarly, there is growing interest in utilizing ROV to provide oyster farmers with critical crop information, possibly improving their management and yield for greater profits.

The use of ROV to collect data is far from optimized and environmental conditions may constrain how they are used to collect data. For example, under low visibility, ROV pilots may want to position the system near the sediment-water interface of oyster leases where the animals reside to use optical sensors. However, ROV have been observed to disturb the bed and transport overlaying sediments while surveying on-bottom oyster leases, creating sediment plumes that diminish water quality and reduce light penetration. These plumes have the potential to negatively affect organisms, especially if the concentration of sediments is high and sustained for long periods of time. Importantly, the plumes may impede collection of image and water quality data, undermining their intended use. Research is needed to understand ROV impacts to aid their use and adoption within the aquaculture industry.

Our broader research team has developed sensing procedures for ROV to survey crop inventories, substrate characteristics, and water quality within on-bottom lease. We monitored sediment plumes created by a ROV collecting this crop/lease information. A DJI Phantom 4 unmanned aerial vehicle with a Micasense RedEdge MX Multispectral camera captured near-infrared (NIR) imagery of reflectance changes caused TSS presence at the water surface and a Nortek Signature1000 acoustic doppler current profiler to monitored backscatter intensity as an indirect indicator of TSS concentration throughout the water column. Together, we were able to measure changes in TSS caused by ROV induced sediment plumes before, during, and after impact. The resulting NIR imagery was used to create predictive maps of TSS throughout the water surface. Backscatter measures were used to create a time-series and 3-dimensional maps of TSS in the water column. Then TSS values within surface images, time series, and 3D maps were compared before, during, and after impact individually through analysis of variance. Information gathered from this study can be used to optimize ROV development for aquaculture applications to reduce water quality impact and improve sustainability measures of such technologies.

IMPROVING SOCIAL DIMENSIONS IN THE SEAFOOD SECTOR THROUGH THE PROMOTION OF GENDER EQUALITY

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While 50% of the global seafood workforce is women, women and genderqueer people are significantly underrepresented in management and decision-making roles. Further, women and genderqueer people's contributions to the sector are often ignored, unseen, and unacknowledged. Seafood and Gender Equality (SAGE) promotes gender equality in the global seafood sector, leading to a more resilient industry, thriving communities, and abundant resources for future generations. SAGE's flagship Gender Equality Dialogues (GED) program promotes innovation and workforce development in the sector by co-creating space for industry leaders to develop actionable gender equality commitments. The GED, led by SAGE's Director of Gender Strategies Becca Williams, completed a pilot cohort earlier this year. In this session, Rebecca will discuss the key takeaways from the pilot cohort, including the collaborative process for developing robust public-facing gender equality commitments.

The success of the GED can serve as a starting point for the sector to integrate gender and social equity into programs and operations. Specifically, the GED underscores the necessity of seafood companies and non-profit organizations applying a gender lens and adopting internal facing gender equality measures. It is not enough for seafood companies and organizations to apply this gender lens only when looking down their supply chain. In order for these adaptations to have long-lasting impacts, the industry itself must first do the difficult work of making strides internally toward gender equality. Further, many seafood companies and organizations are working to achieve SDG 14, Life Under Water. The UN has stipulated that gender equality is a necessary precondition for realizing all the sustainable development goals. Notably, gender is not integrated into SDG 14, which makes this work all the more critical. In addition to building and retaining a diverse and inclusive workforce, the promotion of equity within the seafood sector has significant implications for understanding and improving social and gender conditions, while also serving as a catalyst for mitigating the impacts of climate change, for decades to come.

PRODUCTION AND FIELD EVALUATION OF EASTERN OYSTERS *Crassostrea virginica* GENOMICALLY SELECTED FOR ENHANCED GROWTH AND DERMO RESILIENCE FOR COASTAL BREAKWATER RESTORATION

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The DARPA Reefense program seeks to create self-repairing, hybrid reef-mimicking structures to protect both civilian and Department of Defense infrastructure along coastlines. An adaptive biology component of this effort focuses on genomic selection to boost oyster resilience against dermo disease by 20% and enhance growth rates by 15%. Initial broodstock oysters were sourced from wild populations in St. Andrew Bay, Florida, and an existing aquaculture line representing populations from Louisiana, Alabama, Florida, and Texas (LAFT). These oysters were subjected to laboratory dermo challenges for both phenotypic and genomic selection, with genomic estimated breeding values (GEBVs) generated from the relationship between single nucleotide polymorphisms (SNPs) and dermo resistance. The Florida broodstock was used to produce a genomically selected line using the top GEBVs, a phenotypically selected line using traditional phenotypic performance, and a genomic control using oysters with average GEBVs. A fourth genomically selected line was produced from the LAFT line. All lines were deployed at sites in Alabama, Louisiana, and Florida, but the LAFT line was not permitted in Florida due to regulatory restrictions. Growth and survival was monitored monthly and dermo infection assessed quarterly. Growth varied among sites, but not among lines except the LAFT line generally grew faster as the only F1 line selected for faster growth. Field survival varied among sites related to differences in environmental condition, stock origin, and dermo pressure. In contrast, lab-based dermo challenges clearly indicated that the genomically selected FL oysters displayed higher dermo resistance compared to controls, while the LAFT line showed the least resistance. Results from the lab challenge and growth metrics informed a second round of GEBV calculations that were used to select broodstock for F2 production. In the F2 generation, the LAFT line was replaced with a wild control using oysters collected from the original St Andrew Bay population and is currently undergoing the same field challenges. Lab challenges to dermo were completed in July 2024 and demonstrated significantly greater survival in selected lines ranked as follows: genomically selected (79%) > phenotypically selected (51%) = genomic control (43%) > wild control (22%). Although field trials are not complete, lab challenge results were used to inform the selection of an F3 generation. Field performance is confounded by many other factors affecting survival in addition to disease yet results clearly indicate the improved performance provided by genomic selection.

USING UNOCCUPIED AERIAL SYSTEMS TO MONITOR ALGAL BLOOMS ACROSS SEASONS IN CATFISH AQUACULTURE PONDS

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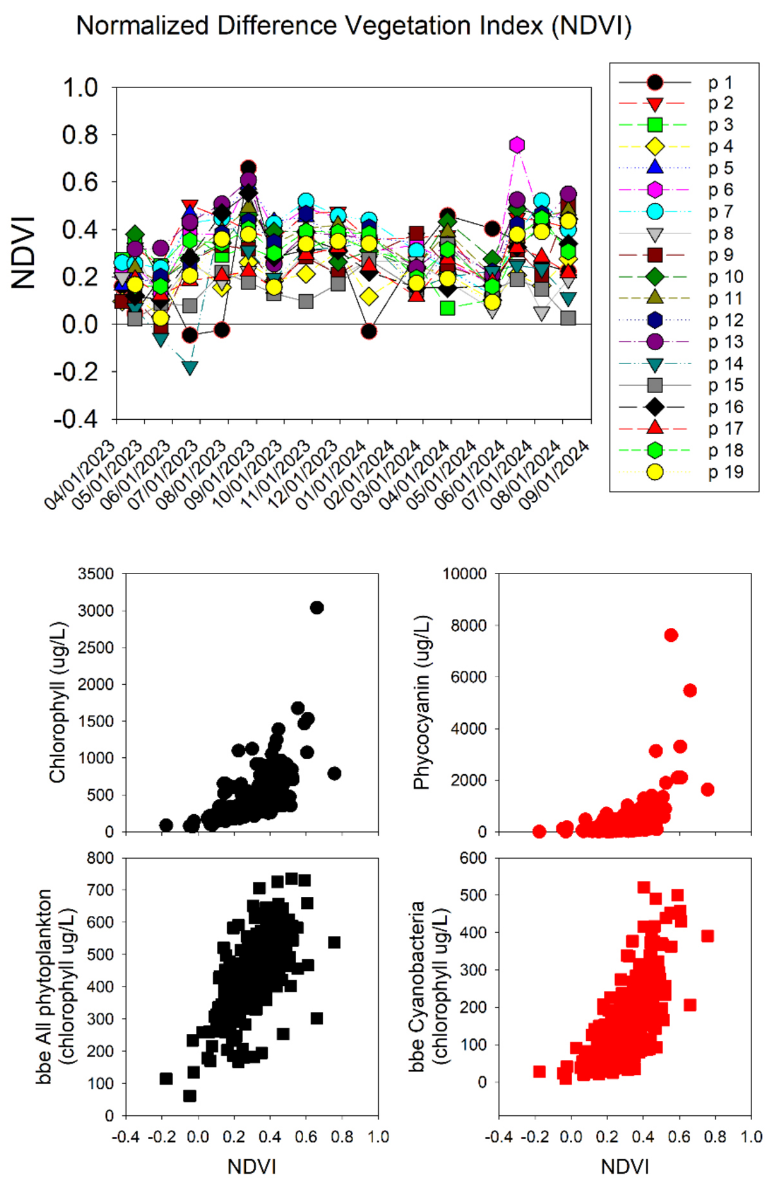
Predicting blooms of noxious cyanobacteria is critical for the protection and management of our freshwater resources. Although many tools and approaches exist for water quality monitoring, the integration of unoccupied aerial systems (i.e., drones) with geographical information systems (GIS) has opened the doors for relatively large-scale and rapid detection of cyanobacteria of small waterbodies when compared to standard lakeside sampling and satellite-based remote sensing methods. To evaluate the utility of using drones to monitor water quality in hypereutrophic ponds where phytoplankton are generally abundant and cyanobacteria dominate during the warm growing season, we conducted missions with an eBee Ag drone with a Duet M camera over 19 aquaculture ponds at one farm each month from June 2023 to August 2024 while simultaneously collecting water quality data for algal pigments (chlorophyll; all phytoplankton) and phycocyanin (cyanobacteria) and two measurements water transparency (Secchi depth and total suspended solids). Our results show strong positive correlations between the normalized difference vegetation index (NDVI) derived from drone imagery and all studied water quality parameters despite fairly large seasonal variation in slopes between NDVI and each studied water quality parameter that is likely mediated by the presence of phytoplankton deeper than the drone cameras are capable of measuring. Our results highlight the value of using drones to monitor surface water quality in aquatic systems for algal blooms that range widely in productivity but show that seasonal variation should not be overlooked considering that phytoplankton communities change in abundance and species composition over time.



NOAA FISHERIES ALASKA FY24 AQUACULTURE ACCOMPLISHMENTS

Hannah Wilson*, Alicia Bishop, James Currie, Jordan Hollarsmith, Rebecca Cates, Henry Fleener, Alix Laferriere, and Angela Korabik

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NOAA Fisheries Alaska (Alaska Regional Office and Alaska Fisheries Science Center) has continued coordinated efforts to prioritize projects and actions to support the growing Alaska aquaculture industry. We achieved a number of notable accomplishments this year in our Aquaculture Program, building off existing research and partnerships as well as starting new efforts to further our impact. This presentation provides a summary of the accomplishments for our identified priority areas.

Marine aquaculture—also referred to in Alaska as mariculture—helps support Alaska's blue economy, contributing to sustainable seafood, and local economies. Seaweed and shellfish aquaculture have been shown to provide ecosystem benefits including creating habitat for mobile fish and invertebrates species. Aquaculture also establishes economic opportunities by creating jobs, supporting eco-tourism, and other pathways.

Alaska aquaculture has room to grow, and there are many collaborative efforts underway to promote and expand this sustainable industry. This presentation will highlight a variety of projects and actions undertaken by the Alaska Regional Office and Alaska Fisheries Science Center in 2024 to support of all four goals identified in the 2023-2028 NOAA Aquaculture Strategic Plan:

- Manage Sustainably and Efficiently - Improve the regulatory processes for sustainable coastal and marine aquaculture through collaboration with partners.
- Lead Science for Sustainability - Use world-class science expertise to meet management and industry needs for a thriving seafood production sector and share this knowledge broadly.
- Educate and Exchange Information - Build awareness and support for coastal, marine, and Great Lakes aquaculture through two-way communication with diverse stakeholders and partners.
- Support Viability and Growth - Support workforce development, with a commitment to diversity, inclusion, and accessibility, based on local communities' needs, interests, and capacities.

NOAA Fisheries Alaska Regional Office and Alaska Fisheries Science Center will continue to play a significant role in the management, policy, and research that helps build a sustainable Alaska aquaculture industry. Implementing our *Aquaculture Action Plan* and *Aquaculture Research Strategic Plan* helps our agency prioritize efforts based on our agency's strengths and management and industry needs, focusing our efforts to best serve Alaska.

FEED-RELATED AND IRON-RESPONSIVE ANEMIA IN CATFISH

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Channel catfish anemia (CCA), colloquially referred to as "no blood disease" or "white-lip," is more accurately described as idiopathic catfish anemia, as hybrid catfish are also affected. Normal packed cell volumes (PCVs) in commercially raised catfish typically range from 20–40%, with higher values observed in summer. However, anemic fish often exhibit critically low PCVs of 10% or less. Clinically, affected fish display lethargy and respiratory distress under adequate dissolved oxygen concentrations. Larger, food-sized fish are most affected, with severe cases in Mississippi typically occurring in late fall and, to a lesser extent, in spring. Despite extensive efforts, the etiology of CCA remains undetermined. Feed-related anemia has been documented in channel catfish in Alabama and Georgia and possibly in Louisiana. For example, in 1983, feed-related anemia was reported on 39 of 166 catfish farms in west-central Alabama, with weekly mortality rates reaching 5%. Losses ceased when fish were temporarily fed feed from a different manufacturer. Conversely, feed-induced anemia has not been reported from feed mills in Mississippi. Instead, it is speculated that anemia observed in Mississippi catfish operations is related to iron deficiency, despite dietary iron levels exceeding NRC recommendations. In these cases, iron-fortified diets restored PCVs within 2–3 weeks and significantly reduced mortality, suggesting the condition may be related to unknown interference with iron metabolism. This presentation will explore the characteristics of feed-related and iron-responsive anemia, with emphasis on clinical observations, potential etiologies, and management strategies.

EVALUATION OF THE ROLE OF IMMUNE SERA IN PROVIDING PROTECTION AGAINST *Aeromonas hydrophila* IN CHANNEL CATFISH (*Ictalurus punctatus*)

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Virulent *Aeromonas hydrophila* (vAh) has attributed nearly $35 million dollars in economic losses within the US farm-raised catfish industry. This bacterial pathogen infects both channel and hybrid catfish causing internal and external hemorrhaging, exophthalmia and death. During an outbreak, farmers can lose over 50% of a harvest yield in less than a week, thereby increasing the urgency for more effective preventative measures. One affordable and efficacious candidate for vAh control involves the utilization of a bacterin vaccine, which is comprised of killed vAh bacteria. This approach should elicit a robust immune response while minimizing regulatory hurdles associated with other vaccine preparations such as live attenuated or modified vaccine preparations. Recently, our research team formulated and tested an oral bacterin vaccine against various vAh strains with and without the inclusion of an adjuvant. At 3 and 12 weeks, the bacterin vaccines showed protection against a Mississippi and Alabama vAh isolate and cross protection was demonstrated. Antibody presence was proposed as a potential mechanism of protection; thus the aim of the present study was to assess whether immune serum could provide protection against vAh. To evaluate the ability of immune serum to provide protection, we used 3 vAh strains (ALG-15-097, S14-452, ML09-119) to generate serum following parenteral immunization with formalin killed bacterins. Briefly, channel catfish (~20g) were IP injected and boosted at 9 weeks with formalin killed vAh strains. Fish were bled and immune serum was collected at 9 and 12 weeks post injection. Following collection of the anti-vAh serum, we conducted passive immunization in (~10 g) channel catfish with the following groups: control sera, heat-inactivated control sera, vAh immune sera, and heat-inactivated vAh immune sera. Three trials were conducted. For trials 1 and 2, fish were injected with 100µL of antisera and heat-inactivated antisera (T1: S14-452; T2: ML09-119) and were exposed with the corresponding vAh strain 24 h post-injection. The final trial evaluated cross-protection potential with the following groups: heat-inactivated S14-452 anti-serum, heat inactivated ML09-119 anti-sera, & control sera, and naive S14-452, ML09-119, & control sera then were immersion challenged using the fin clip model to ALG-15-097 (1.59 x 10^7 CFU/mL) 24 h post-injection. In all three trials, 100% protection was observed in all groups injected with anti-vAh or heat-inactivated anti-vAh sera suggesting immune serum provided protection against homologous and heterologous isolates of virulent *A. hydrophila* in channel catfish fingerlings. Results of this study help define the role of immune serum in providing protection against vAh.

PATERNAL IMPACTS ON INDUSTRY-RELEVANT OFFSPRING PERFORMANCE TRAITS USING BLUE CATFISH, *Ictalurid furcatus* CRYOPRESERVED SPERM

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As the largest aquaculture industry in the U.S., catfish farming accounts for ~75% of total U.S. finfish aquaculture production, in which the channel catfish female by blue catfish male hybrid constitutes nearly 60% of the harvest. A major bottleneck for hybrid catfish breeding is high-quality sperm production as blue catfish reach sexual maturity in 4 to 7 years, and sperm is collected through a lethal testis removal procedure. The fact that sperm can only be obtained once from males creates a substantial investment in sperm production. Great variability has been observed between male sperm quality, cryopreservation success, and offspring performance from specific sires. Therefore, a major barrier to hybrid production is obtaining high-quality male sperm. Our objectives were to (i) examine parental contributions to larval morphological development and survival during early life using cryopreserved sperm, and (ii) identify fatty acids that predict sperm quality and cryotolerance to support hatchery production.

Sperm samples were collected from 44 males. Testes were dissected, weighed, and sperm extracted. An aliquot of sperm from each male was used for evaluation of fresh quality, while another aliquot was cryopreserved. Sperm kinematics and health indices were then quantified on fresh sperm and after cryo-storage. Sperm samples were also collected for fatty acid (FA) analyses. Cryopreserved sperm were thawed, then Males 1-15 were used to fertilize Female 1, Males 16-30 were used to fertilize Female 2, and Males 31-44 were used to fertilize Female 3, creating 44 families. Hatching success was quantified and larvae reared in triplicate (n = 50/tank) from each family. Survival, weights, and morphometrics were collected from 0-40 days post-hatch.

Preliminary results show that maternal and paternal variance both significantly contributed to body size in developing larvae, with increased paternal variance (increased coefficient of variation) throughout ontogeny (Fig. 1A). Relative FA concentrations of the fresh and cryopreserved groups showed significant differences (P<0.05) for saturated, MUFA, and n-3 FA with no differences in the n-6 and PUFA (Fig. 1B). Further data analyses will be presented. Together, these data will emphasize the need for high-quality male and female gametes and will result in a complete repertoire of lipid biomarkers for sperm quality and cryotolerance.

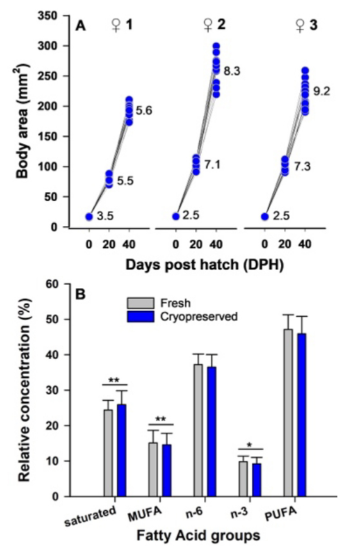

Fig 1. (A) Body area for 44 hybrid catfish families. The coefficient of variation is shown at each timepoint. (B) Fatty acids were compared between fresh and frozen-thawed sperm.

AQUACULTURE IN THE CLASSROOM: HOW SCHOOLS AND AQUACULTURE PROGRAMS CAN WORK TOGETHER FOR A SUSTAINABLE FUTURE

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Workforce development is a vital strategy for addressing the increasing demand for skilled professionals in a rapidly expanding aquaculture industry. The K-12 program at the Virginia Seafood Agricultural Research and Extension Center (VSAREC) aims to inspire and prepare students of all ages for careers in aquaculture. This initiative begins with introducing them early on in their education to aquaculture with a focus on experiential learning and interdisciplinary teaching. Through a series of engaging tours, outreach events, and in-person classroom visits students can explore the scientific foundations of aquaculture. These hands-on learning experiences showcase the scientific, technical, and business aspects of the industry from sustainable farming practices to innovative technologies driving advancement. The goal of this program is to not only expand the reach of aquaculture education but also to inspire students to consider careers in this critical industry.

Through partnering with local schools and community groups, over 4,000 students have been educated in the innovative efforts made at VSAREC to support the industry. A long-running partnership with the Virginia Governors School has now blossomed into a student-led Aquaponics project. This interdisciplinary, hands-on learning is pivotal for increasing engagement and understanding while also allowing students to explore cross-curricular subjects they may have never heard of. Through aquaponics, students can explore data analysis, graphing, food security, and resource management through a single system.

Additionally, the program fosters connections between industry professionals and undergraduates from Historically Black Colleges and Universities (HBCUs) through the Aquaculture Ambassadors internship program in partnership with Virginia Sea Grant. These internships provide students with hands-on experiences in aquaculture research, water quality management, aquaponics systems, and daily procedures at an aquaculture facility. These students are paid, and offered complementary housing to help alleviate potential financial barriers for students.

The goal of this program is to expand the reach of aquaculture education and inspire students to pursue careers in a critical field that will only continue to grow. By ensuring that students are equipped with technical knowledge and hands-on skills, the program is fostering a new generation of interested students ready to contribute to the sustainability of aquaculture. From community events, facility tours, mentorships, internships, and beyond; the growing interest in aquaculture for students will create a stream of prepared and enthusiastic individuals ready to contribute to industry’s advancements.

EARTH OCEAN FOOD SYSTEMS: AN APPROACH TO REGENERATIVE AQUACULTURE THAT SUPPORTS AQUATIC FOOD SYSTEMS FOR ECOLOGICAL RESTORATION, HUMAN HEALTH, AND COMMUNITY FOOD SOVEREIGNTY

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Earth Ocean Food Systems (“ETHOS”), founded in 2023, is a non-profit organization committed to advancing ecologically sustainable marine and freshwater food systems. With a focus on Small Island Developing States (SIDS), ETHOS conducts R&D and education to connect innovative aquaculture initiatives with funding for their food sovereignty, food justice and community engagement programming.

ETHOS’s “Hands Across the Water” is a cohort-based program that engages funding, science, and practitioner communities in interactive exploration of values-based, transdisciplinary work in aquatic food systems. ETHOS leads unique, bi-annual field-based excursions to partner sites to showcase community-based and food sovereignty-focused solutions that integrate sustainable seafood production, increase local economic activity, and are ripe for expansion or replication. As ETHOS cohorts are established, the growing global-to-local network of industry investors, researchers, and producers collectively contribute to more just, sustainable food systems. This results in immediate, local-level impacts as well as learning-communities that continually explore globally valuable regenerative food production.

ETHOS is committed to furthering research and development of values-based aquatic food systems initiatives and to amplifying stories of aquaculture development worldwide that exemplify sustainable and effective food production, ecological and economic sustainability, and social justice. The ETHOS approach introduces new models of engagement by connecting external resources with local needs through a people-to-people approach.

GROWTH PERFORMANCE EVALUATION OF IMPROVED AND WILD NILE TILAPIA FINGERLINGS UNDER POND REARING CONDITION

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The main objective of this study was to compare the growth performance and economic analysis of farmed and wild Nile tilapia fingerlings. For this fingerling were produced from four Nile tilapia strains obtained from different areas. Seventy-five mixed-sex fingerlings per strain with mean body weight 8.03±0.13 g were randomly selected and stocked in hapa in triplicates at a stocking density of 5.7fish/m3 and reared for 90 days. The fish were fed three times a day with 30% crude protein diet at a feeding rate of 5% body weight.

Body weight samples were taken bi-monthly. Five fish were slaughtered for fillet sample for approximate composition analysis. The results showed that the Farmed strain had (*P<0.05*) the highest mean values of final body weight, weight gain, specific growth rate, protein efficiency ratio, and best feed conversion ratio than the Koka and Ziway strains, but comparable with Chamo strain. The Chamo strain had (*P<0.05*) the highest crude lipid and the lowest crude protein among the tested strains. The Farmed strain also had (*P<0.05*) the highest profitable strain than the rest. In conclusion, the Farmed strain had the best performed strain with better profitability strain followed by the Chamo strain.

Table 1:Growth performance parameters of the 4 Nile tilapia strains (mean ± SE).

Parameter	Farmed	Chamo	Koka	Ziway
IMBW (g)	8.13 ± 0.15^{a}	8.20 ± 0.06^{a}	8.03 ± 0.09^{a}	7.83 ± 0.19^{a}
FMBW (g)	44.43 ± 0.41^{a}	41.53 ± 3.14^{a}	33.13 ± 1.43^{b}	24.87 ± 0.27^{c}
MBWG (g)	36.3 ± 0.41^{a}	33.33 ± 3.12^{a}	25.10 ± 1.45^{b}	17.03 ± 0.09^{c}
DGR(g/day)	0.40 ± 0.00^{a}	0.37 ± 0.03^{a}	0.28 ± 0.02^{b}	0.19 ± 0.00^{c}
SGR (%/day)	1.89 ± 0.01^{a}	1.80 ± 0.09^{a}	1.56 ± 0.06^{b}	1.29 ± 0.07^{c}
FCR	2.45 ± 0.03^{a}	2.57 ± 0.11^{ab}	3.02 ± 0.19^{b}	3.82 ± 0.05^{c}
PER	1.36 ± 0.02^{a}	1.29 ± 0.05^{ab}	1.12 ± 0.07^{b}	0.87 ± 0.01^{c}
FCF	1.7 ± 0.04^{ab}	1.78 ± 0.02^{a}	1.61 ± 0.02^{b}	1.64 ± 0.03^{ab}
SR (%)	98.7 ± 1.33^{a}	100 ± 0.00^{a}	98.7 ± 1.33^{a}	97.3 ± 1.33^{a}

Mean values in a row sharing the same superscript are not significantly different (P≥0.05)

Table 4: Economic analysis of four Nile tilapia strains (mean ± SE)

Parameters	Farmed	Chamo	Koka	Ziway
Total production cost (TC)	15.1 ± 1.9^{a}	14.1 ± 1.2^{ab}	11.4 ± 1.5^{b}	8.7 ± 0.7^{c}
Fish sale (FS)	19.4 ± 0.5^{a}	18.2 ± 0.3^{a}	14.5 ± 0.9^{b}	10.9 ± 0.4^{c}
Gross profit margin (P)	4.4 ± 0.6^{a}	4.1 ± 0.9^{a}	3.1 ± 0.2^{b}	2.2 ± 0.1^{c}
Cost benefit ratio (CBA)	1.29 ± 0.01^{a}	1.29 ± 0.02^{a}	1.28 ± 0.1^{a}	1.26 ± 0.01^{b}
Return on Investment (RoI)	29.1 ± 1.6^{a}	28.8 ± 0.1^{a}	27.6 ± 1.1^{ab}	25.8 ± 1.2^{b}

Means in each row sharing the same superscript are not significantly different (P ≥ 0.05).

COMPOSITION OF CALIFORNIA'S AQUACULTURE INDUSTRY: DISEASE MANAGEMENT STRATEGIES AND IMPLICATIONS FOR ANIMAL WELFARE

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California aquaculture is a growing industry best characterized by its diversity of production systems, cultured species, and final products. The industry comprises four major sectors (i.e., finfish, shellfish, macroalgae, and natural resource agency), which raise aquatic organisms for a variety of purposes, such as sustenance, conservation, recreation, and scientific research. Commercial aquaculture in California primarily comprises finfish and shellfish for human consumption; however, commercial producers also raise sportfish for recreational fishing, and niche seafood products like caviar. Natural resource agencies, such as the California Department of Fish and Wildlife (CDFW), are also significant aquaculture producers that raise various species of salmonids for conservation.

Regardless of the production purpose, all aquaculture producers must actively work towards maintaining the health and welfare of animals in their care. The diversity within California's aquaculture industry requires an equally diverse set of disease management strategies that address needs of each sector while maintaining adequate animal welfare. A recent survey on aquatic animal health indicates that California finfish and shellfish facility managers are aware of disease challenges and use a preventative approach to reduce the likelihood of disease outbreaks. Disease prevention strategies such as biosecurity protocols are important for maintaining animal health and welfare within the facility. Biosecurity and disease-response protocols are also important components of disease management, and their standardization across each aquaculture industry sector will enhance the disease prevention capabilities of the industry. Our survey results suggest that when disease is detected, non-prescription antimicrobial products (i.e., hydrogen peroxide) are used most frequently for disease treatment (71% commercial, 17% commercial shellfish, 86% CDFW), while prescription antibiotics are only utilized following veterinary review and approval (18% commercial finfish, 0% commercial shellfish, 71% CDFW). Overall, a comprehensive disease management plan is essential for improving animal health and for meeting the high standards of welfare that the aquaculture industry strives to maintain.

GREAT LAKES AQUACULTURE DECISION-MAKER DAYS

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The future of a sustainable aquaculture industry depends on the cultivation of an aquaculture literate public and informed decision-makers who can make educated decisions that respond to the needs and challenges of the industry. More and better-informed decision-makers have the potential to increase the viability and profitability of aquaculture businesses by:

- Reducing barriers to access capital for business development and expansion,
- Opening pathways to federal crop insurance for finfish production and expanded access to disaster relief programs,
- Reducing redundancies in the regulatory environment, and
- Increasing inclusion in state agriculture and local food marketing campaigns.

While relatively small, aquaculture in U.S. Great Lakes states raises several dozen species of finfish and shellfish in land-based systems for a diversity of markets including food, stocking/recreation, ornamentals, and bait. Here, the aquaculture industry has traditionally been left out of conversations about agriculture, potentially hampering conditions or policies that could support the advancement and growth of aquaculture businesses and markets for aquaculture products.

In 2023, Minnesota, New York, Ohio, and Wisconsin Sea Grant programs proposed piloting Great Lakes Aquaculture Decision-Maker Days as a strategy to open a seat at the table for aquaculture in the region. Each state was charged with organizing their own single-day event, visiting at least one fish farm and inviting a combination of legislators, regulators, non-regulatory agencies and support organizations, educators (including Extension), and aquaculture producers as participants. While Sea Grant programs acted as conveners and hosts, these events provided a forum for producers to talk about what aquaculture is, why they farm aquatic plants and animals, the impacts of their businesses, and open discussions about the opportunities and challenges of the industry.

We will share details and lessons learned about the planning process, examples of program materials provided to participants, similarities and differences between these state-based events, short-term outcomes, and plans for continuing to facilitate the building and sustaining of relationships for longer-term impacts.

WILLINGNESS TO PAY FOR R.A.S. ATLANTIC SALMON BY THE U.S. CONSUMER

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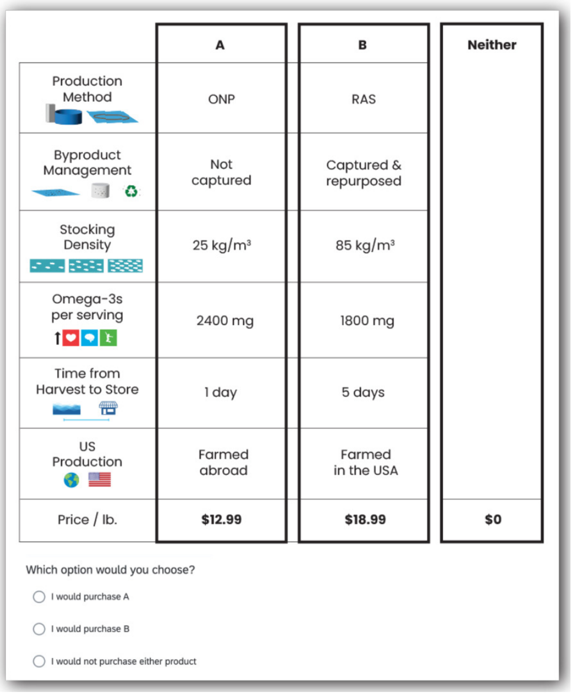
The marine forms of omega-3s have been strongly evidenced to decrease the risk of cardiovascular disease, the leading cause of death in the United States. One of the richest sources of EPA and DHA in the U.S. diet is farmed Atlantic salmon, which has shown a steady consumption growth in the past decade. The U.S. shows the most production potential for farmed salmon produced with an emerging production method, recirculating aquaculture systems (RAS), which directly addresses the environmental challenges associated with the traditional farming method using open-net pens. However, little is known about whether U.S. consumers are willing to pay the premium required to produce RAS salmon. We conduct a hypothetical choice experiment across all regions of the U.S. (n = 2857) to estimate consumers' willingness-to-pay for RAS and other attributes valued by producers and consumers. Our results show that U.S. consumers are willing to pay a 15% premium for RAS, but only after prioritizing freshness, U.S. production, and omega-3s. Byproduct recycling and stocking density did not significantly influence decisions. These WTP estimates can be used to understand the economic viability of RAS and illuminate the path to EPA and DHA nutrient security in the U.S.



	A	B	Neither
Production Method	ONP	RAS	
Byproduct Management	Not captured	Captured & repurposed	
Stocking Density	25 kg/m³	85 kg/m³	
Omega-3s per serving	2400 mg	1800 mg	
Time from Harvest to Store	1 day	5 days	
US Production	Farmed abroad	Farmed in the USA	
Price / lb.	**$12.99**	**$18.99**	**$0**

Which option would you choose?

- I would purchase A
- I would purchase B
- I would not purchase either product

EFFECTS OF NIGER SEED CAKE *Guizotia abyssinic* INCLUSION IN FISH FEED AS A MAJOR PROTEIN SOURCE ON ANION DYNAMICS IN NILE TILAPIA *Oreochromis niloticus* LETTUCE AQUAPONICS SYSTEM

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Aquaponics systems integrate fish and plant culture, relying heavily on nutrients derived feed, typically fish meal, which is expensive and scarce. This study investigates the use of Niger seed cake as a replacement for fish meal and its impact on anion dynamics within a Nile tilapia-lettuce aquaponics system. Five experimental treatments were tested. Overall, the study demonstrated that Niger seed cake can effectively replace fish meal in aquaponics systems with minimal impact on anion dynamics, making it a viable, sustainable alternative protein source.

Fishmeal is a good protein source that could help the performance of fish because it has high protein source. But, it is very expensive to afford for developing countries like Ethiopia (Akewake Geremew et al, 2015). So, replacement of fishmeal with cheaper ingredients of plant origin in fish feed is necessary. Niger *Guizotia abyssinica* belongs to the family *Compositae* and the genus *Guizotia*, has only six species, of which five are native to Ethiopia (Baagoe, 1974). It is one of the sources of edible oil. The waste after oil extraction is free from any toxic substance and contains approximately 30% protein and 23% crude fibre (Getinet and Sharma 1996). According to Rumsey (1993), increased use of plant protein supplements in fish feed can reduce the cost of fish meal. According to Abebe Tadesse (2017), the proportion of Niger seed cake should not be more than 45%.

Table 1 Proportion of ingredients in the formulated feed (Abebe Tadesse, 2017)

Ingredients (gKg-1)	Treatment				
	Ctrl	TA	TB	TC	TD
Niger cake		125	250	375	425
Fishmeal	550	425	300	175	125
Meat bone meal	150	150	150	150	150
Wheat bran	100	100	100	100	100
Wheat grain	150	150	150	150	150
Limestone	7	7	7	7	7
DiCalcium phosphate	10	10	10	10	10
Vitamin-mineral premix 1	3	3	3	3	3
Fish oil (mlkg-1)	2	2	2	2	2
Soya bean oil (mlkg-1	20	20	20	20	20

TIDAL HEIGHT IMPACTS ON THE COMMERCIAL SHELF-LIFE AND STRESS RESPONSE OF TWO CLAM SPECIES ENDEMIC TO CALIFORNIA *Leukoma staminea* AND *Tivela stultorum* AND THE INTRODUCED JAPANESE "MANILA" LITTLENECK *Venerupis philippinarum*

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California is the leading consumer of seafood in the U.S. and relies on the aquaculture industry to provide consistent, high quality farmed shellfish. Aquaculture in California is focused on the non-native Manila littleneck clam (*Venerupis philippinarum*), a species with established markets and a suitable shelf-life – i.e., time a clam remains edible after leaving the farm. Shelf-life duration is an important factor in determining the commercial viability of a species, and it can be influenced by grow-out conditions, harvest methods, and post-harvest storage or transportation conditions. In the current study, we aim to assess the viability of commercially culturing two of California's endemic clam species - the Pacific littleneck clam (*Leukoma staminea*) and the Pismo clam (*Tivela stultorum*) - on a commercial shellfish farm in Morro Bay, California. Specifically, we are investigating whether the intertidal height at which clams are out-planted on a farm (i.e., low vs. high intertidal) influences shelf-life or environmental stress tolerance in these two species of endemic clams, with a focus on comparison to the commonly cultured non-native Manila littleneck. To this end, we conducted a baseline determination of shelf-life among the three species of clams following adjustment to common garden conditions at the Cal Poly pier. Clams (n=30/species) were held in either 4˚C air emersion (to imitate farmed shellfish transport and storage conditions) or 15˚C seawater submersion (control). Individuals were then monitored daily for survivorship until all endemic clams died, and the LT_{50} (time to 50% mortality) could be determined for each species. To date, we have found that native Pacific littleneck has an LT_{50} of 9 days, whereas the non-native Manila littleneck has an LT_{50} of 23 days. Pismo clam data collection is ongoing. In addition to shelf-life determination assays, we harvested hemolymph tissues from each of the three species of clams (n=18/species) following 72h of emersion stress. We will use these samples to compare baseline immunocompetence between the three species via measurements of total hemocyte counts (THC) and plasma lysozyme activity, bacteriolytic elements central to the innate immune response of invertebrates. Following these baseline assessments, we will outplant clams from each species at two tidal heights (+2 MLLW and +0 MLLW; n=120 clams/species/tidal height) and track growth and survival across a 6 month monitoring period. At the end of this grow-out period, these clams will be used to evaluate whether intertidal position has an effect on shelf-life or environmental stress tolerance (measured as survival during exposure to 4˚C air emersion or to combined heat stress and hypoxia) in any of these species. Understanding the factors that impact shelf-life and environmental stress resilience in emerging native clam species is crucial for informing aquaculture practices, and the data we collect will be directly disseminated to local and regional shellfish farmers through existing relationships.

AUTONOMOUS NAVIGATION AND INSPECTION IN AQUACULTURE: RECENT BREAKTHROUGHS AND FUTURE DIRECTIONS FOR ROBUST OPERATION

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Aquaculture is an industrial sector of immense growth, with the production taking place further from the coast, in more exposed areas, in larger cages, and with more biomass. Underwater autonomy is at the forefront for maintaining this growth in the near future in a sustainable way, by improving employee and environmental safety, as well as efficiency. Fundamental problems, for autonomous aquaculture operations involving underwater robots, include navigating to target positions and inspecting target structures with strong safety guarantees. In a domain with poor sensing conditions, disturbances, currents, dynamic and deformable surroundings, and uncertain sensing and controls, most state-of-the-art methodologies struggle immensely.

In SINTEF Ocean, having experience with the challenges of underwater robotics, especially in the context of industrial scale fish farming in Norway, we have focused on addressing the fundamental problem of robust autonomy. Our goal is to both provide novel general methodologies that scientifically improve the state-of-the-art in multiple fronts, and adapt solutions tailored to aquaculture industrial problems. The past years we have developed motion planning solutions that address a subset of important challenges that enable robots to move and monitor their surroundings with efficiency and strong safety guarantees, under the presence of dynamic and deformable obstacles, unpredictable disturbances, highly uncertain motion and localization capacity, including the intersection of all aforementioned challenges.

Moreover, while considering conventional robotic or remotely operated platforms used in fish farms currently (Fig. 1), we develop novel solutions and methodologies that enable safe operations of bioinspired articulated robotic platforms (Fig. 2); an emerging trend that may offer new capacity for livestock monitoring and behaviour understanding.

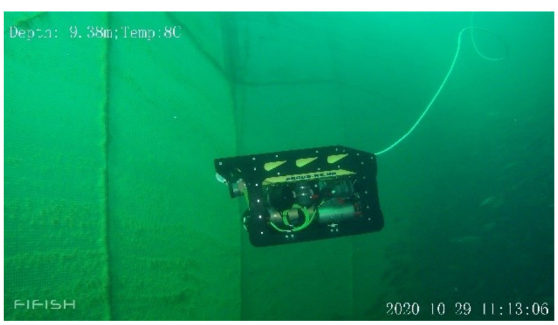

Figure 1: The ArgusMini, a conventional ROV inspecting aquaculture infrastructure.

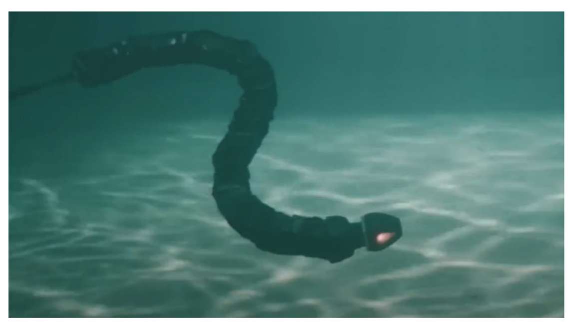

Figure 2: The Mamba, a bioinspired underwater snake robot while swimming.

THE FIRST VITRIFICATION IN NATURAL SPAWNING SPERM OF BIVALVE-DWARF SURFCLAM *Mulinia lateralis* A POTENTIAL MODEL SPECIES FOR BIVALVE RESEARCH

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Genetic improvement programs have been initiated in economic marine bivalve species to improve production and ensure the long-term sustainable development of industry. The potential for these programs will be further enhanced if the existed key bottlenecks (e.g. asynchronized spawning, short window period of natural spawning and risk of maintaining broodstock alive and healthy) could be addressed. Sperm cryopreservation has been widely acknowledged as an effective and reliable technique to address these bottlenecks. However, this technique has not been applied in bivalves. The key reason is the lack of sufficient information on the long-term fitness of resultant progenies and across generations. Investigations on performance of economic important traits and generations are time-consuming and challenging in farmed marine bivalve species due to long generation interval and/or high costs and challenges to manage in the open marine environment. All these obstacles could be solved if a model species applied.

The dwarf surfclam *Mulinia lateralis* is a dioecious marine bivalve species which has the characteristics for model species in bivalve. In order to use this species to understand the effects of bivalve sperm cryopreservation on performance of resultant progeny and/or generations, the first step is to develop a sperm cryopreservation technique. Sperm vitrification is a simple and cost-effective method which is easy to standardize in hatchery, whereas this method has not been succeeded in bivalve. Our research has firstly developed a non-programmable (commonly used in marine bivalve species) sperm cryopreservation technique in dwarf surfclam. Then, the cryodamages on sperm quality were systematically investigated by transcriptome and DNA methylation along with cellular parameters. Finally, sperm vitrification in dwarf surfclam has been successfully developed with the D-stage larval rate about 65% achieved using post-thaw sperm at a sperm to egg ratio of 1000:1.

TABLE 1. Comparison of sperm quality parameters between the control (fresh sperm) and vitrified sperm.

Sperm quality	Group	
	Control	Vitrification
D-stage larval rate (%)	81.33 ± 3.21	64.77 ± 9.65
Superoxide dismutase (U/mL)	12.04 ± 1.60	2.50 ± 0.66*
Catalase (U/mL)	6.63 ± 0.23	5.58 ± 0.62*
Glutathione (μM)	1.87 ± 0.14	0.96 ± 0.15*
Lipid peroxidation (nmol/mL)	204.35 ± 21.13	245.27 ± 7.39*
Reactive oxygen species	1612.87 ± 148.37	1890.00 ± 210.26*
Plasma integrity (%)	81.08 ± 0.66	51.42 ± 1.52*
Mitochondrial function (%)	83.04 ± 0.90	47.87 ± 1.12*
Acrosome integrity (%)	80.89 ± 0.82	49.57 ± 1.47*

BEHAVIORAL RESPONSE OF NILE TILAPIA (*Oreochromis niloticus*) TO SIMULATED AVIAN PREDATION: EFFECTS OF HYPOXIA AND FEED AVAILABILITY

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Avian predation is a natural cause of fish mortality that could affect the fish population and has led to significant economic losses in aquaculture. Most fishes actively swim to the water surface either to gulp atmospheric oxygen during hypoxia or pick pelleted feed during feeding episodes, this phenomenon increases their exposure and vulnerabilities to avian predators. Fish exhibit complex behaviors in response to avian predation risk. While chemical cues have been studied for understanding antipredator behavior, the responses of Nile tilapia to predation risk in the wild and aquaculture have received little attention. This study aimed to investigate the behavioral responses of Nile tilapia (*Oreochromis niloticus*) to simulated avian predation under varying environmental conditions, specifically focusing on the effects of hypoxia and feed availability. In this study, fifty unsexed tilapia of the same size were obtained during the trials. During the feeding trial, five different floating feeds were administered to the fish, and the time taken for them to feed was monitored using a stopwatch. The feeding trial was conducted both without predators (control) and with avian predators placed at varying distances (1m and 30cm) from the water surface. For the hypoxia trial, nitrogen gas was bubbled into the glass tank to displace oxygen until extreme hypoxia was achieved. The dissolved oxygen (DO) levels were measured at regular intervals using a multiparameter probe. The time taken to perform the avoidance response (ASR), the duration of ASR, and the number of fish performing ASR were measured in response to the avian predator under hypoxia conditions. These trials were replicated three times. The presence of an avian predator significantly affected the fish's feeding behavior, with different responses observed based on the fish's size. Additionally, the distance of the avian predator from the water surface affected the fish's aquatic surface respiration behavior. These findings highlight the intricate interplay between hypoxia, predation risk, and fish behavior, suggesting the need for further research on how the individual differences among fish groups interact and shape the predator-prey relationship.

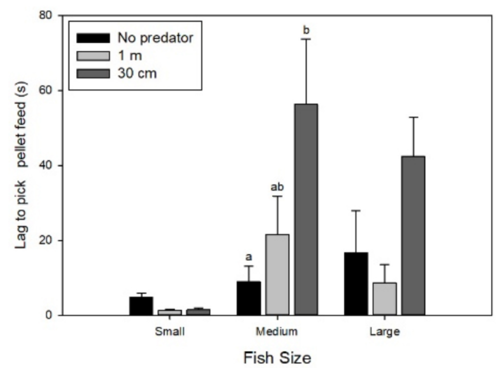

Figure 1: Lag to pick the first feed with avian predator simulated at different distances from the water surface.

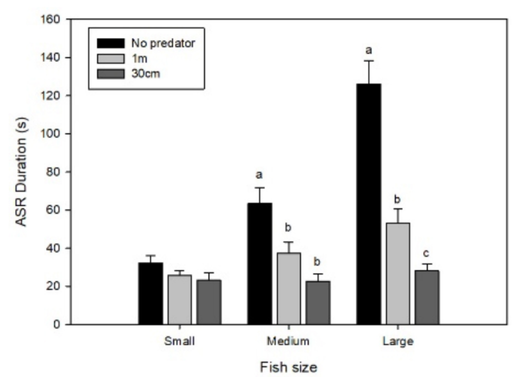

Figure 2: ASR duration of *O. niloticus* with avian predator simulated at different distances from the water surface.

EVALUATING AQUANAT SYNERGY™ *IN VITRO* AND WHEN SUPPLEMENTED IN CHANNEL CATFISH *Ictalurus punctatus* DIETS

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Essential oils and organic acids have shown the potential to inhibit the proliferation of pathogenic bacteria and modulate the fish immune system. The present study evaluated the blend of protected essential oil and organic acids [P(EO+OA); AquaNat Synergy™] *in vitro* against common pathogenic bacteria that afflict the catfish industry, and *in vivo* during a feeding trial using channel catfish juveniles. Minimum inhibitory and bactericidal concentrations (MIC and MIB) were established using the P(EO+OA) against *Aeromonas hydrophila*, *Edwardsiella ictaluri*, *E. piscicida* and *Streptococcus iniae*. Serial dilutions starting from the MIC values were tested with *E. piscicida* and *A. hydrophila* to assess biofilm formation, with *Streptococcus iniae* to evaluate hemolytic activity, and with *E. ictaluri* to quantify gene expression of virulent genes. Interestingly, concentrations lower than the MIC disrupted the normal metabolism of pathogenic bacteria by significantly reducing all the aforementioned parameters. The *in vivo* study evaluated the supplementation of the P(EO+OA) in diets for juvenile catfish at 0, 300, 600 and 1,500 mg/kg. A total of 840 channel catfish juveniles were equally distributed in 28 aquaria (30 fish/tank; n=7) operating as a recirculating aquaculture system. Fish were fed twice daily with rations corresponding to a percentage of the biomass, which was adjusted biweekly by group weighing each tank. The feeding trial was carried out for 124 days, and at the end of the study, production performance and condition indices were evaluated. Blood and intestine samples were collected for hematology, intestinal histology and expression of immune-related genes. Fish were fed their assigned experimental diets for an additional week and digesta from the posterior intestine were sampled 20 h post-feeding for DNA extraction and 16S rRNA gene sequencing on an Illumina MiSeq. The remaining fish from the feeding trial were subjected to a bacterial challenge by bath exposure using a LD50 dose of *Edwardsiella ictaluri*. No differences were observed for production performance, condition indices, expression of genes related to the antioxidant system from the intestine, and blood/plasma parameters. However, a lower expression of TNF-α and a higher expression of IL-6 was observed in the posterior intestine of fish fed P(EO+OA), 300 and 1,500 mg/kg respectively, when compared to the control group. No differences were observed for α- and β-diversity, but a higher relative abundances of bacteria from the genera *Enterococcus* and *Romboutsia*, and *Plesiomonas, Weissella* and *Shewanella* were observed in fish fed the 600 and 1,500 mg/kg of P(EO+OA) diets, respectively. In addition, a higher survivability was observed for fish fed 1,500 mg/kg of P(EO+OA) when compared to the control group after the *E. ictaluri* challenge. The commercial product AquaNat Synergy™ presented to be a promising feed additive for aquaculture by potentially decreasing virulence factors *in vitro*, as well as modulating the intestinal microbiota and immune-related genes of channel catfish juveniles during the feeding trial. In addition, highest dose of P(EO+OA) appeared to confer protection during the bacterial challenge, which shows potential to be applied in practical conditions as a prophylactic strategy when epizootic outbreaks are more likely to occur.

PERFORMANCE OF F1 GENERATIONS FROM HEAT SHOCK SURVIVORS OF NORTHERN QUAHOGS *Mercenaria mercenaria*

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Aquaculture of the northern quahogs *Mercenaria mercenaria* is an important industry in Florida and involves over 350 small family-based farms. Summer heat is a major environmental challenge for the sustainability of the industry. For aquatic organisms, water temperature is a critical factor in controlling growth, metabolic rates, and immune responses. Breeding of quahogs for heat tolerance would be an effective way to overcome this challenge and support industry development. Three current farm stocks from Cedar Key, Florida were challenged with heat shock by increasing water temperature at 1°C per day from 24°C to a final temperature of 35°C based on our pilot experiment. The survivors from the heat shock challenge were used as broodstock to produce F1 offspring generation by mass spawning. The survival and growth of the F1 generation in parallel with their control in aquaculture leases were evaluated at different stages. During six months of field nursery from September to March, no differences were found in growth between heat shock and control lines ($P \geq 0.253$, shell metrics and body weight), but the heat-shock line showed significantly higher survival than the control line (82.9% vs. 74.0%, $P = 0.017$), indicating the potential of the heat shock line. Throughout the initial six-month grow-out period, the monthly water temperatures ranged from 14.0 to 26.4□ (min 5.8□ and max 29□). During a seven-month grow-out period from March to November, no difference was found in survivals between the heat-shock line and the control, but the body sizes/weights in the heat-shock line were significantly higher than the control. After about 20 months of field culture, the F1 offspring (heat shock and control) were harvested and brought back to the laboratory for a second heat shock challenge. Comparison of immune response and survival were evaluated, and the data is in the process of analysis. The survivors of quahogs of heat shock and control lines will be used to produce F2 generations with the aid of genomic selection.

INVESTIGATING GENE EXPRESSION PLASTICITY IN PACIFIC OYSTER (*Magallana gigas*) LARVAE SUBJECTED TO OCEAN ACIDIFICATION CONDITIONS

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In the Anthropocene, human activities generating excess CO_2 emissions have resulted in ocean acidification (OA), prompting the need for new management approaches for commercial production of some marine species, including oysters. On the U. S. Pacific coast, seasonal upwelling of deep, acidified seawater forces oyster hatcheries into buffering of incoming seawater to mitigate larval production losses. In this study, we sought to quantify and evaluate changes in gene expression across early developmental stages in larvae of the widely cultivated Pacific oyster, *Magallana gigas* (quondam *Crassostrea gigas*) when exposed to OA conditions.

Larvae used in this study were hatchery produced from broodstock oysters that had been exposed to both OA conditions as larvae and heat stress as adults over the period 2019-2023. A control group consisted of sibs from the same stressed pooled families that had not been exposed to OA or heat stress conditions. The fastest growing individuals from both the stressed and non-stressed parental groups were selected for spawning. Developing embryos and larvae from these two parental groups were then exposed to either OA (7.5-7.6 pH) or ambient seawater conditions (8.0-8.1 pH) until 35-days post fertilization (dpf). We found that shell growth was negatively affected by the OA treatment regardless of parental source. At 10 dpf, progeny of stressed parents grew larger relative to offspring produced from non-stressed parents; however, less progeny from stressed parents successfully developed into spat (juveniles) at 35 dpf under both rearing conditions.

Sequence libraries were prepared from larvae produced from each parental and seawater treatment group across different developmental stages following exposure to OA or ambient seawater conditions, using Illumina paired-end mRNA-seq. Transcriptome analysis was used to investigate plasticity in gene expression to OA across developmental stages of larval *M. gigas* up to 10-days post fertilization. The results of this study could lead to the development of sensitive molecular tools to monitor the responses of Pacific oyster larvae to OA stress under both hatchery and field conditions.

CRYOPRESERVATION OF *Tetraselmis* SPECIES: DEVELOPMENT OF VIABILITY ANALYSIS AND PROTOCOL APPLICATION ACROSS SIX SPECIES

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Microalgae are essential food for the aquaculture of molluscan shellfish and larval finfish, and the species from genus *Tetraselmis* are excellent candidates due to their ease of culture, biochemical composition, and high levels of EPA, ARA, 24-meth sterols, starches, and existing lipids. This genus of microalgae is also a candidate for biofuels and pharmaceuticals due to its anti-inflammatory, antimicrobial, and antioxidant properties. Over time, microalgae cultures are susceptible to risks, such as contamination, degradation, mutation, or complete loss of cultures; Therefore, cryopreservation could be applied to preserve the germplasm in perpetuity. In our previous work, effective protocols for the cryopreservation of *Tetraselmis suecica* had been developed (*Aquaculture* 2023, 566, 739172) where neutral red was used to stain cells for viability analysis. However, due to the large number of samples involved in protocol development, staining and microscope counting for viability analysis became the biggest obstacle. Flow cytometry is an important tool for single-cell sample analysis using fluorescent dyes which could be applied to viability analysis.

The goal of this study is to cryopreserve *Tetraselmis* species with a focus on the development of viability analysis and application of the existing protocol for *Tetraselmis strata* across six *Tetraselmis* species. The objectives are to: 1) Develop an effective method for viability analysis of *Tetraselmis* species using flow cytometry and fluorescent dye of Propidium Iodide (PI); 2) Apply the existing cryopreservation protocol for *T. striata* to other species in this genus; 3) Evaluate the effect of microalgae age and lipid content on cryopreservation over 14 days, and 4) Verify the cryopreservation protocols across the six species.

Staining of PI at concentrations ranging from 0 to 80 μg/ml for 10 min indicated the effective concentration was 30 μg/ml or beyond for viability analysis, and this concentration is applicable for six *Tetrasemis* species. This study is still in progress. The quick and accurate method for viability analysis using flow cytometry and PI can speed up the post-thaw sample viability process for this project. Cryopreservation protocols to be established in this study would be useful for long-term germplasm preservation for the aquaculture industry and stock centers.

ENHANCED BIOAVAILABILITY OF BIOACTIVE COMPOUNDS OF CITRUS PULP INCREASED RESISTANCE OF CHANNEL CATFISH, *Ictalurus punctatus* TO *Aeromonas hydrophila* INFECTION

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Citrus processing sector generates tremendous amounts of by-product that contains variety of bioactive compounds such as phytochemicals, including flavonoids, carotenoids, apocarotenoids, terpenes, limonoids, and other health-promoting nutrients. However, many bioactive compounds in plant cells need to be bioaccessible and bioavailable to have any beneficial effects to the host. To enhance bioavailability of bioactive compounds, two types of citrus pulps (orange and lemon pulp) were modified by processing with either thermochemical or supermasscolloidization techniques. This study was conducted to determine the protective effect of dietary supplementation of modified citrus pulp against virulent *Aeromonas hydrophila*, one of the most economically important bacterial diseases affecting the channel catfish, *Ictalurus punctatus* industry in the United States. A 10-week study was conducted to evaluate the effect of dietary supplementation (2%) of lemon pulp or orange pulp. The study evaluated the results of two different techniques as well as the unprocessed form on growth, feed utilization, body proximate composition, hematology, serum biochemistry, immune responses and disease resistance of channel catfish (11.36 ± 0.09 g) in triplicate aquaria fed to apparent satiation twice daily. Fish fed diets supplemented with modified orange pulp treated with either thermochemical or supermasscolloidization and lemon pulp that received thermochemical treatment had significantly better survival rate against *A. hydrophile* challenge than fish fed either untreated citrus pulp or the control (not supplemented) without any negative effect on fish performance. Supplementation of both modified orange pulp significantly increased complement activity of fish compared to fish fed untreated orange pulp or control diet. However, fish fed lemon pulp that received supermasscolloidization had significantly lower weight gain, feed intake, and hematological values than fish fed control diet. Dietary supplementation of modified citrus processing waste, orange waste in particular, in aquafeeds may prove cost effective and beneficial in increasing resistance of channel catfish against bacterial infection.

MARINE INVERTEBRATE GILL MICROBIOMES: ARE THEY INFLUENCED BY THE HOST'S ENVIRONMENT OR SPECIES-SPECIFIC?

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Organisms in the Chesapeake Bay face rapid changes of salinity, water temperature, and dissolved oxygen levels in the summer months due to seasonally increased volumes of hypoxic water. We know that these environmental changes produce a large variation in microbiomes collected from the water column. However, we do not know the extent to which they alter microbiomes within host species with distinct life histories and differing salinity tolerances. Two organisms with habitats in Chesapeake Bay that tolerate salinity variability through osmoregulation are the American Blue Crab (*Callinectes sapidus)* and the Eastern Oyster *(Crassostrea virginica*). Additionally, these organisms are extremely important resources to the economy of Maryland. A few studies suggest that oyster microbiomes vary across individuals exposed to the same environmental conditions. However, we hypothesize that this may not apply to other species.

To test this, we used two species with different life histories: a sessile species (the Eastern Oyster, *Crassostrea virginica*), and a horizontally migratory species (the American blue crab, *Callinectes sapidus*). Each of these species will have their gill microbiomes undergo 16S rRNA amplicon sequencing to discover potential endosymbionts. We will examine whether the gill microbial communities extracted from the same species will be similar when samples were collected close to each other and become more dissimilar when their hosts were collected further away from each other. We expect to see a more randomized microbiome across oysters; however, we anticipate seeing a more similarities amongst gill microbiomes from the American Blue Crab since these organisms have more control over which environments they inhabit. In so far as American Blue Crabs and Eastern Oysters do not select their microbiome, we would expect to see similar gill microbiomes from hosts living in similar environmental conditions. Additionally, we anticipate dissolved oxygen to be a major factor that contributes to gill microbiome changes seen between samples extracted from the same species but in separate locations. We are currently analyzing sequence data to see if our data follows these anticipated results.

ACTIVIDAD ENZIMATICA EN EL DETRITÍVORO *Prochilodus mariae* ALIMENTADO CON DIETA DE FUENTES C_3

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La fuente de carbono en ambiente natural para detritívoros de la familia Prochilodontidae proviene de plantas C_3, como registrado en Bolivia, Venezuela y Brasil. En Colombia, el coporo *Prochilodus mariae*, especie nativa de la cuenca del Orinoco, tiene representatividad en las pesquerías mas no en cultivo, tal vez, por la fuente de carbono del alimento comercial, aunado a la actividad enzimática para aprovechar dietas enriquecidas proteicamente, lo que difiere del detrito consumido en ambiente natural.

La actividad de las enzimas digestivas fue determinada en ejemplares con peso de 126.6±38.77 g. Los peces en una jaula y durante 15 días fueron adaptados a una dieta isotópica C_3 con 34% de proteína y 4.000 kcal, suministrada a las 8:00 y a las 14 horas hasta aparente saciedad. Posteriormente, los peces fueron distribuidos aleatoriamente en ocho tanques circulares de 500 l., con recirculación y temperatura constante. La dieta se ofreció durante 30 días; una hora antes y una hora después de alimentar, se recolectaron cuatro peces por tanque/día, así durante los días 28,29 y 30. Luego de la eutanasia, tejidos de intestino anterior y ciegos pilóricos fueron almacenados en nitrógeno líquido. Para la determinación de la actividad enzimática se usó bradfford para proteína total, caseína para proteasa alcalina, kit comercial CNPGR Liquiform para amilasa y Liquiform de laboratorio LABTEST para lipasa. Las lecturas del homogenizado se realizaron en espectrofotómetro GENESYSTM 20 CIENYTEC®.

Para la proteasa alcalina se estimó una actividad de 0.042±0.06 UPA mg^{-1} de proteína total. Para la amilasa fue de 2022.971±1.7926 UA mg^{-1} de proteína total y para la lipasa fue de 2.319±1.7 UL mg^{-1} de proteína total. La actividad de la amilasa (1729±153) y de la lipasa (3.114±0.339) fue superior ($P<0.05$) en los ciegos pilóricos, mientras que en la proteasa (0.054±0.019 y 0.037±0.009) no se presentó diferencia significativa entre tejidos.

Se estableció que la amilasa fue la enzima con mayor actividad, no obstante, a nivel intestinal fue afectada por la interacción entre horario y momento respecto a la alimentación. En la mañana la actividad fue significativamente ($p<0.05$) mayor en el momento postprandial (3072) y prepandial (1777). En la tarde también la actividad postprandial (1959) fue mayor a la prepandial (1757) sin embargo dicha diferencia no fue significativa ($p>0.05$). Estos resultados pueden ser usados como insumos para la elaboración de la dieta atendiendo los requerimientos nutricionales de la especie.

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LARVICULTURE UNDER CONDITIONS OF HIGH SALINITY: SNUBNOSE POMPANO *Trachinotus blochii*

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This study aimed to estimate the survival rate and growth performance of Snubnose pompano (*Trachinotus blochii*) larvae at various salinity levels: 25‰, 33‰, 39‰, and 42‰. The experiments were conducted in triplicate, each with 1000 larvae per tank. The control group was maintained at a salinity level of 42‰ and a temperature range of 28.3°C–31.0°C. Eighteen hours post-hatching, larvae were transferred directly to different salinities (25‰, 33‰, 39‰, and 42‰), and their survival rate and growth performance were monitored. The results indicated that the optimal salinity for larval survival ranged from 33‰ to 39‰, with 100% mortality observed within 4 days post-hatching (dph) at a salinity level of 42‰. While growth performance did not significantly differ among the experimental groups, there was a notable difference in the control group. After 21 days, stronger cannibalism was observed in the 25‰ groups compared to the other groups. In conclusion, this study suggests that pompano larviculture is feasible within salinity ranges of 33‰–39‰.

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STATUS AND OUTLOOK OF LARVICULTURE DEVELOPMENT IN SAUDI ARABIA

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This study assesses the state of larviculture among the leading aquaculture companies in Saudi Arabia, focusing on informing future sustainability strategies. Evaluations were performed on freshwater and marine fish hatchery facilities. The factors estimated were production capacity, existing infrastructure, main culture fish species, broodstock and seed stock source, and a range of water temperature and salinity. Most fish hatcheries lacked broodstock capacity. The live food production section was absent in mariculture hatcheries. Most of the culture species in hatcheries were tilapia (*Oreochromis* spp.), common carp (*Cyprinus carpio*), Asian sea bass (Lates calcarifer), Gilt-head bream (*Sparus aurata*), Sabaki tilapia *(Oreochromis spilurus*), and Sobaity seabream (*Sparidentex hasta*). The source of seedstock in most hatcheries depended on imports or other hatcheries. The sustainability strategies can be focused on increasing broodstock capacity, live food production, and new culture species (such as rainbow trout *Oncorhynchus mykiss*).

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SHRIMP SHAPES A RESISTANCE TRAIT AGAINST VIBRIOSIS BY MEMORIZING THE COLONIZATION RESISTANCE OF INTESTINAL MICROBIOTA

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Vibriosis is one of the most serious diseases that commonly occurs in aquatic animals, thus, shaping a steady inherited resistance trait in organisms has received the highest priority in aquaculture. Whereas the mechanisms underlying the development of such a resistance trait are mostly elusive.

In this study, we constructed vibriosis-resistant and susceptible families of the Pacific white shrimp *Penaeus vannamei* after four generations of artificial selection. Microbiome sequencing indicated that shrimp can successfully develop a colonization resistance trait against *Vibrio* infections. This trait was characterized by a microbial community structure with specific enrichment of a single probiotic species (namely *Shewanella algae*), and notably, its formation was inheritable and might be memorized by host epigenetic remodeling.

Regardless of the infection status, a group of genes was specifically activated in the resistant family through disruption of complete methylation. Specifically, hypo-methylation and hyper-expression of genes related to lactate dehydrogenase (LDH) and iron homeostasis might provide rich sources of specific carbon (lactate) and ions for the colonization of *S. algae*, which directly results in the reduction of *Vibrio* load in shrimp. Lactate feeding increased the survival of shrimp, while knockdown of LDH gene decreased the survival when shrimp was infected by *Vibrio* pathogens. In addition, treatment of shrimp with the methyltransferase inhibitor 5-azacytidine resulted in upregulations of LDH and some protein processing genes, significant enrichment of *S. algae*, and simultaneous reduction of *Vibrio* in shrimp.

Our results suggest that the colonization resistance can be memorized as epigenetic information by the host, which has played a pivotal role in vibriosis resistance. The findings of this study will aid in disease control and the selection of superior lines of shrimp with high disease resistance.

INTEGRATION OF CHROMATIN ACCESSIBILITY AND GENE EXPRESSION IDENTIFIES OSMOREGULATION-RELATED TRANSCRIPTION FACTORS IN PENAEID SHRIMP

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The Pacific white shrimp, *Penaeus vannamei*, is a euryhaline species that can tolerate extremely low salinity levels. Whereas, molecular mechanisms underlying low salinity tolerance, including upstream functional regulators, remain elusive in penaeid shrimp. In this study, *P. vannamei* was exposed to low salinity stress through degrading salinity from 30‰ to 3‰ gradually, and potential osmoregulatory mechanisms were investigated by ATAC-seq and RNA-seq analyses.

As expected, the chromatin accessibility was positively correlated with the gene expression levels. Whereas only a part of differentially expressed genes (DEGs, 36.8%) was associated with the differential chromatin accessibility; and the expression of these genes may be regulated by cis-regulatory elements, e.g. transcription factors (TFs). These genes were enriched in betaine synthesis pathway and PI3K-Akt signaling pathway and so on.

Based on the sequences of differential accessible regions, nine TFs and their potential binding sites (TFBS) were identified throughout the genome. Among the nine TFs, seven of them were newly identified to be associated with salinity adaptation. Characteristics of these nine TFs, including potential functions, expression profiles, consensus TFBS motifs, and the functional enrichment and expression profiles of their potentially targeted genes, were thoroughly investigated. Among these TFs, ZBTB has the largest number of specific target genes and functional enrichment of target genes showed pathways that closely associated with salinity adaptation, including amino acid metabolism and lipids metabolism pathways.

Knocking down the expression of ZBTB resulted in the significantly increasing mortality rate under both acute and chronic low-salinity stresses. DAP-seq sequencing and analysis of ZBTB confirmed the locations of TFBSs and targeted genes, which provided a comprehensive perspective for understanding the regulatory network of this TF.

These findings provide insights into the regulatory mechanism of salinity tolerance to improve genetic breeding and desalination aquaculture of penaeid shrimp.

GROWTH AND PHYSIOLOGICAL RESPONSE OF NILE TILAPIA (*Oreochromis niloticus*) FED A DIETARY SAPONIN

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Aquafeed is a crucial indicator of success in aquaculture, especially for commercial aquaculture. This is because the cost of feeding accounts for more than 70% of the total production costs in each production cycle. Currently, challenges in commercial aquaculture and efforts to address these issues include the selection of additives to promote growth and improve feed efficiency as a means to reduce feed costs. Saponins are natural compounds found in various plants. They have a glycoside structure, saponins extracted from soapbark tree (*Quillaja saponaria*) and Mojave yucca (*Yucca schidigera*) have proven to be very promising ingredients for aquafeeds as natural growth promoters in aquatic animals.

The current work was designed to investigate the dietary effects of saponin on tilapia (*Oreochromis niloticus*). Fish with initial average weight 34.99±1.00 g/fish were randomly divided into five treatments in four replicates. Four experimental diets were designed containing different inclusion of saponin as 0 (control), 200, 400, 600 and 800 mg kg^{-1} of feed) and fish were fed with satiation two times a day in RAS system for 90 days.

At the end of the trial, the results showed significant differences ($p<0.05$) in specific growth rate (SGR) in fish fed with dietary saponin 400 mg kg^{-1}. The feed utilization, digestive enzyme activity, serum biochemical parameters, body composition, innate immune, and apparent digestibility coefficients of crude protein were not significant differences ($p>0.05$). Conversely, fish treated with diet contained saponin at 400 mg kg^{-1} of feed showed slight increase trends in PER and PG. The histopathological observation results in both liver and posterior intestine areas indicated no observable significant difference among the experimental groups. Moreover, fish fed control diet and inclusion of saponin at 400 mg/kg^{-1} was observed increase intestinal villi height. It is concluded that fish fed with diet supplemented saponin at 400 mg kg^{-1} tended to have better growth performance, feed utilization and physiological response.

Table 1. Growth performance and feed utilization of tilapia fed diets containing different levels of saponin for 90 days.

Parameters	Level of saponin supplementation (mg Kg^{-1})					P-value
	0	200	400	600	800	
SGR	1.84±0.02^{abc}	1.86±0.04^{ab}	1.89±0.02^{a}	1.82±0.02^{bc}	1.80±0.02^{c}	0.048
FCR	1.12±0.14	1.15±0.14	1.06±0.13	1.04±0.06	1.12±0.08	0.620
PER	2.82±0.37	2.73±0.30	2.96±0.40	2.97±0.18	2.75±0.18	0.690
PG	1.88±0.10	1.85±0.14	1.99±0.23	2.05±0.18	1.75±0.16	0.157
SR	100	100	100	100	100	-

Data were presented as mean ± SE. Means having the same letter in the same row are significantly differed at $p<0.05$.
SGR: Specific growth rate, FCR: Feed conversion ratio, PER: Protein efficiency ratio, PG: protein gain, SR: Survival rate.

Table 2. Digestive enzyme activity and lysozyme activity of tilapia fed diets containing different levels of saponin for 90 days.

Parameters	Level of saponin supplementation (mg Kg^{-1})					P-value
	0	200	400	600	800	
Protease	1.98±0.16	1.72±0.17	1.92±0.13	1.71±0.24	1.83±0.29	0.313
Lipase	40.721.73	37.19±2.93	41.34±2.03	38.59±4.71	36.11±3.77	0.160
Amylase	149.38±4.41	136.18±7.70	151.29±6.95	140.53±8.73	134.64±7.35	0.186
Lysozyme	0.63±0.02	0.64±0.08	0.66±0.13	0.62±0.06	0.61±0.05	0.889

THE POWER OF COLLABORATION: LEVERAGING SHARED LEARNINGS AND NEEDS TO BUILD A U.S. AQUACULTURE WORKFORCE PATHWAYS MAPPING TOOL

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The growth of the U.S. aquaculture industry has elevated both the need for, and the attention paid to, workforce development initiatives. Yet, while there is broad recognition around the importance of workforce development, industry and educator efforts to encourage greater participation in the sector are not often aligned across the workforce pathway.

Over the past three years, Meridian Institute has been facilitating conversations and action around the ways workforce development can support the long-term sustainability of responsible U.S. aquaculture. As a non-profit that specializes in serving as a trusted third party in collaborative decision-making processes, Meridian has focused on fostering, elevating, and leveraging connective opportunities for the aquaculture sector to strengthen its own workforce development capacity.

Our engagement has revealed that when it comes to workforce development, trainees, educators, and industry often lack a shared understanding on: (1) the realm of career options in U.S. aquaculture, (2) the skills needed to succeed in these jobs, and (3) career pathways through the industry. In response to this need, Meridian has been working to build an interactive workforce pathways mapping tool that guides decision-making for trainees, educators, and industry alike.

Our work focuses on how collaborative processes were critical to this tool's development, including synthesized interview research, insights from a stakeholder community of practice on aquaculture DEI (diversity, equity, and inclusion), roundtable dialogues on workforce development, and an advisory panel informing tool design. We will also highlight the tool's potential to facilitate further collaboration — whether through iterative development or by identifying opportunities for multiple entities to jointly address the sector's workforce challenges.

REVERSE TRANSCRIPTASE RELATED PROTEINS ARE RECRUITED BY MULTIPLE AQUATIC ORGANISMS TO SERVE IN ENVIRONMENTAL STRESS RESPONSE PATHWAYS

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Reverse transcriptase-related (RVT) proteins are a distinct class of domesticated reverse transcriptases (RTs) with unusual properties. This is the only RT type found in bacteria, fungi, protists, plants and invertebrates from aquatic and soil-dwelling environments. RVTs are encoded by cellular single-copy non-mobile genes preserved by natural selection, potentially performing biological function that is applicable to both prokaryotes and eukaryotes. Domestication of RT domain that makes DNA using RNA templates is extremely rare in eukaryotes, although it was described several times in prokaryotes. Our group aims to reveal cellular function(s) of RVT proteins.

We focused on three free-living organisms often dwelling in activated sludge with active *rvt* genes in their genome (the filamentous gliding bacterium *Herpetosiphon aurantiacus*, the model ascomycete fungus *Neurospora crassa,* and the bdelloid rotifer *Adineta vaga*). Free-living organisms can be frequently exposed to hazardous pollutants including transition metals, antibiotics and other chemicals that disrupt protein synthesis. Therefore, those organisms need potent mechanisms to cope with such stresses. We show that the examined organisms display signs of altered growth and behavior after exposure to increased concentrations of several metal ions (Ni^{2+}, Co^{2+}, Fe^{2+}, Zn^{2+}) or antibiotics (eg. Blasticidin S) in growth media and are characterized by strongly induced expression of *rvt* genes.

To discover the cellular function of RVT proteins, we applied various comparative genomic, transcriptomic, biochemical, structural, and functional approaches, as well as bioinformatic analyses of genomic and environmental datasets. Comparing results obtained for chosen bacterial, fungal and animal models, we conclude that RVT is involved in response to diverse environmental stresses via template-independent polymerization. Furthermore, when recombinant RVT from *H. aurantiacus* was expressed in *E. coli,* bacterial clones demonstrated a notable improvement in survival on iron-rich medium. This ability to work in a heterologous system can make RVT proteins a useful tool for biotechnological applications.

ALTERNATIVE POND-BASED PRODUCTION SYTEMS FOR REARING OF FINGERLING LARGEMOUTH BASS *Micropterus nigricans*

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Current production of Largemouth Bass (LMB) in the Midwest is carried out in ponds, which typically leads to high variation in survival and growth. This is due to increased cannibalism, predation, and inconsistency in feed consumption associated with low-density ponds. This study sought to evaluate two alternative pond-based production systems for the rearing of LMB. Specifically, the aim of this study was to evaluate two alternative pond-based production systems (split ponds and pond-side tank culture) on LMB quantified production parameters.

A total of 9 systems were utilized, with 3 replicates of each treatment (split pond, SP; pond side tank, TK; & full pond system; FP). Each pond utilized was ~ 0.1 acre, with a maximum depth of 1.5 m. The SP treatments were equipped with a T-shaped baffle to hold the LMB in < 25% of the pond's area and direct the flow of water in a U-shaped fashion around the non-fish side before returning to the fish-side. A TK consisted of a 4.6 m^3 plastic tank set on the side of the pond, supplied with blower aeration and water from an adjacent full pond. Each system was stocked with 1,250 feed-trained LMB fingerlings in June 2024 at an average size of 58.02 mm (± 2.71) and 2.18 g (± 0.39). Fish were fed twice daily with a restricted feeding rate. The feeding amount was adjusted daily based on an assumed FCR of 1, and the rate adjusted biweekly based on the subsampling of 25 LMB from each system. Final data collection gathered total count of surviving fish and length/weight data for 100 fish from each system.

The study was concluded in late September 2024, with data still being processed. Initial data analysis revealed noteworthy trends in production differences between the alternative rearing systems and the FP treatment. The TK treatment showed numerically lower final weight, final length, specific growth rate, production efficiency, and survival of LMB compared to the FP and SP treatments. With regards to the FP and SP treatments, the survival, production, and growth parameters were found to be similar between systems. An important trend observed was that the coefficient of variation of fish length (Figure 1) and weight decreased numerically as the area of the rearing system decreased. While this decreased variation occurred with decreased growth and survival in the TK treatment, the SP treatment was able to achieve more uniform growth than the FP, with similar production parameters. Uniformity of growth in desirable in LMB rearing, as there is a reduced occurrence of cannibalism, and a more synchronized achievement of market-size in the farmed fish. More details will be presented in the oral presentation.

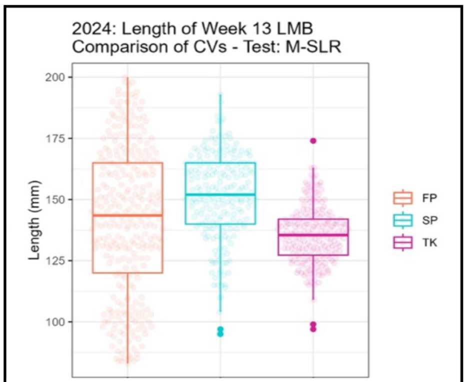

Figure 1. Final length distribution of LMB at the conclusion of the study.

DO DISCARDED SEA SCALLOP SHELLS SEQUESTER ATMOSPHERIC CO_2? HOW GREEN IS THE UNITED STATES SEA SCALLOP FLEET?

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The Atlantic Sea Scallop (*Placopecten magellanicus*) is one of the most important commercially harvested species in the United States, with annual landings over $400 million USD. Within the US fleet, most vessels are based out of New Bedford, MA where vessels primarily fish on Georges Bank and neighboring areas, requiring the combustion of fossil fuels which increases atmospheric greenhouse gas emissions. Yet, unlike other extractive fisheries, the U.S. scallop fleet shucks all scallops at sea, which returns the shells to the seafloor. In turn, these shells are composed of calcium carbonate, which can increase oceanic total alkalinity and subsequently sequester atmospheric CO_2, as they dissolve. At present, no studies have examined the fuel efficiency or amount of atmospheric CO_2 theoretically sequestered by shells discarded by the scallop fleet. Thus, this presentation will present analyses of the fleets greenhouse gas emissions and incorporate our initial estimates of the quantity of discarded shell material and its contribution to increasing oceanic total alkalinity and subsequent drawdown of atmospheric CO_2.

A MIXED STUDY ON THE IMPACTS OF THE AGGRESSIVE EXPANSION OF THE NIS WATER HYACINTH *Echhornia crassipes* ON AQUACULTURE IN NIGERIA

Markose Chekol Zewdie*, Okomoda Victor Tosin, Sunil Siriwardena, Akintola Shehu Latunji, Rodrigue Yossa, James Fasakin, and Leila Basti

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Fish farming is an important agricultural activity that contributes to Nigeria's economy, and is set to improve the nation's food security, employment, and income generation. Despite its potential, aquaculture faces several challenges including inadequate infrastructure, limited access to resources, climate change, and environmental issues that affect the productivity and sustainability of fish farms. Among these challenges, an aggressive expansion of water hyacinth has been negatively impacting the Nigerian aquaculture. It creates dense mats that block sunlight, reduces oxygen levels, restricts fish movement and access to food, causes fish mortalities, and increases operational costs for fish farmers. Addressing the adverse impacts of this invasive non-indigenous species (NIS) on aquaculture in Nigeria is therefore important. Effective control methods such as mechanical excavation or biological control are widely recognized, but their success heavily depends on community support and financial contributions.

Therefore, the objective of this study was to investigate perceptions of communities of water hyacinth's environmental and economic impacts, focusing on its implication for aquaculture farming. Specifically, this study was designed to answer the following research questions: 1) How local communities perceived the impact of water hyacinth on their livelihoods and environment? 2) What are the pros and cons of water hyacinth expansion in Nigeria? 3) Is controlling water hyacinth aggressive expansion in Nigeria needed? 4) Are farmers willing to financially support programs aimed at controlling or excavating water hyacinth?

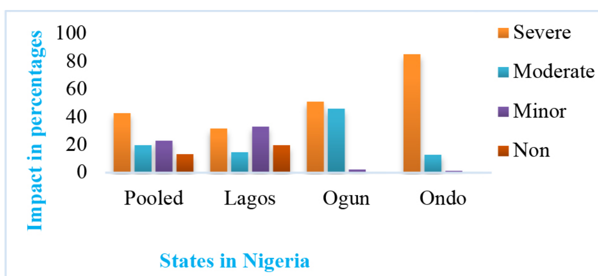

Figure 1: Farmers' Perception of Water Hyacinth Impacts

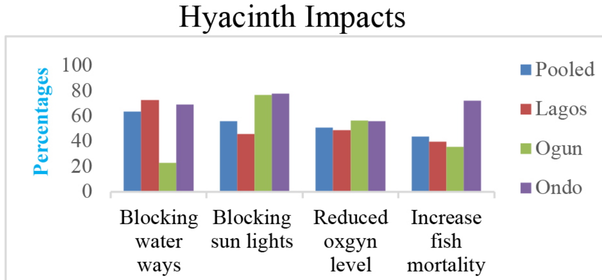

Figure 2: The Impacts of Water Hyacinth on Nigeria's Aquaculture Sector

TRANSCRIPTOME AND DNA METHYLOME SIGNATURES OF HIGH REPRODUCTIVE PERFORMANCE IN CRYOPRESERVED BLUE CATFISH SPERM

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Catfish is the most important species in US aquaculture, accounting for 70% of freshwater production. The production of hybrids of female channel catfish (*Ictalurus punctatus*) and male blue catfish (*I. furcatus*) constitutes over 50% of the total harvest due to their superior production traits and enhanced disease resistance. However, hybrids cannot be produced naturally, and male must be euthanized for sperm collection. An appropriate storage condition is essential to preserve the sperm's ability to fertilize eggs during the female spawning season. Cryopreservation is a widely used method for sperm storage. However, it has been shown to affect sperm gene expression in many vertebrate species. A high degree of individual variability among cryopreserved sperm was reported, resulting in huge variations in hatch rate. Since high-quality gametes are the prerequisites for hybrid catfish reproduction, understanding the molecular mechanisms associated with sperm quality is critical for the accurate prediction of hatching rate and offspring performance in hatchery environments.

In this study, sperm collected from 28 adult male blue catfish has been experienced a cryopreserved process. After cryopreservation, the sperm was thawed and fertilized with eggs from 3 female channel catfish to test the embryo hatching rate. The average embryo hatching rates ranged from 14% to 63%. RNA-seq and DNA methylome sequencing experiments were performed to investigate the gene expression and epigenetic profiles of each sample. After sequencing adapter and low-quality reads removal, the RNA-seq reads from 28 samples were aligned to the blue catfish genome, achieving an average mapping rate of 81.98% (from 70.7% to 91.7%). A total of 33,043 expressed genes remained after filtering out those with low expression level (RPKM < 1) in more than 6 samples. Differential expressed gene analyses showed that 1,400 genes were up-regulated and 4,611 genes were down-regulated in sperm with high embryo hatching rates. Compared to the sperm with low hatching rates, genes involved in cytokine-cytokine receptor interaction, regulation of actin cytoskeleton and calcium signaling pathway were significantly up-regulated in high hatching rate sperms, suggesting that the sperm with stronger immune responses and enhanced cell movement are more likely to result in hatching success. In contrast, up-regulated genes in sperm with low hatching rates were enriched in RNA degradation, mitophagy and autophagy pathway, suggesting that those may experience decreased motility due to the reduced energy and protein production. Our findings provide valuable insights into the molecular mechanisms behind male reproduction success. By identifying these key genes, we will be able develop biomarkers to predict hatching success and potentially improve breeding strategies in catfish aquaculture. Our research contributes to a deeper understanding of reproductive performance on the male side and paves the way for efficient hybrid catfish production.

THE DESIGN OF A KELP FARM FOR THE UNIVERSITY OF NEW HAMPSHIRE OFFSHORE AQUACULTURE SITE

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The University of New Hampshire (UNH) presently holds two adjacent offshore aquaculture permits with a total area of 52.6 hectares. The permitted sites are approximately 5 km from shore in a depth of 35 m of water. At this location, UNH is licensed to grow and sell several species of shellfish and macro-algae. Being offshore, however, aquaculture systems must be designed to withstand extreme events and to minimize harmful interaction risk that exists with large marine mammals, especially, the North Atlantic right whale (NARW).

The objective of this presentation is to describe the engineering procedures for the whale-safe design of an offshore kelp farm. The approach builds upon the experience of two successful, smaller kelp farm deployments, using (1) composite rods as a replacement for mooring rope, (2) high density polyethene (HDPE) pipe as a kelp growth substrate and (3) weak links attached to specific flotation elements. The offshore kelp farm design incorporates a planar assembly for near-surface kelp growth on HDPE pipe, pretensioned with components having bending resistance, like the composite rods described in [1]. The composite rods were also used in the mooring components as a rope replacement. The system design for the UNH offshore site utilized wave and current parameters described in [2] with a kelp aggregate modeling approach validated in [3]. The presentation will also describe the application of a preliminary whale loading numerical routine applied to this specific structure. The intent was to design the offshore kelp farm to satisfy the tenets of the Marine Mammal Protection and Endangered Species Act and to yield up to 12,000 kg of fresh product, while absorbing coastal nutrients.

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[2] Sunny, R.C., Fredriksson, D.W., Tsukrov, I., Zhu, L., Bowden, M., Chambers, M., Silkes, B. 2024 (in revision). Design considerations for a continuous mussel farm in New England offshore waters. Part I: Development of environmental conditions for engineering design. Aquacult. Eng.
[3] Zhu, L., Patwary, M.M.R., Sunny, R.C., Tsukrov, I., Fredriksson, D.W. 2024. (in Revision). Hydrodynamic modeling of kelp (Saccharina latissimi) Farms: From an aggregate of kelp to a single line cultivation system. Ocean Eng.

MERCK ANIMAL HEALTH: CONNECTING AQUACULTURE AND CONSERVATION THROUGH TECHNOLOGY

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Meeting the global demand for seafood is more essential than ever as the world's population continues to increase. Not only is the need for protein increasing but so are the demands of consumers who expect more accountability and transparency in how their seafood is produced. Consumers today expect producers and suppliers to meet their seafood demands, and to do so in a manner where production practices are ethical and humane and protective of the planet's natural resources.

How can these separate and complicated demands be supported as stakeholders meet the growing need for food security? Many of the answers lie in today's advancements and adoption of technology.

Merck Animal Health, known as MSD Animal Health outside of the United States and Canada, has expanded its biopharma portfolio to include innovative vaccines, DNA-based traceability solutions for aquaculture producers, processors, and retailers as well as technology valued by conservationists that provides insightful data used to manage fish populations in critical habitats throughout the world.

Meeting today's protein and seafood demands and the high expectations of consumers and conservationists, requires a new and holistic approach to seafood health and production that is accomplished through a portfolio of biopharma and technology solutions.

VACCINE OPTIMIZATION AND VIRULENCE MODULATION OF VIRULENT *Aeromonas hydrophila* WITH MUTATIONS IN RTX TOXIN GENES

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Aeromonas hydrophila causes Motile Aeromonad Septicemia (MAS) in channel catfish. In 2009, a clonal type emerged, designated virulent *A. hydrophila* (vAh), that led to the loss of 5.5 million pounds of fish in the US and continues to threaten the aquaculture industry. Comparative genomic analysis revealed that RTX toxins, encoded by *rtxC* and *rtxA*, are unique to vAh compared to other sequenced *A. hydrophila* strains. *vAhΔrtxC*, *vAhΔrtxA*, and double-deletion *vAhΔrtxA-C* mutants were constructed using the in-frame deletion method. RTX-deficient mutants demonstrated efficacy as live attenuated vaccine candidates due to reduced virulence and strong protection against wild-type vAh (Figure 1). This study aimed to optimize vaccine delivery via immersion exposure and assess the role of RTX toxin in vAh virulence.

The ability of the mutants to replicate intracellularly was compared to wild-type strain using the J774A.1 murine macrophage cell line, while the invasion was compared using EPC fathead minnow epithelial cell line. Interestingly, intracellular replication in J774A.1 cells decreased from 1 to 3 hours post-infection in RTX-deficient strains compared to wild-type, but from 3 to 6 hours, RTX-deficient strain replication remained constant while wild-type strain decreased. Similarly, RTX-deficient vAh had significantly reduced invasion of EPC cells at 3 hours post-infection compared to wild-type, but there was no difference at 6 hours post-infection.

Growth of vAh under iron restriction increases virulence. As previously reported, we found that growing vAh in the presence of iron chelator deferoxamine mesylate (DFO) caused significantly increased mortalities following immersion exposure. However, RTX-deficient vAh remained attenuated when grown with iron restriction. Furthermore, RTX-deficient vAh provided 100% protection at 21 days post-vaccination against immersion challenge with wild-type vAh grown under iron restriction, compared to 42.5% mortalities in sham-vaccinated fish.

This study highlights that RTX toxin played a role in early cellular invasion and intracellular replication of vAh, and disruption of RTX toxin demonstrated its protective efficacy as a vaccine against MAS, including when grown under iron restriction.

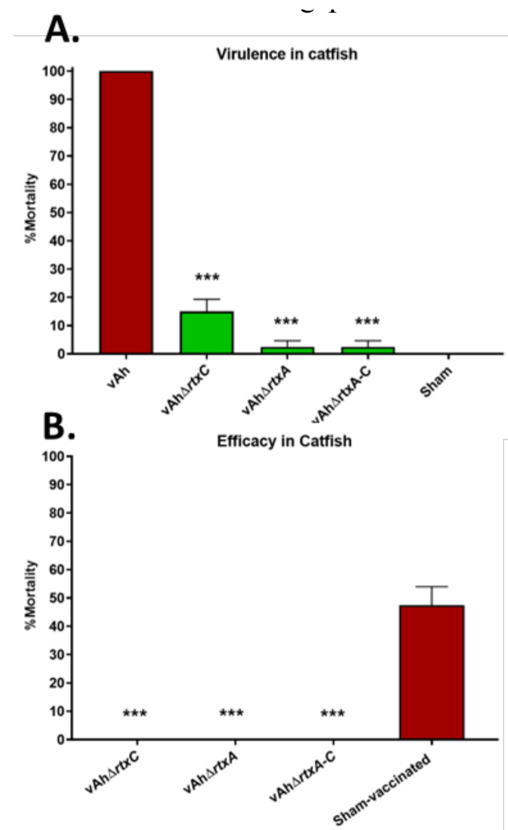

Figure 1. Virulence and vaccine efficacy in channel catfish. (**A**) Mean percent mortalities in catfish. (**B**) Percent mortalities in catfish immunized with vAhΔ*rtxC*, vAhΔ*rtxA,* and vAhΔ*rtxA-C*, and infected with vAh at 21 days post-infection.

RECIRCULATING AQUACULTURE SALMON NETWORK (RAS-N)

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Over 90% of the ~500,000 metric tons of Atlantic salmon consumed in the US annually are imported from overseas, for a value of around $3.6 billion. As the challenges surrounding domestic production of salmon in floating coastal net-pens mount, we have witnessed in the US increased interest and major investments in land-based, environmentally-friendly production of salmon, using recirculating aquaculture systems (RAS). Responding to these trends, NOAA/National Sea Grant funded the RAS-N collaborative hub, consisting of research and industry partners. RAS-N's overarching goal was to establish a holistic hub of knowledge that will integrate past, current and future research as well as extension, outreach, education, training and workforce development to promote the successful growth, stability and economic feasibility of the Atlantic salmon RAS sector and, more broadly, US aquaculture. A major deliverable of RAS-N was to engage in dialogue with industry in order to identify gaps in knowledge and impediments to the development of the industry, ultimately resulting in prioritized R&D needs. Six working groups were established consisting of researchers and industry stakeholders, which periodically met virtually and in-person to discuss program objectives and develop strategies to achieve them. Joint public-private panels were conducted at RAS-N and other national meetings in an effort to develop broad and inclusive priorities. These activities culminated in the generation of a "Concept Paper" aimed at helping policymakers, federal and state agencies and industry identify and responsibly allocate resources to build capacity and promote an economically feasible and environmentally sustainable land-based Atlantic salmon industry in the US. The following 8 stakeholder-driven objectives were identified:

1. Understand and mitigate off-flavors in RAS platforms
2. Establish domestic, year-round egg production from North American strains
3. Understand/reduce early maturation, develop methods for reproductive sterility
4. Develop RAS-specific and alternative feeds
5. Study/optimize microbiome in salmon RAS for efficient biofiltration, waste treatment/conversion, containment, fish health and environmental compatibility.
6. Engage in economic and market analysis
7. Develop education programs - K-16, RAS Certificates, workforce development
8. Create effective extension programs – outreach, community engagement, public awareness, technology transfer

The RAS-N hub led to the establishment of a broader research consortium, funded in 2021 by USDA-NIFA, to implement the RAS-N findings and recommendations. This national program, "Sustainable Aquaculture Systems Supporting Atlantic Salmon" (SAS^2), is a multidisciplinary, synergistic, "hands-on" partnership in which leading aquaculture scientists, in collaboration with all major US producers, carry out research focusing on the above industry-identified impediments to the expansion of the salmon RAS industry.

STERILITY INDUCTION BY A TRANSIENT GENE SILENCING TECHNOLOGY

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Farming reproductively sterile fish is the most proficient genetic containment strategy to prevent domesticated escapees from propagating or interbreeding with wild stocks. It also overcomes the challenge of early maturation observed in land-based aquaculture. Sterility carries ecological significance along with economic benefits. Sterilization impedes energy input toward gonadal development, enhancing muscle growth (flesh/fillet yield) and promoting overall performance and health. Sexual maturation is accompanied by deterioration of flesh quality and suppresses the immune system, which increases susceptibility to stress and disease, causing significant economic consequences for aquaculture. Additionally, sterility is a practical means for producers to protect their selectively-bred IP strains from unauthorized propagation.

Aquaculture is becoming increasingly crucial to resolving the current and projected shortfalls in aquatic food production. Thus, an effective environmentally-friendly containment strategy for large-scale commercial aquaculture operations is urgently needed to achieve the environmental sustainability of this industry. We developed a bath-immersion method to produce sterile fish by irreversibly disrupting primordial germ cell (PGC) migration and development. We demonstrated that the Vivo molecular transporter effectively carries a transient gene silencing Morpholino oligomer (MO) across the chorion, entering the embryo and reaching the target PGCs. Indeed, immersion of salmonid eggs in the Vivo-conjugated MO targeting *deadend* (*dnd*), an essential gene in PGC development, effectively disrupted germ cell development and resulted in reproductively sterile fish with gonads that are deprived of germ cells. This technology was successfully applied to salmonid species without introducing any genome modification. Using Dnd-MO-Vivo, 84% and 75% sterility induction was achieved in rainbow trout and Atlantic salmon, respectively. As a substitute for Vivo, we developed a ZP9 molecular transporter that enables the fluorescence labeling of the MO conjugate to advance the immersion technology. Using fluorescence screening, our ongoing experiments focus on optimizing bath immersion and developing a strategy to selectively sort eggs that have sufficient uptake of Dnd-MO to induce sterility (Fig 1).

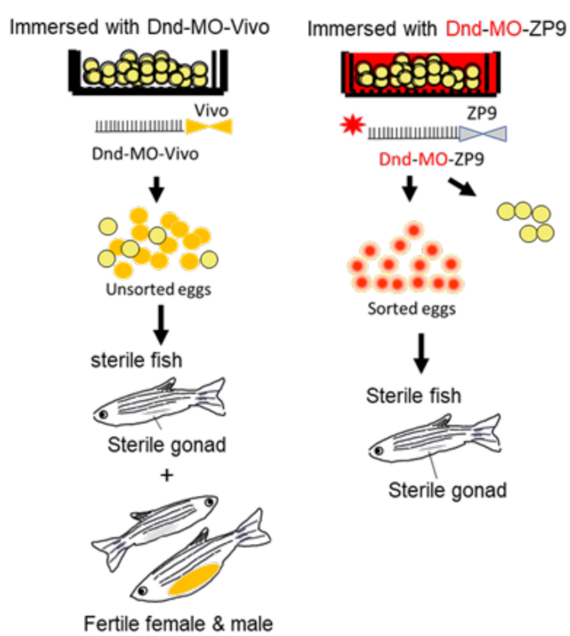

Figure 1. Fluorescence-labelled Dnd-MO-ZP9 assists the optimization of the bath immersion technology and enables the sorting of treated eggs that have sufficient uptake of Dnd-MO to induce sterility.

The *RTE-3_Lva* NON-LTR RETROTRANSPOSON IS A POTENTIAL SEX MARKER FOR THE PACIFIC WHITE SHRIMP *Penaeus vannamei*: TOWARDS AN ALL-FEMALE SHRIMP INDUSTRY

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While studying Infectious hypodermal and hematopoietic necrosis virus (IHHNV), now called *Decapod penstylhamaparvovirus 1,* one of the major viral pathogens of penaeid shrimps that can result in runt deformity syndrome (slow growth), a Type-A non-infectious endogenous IHHNV related sequence (DQ228358, 4,655bp; Tang & Ligthner 2006) was previously identified in the genome of *P. monodon* from Madagascar and demonstrated integrated into an *RTE-like* non-LTR retrotransposon. The 3'-flanking sequence of the integrated IHHNV, nucleotides 3262-4655 of DQ228358, shows 98% identity to nucleotides 1531-2924 of a *P. monodon* repeat family *RTE-2_PMon* (3,656-bp) which shares 85% sequence identity along the whole length with *RTE-3_LVa* non-LTR retrotransposon (3,654-bp; www.girinst.org).

RTE-3_LVa was characterized from a pilot genome sequence (total length of ~470 Mb) from the first SPF *P. vannamei* produced by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP) maintained in Kona and Oahu, Hawaii, USA. Thirteen microsatellites isolated from ovary of SPF *P. vannamei* are homologous to *RTE-3_LVa*, two located onto the sex linkage group 4 (LG4, *ShrimpMap*2) of SPF *P. vannamei*.

Homology searches using the whole genome sequences databases in GenBank revealed that *RTE-3_LVa* has many copies in various scaffolds of *P. vannamei* breed Kehai No.1 assembly (ASM378908v1, ~1.86 Gb), including in LG18 associated with sex differentiation. PCR amplification of DNA from adult SPF *P. vannamei* using primers from two of the microsatellites similar to *RTE-3_LVa* showed sex-specific bands, suggesting that *RTE-3_LVa* is a potential sex marker for shrimp. Results should be confirmed in cultured and wild shrimp.

RTE-3_LVa is also present in various chromosomes of other penaeid species like *P. monodon* from Thailand (NSTDA_Pmon_1, GCF_015228065, 2.39 Gb). Considering the variability in genome sizes of current penaeids assemblies [*P. monodon* from China and Vietnam (~1.4-~1.6 Gb), *P. chinensis* from China (~1.6 Gb), *P. indicus* from India (~1.6 Gb), *P. japonicus* from China and Japan (~1.7 Gb)], which are smaller than the expected ~2.87 Gb genome size of SPF *P. vannamei* from a breeding company in Florida, USA, a new, continuous, whole reference genome sequence is urgently needed from both the founding parents of the SPF *P. vannamei* breeding program of the USMSFP and wild *P. vannamei* to study organization and evolution of integrated viruses like IHHNV, expression of *RTE-3_LVa*, and mechanisms of sex determination and differentiation. This research will lead to sustainable production of "ALL-FEMALE *P. vannamei*".

AN ECOLOGICAL APPROACH FOR RESTORATION AND MAINTENANCE OF COASTAL WATER QUALITY THROUGH ADOPTION OF SHELLFISH AQUACULTURE COMBINED WITH UPSTREAM NUTIENT CAPTURE AND RESUSE

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Nearshore marine waters and estuaries are deteriorating globally, particularly from nitrogen pollution that leads to severe hyper-eutrophication causing a cascade of adverse impacts to ecosystem function, including critical habitat disruption and fishkills from hypoxia. Nitrogen is the limiting factor in marine waters of which excessive amounts lead to these problems.

A dynamic strategy is being developed and demonstrated on Cape Cod in Massachusetts to address this problem in a cost-effective, energy efficient and resource conserving manner that minimizes capital and operating expense and climate impacts. The strategy prioritizes the use of commercial shellfish aquaculture and nutrient capture and reuse via urine diversion (UD), collection and application as agricultural fertilizer. Urine contains 80% of the nitrogen in human waste or about 3 kg-N/person/yr in the USA, based on research conducted by The Green Center in conjunction with the Massachusetts Septic System Test Center. Urine is generally free of contaminants and is pasteurized prior to application to crops such as hay fields or vegetables. As the cost of chemical fertilizer has escalated, urine has become a more desired alternative.

In the case of shellfish aquaculture in estuaries, the nitrogen Total Maximum Daily Load is first determined for a healthy ecosystem followed by a n assessment of the estuary's carrying capacities – allowable area, production capacity, ecosystem sustainability and social acceptance -- for shellfish aquaculture. The eastern oyster, *Crassostrea virginica*, is the preferred shellfish due to its rapid growth and marketability. At harvest size of 7.5 cm, one contains 0.28 g-N, per findings on Cape Cod or can assimilate about 700 kg-N/ha/yr. Therefore, for example, if 1,000 kg-N/yr needs to be removed from an estuary to meet its nitrogen TMDL, in addition to one hectare of oyster culture, about 100 people would be needed to adopt UD technologies in its watershed. UD fixtures and plumbing needs are easily adopted in most homes and are much lower cost than that of sewer infrastructure both in terms of construction and operation needs.

In Falmouth, Massachusetts with 33,000 year-around residents, a three-year, pilot shellfish aquaculture program was recently completed via a public-private partnership by the Town with three growers, using 0.6 ha in one estuary. In 2025, the Town next plans to expand to 6 ha in a few of its 15 estuaries all with degraded water quality due primarily to septic effluent. The Town is also obtaining permits from the state and designing a UD pilot project to start in 2025. This ecological, cost-effective, climate friendly hybrid approach has enormous promise.

ADDDENDUM

PRODUCTION PERFORMANCE AND DISEASE RESISTANCE OF CHANNEL CATFISH JUVENILES *Ictalurus punctatus* FED DIETS CONTAINING DIFFERENT LIPID SOURCES

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Alternatives lipid ingredients can impact the growth performance and disease resistance in farmed fish. Vegetable oils and terrestrial animal fats have been commonly used to manufacture aquafeeds and they can heavily impact the physiological and immune responses of farmed fish. On the other hand, single cell ingredient, *Schizochytrium* sp. meal can be a sustainable alternative to fish oil due to its high content of docosahexaenoic acid.

Five experimental diets (catfish oil, soybean oil, lard, menhaden oil, and *Schizochytrium* sp. meal) were formulated to be isonitrogenous (36%) and isolipidic (7.8%). Six hundred channel catfish (initial weight ~5 g) were randomly distributed across 20 aquaria (110 L, 30 fish/tank, n=4) and fed the experimental diets to apparent satiation for 10 weeks. The tanks operated as a recirculating aquaculture system and the dietary treatments were assigned in a completely randomized design. On the last day of the feeding trial, fish were weighed to calculate production performance and sampled for condition indices and hematology. For the bacterial challenge, the remaining fish were transferred to and acclimated in fiber glass tanks (22 L) operating as a flow through system and challenged with an LD50 of *Edwardsiella ictaluri* through immersion (6.2×10^6 CFU/mL). Data were subjected to analysis of variance in one-way ANOVA, post-hoc testing was performed using Tukey's test. Fish grew on average 832.6% when compared to the initial weight.

There were no significant differences in the production performance variables, except for feed efficiency ($P = 0.023$), in which the menhaden treatment was more efficient compared to the lard treatment. However, fish fed diets containing lard had significantly higher survival after the bacterial challenge when compared to the fish fed diets supplemented with soybean oil (Figure 1). It can be concluded that terrestrial animal fat reduced channel catfish feed efficiency; however, it did increase their resistance against *Edwardsiella ictaluri*.