Aquaculture 2022

Come one, Come all, for Aquaculture Large and Small

February 28 - March 4, 2022

Town and Country Resort & Conference Center

San Diego, California

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WELCOME

You made it! We're so glad to have you here IN PERSON! Welcome to AQUACULTURE 2022, the Triennial. On behalf of the Fish Culture Section of the American Fisheries Society, the World Aquaculture Society, the National Shellfisheries Association, and the National Aquaculture Association, it is my pleasure to welcome you to San Diego. As Ron Burgundy (played by Will Ferrell) boldly states in the movie *Anchorman*, "San Diego. Mmmmmm. Drink it in. It always goes down smooth."

This year's theme is "Come one, Come all, for Aquaculture Large and Small." This is a nod to the fact that the Food and Agriculture Organization of the United Nations has adopted 2022 as the "International Year of Artisanal Fisheries and Aquaculture," and, as such, reaffirms the importance of sustainable aquaculture to food security, recognizing that aquaculture is already making a significant contribution to the global seafood supply. Dr. Roz Naylor, founding Director of the Center on Food Security and the Environment at Stanford University, re-examines the question "How Sustainable Is Aquaculture?" in our esteemed plenary session. Roz is lead author on "A 20-year retrospective review of global aquaculture" published in *Nature* last year.

This year's Triennial is co-sponsored by the United States Aquaculture Society (a chapter of the World Aquaculture Society), the California Aquaculture Association, and the Aquaculture Suppliers Association. Our tradeshow vendors are very excited to be here and have long awaited the opportunity to rub elbows with you all again. Make sure that when you stop by their booths to stamp your scavenger hunt sheet or grab a free pen, you ask them about their state-of-the-art products and services as they are tried-and-true reliable suppliers and supporters of aquaculture.

As always, students will have the chance to shine with Student Spotlight awards and presentations, a field trip to Hubbs-Sea World, the student-mentor breakfast, scavenger hunt, and the always much anticipated student reception. Both the National Aquaculture Association and the National Shellfisheries Association will be hosting their (in) famous auctions full of great prizes (and tomfoolery).

The Town and Country Resort has been totally renovated since the last time the Triennial was held here in 2010. In 2020, the resort completed a multi-million dollar renovation including all guestrooms, meeting space, and the introduction of a host of new resort amenities, including three new dining outlets, a sun-drenched pool deck, and a three-acre Riverfront Park.

All good things take a lot of time and effort, and our Steering Committee (Mike Masser, Sandy Shumway, Mick Walsh, Paul Zajicek), Program Committee (Co-Chairs, Jay Parsons and Sandy Shumway, Steve Allen, Jeff Heindel and Jim Tidwell), and Student Committee (Adam Daw, Henry Fleener, Matthew Smith, Laura King, Hannah Collins, and Alexandria Marquardt) are a testament to this. None of this would be possible without the tireless work of John and Noah Cooksey who ensure the meeting goes seamlessly. If you see any of these fine folks as you walk through the tradeshow, get on a reception bar line, or take a seat in a presentation, make sure to express your appreciation for their devotion to this great event.

It has been a truly unusual year, but I am proud to see how we have all come together to engage in this week of research-sharing, product-testing, networking, and all-around good time.

Thanks for your support!

Michelle L. "Mick" Walsh, Steering Committee Chair
Fish Culture Section of the American Fisheries Society, Past President

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Aquaculture 2022

Come one, Come all, for Aquaculture Large and Small

ABSTRACTS

AN ESTABLISHED CATFISH CELL LINE TO AID IN FISH VIRUS STUDIES

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Catfish industry is a major contributor to the economic sustainability of the southern United States. Viral diseases are a major concern in the hatchery and nursery phases of catfish rearing. The associated fish mortalities can affect availability of catfish fry and fingerlings impacting the viability of farm operations. Since treatment options are limited in the case of several viral diseases, early disease diagnosis and prophylactic measures are key to successful fish health management. For the emergent, emerging, and any uncharacterized fish viruses, the pathogenicity, potential host range, and viral inhibition methods need to be studied. The ability to propagate fish viruses *in vitro* using cell cultures is imperative in advancing virus research and to facilitate pathogen-targeted management strategies including vaccines and antiviral agents. Though cell lines are a very relevant research tool in virology, cell lines originated from ictalurid catfish are limited. The ictalurid cell line (channel catfish ovary (CCO - ATCC® CRL-2772)) previously available from ATCC cell repository has recently been reported as cross-contaminated by brown bullhead (BB) cells. Lack of host-specific cell lines and contamination issues necessitated initiation of cell cultures from the fin tissues of hybrid catfish (♀ channel catfish, *Ictalurus punctatus* x ♂ blue catfish, *I. furcatus*). A combination approach involving tissue explantation and enzymatic digestion methods were employed to develop the hybrid catfish fin (HCF) cell line. The fin cell cultures were passaged over 100 times and transitioned into an established cell line (Figure 1). The HCF cell line has been characterized, maintenance conditions optimized, species of origin molecularly authenticated, and its susceptibility to fish viruses evaluated. Susceptibility of HCF cell line to catfish viruses demonstrated the potential of these cells to propagate ictalurid viruses. This established ictalurid catfish cell line could serve as an efficient tool for virus studies, antiviral agent screening, and vaccine development benefitting catfish aquaculture.

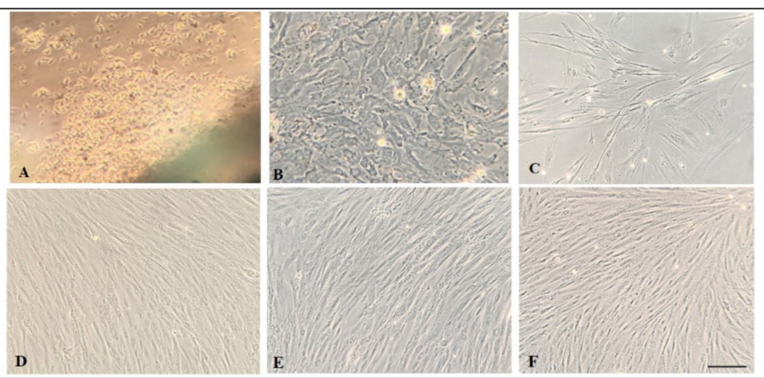

Figure 1. Growth of cells from enzymatically digested fin explants of hybrid catfish in L-15 media with 20% FBS at 25°C. (A) cells emerging from fin explant two days post-incubation; (B) passage 1 (P1); (C) P9; (D) P65; (E) P81 and (F) P96 (Magnification 10X, Zeiss Primovert) (scale bar = 10 µm).

INSECT MEAL AS COMPLEMENTARY INGREDIENT IMPROVES THE UTILIZATION OF SOY BASED DIETS AND REDUCES THE MORTALITY OF RAINBOW TROUT CHALLENGED WITH *Flavobacterium psychrophilum*

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Aquaculture is the fastest growing agricultural sector, supplying about half of the global fish production. Soybean meal (SBM) is a major ingredient in aquafeed industry and high inclusion of SBM exhibit soybean induced enteritis in carnivore fish including rainbow trout (*Oncorhynchus mykiss*). Our goal was to evaluate the effects of whole/defatted black soldier fly larvae (WB/DB) as complementary feed ingredient in soybean meal based diets on growth performance and disease resistance in rainbow trout.

Experiment was conducted in two phases, Phase I: feeding trial for 10 weeks. Phase II: bacterial (*Flavobacterium psychrophilum*) challenge study for 4 weeks. Six experimental diets (42% crude protein and 20% lipid): fish meal based diet (FM), SBM based diet (SBM), SBM+2.5% and 5% WB (WB2.5 and WB5) and SBM+2.5 and 5% DB (DB2.5 and DB5) were fed twice at satiation level for 10 weeks. Each diet was fed with four replicate 30 fish (~5 g) per tank (60-L) in recirculatory aquaculture system Supplementation of WB and DB improved ($p<0.05$) the performance rainbow trout (Figure 1). Feed efficiency was negatively corelated with growth performance. Fish fed diet WB5 had the lowest cumulative percent mortality (CPM) at 32.17%, while fish fed diet SBM had the highest mortality (64.10%). Mortality of fish in group WB5 and DB5 were significantly lower than group SBM ($p<0.05$) (Figure 2). Histological and immunological parameters will be presented for pre/post challenge study. Conclusively, inclusion of insect meal improved the production performance and enhances the resistance against the cold water disease for sustainable trout production.

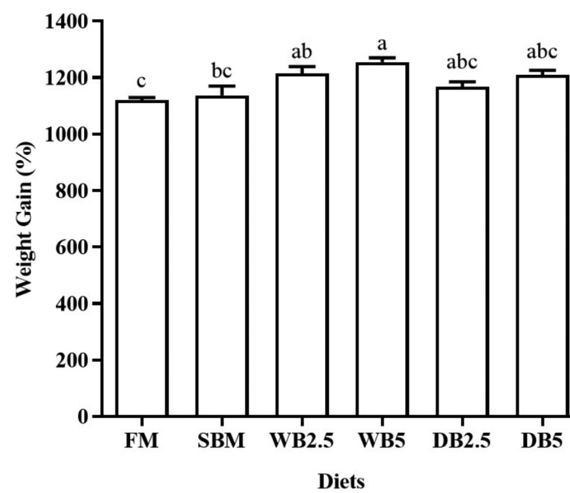

Figure 1: Results of growth performance

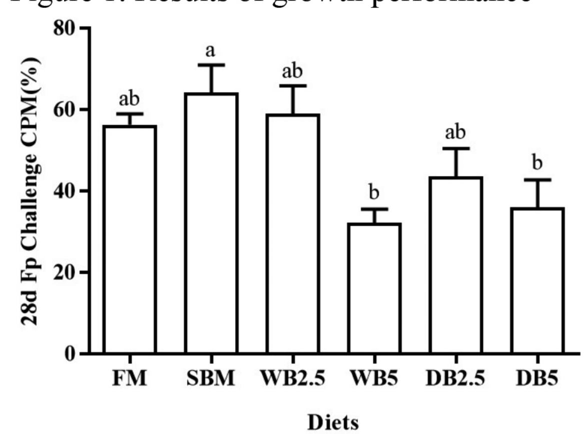

Figure 2: Results of bacterial challenge study

EFFECT OF *Yucca schidigera* EXTRACT AND *Bacillus subtilis* ON THE AMMONIA REDUCTION IN EFFLUENT AND ANTIOXIDANT ACTIVITY OF NILE TILAPIA (*Oreochromis niloticus*)

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Tilapia (*Oreochromis niloticus*) farming effluents contain high level of ammonia, which poses a significant threat to fish and the surrounding environment. *Yucca schidigera* extract (YE) has been found to reduce ammonia pollution resulting from fish farming. On the other hand, dietary supplementation of *Bacillus subtilis* was found to improve anti-oxidation activity, and water ammonia level for fish. Therefore, the aim of the present study was to assess an inclusion of *Yucca schidigera* extract and *Bacillus subtilis* separately or in combination on anti-oxidation activity and ammonia reduction in the effluent of Nile tilapia fingerlings.

Fish were divided into 4 groups and fed for 60 days with four experimental diets which supplemented with 0.2 gkg-1diet of YE and 2 g kg-1diet of *Bacillus subtilis* (B) separately or in combination (YEB) and the control diet was left without addition (CON).

Results revealed the positive role of YE and B on total ammonia nitrogen (TAN) reduction (Table 1); however combination of both YE and B had less efficiency. The addition of YE or B in tilapia diets reduced TAN removing by 50% compared with the control. Superoxide dismutase (SOD) showed the best activity in YEB treatment followed by YE and B treatments while the lowest activity was recorded in the control group. Fig. 1 illustrates the effect of YE and B on anti-oxidative activity of Nile tilapia. The highest activity of Malondialdehyde (MDA) was reported in the control group while the lowest value was found in YEB group followed by YE and then B group. Glutathione peroxidase (GPx) and total antioxidant capacity (TAC) showed the highest activity in YEB followed by B treatment, while the lowest activity was recorded in YE and CON. It could be concluded that Yucca extract and bacillus can efficiently reduce ammonia in Nile tilapia effluent and boost their antioxidative activity.

Table 1. Effects of experimental diets on antioxidant activity of Nile tilapia

Treatment	SOD	MDA	GPx	TAC
CON	16.63±0.55^{c}	4.07 ±0.15^{a}	0.23±0.03^{c}	0.25±0.04^{d}
YE	24.17 ±0.25^{b}	3.27 ±0.15^{b}	0.33±0.02^{c}	0.60±0.10^{c}
B	25.30 ±0.66^{b}	2.400 ±0.10^{c}	0.63±0.06^{b}	1.33±0.15^{b}
YEB	31.63±1.19^{a}	1.03±0.15^{d}	1.77±0.15^{a}	2.27±0.14^{a}

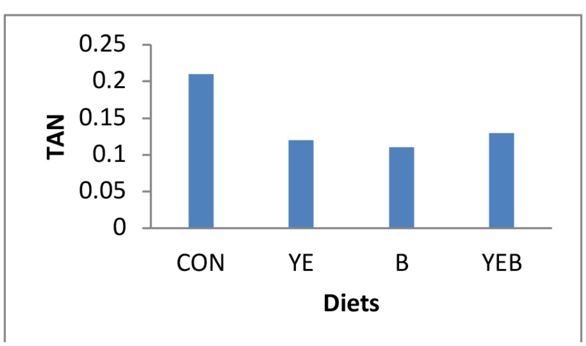

Fig. 1. Effects of experimental diets on TAN of effluent of Nile tilapia

DISEASE-RELATED ECONOMIC LOSSES ON COMMERCIAL CATFISH FARMS: A SEVEN-YEAR CASE STUDY FROM ALABAMA, USA

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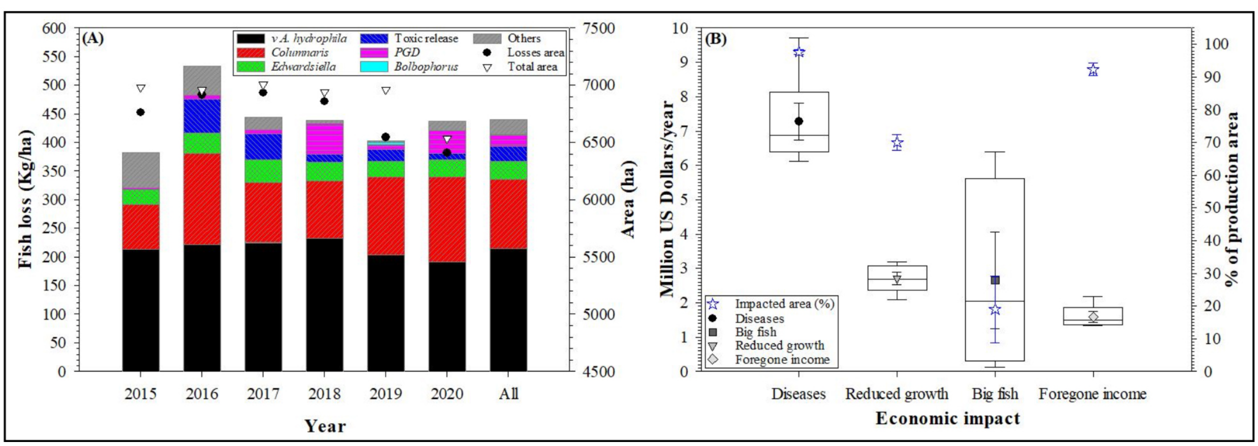
The U.S. farm-raised catfish industry faces serious challenges such as disease losses, competition from cheaper fish products, high feed prices, and big fish (not accepted by fish processors). Alabama ranks second in U.S. food-size catfish production. The Alabama Fish Farming Center (AFFC) diagnostic laboratory has recorded many catfish diseases in western Alabama, where most commercial catfish production is located in the state. The primary diseases resulting in significant catfish losses as reported by the AFFC include virulent *Aeromonas hydrophila* (vAh), *Edwardsiella ictaluri*, *Bolbophorus damnificus*, columnaris disease, proliferative gill disease (PGD), toxic releases, and others (such as ich, water quality, bird predation). Identifying regional prevalent catfish diseases and understanding their economic impact on catfish production is becoming crucial. The objectives of this study were to determine statewide production losses caused by diseases, identify the prevalent catfish diseases affecting Alabama, and determine disease-related economic impacts. The AFFC conducted annual surveys of all commercial catfish producers in Alabama during 2015–2021 by mail and/or telephone interviews to achieve these objectives.

The annual number of survey respondents ranged from 66–74, with a total of 418 respondents (55.3% by phone, 44.5% by mail, and 0.2% by email). The annual survey response rate ranged between 95.6% and 100% (mean ± *SE* = 98.1 ± 0.6%). Reported catfish production area ranged from 6,534–7,006 (6,897 ± 73.1) ha/year. The overall rate of fish deaths caused by diseases was 440.52 kg/ha (Fig. 1A). The highest statewide disease losses in metric tonnes (MT) were caused by vAh (1,447.5 ± 58.1 MT/year), followed by columnaris disease (812.6 ± 84.2 MT/year), followed by *Edwardsiella* (217.5 ± 16.9 MT/year). The annual rate of total economic impacts ranged between 959.1–2,660.4 (1,855.8 ± 228.5) $/ha — 58% of which was due to diseases (Fig. 2A).

Figure 1. (A) Annual and across-study statewide rate of catfish losses (kg/ha), as reported by catfish producers, classified by disease. (B) Annual statewide economic impacts ($/year) on catfish production and percentage of production area impacted by each economic impact element (vertical lines represent medians; symbols with error bars represent means ± standard errors).



THE NOAA COASTWATCH PROGRAM – CAPACITY BUILDING AND EDUCATION IN OCEAN SATELLITE DATA

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The NOAA CoastWatch/OceanWatch/PolarWatch program (hereafter "CoastWatch") assists a wide range of stakeholders in their use of ocean and aquatic satellite data along the research-to-applications value chain, from observations to decision making. CoastWatch is a value-added data provider that offers a range of services centered on satellite product development, data distribution and user capacity building, tool development and sometimes direct collaboration on projects and applications. Our overall goal is to remove the barriers that lead to underutilization of environmental satellite data in research, operational, and commercial applications.

Identifying and using satellite data products appropriate for a given application can be challenging for users outside of the satellite community. The NOAA CoastWatch satellite course was developed to build capacity within NOAA and beyond by providing the background knowledge, skills, and resources for using satellite data to our users who are less familiar with satellite products.

CoastWatch and NOAA SeaGrant are working on a needs assessment for aquaculture practitioners and are developing new course modules on water quality and harmful algal blooms. We plan to offer courses targeting audiences engaged in aquaculture to familiarize government, research, and industry users in the aquaculture fields with ocean satellite data products and their potential for informing farm sitings and farm operations.

The course materials include lectures, self-paced tutorials and tool demonstrations. Course resources are publicly available here: https://coastwatch.gitbook.io/satellite-course/ . The course is followed by a hands-on workshop where participants apply what they have learned to their own projects. Our goal is that upon course completion the participants can apply satellite data to projects using their choice of software (e.g. R, python, ArcGIS).

Courses can be taught in person or virtually, and are tailored to specific audiences, based on participants' interests, needs and technical level. Surveys are conducted before and after each course to generate metrics of course effectiveness and to inform NOAA developers of satellite product needs and gaps.

THE EFFECTS OF DISSOLVED CARBON DIOXIDE ON THE FORMATION OF VATERITE IN THE OTOLITHS OF AQUACULTURE RAINBOW TROUT *Oncorhynchus mykiss*

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Otoliths are dense calcium carbonate structures found within the cranium, that put pressure on cilia and stimulate sensory hair cells, enabling a fish to hear. The chemical structure of otoliths is typically aragonite, which is a more dense form of calcium carbonate that allows the otolith to put pressure on the cilia that enable the fish to hear. Aragonite otoliths can shift to the less dense polymorph, vaterite. The otolith then becomes irregularly shaped and impairs the fish's ability to hear. Previous studies found that otoliths can shift their chemical structure, especially in recirculating aquacultured settings. Many studies have suggested possible factors such as increased growth, temperature, and genetic predisposition. However, evidence suggests that increased levels of dissolved carbon dioxide (DCO2) may be the causative agent in the shift of otolith composition.

A preliminary study was conducted to demonstrate the effects of DCO2 in juvenile rainbow trout (Oncorhynchus mykiss), which are commonly aquacultured. Nine fish tanks (110L) were set up to test the effects of DCO2 on the otolith's structures in rainbow trout. CO2 was dispersed through airflow meters, and three tanks each received 0mg/L, 15mg/L, and 30 mg/L of additional CO2. Weight and length were measured every two weeks. Sagittal otolith samples were taken at the start of the experiment and every 4 weeks over the course of the 8-week experiment.

A Micro Raman Spectrometer (WiTec Alpha 300 Raman, equipped with a 785 nm excitation laser) was used to determine the chemical structure of the otoliths. The number of fish in each treatment displaying the vateritic signature (Figure 1) was quantified and analyzed using an ANOVA for each sampling point (start, 4, and 8 weeks). Vateritic otoliths were seen as early as 4 weeks in the highest CO2 treatments. Growth, both in terms of length and weight, were also significantly affected by higher levels of DCO2. The analysis of this preliminary data strongly suggests that DCO2 is a causative agent for vateritic otoliths in recirculating aquaculture systems and that more emphasis should be placed on the significance of DCO2 to improve the welfare of aquacultured fish. This may be especially important in fish used for restocking programs and may help to improve the success rate.

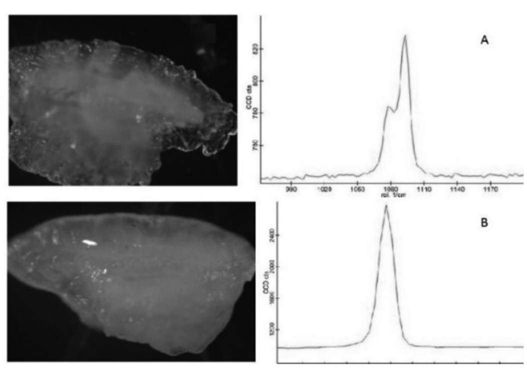

Figure 1. Raman spectrometer output of whole rainbow trout otoliths. A) Plot of Raman shift shows characteristic double peak (or peak with a spur), indicating presence of vaterite. B) Plot demonstrates single peak, characteristic of aragonite

HORMONAL INDUCTION OF SPAWNING IN TRIPLETAIL

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The tripletail, *Lobotes surinamensis*, is a pelagic fish commonly found in coastal waters of the Gulf of Mexico and the US South Atlantic region. The species is one of the emerging candidates for the US marine aquaculture industry due to its fast growth rate and praised flesh quality. Efforts to develop aquaculture methods for tripletail to date have included studies of captive spawning, larval culture, and pilot growout trials conducted at low density. Spontaneous spawns of tripletail are infrequent and display low or no fertility. Hormonal induction of males and females selected at advanced stages of oocyte pre-maturation using gonadotropin releasing hormone analog (GnRHa) implants led to large egg releases but fertility remained very low, suggesting inhibitions affecting spawning in culture also impact response to GnRHa treatments.

In this work, the effectiveness of various hormonal induction treatments was evaluated in two series of trials. All trials employed tripletail broodstocks maintained under a photothermal cycle simulating conditions in Mississippi coastal waters during the spring and summer maturation periods. Hormonal therapies were administered to single pairs featuring one female selected with fully grown oocytes and one male. Two pairs were selected among the broodstock every week, administered one of the tested treatment, and isolated in one of two spawning tanks for monitoring of spawning activity and spawn parameters for 5 days. Each treatment was evaluated using a total of 5 to 7 pairs in both series of trials. The first experiment was conducted in 2019 and 2020 and evaluated (i) a single injection of chorionic gonadotropin (hCG administered at 1,100 IU.kg^{-1} for females and 550 IU.kg^{-1} for males), (ii) a GnRHa EVAC implant (75 mg.kg^{-1} for females, 55 mg.kg^{-1} for males), (iii) a GnRHa EVAC implant as in treatment (ii) administered with a single injection of 5 mg.kg^{-1} domperidone, (iv) a treatment identical to (iii) but administered a week following the hCG injection of treatment (i), (v) control (not treatment). The second experiment was completed in 2021 and compared pairs receiving treatment (iii) of experiment 1 to pairs treated with a GnRHa EVAC implant and an injection of 10 mg.kg^{-1} domperidone.

None of the control pairs and those treated with chorionic gonadotropin spawned. Pairs treated with GnRHa implants only produced spawns with no or very low fertility (average 0.6 ± 1.3%), consistent with previous attempts to induce spawning with this treatment in tripletail. Administration of GnRHa implants with 5 mg.kg^{-1} domperidone lead to a major increase of fertility (42 ± 32.2% in experiment 1). The treatment with a higher dose of domperidone in experiment 2 improved further the fertility of spawns (65.2 ± 34.5% at 10 mg-kg^{-1} *versus* 42 ± 32.2% at 5 mg.kg^{-1}). The number of egg releases, fecundity and viability to hatch and post hatch were also improved when domperidone was administered at 10 mg.kg^{-1}.

TECHNOLOGY PUSH POLICY AND THE NORWEGIAN AQUACULTURE INDUSTRY: THE CASE OF DEVELOPMENT LICENSING SCHEME

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The point of departure for this study is the restructuring dynamics of the Norwegian Salmon farming (*Salmo salar*), which despite growing into one of the most profitable aquaculture industries in the world, has in recent years seen its growth curtailed due to challenges related to parasitic sea lice infections, and escape of farmed fish and mortality. To address these challenges, the Norwegian authorities launched in 2015 a new type of innovation policy instrument: development licences, to foster radical innovations focusing on the development of disruptive fish-farming technologies. Based on mapping of the technology development projects as well as in depth interviews with actors involved in these projects we show how the targeted industrial and/or innovation policy response by Norwegian authorities, is re-shaping the structure of the Norwegian aquaculture industry. We argue that in the short term, the scheme appears to have succeeded in addressing not only the market failure but also the structural innovation system failures primarily associated with capabilities and network failures as it clearly played a crucial role in facilitating both the development of new knowledge and capabilities among the traditional aquaculture players. The scheme is also changing the structure of the Norwegian aquaculture innovation networks (and/or the development of new innovation eco-system) by facilitating entry of new actors and competences from adjacent sectors and strengthening ties between the actors involved in the network. However, overall success of the innovation policy instrument ultimately hinges upon sufficiently addressing some aspects of the institutional failures in the sector.

CAN SPECIES OTHER THAN *Crassostrea gigas* VECTOR OSHV-1 IN SAN DIEGO BAY?

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Ostreid herpesvirus-1 (OsHV-1) and its variants cause mass mortalities in all life stages of the Pacific oyster, *Crassostrea gigas*, and has plagued the aquaculture industry globally. OsHV-1 was originally detected in France in the 1990's, and in 2008 a genetically distinct variant of this virus emerged in correlation with increased mortality events. This variant and others, referred to collectively as "microvariants" (µvars), have spread globally, and in 2018 an OsHV-1 µvar was detected in San Diego, CA (SD µvar) along with mass mortalities of *C. gigas*. Containing the spread of OsHV-1 and its microvariants is crucial to the livelihood of the Pacific oyster aquaculture industry in the United States. However, the spread and emergence of OsHV-1 microvariants despite regulation indicates potentially overlooked sources of infection. One possibility for this spread is through vector species, as OsHV-1 is not limited to *C. gigas* as a host. Other species of bivalves are known to become infected with OsHV-1, including the eastern oyster (*C. virginica*), the bay scallop (*Argopecten irradians*), and OsHV-1 can be carried by the European green crab (*Carcinus maenas*). In San Diego Bay, both native and non-native bivalve species are present in addition to an aquaculture nursery; the ability of these species to become infected with, or vector, OsHV-1 is currently unknown. The goal of this research is to test the ability for potential aquaculture species in the San Diego Bay to vector OsHV-1 to naive *C. gigas* or other native species. Pacific oysters (*Crassostrea gigas*), red abalone (*Haliotis rufescens)*, kumamoto oysters (*Crassostrea sikamea)*, and manila clams (*Venerupis philippinarum*) will each be injected with the SD µvar. After injection, animals will be allowed to incubate for 24-48 hours to shed virus into the water, during which time they will be monitored for mortality. If sufficient viral shedding is detected, water containing shed virus will then be used to attempt infection of naive juvenile *C. gigas*. The juvenile oysters will be monitored daily for mortality, and samples will be taken to test for OsHV-1 DNA in the oyster tissue. Results from this experiment will provide insight into potential vector species for OsHV-1 in San Diego Bay, as well as species that may be at risk of OsHV-1 infection. These results will be useful when implementing aquaculture management decisions for the San Diego Bay and other areas of the US west coast.

TEMPERATURE EFFECTS ON LARVAL DEVELOPMENT OF FLORIDA *Micropterus salmoides floridanus* AND NORTHERN *Micropterus salmoides salmoides* LARGEMOUTH BASS: IMPLICATIONS FOR INTENSIVE INDOOR AQUACULTURE PRODUCTION

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Largemouth bass (LMB), *Micropterus salmoides* is the most popular sportfish in the US, with a rapidly expanding global food fish market. Farmers traditionally raise LMB in earthen ponds, however, they are often plagued with high mortality at the larval stage. Thus, circumventing these initial pond stages to complete indoor intensive culture would streamline production and minimize risks. Therefore, our objectives were to (i) identify the optimal thermal regime for rearing LMB in an indoor recirculation aquaculture system (RAS); (ii) assess the performance of Florida vs. Northern LMB for RAS culture; and (iii) elucidate thermally induced phenotypic changes and inter-linked expression of targeted genes involved in early development.

Florida and Northern LMB were reared at 21°C, 24°C, and 27°C using RAS technology. Fish were randomly sampled at 2 to 28 days post-hatch (DPH) for total length (TL), body area (BA), myotome height (MH), eye diameter (ED), jaw length (JL), yolk area (YA), and oil droplet area (ODA). Yolk utilization efficiency (YUE) and yolk utilization rate (YUR) were calculated. Weight was also determined at 29 DPH along with survival and expression of targeted genes.

There was a significant temperature effect for all morphometric traits, where both sub-species increased in size over the temperature gradient, with the largest traits (TL, BA, MH, ED, JL) detected at 27°C (Fig. 1AB). Northern LMB larvae were typically larger with respect to morphometric traits and weighed more after 29 DPH (Fig. 1C). LMB reared at 21°C had higher survival than those reared at 24 or 27°C, and Northern (17%) had higher survival than Florida bass (11%). Gene expression and yolk results are ongoing. These results suggest that 1) rearing larval LMB at 27°C improves growth performance during early ontogeny, and 2) there is supporting evidence that Northern LMB is selected for faster growth when reared in an indoor RAS.

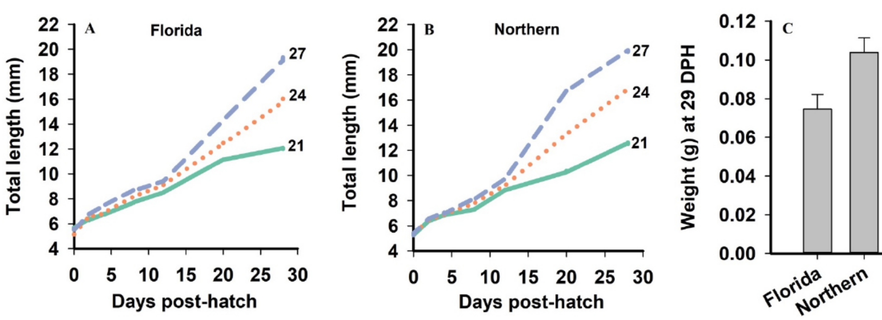

Fig. 1. Effect of temperature on total length of Florida (A) and northern (B) largemouth bass. Impact of sub-species on weight at 29 DPH (C).

WARMWATER MARINE FINFISH IN THE SOUTHERN TIER STATES: CONSUMER PREFERENCE SURVEY RESULTS

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The United States Department of Agriculture-Agricultural Research Service (USDA-ARS) has devoted substantial effort into the development of aquaculture technologies for 20 selected warmwater marine finfish species (Almaco Jack, Atlantic Cod, Black Drum, Black Sea Bass, California Flounder, California Yellowtail, Cobia, Florida Pompano, Greater Amberjack, Olive Flounder, Red Drum, Red Snapper, Sablefish, Southern Flounder, Spotted Seatrout, Spotted Wolffish, Striped Bass, Summer Flounder, Tripletail, and White Sea Bass). Successful commercialization of these species hinges on the availability of readily accessible information on the size of markets, consumer preferences, and market opportunities. However, information on the existing markets and marketing of warmwater marine finfish is currently deficient. This study attempts to address this knowledge gap by assessing and summarizing consumer preferences for 20 locally available warmwater marine finfish species identified as species of interest in southern tier states in the US. A total of 817 surveys (with approximately equal quotas from each state) were collected from seafood consumers across nine southeastern states (Virginia, North Carolina, South Carolina, Texas, Florida, Georgia, Louisiana, Alabama, and Mississippi) using the online Qualtrics platform.

Ninety-four percent of respondents identified a marine fish species as their favorite fish, while 89% of respondents indicated a marine finfish was their most consumed fish species (Table 1). Results indicate that among the identified species of interest, red snapper and spotted wolffish are the most and least recognized species, respectively. While consumer knowledge and preferences for each species vary across states and other socio-demographic characteristics, being available for sale and the opportunity to taste in supermarkets are critical to increasing consumption among consumers. While salmon and tuna were most often listed as favorite or most consumed, 59% and 49% of respondents indicated a marine species other than salmon and tuna was preferred. Findings provide a better understanding of the existing geographic markets and consumer preferences which are vital to the identification and development of new market opportunities for warmwater marine finfish and the improvement and expansion of aquaculture production of marine finfish in the US.

Table 1: Respondents' favorite and most consumed fish by species category

Species Categories	Favorite	Most Consumed
Marine Species (MS)	93.6%	88.6%
Freshwater Species	42.2%	45.2%
Shellfish	17.2%	14.6%
Salmon & Tuna	74.9%	74.7%
MS other than Salmon & Tuna	58.8%	49.4%

THE SYNERGISTIC EFFECT OF COMBINED THERAPEUTIC AND SINGLE ACUTE DOSES OF PITUITARY AND OVAPRIM HORMONE ON LATENCY, FERTILITY, HATCHABILITY, AND SURVIVAL OF AFRICAN CLARIID CATFISH - *Clarias Gariepinus*

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The hatchability of *Clarias gariepinus* using Pituitary Extract and Ovaprim Hormones was carried out at the Hatchery unit of the Fisheries Department's fish Farm of the Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. twelve catfish brood stocks comprising eight females and four males were used for the breeding exercise. Equal numbers of one male to two females were used across all batch treatments (T1-T3). Batch T1 was administered 100% Ovaprim, T2 received 100% Carp Pituitary Extract-CPE, T3 a combination of both synthetic (50% ovaprim) and CPE (50% CPE). T4 was without any hormonal treatment. The latency periods and temperatures were 12h, 16h, 10h and 0h at 27.8°C, 26.9°C, 27.5°C and 26.6°C, respectively, for T1, T2 T3 and T4. T3 had the highest fertilization and hatchability rates of 78%, and 85% respectively followed by T1(7%, 66%), T2(61%, 56%) and lastly T4(26%, 28%). The hatching duration was equally fastest with T3(18h), followed closely by T1 and T4 at 20h and lastly 23h for T2. Similarly, the survival rates from the highest to the lowest were T3(87.77%), T1(84.44%), T4(81.11%) and T2(76.66%). The mean weight gain and specific growth rate in T3 were equally higher with 0.39g and 10.07% followed by T2 (0.39g & 9.63%), T4 (0.33g & 9.54%) and lowest with T1 having 0.32g and 9.44% respectively. The result also indicated that it was more economical to use ovaprim costing (₦700, 1.71USD) compared to ₦1382.4 (3.37USD) with pituitary extract. However, the results showed that both CPE and ovaprim promoted spawning in African catfish. The synergy (T3) had the best fertility, hatchability, and survival rates, and gave the best growth performance. In conclusion, the combined therapeutic dose yielded the overall best in all four parameters of African Clariid Catfish *C. gariepinus* production and is hereby recommended to fish breeders.

Table 1: Induced ovulation and spawning of *Clarias gariepinus* using Synthetic (ovaprim,T1) and non-synthetic (CPE,T2), synergy,T3 and no hormones (T4)

Parameter	**T1**	**T2**	**T3**	**T4**
ABW(g)	1450	1500	1350	1350
Fertility Rate (%)	72.00	61.00	78.00	26.00
Hatchability (%)	66.00	56.00	85.00	28.00
Hatching/Incubation Period(hrs)	20.00	23.00	18.00	20.00
Latency(hrs)	12.00	16.00	10.00	-
Survival (%)	84.44	76.66	87.77	81.11
Initial weight(g)	0.020	0.020	0.020	0.020
Final weight(g)	0.34	0.36	0.41	0.35
Mean weight gain	0.32	0.34	0.39	0.33
Specific growth rate	9.44	9.63	10.07	9.54

THE COMPLETE GENOME OF AN ENDOGENOUS NIMAVIRUS (*Nimav-1_LVa*) FROM SPECIFIC PATHOGEN-FREE SHRIMP *Penaeus vannamei* – THE NEED FOR REFERENCE GENOMES OF PENAEIDS AND OTHER CRUSTACEAN GENOMES

Acacia Alcivar-Warren1* and Weidong Bao2

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White spot syndrome virus (WSSV), the lone virus of the genus Whispovirus under the family Nimaviridae, is one of the most devastating viruses affecting the shrimp farming industry (https://pubmed.ncbi.nlm.nih.gov/31947590/). Knowledge about this virus, in particular its evolution history, has been limited, partly due to its large genome and the lack of other closely related free-living viruses for comparative studies.

A reconstructed full-length endogenous nimavirus consensus genome, *Nimav-1_LVa* (279,905 bp) was found in the genome sequence of the Kehai isolate of *Penaeus vannamei* from China (GCA_003789085.1; breed Kehai No. 1, cultured shrimp imported from USA; 1.6 Gb) in which ~12 copies of *Nimav-1_LVa* exist. This endogenous virus seems to insert exclusively into the telomeric pentanucleotide microsatellite (TAACC/GGTTA)n. 117 putative genes are predicted. Sequence analysis of these genes indicates that there are four more recognizable nimaviruses core/ancestor genes, wsv112 (dUTPase), wsv206, wsv226 and wsv308 (nucleocapsid protein), making the total number to be 43. Some *Nimav-1_LVa* contain introns, such as g012 (IAP), g046 (CHH), g155 (innexin), g158 (BI-1-like). More than a dozen *Nimav-1_LVa* genes are involved in the pathogen-host interactions. We hypothesize that g046, g155, g158 and g227 (semaphorin 1A like) are recruited host genes for their roles in immune regulation.

Availability of *Nimav-1_LVa* sequence will help understand the genetic diversity, epidemiology, evolution, pathogenicity, and virulence of WSSV. Future studies will focus on the possibility that *Nimav-1-LVa* represents a free-living virus, yet unidentified but infecting crustaceans, because almost identical *Nimav-1_LVa* sequences were also found in genome sequences from black tiger shrimp *P. monodon* isolate Shenzhen from China (GCA_002291185.1, wild shrimp; 1.4 Gb) and *P. japonicus* Guanxi isolate from Japan (GCA_002291165.1; wild shrimp; 1.6 Gb). In addition, three relatives of *Nimav-1_Lva* are detected in *P. monodon: Nimav-1_PMo, Nimav-2_PMo* and *Nimav-3_PMo*. So far, *Nimav-1_LVa* sequences have not been found in the genomes of *P. monodon* isolate 26D (GCA_007890405.1; cultured shrimp from Vietnam, originally from Australia; ~1.6 Gb), *P. monodon* isolate SGIC_2016 from Thailand (GCA_015228065.1; cultured shrimp from Surat Thani, Thailand; ~2.4 Gb), and *P. chinensis* isolate QD-2010 from China (GCA_016920825.1; wild shrimp; ~1.6 Gb).

Future research should focus on (a) sequencing fully assembled reference genomes for all Penaeid shrimp, (b) determine, by FISH, the chromosome location of the integrated *Nimav-1_LVa* or its relatives in crustacean genomes, and (c) survey the existence of Nimavirus in more Crustacean species. Search and isolate the free-living *Nimav-1_LVa* virus, if possible.

INTERACTIONS OF METALS, GLYPHOSATE, PARASITES AND EPIGENETICS IN SHELLFISH AND FISH: A REVIEW

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Current studies indicate that parasites, environmental pollutants, and other pathogens interact with each other in aquatic organisms. Parasites impact host's growth, reproduction, immunity, and behavior, while some metals are endocrine disruptors. Some metals are chelated by the herbicide Glyphosate (N-phosphonomethyl-glycine). Metal bioaccumulation and parasitic diseases could vary in different species depending on some environmental factors, such as temperature changes or contaminants. Little is known about the interactions of metals, Glyphosate-based herbicides (GBHs), parasites, and epigenetics in aquaculture species. GBHs can modify toxicity and epigenetic marks in shellfish and fish. Recent findings of portions of the SARS-Cov-2 coronavirus RNA in bivalve mollusks and in wild carp of Wuhan, China, as well as the potential association of parasite co-infection with a reduced risk of severe COVID-19 in African patients, suggest the need for a more holistic approach to studying interactions of metals and other contaminants in parasites and their hosts.

A review of the interactions of metals, GBHs, parasites, and epigenetic marks in shellfish and fish is presented. The glyphosate-associated Shikimate pathway was first reported in apicomplexan parasites in 1999. Cadmium (Cd) is highly toxic to earthworm *Eisenia fetida,* and the presence of glyphosate markedly reduced the acute toxicity of Cd to earthworm. GBHs impact the gut microbiome of earthworm species (*Alma millsoni, Eudrilus eugeniae,* and *Libyodrilus violaceus*) - a major shift reported in bacterial populations in exposed earthworms with Proteobacteria becoming the dominant phylum. Affected bacteria were mostly from the genus Enterobacter, Pantoea and Pseudomonas, while they were present at a minor abundance in unexposed earthworms. Mortality rate and accumulation of Cd in the earthworms decreased with the increase of the glyphosate/Cd molar ratio; longer exposure glyphosate alleviated the weight loss of earthworm and the total Cd absorption. Glyphosate can generate oxidative stress in *Danio rerio* by interfering with mitochondrial metabolism, impairing mitochondria in oocytes, and adversely affecting ovarian maturation. Arsenic affects resistance to cestodes in brine shrimp. GBH Roundup® Biactive modifies Cd toxicity to *Daphnia carinata*. Exposure to GBH Roundup® Original on hemocytes of the snail *Biomphalaria glabrata* infected by the platyhelminth *Echinostoma paraensei* showed that the frequencies of dead hemocytes were lower in the infected group and higher in pesticide treated groups, suggesting impairment of the internal defense system of *B. glabrata* making the snails more vulnerable to parasitic infections.

Studies are needed to understand the synergistic interactions between metals, pesticides, parasites, and epigenetics in shellfish and fish using the tools of One Health.

THE COMPLETE GENOME OF AN ENDOGENOUS NIMAVIRUS (*Nimav-1_LVa*) FROM SPECIFIC PATHOGEN-FREE SHRIMP *Penaeus vannamei* – THE NEED FOR REFERENCE GENOMES OF PENAEIDS AND OTHER CRUSTACEANS

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ENDOGENOUS VIRAL ELEMENTS (EVE) OF *Decapod penstylhamaparvovirus 1* (INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS, IHHNV) – IMPLICATIONS FOR SHRIMP DIAGNOSIS

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Shrimp endogenous viral elements (EVE) have been reported in White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), now renamed *Decapod penstylhamaparvovirus 1*. IHHNV is a ssDNA virus in the family Parvoviridae, one of the smallest penaeid shrimp viruses encoding three ORFs and listed by the World Organisation for Animal Health (OIE). It was first reported in the 1980s in Hawaii, USA (reference genome AF218266.2, 3909 bp). This virus was found integrated in the *P. monodon* genome collected from Australia, Thailand, Africa (Tang et al. 2003; Tang & Lightner. 2006). In some African isolates, IHHNV (DQ228358) was found inserted into *RTE-2_PMo* non-LTR retrotransposon in Repbase (www.girinst.org), and these DQ228358-like insertions are absent in other *P. monodon* isolates. We suspect IHHNV have also endogenized *P. vannamei* and some IHHNV-EVEs remain in some *P. vannamei* genomes, introduced to the American continent in the 1970s. IHHNV causes high mortality in *Penaeus stylirostris*; but no mortality has been reported in *P. vannamei* and *P. monodon* where is known to cause slow growth and deformities in the exoskeleton (called Runt deformity syndrome), causing serious economic losses. Homology searches using the 10 whole genome sequence (wgs) databases for five penaeid species in GenBank revealed that the sequences of infectious IHHNV isolates from *P. stylirostris* of Hawaii (AF218266.2), *P. vannamei* of Ecuador (AY362548, 3775bp and OL598344, 3203bp) and Australia (EU675312, 3832bp) are integrated in chromosome_35 of *P. monodon* genome from Thailand (NSTDA_Pmon_1, GCF_015228065.1, 2.39 Gb). These IHHNV-EVE-related sequences are also present in *P. vannamei* breed Kehai No.1 LVANscaffold_759 of *P. vannamei* genome farmed in China (assembly ASM378908v1, ~1.86 Gb). Non-infectious IHHNV-EVEs have also been found within the shrimp genome in populations of *P. monodon* from Africa (Type A from Madagascar: DQ228358, 4655bp) and Australia, and they are also integrated in both chromosome_35 of *P. monodon* genome from Thailand and the genome of *P. vannamei* breed Kehai No.1 LVANscaffold_759, to be confirmed once a new, continuous, fully assembled reference genome for SPF *P. vannamei* from the USA is available (expected size: 2.87 Gb). A new reference genome for *P. vannamei* is urgently needed to help improve accuracy in diagnosis of IHHNV/ *Decapod penstylhamaparvovirus 1* (OIE PCR, multiplex PCR, IQ 2000 LAMP).

THE *NonLTR-1_LVa* NON-LTR RETROTRANSPOSON FROM THE FIRST SPECIFIC PATHOGEN-FREE (SPF) SHRIMP *Penaeus vannamei* PRODUCED IN THE UNITED STATES IS SIMILAR TO A RETROTRANSPOSON PUTATIVELY ASSOCIATED WITH ABDOMINAL SEGMENT DEFORMITY DISEASE (ASDD) OF FARMED *P. vannamei* FROM THAILAND

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Abdominal Segment Deformity Disease (ASDD) of farmed Pacific white shrimp *Penaeus vannamei* from Thailand was suggested associated with a partial non-long terminal repeat (non-LTR) retrotransposon sequence (NLRS) (KC179708, 4,101bp; Sakaew et al. 2013). ASDD was initially associated with the presence of viral-like particles seen by electron microscopy in ventral nerve cords of affected shrimp. Using the NLRS probe, in situ hybridization signals were detected in abdominal-ganglion neurons of ASDD shrimp's distorted abdominal muscles, but not normal shrimp. ASDD appeared related to inbreeding and long-term use of eyestalk-ablated female broodstock used in commercial hatcheries, and increased prevalence in mysis stage offspring from those broodstock.

A search in the Rebpase database (www.girinst.org) revealed that KC179708 represented part of the representative full-length of this non-LTR retrotransposon family, designated as *NonLTR-1_LVa* in Repbase [Bao 2015, Repbase Reports 15(4), 1579]. Nucleotides 3-4,101 of KC179708 shows 96.9% identity to nucleotides 1,974-6,062 of *NonLTR-1_LVa* consensus sequences (6,180bp), a young non-LTR family reconstructed from multiple members of only 2% divergency from the consensus. *NonLTR-1_LVa* was characterized in the genome of the first SPF *P. vannamei* domesticated by the breeding program of the US Marine Shrimp Farming Program (USMSFP) maintained in Kona, Hawaii, USA. *NonLTR-1_LVa* contains 50% CGs, two ORFs, and conserved protein domains like R1-I-EN from superfamily EEP (exonuclease/endonuclease/phosphatase) and reverse transcriptase (RT, RNA-dependent DNA polymerase) from RT-like superfamily.

Homology searches using the whole genome sequence databases in Genbank identified multiple copies of *NonLTR-1_LVa* in five penaeid species. It is an intrinsic part of penaeid shrimp genomes, comprising a significant part of the whole genome. In the genome sequence of muscle DNA from a male *P. vannamei* farmed in China, breed Kehai No. 1 (assembly ASM378908v1, 1.86 Gb), more than 250 loci are found inserted with relatively young copies of *NonLTR-1_LV*a (<95% identity to its consensus), excluding 4 times more loci with insertions of older *NonLTR-1_LVa* or of its sibling families. *NonLTR-1_LVa* was found in *P. vannamei* transcriptomes from various developmental stages (nauplii, mysis, postlarvae) and adult tissues (hepatopancreas, muscle, eyestalk, testis, ovaries), with no major increase in expression in ovaries after eyestalk ablation. It remains to be determined if the expression of *nonLTR-1_LVa* increase the incidence rate of ASDD.

MANGROVE SHELLFISH SESSION: A ONE HEALTH PERSPECTIVE ON ANTIMICROBIAL RESISTANCE, FOOD SAFETY AND FOOD SECURITY

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Programa 'UNA SALUD / ONE HEALTH Epigenomics and Microbiomes:
Somos lo que comemos / We are what we eat'
Fundación para la Conservación de la Biodiversidad (FUCOBI),
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The MANGROVE SHELLFISH session will address the following topics: mangrove biodiversity, conservation and reforestation; the Mangrove Epigenome (MangroveENCODE) Project of the FUCOBI Foundation of Ecuador: A One Health approach to Conservation of Healthy Mangroves, to produce healthy shellfish and fish, to protect people's health long-term; genomes, transcriptomes, and epigenomes of mangrove trees, epigenetic regulation in shellfish; transposable elements (TEs) in mangroves and shellfish (shrimp, oysters) genomes; Gypsy long terminal repeat (LTR) retrotransposon and mangrove resilience (pH and temperature); greenhouse gas emissions and carbon sequestration (blue carbon) in response to climate change and human management in coastal wetlands / mangroves; the status of mangroves in Ecuador; status of mangrove forests in Honduras: metals and pesticides; mangrove microbiomes; antimicrobial resistance (AMR) of pathogens in mangrove sediment; endocrine disrupting chemicals (EDCs) like heavy metals, pesticides, antimicrobial herbicide glyphosate, metals chelated by glyphosate, bacterial transgene *Bacillus thurigiensis*, *Vibrios* spp. and plastics in mangrove sediment and shellfish (clams, shrimp); Shrimp Scampi: a citizens science project to examine the levels of endocrine disrupting chemicals (metals, glyphosate, bisphenol A) in frozen seafood sold at US supermarkets; microplastics and organic pollutants in mangrove sediments and marsh clam (*Polymesoda expansa*) from selected coastal areas of Negros Oriental, Philippines, as indicators of coastal pollution, among others. A female researcher, Suhua Shi from China, will receive a recognition as '2022 Outstanding ONE HEALTH Researchers in Aquaculture' by the Foundation for Conservation of Biodiversity (FUCOBI) of Ecuador in recognition of her research on mangroves genomes, transcriptomes and epigenomes.

A REVIEW OF WHITE SPOT SYNDROME VIRUS (WSSV) FROM ECUADOR, ENDOGENOUS VIRAL SEQUENCES (EVE) OF WSSV (WSSV-EVE), AND ENDOGENOUS NIMAVIRUS *Nimav-1_LVa:* THEIR INTEGRATION IN THE GENOMES OF THE FIRST SPECIFIC PATHOGEN-FREE (SPF) *Penaeus vannamei* DOMESTICATED IN THE UNITED STATES AND *P. vannamei* FARMED IN CHINA

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It was previously reported that mortalities of cultured shrimp, *Penaeus vannamei*, induced by white spot syndrome virus (WSSV) [Nimaviridae(1); Whispovirus(1); WSSV(1)] have occurred in Ecuador since May 1999 (Rodriguez *et al*. 2003) confirmed by histopathology and PCR, and showed 'an apparent association between lower temperature and increased mortality rates in commercial ponds'. However, it's possible that "asymptomatic" WSSV may have been present in Ecuador before 1999. Low survival in semi-intensive ponds is rarely examined by histology and therefore the start of the epidemic might have been missed. Here, we report detection of WSSV in wild and farmed *P. vannamei* of Ecuador since 1996 using histopathology and confirmed by *in situ* hybridization (Flegel, *pers. comm.*) techniques. WSSV was first detected by PCR in 5 of 38 (13%) juveniles from Bonanza, Isla Puna, Salinas River Estuary, Guayas province of Ecuador (samples provided by Empacadora Nacional in 1996, placed in liquid nitrogen immediately after collection, until DNA isolation and PCRs were performed). All 27 wild broodstock from Puerto Cayo, Manabí province, collected in 1996 and 25 broodstock from Puerto Cayo collected in 2000 were negative (0/52) by PCR, suggesting geographic differences in virus prevalence.

The sequence of WSSV from *P. vannamei* of Ecuador collected in 2015 (WSSV-EC-15098, MH090824, 288,997bp) was recently published. WSSV-like sequences (WSSV-EVE, Utari *et al*. 2017) are found in genomic libraries and expressed sequence tags isolated 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, USA since late 1980's. DNA from juveniles of SPF Kona Line (characterized by high susceptibility to all shrimp viruses but high growth performance) was subject to a pilot whole genome sequencing using PacBio SMRT method. From ~424 Mb genomic sequence, 312 diverse repetitive families were characterized including a nimavirus, *Nimav-1_LVa,* of 279,905bp (Bao *et al*. 2020: https://pubmed.ncbi.nlm.nih.gov/31947590/) and are deposited in Repbase (www.girinst.org). *Nimav-1_LVa* is supported by 3-6 copies throughout the whole length, all flanked by the putative telomeric (GGTTA)n, with some ORFs significantly homologous to WSSV isolate of *P. japonicus* from China (CN01) collected in 1994 (NCBI reference sequence NC_003225.3, 309,286bp). Homology searches using 11 whole genome shotgun (wgs) databases in GenBank revealed that NC_003225.3 and MH090824 are not integrated in the draft genome sequence of *P. vannamei* farmed in China (assembly ASM378908v1; ~1.7Gb), but *Nimav-1_LVa* is integrated in scaffold Penaeus vannamei breed Kehai No.1 LVANscaffold_3666 (QCYY01003664, 990704bp, 428 matches). Because the genome size of SPF *P. vannamei* is expected to be ~2.87 Gb, access to a full, contiguous, whole reference genome for *P. vannamei* is urgently needed from both the founder SPF stocks and wild *P. vannamei* of Ecuador (samples available for research, collected since 1996) to study evolution and pathogenicity of WSSV and *Nimav-1_LVa*.

THE SHRIMP GENOME AND EPIGENOME (ShrimpENCODE) SESSION: IN MEMORY OF DR. DONALD V. LIGHTNER

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The ShrimpENCODE session is in memory of shrimp pathologist Dr. Donald V. Lightner (1945-2021). The session will address the following topics: epigenetic regulation in aquatic micro-invertebrates: a non-canonical system of bacterial origin; genome sequencing and assembly strategies and comparative analysis of the genomic characteristics of Penaeid shrimp species; the genomes, epigenomes, and transcriptomes of *Penaeus vannamei*, *P. monodon*, *P. japonicus*, *P. chinensis*, and *P. indicus*; 312 transposable elements characterized from the first specific pathogen-free (SPF) *P. vannamei* produced by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP); the complete genome of an endogenous nimavirus (*Nimav-1_LVa*) integrated in the genome of SPF *P. vannamei* - the need for fully-assembled, contiguous reference genomes of penaeids and other crustaceans to study virus evolution and pathogenicity; white spot syndrome virus (WSSV) from Ecuador, endogenous viral elements (EVE) of WSSV (WSSV-EVE) and endogenous nimavirus *Nimav-1_LVa*: their integration in the genomes of the original SPF *P. vannamei* produced in the U.S. and *P. vannamei* farmed in China; EVEs of *Decapod penstylhamaparvovirus 1* (Infectious Hypodermal and Hematopoietic Necrosis Virus, IHHNV) – implications for shrimp diagnosis; *P. monodon* endogenous Type-A IHHNV integrated in the genomes of *P. monodon* from Thailand and *P. vannamei* from China; chromosomal locations of *RTE-3_LVa* a non-LTR retrotransposon identified in the first SPF *P. vannamei* produced in the US: a potential sex marker for shrimp; understanding of the shrimp immune response to pathogens from its transcriptome; estimation of linkage disequilibrium and effective population size in a *P. vannamei* population using a novel 50K SNP genotyping array; the hurdles of delivery CRISPR-Cas9 components for gene editing in penaeid shrimps; assessment of the oxidative stress and bio-transformation enzymatic effects of glyphosate exposure on *P. vannamei*; virus interference and occurrence in crustaceans; the *NonLTR-1_LVa* non-LTR retrotransposon from the first SPF *P. vannamei* domesticated in the USA is similar to a retrotransposon putatively associated with abdominal segment deformity disease (ASDD) of farmed *P. vannamei* from Thailand; the developmental biology of penaeid shrimp; and White Paper: the need to sequence the genomes of *Penaeus Fabricius*, 1798 species to confirm taxonomy classification accepted by The World Register of Marine Species (WoRMS), among others.

Two female researchers, leaders of the genome sequencing projects of *P. vannamei* (Fuhua Li, China) and *P. monodon* (Nitsara Karoonuthaisiri, Thailand) will be recognized as 2022 ‘Outstanding ONE HEALTH Researchers in Aquaculture’ by the Foundation for Conservation of Biodiversity (FUCOBI) of Ecuador. Twenty-two students, postdocs and research associates from twelve countries (Belgium, Chile, China, Ecuador, Honduras, India, Mexico, Nigeria, Philippines, Romania, Thailand, United States) will be recognized as winners of the 2022 ‘Johnnie Castro Montealegre Travel Awards’ by the FUCOBI Foundation, to attend AQUACULTURE 2022.

SINGLE OR COMBINED EFFECTS OF INULIN OR MANNAN-OLIGOSACCHARIDE (MOS) ON GROWTH, FEED UTILIZATION, FATTY ACID PROFILES, AND GUT MORPHOLOGY OF CHAMO STRAIN NILE TILAPIA FRY REARED AT SUBOPTIMAL TEMPERATURE

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The present study was designed to investigate the effect of supplementation of inulin and mannan-oligosaccharides (MOS), single or combined, on the growth performance, feed utilization, fatty acid profiles, and gut morphology of Chamo strain Nile tilapia *Oreochromis niloticus* (L.) fry reared at sub-optimal temperature (22-23oC). Nile tilapia fry (initial weight 1.6 ± 0.074g) were fed four diets supplemented with prebiotics at 0 g of prebiotic/kg of fish feed (Diet-T1), 5 g kg-1inulin-supplemented diet (Diet-T2), 6 g kg-1 MOS-supplemented diet (Diet-T3), or a combination of 2.5 g kg-1 inulin and 3 g kg-1 MOS (Diet-T4). Each diet was randomly assigned to three aquaria and hand-fed 8% of their body weight divided into three portions daily. The results showed that Nile tilapia fed Diet-T4 had the highest final weight, weight gain and specific growth rate (SGR) than fish fed Diet-T1, Diet-T2 and Diet-T3 although the effect was not significant ($p > 0.05$). Fish fed Diet-T4 also had the highest FCR than other feeding groups. Fish fed Diet-T4 and Diet-T3 had the highest content of polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA; 22:6 *n*-3) and eicosapentaenoic acid (EPA; 20:5 *n*-3) than Diet-T1 and Diet-T2. Gut morphology parameters revealed significant ($P < 0.05$) increase in villi length (VL), villi width (VW) in fish fed Diet-T4 and Diet-T3 and higher goblet cell number (GCN) in proximal and middle portion of intestine in fish fed Diet-T3. Overall, this study suggested that supplementing fish feed with MOS alone or in combination of with inulin improved growth performance, intestinal morphology and fillet quality of Chamo strain Nile tilapia.

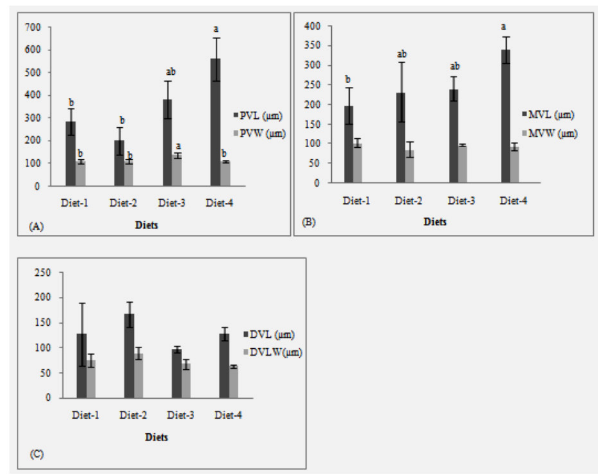

Figure 1. Graph showing proximal villi length (PVL) (A), middle villi length (MVL) (B), and distal villi length (DVL) of small intestine of Nile tilapia in µm

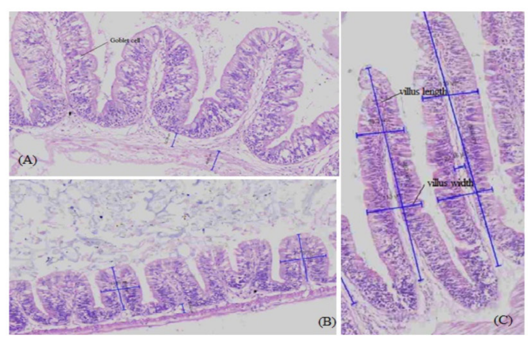

Figure 2. Gut morphology slide samples scanned by MoticEasyScan Pro 6 (USA) (arbitrarily chosen as examples): goblet cell (A), villi length and width (B& C)

REVERSE-GENETICS APPROACH IN DEVELOPING A *Macrobrachium rosenbergii* NODAVIRUS (MrNV)-BASED VIRAL VECTOR FOR AN ORAL DEVELIVERY OF THERAPEUTICS IN SHRIMP

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Shrimp is one of the major aquaculture species that is farmed at an industrial scale in Asia and the Americas. The growth and profitability of shrimp farming is hampered due to viral disease outbreaks and currently, there is no commercially available therapeutic to control these diseases. Despite the advancement of gene-therapy technology such as RNAi, the lack of a cost-effective oral delivery method impedes their applicability to shrimp aquaculture. We present here an orally administered shrimp viral vector capable of delivering a gene payload. As a proof-of-concept study, we reverse-engineered *Macrobrachium rosenbergii* nodavirus (MrNV) making it replication-deficient by replacing its RNA-dependent RNA-polymerase (RdRp) gene with a marker gene, green fluorescent protein (GFP). The recombinant MrNV was produced using a baculovirus (BV) expression system in insect cell, Sf9. The assembly of mature virions of MrNV carrying GFP gene in Sf9 cells was confirmed by transmission electron microscopy and by measuring GFP expression by fluorescence microscopy and flow cytometry.

An MrNV viral vector should meet two criteria: First, the recombinant MrNV (MrNV-GFP_rBV) should be able to successfully deliver and express GFP gene into shrimp cells. Second, the viral vector should be able to deliver the payload via oral route in shrimp. For this, we delivered MrNV-GFP_rBV by injection and oral route by mixing the MrNV-GFP_rBV with in shrimp feeds. Three groups comprising five individual SPF *Penaeus vannamei* shrimp (2.5-3g) were treated with: Group 1- was injected once with ~1 x 107 pfu/mL of MrNV-GFP_rBV; Group 2- was fed with commercial feeds soaked with SF9 cells infected with MrNV-GFP_rBV at 5% of the biomass for 5 days; Group 3 - was fed with the same commercial feed (without the virus) and served as the negative/naïve control. Hemolymph were drawn from shrimp in all three groups after 5 days, hemocytes were separated by centrifugation and seeded in 24-well plate with L-15 media, then viewed under fluorescent microscope.

Results show that hemocytes from both Groups 1 and 2, i.e. rBV-MrNV-GFP delivered by injection and oral feeding expressed GFP (Fig. 1). These results provide a strong evidence that a baculovirus based shrimp noda viral vector can effectively deliver a foreign (GFP) gene *in vivo* by injection and via oral route.

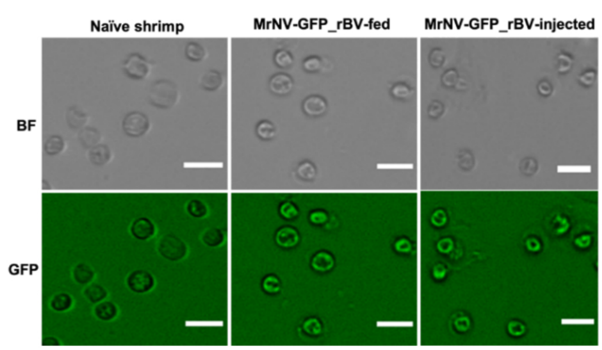

Figure 1. Delivery and expression of GFP in *P. vannamei* hemocytes by the viral vector MrNV-GFP. Bright field (BF) photographs and Green Fluorescent Protein (GFP) expressions in the bottom panel. Scale bar indicated is at 10 µm.

EVALUATION OF AN OPEN-SOURCE FISHMEAL AND FISH OIL FREE LARGEMOUTH BASS FEED

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At the turn of this century the production of farmed largemouth bass (LMB) stood at 178 tonnes. By 2019, worldwide production was above 480,000 tonnes, with most being farmed in China. The massive increase in LMB cultivation has been attributed to technological innovation in the hatchery combined with farmers switching from tilapia due to the higher market price of LMB. As with other aquaculture ventures several issues obstruct future growth of LMB farming. Major impediments include emergence of various diseases, lack of genetically selected strains, and poor quality, production, and distribution of seed. LMB has a high dietary protein requirement, and this is generally supplied in the form of fishmeal (FM). Demand for FM remains elevated, and the future promises higher raw material costs for aquafeed manufacturers. Already feed represents 60% of LMB farming operational costs and, unless cheaper, more sustainable alternative protein-based diets emerge, this too will represent an obstruction to sector growth. To demonstrate the potential for excluding FM from LMB aquafeeds we compared the performance of an open-source, FM and fish oil (FO) free diet (F3) against two commercial, closed formulation LMB feeds (Xinxin or Alltech-Coppens [A-C]).

A 10-week feeding trial was undertaken in a RAS using 3 randomly designated tanks per diet. Fish (15 g, n=20/tank) were fed twice daily to satiation over 5 min. Groups were weighed every 3 weeks and at trial end to monitor feed conversion ratio (FCR) protein efficiency ratio (PER) and weight gain. There were no differences in FCR or PER between diets ($P > 0.05$; Table 1). However, weight gain and survival were lower in fish fed the A-C feed.

Whole body proximate composition of fish at trial end differed ($P < 0.01$; Table 2) reflecting the composition of feeds. There were no differences between feeds for percent apparent digestibility coefficients or phosphorus availability. This study demonstrates that total replacement of dietary FM/FO in LMB feeds is a realistic and economically viable proposition.

	Xinxin	A-C	F3	P value
% wt gain	374[a]	250[b]	398[c]	0.003
K factor	1.19	1.21	1.29	0.330
% survival	98.3[a]	81.7[b]	100[a]	0.001
FCR	0.86	0.93	0.89	0.088
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

	Xinxin	A-C	F3	P value
Moisture	68.6	68.5	68.8	0.62
Protein	18.1[a]	16.0[b]	17.9[a]	0.01
Lipid	7.9[a]	10.7[b]	8.8[a]	0.01
Ash	4.5[a]	4.0[b]	4.0[b]	0.01

Table 2. Whole-body proximate composition of LMB fed commercial and experimental feeds.

CHARACTERIZATION OF THE BAY SCALLOP COCCIDIA (BSC) IN *Argopecten irradians irradians*

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Since 2019, bay scallop populations in New York have been suffering large-scale summer mortalities resulting in over 90% reduction in biomass. These events were associated with heavy infections by an undescribed parasite dubbed Bay Scallop Coccidia (BSC). BSC infects and disrupts multiple scallop tissues including kidney, adductor muscle, gill, and gonad. This presentation summarizes some of our latest findings on BSC and on disease dynamics in the field.

Light and transmission electron microscopy analysis of infected scallop tissues allowed the identification of both intracellular and extracellular stages of the parasite, including sporozoite stages that display structural characteristics of the Apicomplexa (e.g., micronemes). Field surveys performed in 2020 and 2021 demonstrated a strong seasonal signature in disease prevalence and intensity, with severe cases increasing as summer progresses before slightly decreasing (Figure 1), likely as a result of the mortality of most heavily infected scallops. Laboratory investigations underline higher mortalities in most severely infected scallops exposed to ecologically-relevant high temperature, while dissolved oxygen appears to have less effect on disease dynamics. Overall, our results suggest that BSC infection plays a major role in the collapse of bay scallop populations in New York. In this framework, BSC may synergistically interact with stressful environmental conditions to impair the host and lead to mortality. Our current investigations target the characterization of host-parasite interactions, including molecular cross-talks and possibility to breed resistant scallops.

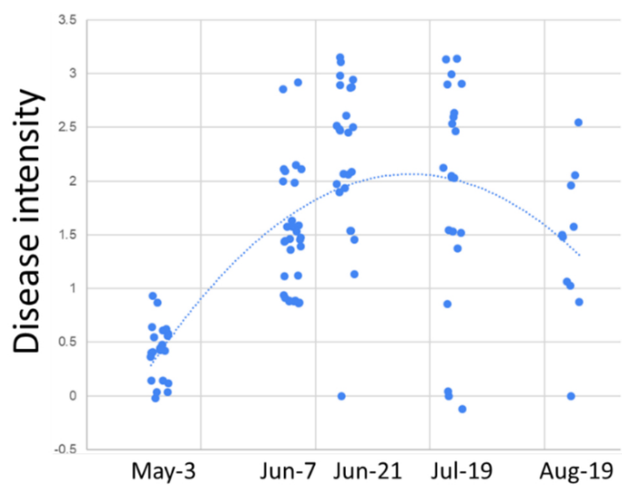

Figure 1. A representative curve showing changes in BSC prevalence and intensity (on a scale ranging from 0 –negative- to 3 –heavily infected) in scallop kidney tissues during summer.

UPDATES FROM THE EAST COAST HARD CLAM SELECTIVE BREEDING COLLABORATIVE

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The hard clam, *Mercenaria mercenaria*, is extensively cultured along the Atlantic seaboard of the U.S. Maintenance and growth of this aquaculture industry relies on hatchery production of seed, and billions of seed clams are produced annually to fulfill aquaculture and restoration needs. Several states regularly suffer losses in hard clam stocks due to disease (e.g. QPX in the Northeast) and environmental stress (e.g. heat waves in Florida). Therefore, the production of quality seed able to survive under harsh biological and environmental conditions represents a priority for the aquaculture community. This collaborative initiative builds on ongoing cooperation and new partnerships among Sea Grant programs, scientists and extension teams in five Atlantic states to develop a hard clam selective breeding program using state of the art genomic tools, for the benefit of clam farmers throughout the region. The team has just completed the sequencing and assembly of the hard clam genome and is currently re-sequencing clams collected from Maine to Florida to gather information about the genetic diversity of the species throughout its natural range in the U.S. Generated genomic data will be used to develop an efficient and cost-effective genotyping platform (SNP array) for *M. mercenaria*. This genotyping platform will enable genome-assisted selection for traits relevant to various regions supporting the growth of the hard clam aquaculture industry. These activities will serve as a basis to establish clam breeding programs linking scientists, extension networks and the industry to provide growers with superior clam stocks.

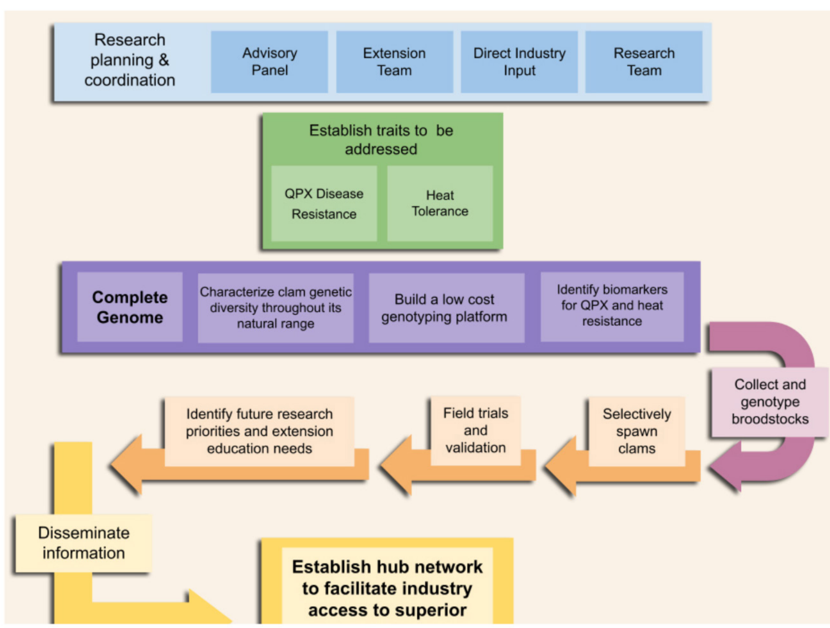

Figure 1: Schematic representation of the hub workflow.

CHARACTERIZATION OF NEUROPEPTIDES IN *Crassostrea virginica* WITH A SPECIAL EMPHASIS ON FEEDING-RELATED GENES

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Suspension-feeding bivalves, including the oyster *Crassostrea virginica,* use mucosal lectins to discriminate and capture food particles via interactions with particle surface carbohydrates. Endogenous factors, and most specifically neuropeptides, affecting the expression of mucosal lectins and feeding processes remain unclear.

The aim of this study was to interrogate the genome of *C. virginica* to identify and characterize putative neuropeptide genes. A special focus was given to genes with potential function in feeding processes. Thus, the main organs of expression of these genes were determined using quantitative PCR. Further, synthetic neuropeptide F (NPF) and visceral ganglia extracts were injected to oysters to evaluate their impact on genes involved in feeding processes and energy homeostasis.

A total of 61 neuropeptide genes, including duplicated genes, were identified in the *C. virginica* genome, and homologs of more than 50% of these have been suggested to play a role in feeding processes in other invertebrates. Gene expression analyses showed that the visceral and cerebral ganglia and the digestive system are the main organs involved in neuropeptide production. Further, the expression of several neuropeptide genes, including NPF and insulin-like peptides, increased after starvation. Finally, the injection of visceral ganglia extracts and synthetic NPF significantly increased the expression of a mucosal lectin and a glycogen synthase, which are known to be involved in particle capture and glucose storage, respectively.

This study presents the first neuropeptidome in *C. virginica* and points to some neuropeptides that are involve in the control of the feeding process and energy homeostasis in oyster.

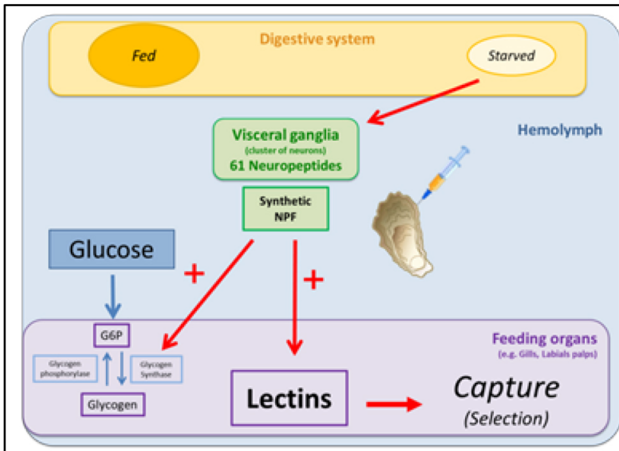

Figure 1. Schematic drawing of the role of neuropeptides in feeding processes and metabolism in *C. virginica*.

PHYSIOLOGICAL COMPENSATION FOR ENVIRONMENTAL SALINITY IN GAR: IMPLICATIONS FOR CULTURE

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There are seven extant members of the Order Lepisosteiformes commonly known as gar distributed in North and Central America. This group of fishes is cultured as a food-fish and for conservation stocking. Members of this family possess many primitive characteristics and span a wide range of habitats, including freshwater rivers, estuaries, and saline coastal marshes. Euryhalinity is important to many populations, but relatively little is known about adaptations to compensate for salinity changes, information important for their culture and introduction to natural habitats. Therefore, several experiments were conducted to better understand physiological changes associated with acclimation to a range of salinity in alligator gar (*Atractosteus spatula*). To guide an understanding of changes in mucosal composition which may be important for general health and immune defenses, mucus peptides were compared between gar acclimated to freshwater (0 ppt) or saline water (20 ppt). Scanning and transmission electron microscopy were used to examine changes in gill ultrastructure. Capabilities to respond to a handling stressor were evaluated using blood measurements of primary and secondary stress responses. In addition, to understand the ability to rapidly compensate for a change in salinity, gill and kidney proteomic responses were evaluated during a time-course of salinity acclimation, alongside blood osmoregulatory measurements. Collectively, results of this study will be discussed in the context of providing a baseline of understanding important for guiding culture practices for gar.

IMPROVING EQUITABLE ACCESS TO LOCALLY FARMED SEAFOOD THROUGH THE SOUTH CENTRAL LOS ANGELES SUSTAINABLE SEAFOOD HUB

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Most Americans do not eat enough seafood to receive health benefits, with greatest deficits among low-income and food-insecure communities. Yet few assessments of seafood preferences and barriers for low-income/Food Insecure (FI) consumers exist. There is thus a critical need to identify community-relevant opportunities to 1) improve public health through expanded education and access to seafood; and 2) determine the role of local marine aquaculture industries in supporting these efforts.

The South Central LA Sustainable Seafood Hub was developed to fill this gap. A pilot of the Hub was established in 2020 to create space for trusted community-based intermediaries to deliver sustainable seafood directly to residents of South Central Los Angeles, a neighborhood impacted by systemic barriers that perpetuate high rates of food insecurity, poverty, and disease risk. The Hub was launched via partnerships between Southern California-based aquaculture producers Holdfast Aquaculture (HA) and Santa Barbara Mariculture (SBM), researchers and educators from University of Southern California (USC) Sea Grant, and Community Services Unlimited (CSU), a 501c3 established in 1977 in South Central Los Angeles (SCLA) with a strong emphasis on promoting food sovereignty in the area. The Hub's pilot efforts focused on 1) understanding existing demand, preferences, and barriers to seafood consumption among SCLA community members; and 2) providing experiential learning opportunities about California aquaculture products through provision of mussels produced by HA and SBM as well as a live-streamed cooking demonstration and Q&A with the producers and CSU.

We will share the outcomes of our exploratory community seafood survey as well as feedback gathered from focus groups of community members who were provided with locally farmed mussels to prepare at home. Lessons learned from successes and challenges of the pilot program will also be discussed. We are seeking to expand this Hub model of a more equitable sustainable seafood network with other community-based organizations, sustainable seafood producers, and other parts of the seafood supply chain to subsidize costs for low-income consumers and build community trust in local sustainable seafood.

OPPORTUNITIES IN THE ORNAMENTAL FISH BUSINESS IN NIGERIA

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Ornamental fish keeping is a popular hobby in developed countries and is gaining popularity in many developing countries. Ornamental fish breeding, wild-fish collection and trade and associated aquarium business provide excellent opportunities in non-food fisheries for employment and wealth creation. Pet fish keeping has made a come-back in Nigeria after about two decade's decline of the hobby.

There is a great opportunity for growth in both in international market and the almost untapped domestic market with the non-existence of aquatic outlets and public aquariums in major cities of Nigeria. This paper takes a close look at the business opportunities presented by this non-food fisheries/aquaculture sector for employment and wealth creation.

HOW MANY PARENTS HAVE YOU GOT? PARENTAL CONTRIBUTION IN A FLAT OYSTER HATCHERY

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A couple of years ago, the flat oyster (Ostrea edulis) population was thriving in the Limfjorden (Denmark), with a sustainably regulated fishery landing up to 320t/year. In late 2019, fishermen reported the first event of mass mortality in some fishing areas, probably due to the proliferation of parasitic pathogens.

Preserving and maximizing genetic diversity in putative conservation programs, including restocking, are therefore now of high importance. A new strategy was implemented in the sole flat oyster hatchery in Denmark for producing parasite-free spat and preserving high genetic diversity.

Hatchery procedures may affect the genetic variation among stocked spat in comparison with natural reproduction in the wild. We tested a cost-effective and easily applicable method to assess the genetic diversity in flat oyster production in the hatcheries. It consists in assigning parental contribution of broodstock to the offspring, reliably, in hatchery conditions.

To test our method, we studied the genetic composition at 17 microsatellite loci of hatchery-produced spat and compared it to that of the wild population, wild spat and adults from the broodstock source in the Limfjord. We found that swarming events were characterized by a single maternal and several paternal contributions, but also that only some potential parents contributed offspring per swarming event and that the number of successful parents varied between tanks in the hatchery. The work has provided a protocol that can give a robust estimation of the number of breeding pairs per swarming event (larval release) and it can provide input for potential conservation/restoration programs that utilize hatchery reared spat to support natural populations.

IMPACTS OF XENOBIOTICS AND MOLECULAR DEFENSE MECHANISM ON MARINE GREEN MUSSEL *Perna viridis* (LINNAEUS, 1758) IN SOUTHERN EAST COASTAL REGION OF BAY OF BENGAL, INDIA

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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 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 using Spectro-Fluorometer. 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. 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*.

EXPRESSION PROFILE ANALYSIS VIA MACHINE LEARNING TO IDENTIFY CRITICAL GENES DETERMINANT OF HYBRID STRIPED BASS *Morone chrysops* X *M. saxatilis* GROWTH

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Hybrid striped bass are the fourth largest finfish aquaculture industry in the U.S. and are reared in other countries worldwide. Decades of research have been conducted on the parent fish of the hybrid striped bass, striped bass (*Morone saxatilis*) and white bass (*M. chrysops*). Numerous genomic data, including recently updated reference genomes and transcriptomes, have been generated for these species and their hybrid offspring. The high volume of this information has increased the focus on developing data analysis to improve genetic breeding efforts. However, analytical approaches that reduce highly dimensional data into information that can be applied to breed consistently high-yielding cultivars are largely unavailable. Here we report a novel machine learning (ML) data analysis pipeline that reduces high-throughput "omics" data to advance breeding hybrid striped bass and their parental fish. Transcriptomes of hybrid striped bass white muscle tissue (n = 40 individuals) collected at final harvest were scanned against a genomic library of 34,000 unique protein motif fingerprints (MFs). Each MF is a twelve amino acids-long fragment that forms quantitative patterns. These data were initially reduced by excluding those that did not significantly differ in expression (mean read count in each of the six reading frames) between any sample or technical replicate thereof, leaving 15,000 MFs of interest. A ML pipeline and cross-validation strategy was applied to further reduce these data by determining MF inclusion or exclusion points to focus on those most critical to growth performance. Trained ML models were used to predict fish growth performance as either superior, inferior, and/or average as determined at two critical time points of production: grading (2-3 months of age) and final harvest (15 months of age). The data analysis pipeline identified fewer than 1,000 unique MFs as highly determinant of grade and/or growth performance at final harvest of hybrid striped bass. When concatenated, these MFs annotated to thirty-four (34) unigenes, all of which can be regarded as potential targets for breeding or other genetic targeting efforts. Moreover, the examination of individual MFs mapped to translated regions of the reference genome assemblies for the parent fish have enabled the determination of instances in which the expression of one allele specific to the striped bass or white bass parent is more influential for growth performance in the hybrid offspring for the first time. This is the only study that has been conducted to date that explores gene expression at the allele-level in hybrid striped bass to understand heterosis. Both the findings of this study and the analysis pipeline used to produce these results can be utilized by other groups concerned with aquaculture or any other animal rearing for agricultural purposes.

IMPACT OF WATER QUALITY ON GROWTH PATTERN AND TISSUES OF A CULTURABLE CICHLID - *Tilapia guineensis* COLLECTED FROM SOME AQUATIC ECOSYSTEMS (TOPO, GBAJI, AGBOJU AND AJEGUNLE) IN BADAGRY DIVISION, LAGOS, NIGERIA

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The study examined the impact of physico-chemical parameters of four aquatic ecosystems in Badagry division, on morphometric distances (total length, standard length and body weight) and tissues (gill, muscle and small intestine) of *Tilapia guineensis.* Samples of water were collected for 18 months, while twenty wild adult *Tilapia guineensis* from each station were examined for histopathology using standard procedures.

Length- weight relationships of *T. guineensis* obtained from all the creeks showed that *T. guineensis* had negative allometry (b<3). Only samples from Ajegunle had condition factor (k) value less than 1.

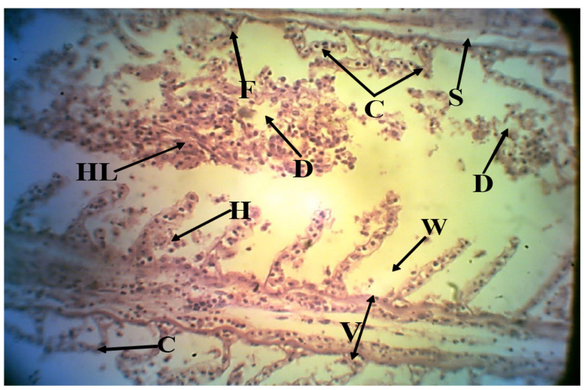

Figure 1: Gill structure of *T. guineensis* from Ajegunle creek showed severe degeneration (D) and hyperplasia (HL) of secondary gill lamellae (D), separation of gill fill mate from basement membrane (S), and curling of secondary lamellae (C) with unclear water channel (W). It is also characterized by hypertrophy of lamellae (H). There is also evidence of partial fusion of lamellae (F) and dilation of gill filament (V)

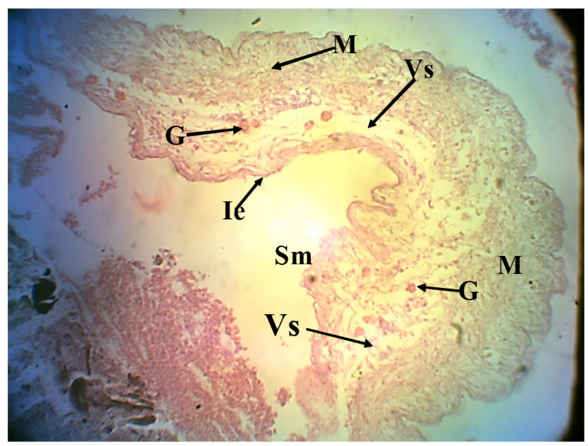

Figure 2 At Ajegunle, cross section of the small intestine showed the intestinal fold (I), Outer muscularis (Om) and inner muscularis layer (Im). There is slight degeneration and vacuolization in Submucosa connective layer (D). However, glandular cell (Gc) are adequately seen. It also showed mild lesion in epithelia lining Serosa(S).

(Continued on next page)

Table 1: Water Parameters and Morphometric Features of *T.guineensis* from the stations

Parameter	Agboju	Gbaji	Topo	Ajegunle
TL (cm)	10.57±1.12^{a}	10.34±1.14^{a}	10.46± 1.32^{a}	8.55± 0.85^{ab}
BW(g)	29.13±8.34^{a}	28.85±8.56^{a}	29.44± 7.85^{a}	26.47± 5.65^{ab}
SL(cm)	8.34± 1.25^{a}	8.64± 1.22^{a}	8.79± 1.29^{a}	6.53± 1.25^{ab}
b value	2.143^{a}	2.132^{a}	2.002^{a}	0.385^{ab}
K value	1.98± 0.83^{a}	2.59± 0.44^{a}	2.34± 0.98^{a}	0.99± 0.54^{ab}
pH	7.14± 0.54^{a}	7.33± 0.48^{a}	7.43± 0.52^{a}	7.56± 0.63^{a}
Temp.(^{0}c)	28.03±2.42^{a}	27.49±□1.99^{a}	27.44± 1.99^{a}	28.45± 2.35^{a}
BOD(mg/L)	248.80±□ 18.57^{ab}	209.68± 2 1.68^{a}	215.46± 24.19^{b}	247.98±28.37^{ab}
Conductivity (µs/cm)	254.27□ ± 42.12^{a}	220.27□ ± 30.97^{ab}	236.60□± 40.48^{ac}	268.30□ ±45.41^{ad}
TDS (mg/L	116.61□ ± 8.78^{a}	135.89□ ± 28.74^{ab}	131.78□± 21.12^{ac}	124.97± □4.38^{ad}
COD(mg/L)	175.90±21.65^{a}	166.21□ ± 20.93 ^{ab}	167.50±□24.60^{ac}	184.46± 25.29^{ad}
DO(mg/L)	6.20□±0.71^{a}	6.53□±1.88^{a}	6.76±□ 0.99^{a}	4.77□± 0.91^{ab}
Hardness(mg/L)	128.85□ ± 14.76^{a}	191.45□ ± 10.56^{ab}	197.37□±5.53^{aa}	238.25 ±18.91^{ac}
Alkalinity(mg/L)	37.98 ±5.63^{a}	39.44□± 6.96^{a}	38.86□ ±6.49^{a}	34.75□ ±4.21^{b}

Mean with same superscript in the row=not significantly different (p>0.05

LAND-BASED ECHINOCULTURE IN CALIFORNIA: DEVELOPING A PROTOCOL FOR THE ENHANCEMENT OF PURPLE SEA URCHIN ROE IN A RECIRCULATING AQUACULTURE SYSTEM (RAS)

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The California sea urchin fishery has been one of the most valuable commercial fisheries in the United States for several decades. The harvest of red sea urchins (*Mesocentrotus franciscanus*) originally began in California as a way to develop fisheries of underutilized species and was also seen as a way to curb destructive grazing on giant kelp (*Macrocystis pyrifera*). Unfortunately, over the last decade, this fishery has rapidly declined due to large-scale environmental stressors and is now considered fully exploited. In contrast to red urchins, purple sea urchins (*Strongylocentrotus purpuratus*) have very limited commercial value due to their smaller size and lesser roe yield. In recent years purple urchins have undergone a rapid population explosion and are now considered a pest as they voraciously overgraze kelp and outcompete other benthic invertebrates. The persistence of kelp loss has led to widespread ecosystem shifts to urchin barrens consisting predominantly of malnourished purple urchins with no economic value. Harvesting these urchins from barrens and enhancing their roe through aquaculture has the potential to transform these destructive grazers into high-quality seafood. Although practiced in several other countries, roe-enhancement aquaculture has not yet been applied in the United States. Using a land-based recirculating aquaculture system (RAS) in conjunction with two different formulations of prepared feed, we demonstrated that the malnourished roe of purple urchins collected from barrens (mean Gonad Index (GI) = 6.8 ± 0.7%) increased threefold, surpassing standard marketable yield (mean GI > 15%) after 9 wk of enhancement. Furthermore, roe enhancement on these prepared diets was significantly greater than on a diet of giant kelp (mean GI = 11.7 ± 1.2%). Analyses of proximate constituents and amino acid composition of gonad tissue also revealed differences among the roe of urchins fed the three diets during the trial period. In particular, one of the two prepared feeds resulted in a significantly greater amount of bitter-tasting amino acids in enhanced gonads compared to urchins fed whole kelp, while the other prepared formulation indicated a more natural flavor profile. Finally, consumption rate trials suggested differences in feeding rates as urchin gonads grew, showing the lowest consumption of feed occurring at the highest GI levels. Our results highlight an untapped potential to quickly produce a highly valued seafood product from seemingly low-value purple urchins. Echinoculture could thereby serve as a tool to help reinvigorate the California urchin industry, restore kelp forests from degraded barrens, and enhance the ecosystem services kelp forests provide to impacted coastal communities.

EXPERIMENTAL REPRODUCTION OF WHITE FECES SYNDROME IN WHITELEG SHRIMP, *Penaeus vannamei*

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White Feces Syndrome (WFS) is an emergent disease of penaeid shrimp (*Penaeus monodon* and *P. vannamei*) that is identified by the presence of floating white fecal strings on pond water in grow-out ponds. Although the clinical manifestations of WFS are well defined, the underling etiology remains obscure. WFS has been associated with several enteric pathogens, including *Enterocytozoon hepatopenaei* (EHP). The association is based on studies that found areas where WFS has been reported, the prevalence and severity of EHP infection are high. In this study, we describe an experimental reproduction of WFS in *P. vannamei* pre-infected with EHP and challenged with a unique isolate of *Vibrio parahaemolyticus* isolated from the gastrointestinal tract of a shrimp displaying WFS. Upon laboratory challenge, shrimp displaying white fecal strings and white discoloration of the gastrointestinal tract were analyzed by histopathology, *in-situ* hybridization and quantitative PCR. Histological analysis confirmed the lesions of EHP and septic hepatopancreatic necrosis in the hepatopancreas of shrimp exposed to both pathogens. Quantitative PCR showed shrimp infected with both EHP and *V. parahaemolyticus* had a significantly higher load of EHP compared to shrimp infected with EHP alone. This is the first demonstration of experimental reproduction of WFS under laboratory conditions when animals are infected with EHP and *V. parahaemolyticus* concurrently. The data revealed a synergistic relation between EHP and *V. parahaemolyticus* isolate that led to the manifestation of WFS. We propose the gross signs of WFS can be used as an indicator of the presence of EHP infection in association with a particular strain of an enteric *Vibrio* spp. in countries where EHP is endemic.

BENEFITS OF ADJUSTING TRACE MINERAL PREMIX IN SHRIMP FEED– A MEXICAN FIELD STUDY

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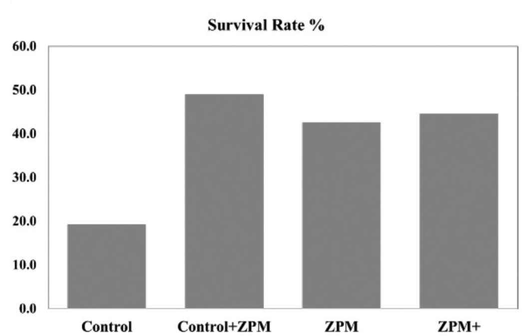
Replacement of inorganic mineral premix (sulfates) with complete metal-AA complexes (i.e. Zinpro Performance Minerals®, ZPM) at 0.5x rate of inorganic, resulted in numerically higher body weight, 8.3% reduced FCR, and reduced mortality of shrimp challenged with *Vibrio harveyi* (Jintasataporn et al., 2015). Metal-amino acid complexes have an absorption advantage over inorganic minerals, because they are minimally antagonized by dietary components such as phytic acid and utilize the AA transporters instead of common metal transporters. Davis et al. (1993) reported that *L. vanammei* required 33 ppm Zn to maintain normal growth and maximize Zn concentration in hepatopancreas, whereas 200 ppm inorganic Zn was required to overcome depressed Zn bioavailability caused by phytates. More recently, Yuan and co-authors reported improved growth performance and health of shrimp when replacing 120 ppm zinc sulphate with 60 ppm Zn-AA complex (Yuan et al. 2020) and 30 ppm Cu sulphate with 15 ppm Cu-AA complex (Yuan et al., 2019a,b). Importantly, increasing Zn-AA complex from 60 to 80 ppm further improved shrimp health-related parameters (Shi et al., 2021). The present study aimed to evaluate benefits of replacing inorganic trace mineral premix with metal-AA complexes (ZPM) on shrimp performance and survival rate under commercial conditions. Shrimp of 0.5 g initial body weight were grown for ~100 days (cycle varying from 95-130 days) in earthen ponds at a stocking density of 100 shrimp per m2 and 5-6 ppt. Feeds were produced at a commercial feed plant in Mexico, following ingredient and nutrient composition of a typical commercial shrimp feed. Four feeds were produced, varying in their trace mineral premix, as described in embedded table. Inorganic trace mineral premix used in the Control diet was combined with ZPM premix (Control+ZPM) or completely replaced with ZPM (ZPM diet). An additional diet was produced to include fortified ZPM premix (ZPM+), by giving special attention to trace minerals critical in the modulation of shrimp immune system (Zn, Se, Cu, and Cr). Replacement of inorganic premix with ZPM doubled shrimp survival rate. Fortifying trace mineral premix by combining inorganic with ZPM (higher total levels) or using ZPM+, tended to further increase survival rates. Regardless of the treatment, adjustment of inorganic trace mineral premix with ZPM increased final biomass and significantly reduced FCR, translating in positive return on investment (ROI, measured by additional income over feed cost) under commercial conditions. Best ROI was found with ZPM+ treatment (>150%).

	Supplemented Trace Mineral, ppm feed			
TM Source	Control	Control+ZPM	ZPM	ZPM+
Inorganic Zn	60.5	60.5		
Availa Zn		60.5	60.5	80
Inorganic Se	0.24	0.24		
Availa Se		0.24	0.24	0.4
Inorganic Cu	21	21		
Availa Cu		21	21	40
Inorganic Mn	47.2	47.2		
Availa Mn		47.2	47.2	40
Inorganic Fe	48.6	48.6		
Availa Fe		48.6	48.6	50
Availa Cr				0.4



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.

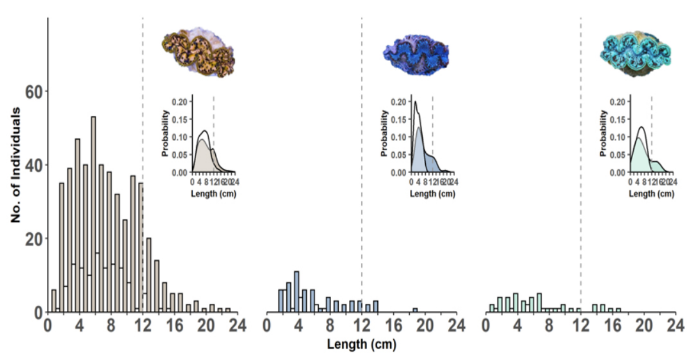

Figure 1. *Population structure differs between MPA and non-protected sites.* Size distributions across all MPA (shaded), and non-MPA (white) sites for *Tridacna maxima* clams with cryptic (brown) and conspicuously-colored (blue and teal) mantles. Size at maturity (i.e., length ≥ 12 cm) is denoted by 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.

MODEST SUSPENDED FLOATING RACEWAY DESIGN, ASSEMBLY, AND PRELIMINARY EVALUATION

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The floating in-pond raceway (IPR) is a channel that floats in a pond with a device that pushes water through system. It has potential to improve presentation of feed to the fish, assure good oxygen concentrations for growth, improve feed efficiency, improve inventory control, minimize predation, facilitate management of disease, and increase access for harvest. Utilization of a grid style airlift (GAL) is a significant improvement in the technology that has resulted in rapid adoption overseas in systems where the IPRS is constructed as a fixed structure in a pond designed for both intensive aquaculture production and waste management.

A modest size floating raceway has been developed by investigators at KYSU based on suspension from a floating frame and equipped with a grid style airlift to induce flow at about 20 exchanges/minute through the system. This design is something that can be constructed on the farm with material costs for the raceway, floatation, air blower, screens, and airlift estimated to be about $2,500. Though the entire unit is 32 feet long, the volume containing fish is about 9 m3 (21x4x4 ft) and is capable of supporting 460 kg at a loading rate of 48 kg/m3 (3 lb/ft3). Floatation and double keyways are built into each unit. These raceways feature a uniform square cross section and hard flat bottom such that a worker in waders can crowd, grade, and/or harvest the fish.

Initial evaluation of these raceways have been with phase I and phase II largemouth bass.

EARNING TRUST IN TECHNOLOGY FOR A SUSTAINABLE FOOD FUTURE

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Those who grow, raise and produce our food rely on technology to provide food that is safe, nutritious, abundant, affordable and produced sustainably. While technological advances can clearly help agriculture meet the growing demand for healthy, affordable food while reducing environmental impact, the technology must be accepted and supported by consumers for it to be viable. Gene editing, for example, shows tremendous promise, including enhancing nutrition, increasing disease resistance in plants and animals, and helping farmers use fewer inputs and natural resources and combat the challenges of a changing climate. The long-term success of gene editing depends on gaining early public support and market acceptance.

Historically, it was assumed that sound science was enough, but The Center for Food Integrity (CFI) peer-reviewed and published consumer trust model shows that communicating shared values is three-to-five times more important to earning trust than competence or sharing facts. New research conducted by CFI in July 2021 with the support of the United Soybean Board (USB) measured consumer attitudes to understand what specifically drives acceptance and rejection of technology use in food and agriculture. The research resulted in a model to classify existing and emerging agricultural technology along a continuum of key factors that drive consumer acceptance (the model is based on work by Siegrist and Hartmann in Nature Food, June 2020 issue). The model identifies aspects of the technology that align with consumer values, as well as aspects that represent obstacles to consumer support. Knowing these acceptance and rejection factors will enable food producers and technology developers to create and implement strategies that are more likely to earn support as new innovations are developed and launched. Several consistent acceptance themes were identified in the 2021 research, including: 1) Belief that food resulting from technology use is safe to consume, 2) Information about the use of technology to produce food is readily available, enabling an informed choice of voluntary exposure, 3) Benefits outweigh perceived risks, 4) Technology can help ensure a consistent supply of food, and 5) Technology promotes greater sustainability by making more with a lesser environmental impact.

U.S. Soy is one example of an industry actively engaging in strategies to earn social license – working to gain acceptance of technology as farmers produce soy for human consumption and as feed for aquaculture and livestock. USB is engaged in the CFI Coalition for Responsible Gene Editing in Agriculture, a collaboration of organizations that have developed trust-building strategies including a responsible use framework. USB and the Coalition created a communications guide that details five research-based approaches to earning trust in gene editing, along with consumer-facing videos, graphics and articles that incorporate these recommendations. Earning market acceptance of gene editing and other innovations will be beneficial as the aquaculture industry engages with an increasingly discerning supply chain, food customer and public that expects sustainably produced food.

SCALABLE AND GENERALIZED APPROACHES FOR STOCK-CENTER-BASED REPOSITORY CAPABILITIES IN AMPHIBIANS

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Amphibian biomedical models provide valuable resources for many branches of human health research and as such have comprehensive national stock centers to maintain and distribute these animals. The axolotl (*Ambystoma mexicanum*) is a model used for research in tissue regeneration to better understand and improve treatment of spinal cord and limb injuries. The clawed frogs (*Xenopus laevis* and *X. tropicalis*) are used to study vertebrate embryology and early development, basic cell and molecular biology, genomics, neurobiology, toxicology and to model human diseases. Production of transgenic and mutant lines in these and other species has led to a rapidly expanding need for a cost-effective and efficient way to maintain the increasing number of lines. Storage of cryopreserved germplasm in repositories can provide a way to protect such lines and reduce the number of live animals held at each center. In collaboration with the *Ambystoma* Genetic Stock Center (University of Kentucky) and the National *Xenopus* Resource (Marine Biological Laboratory), we aim to develop a high-throughput cryopreservation pathway that is both scalable and generalizable (Figure 1), integrating processing and quality management to establish repository capabilities for these species. These would be integrated into a larger repository network developed in cooperation with the USDA National Animal Germplasm Program located in Ft. Collins, CO and the AGGRC. Based on existing processes for reproduction and cryopreservation in these centers, we will employ user-centered design approaches to develop repository programs that serve the needs of the centers and their associated research communities. A major design consideration is that the practices at any center will be in accordance with those of the other centers (to facilitate network activities), and that these processes can be applied generally across a broad spectrum of scale. Thus, this collaborative initiative will consider repository development in a multi-level approach that addresses far more than simple protocol development.

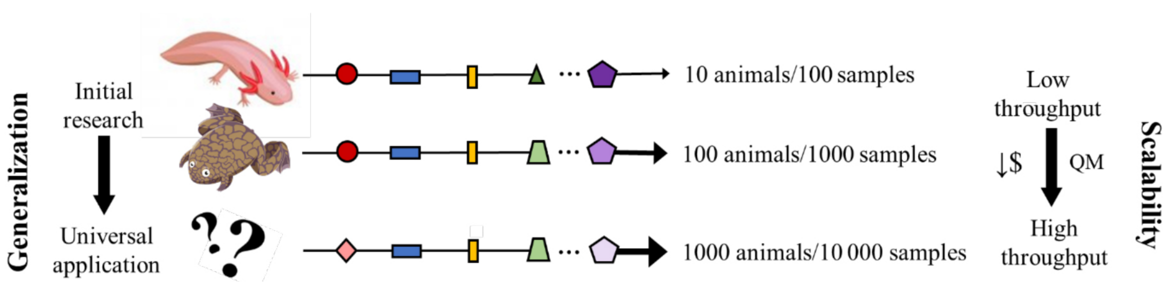

FIGURE 1. Strategy for developing scalable and generalizable germplasm repositories. Different shapes represent refinement of the cryopreservation process, however, each pathway need not be redeveloped from scratch. A generalized and scalable pathway for repositories can be developed for other species with quality management integrated and cost per sample decreased.

MICROBIOLOGICAL DIVERSITY AND COMPOSITION OF WATER AND SEDIMENTS IN THREE FARMS FROM ECUADOR

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It is understood that there is an association between productivity and microbial diversity in aquaculture systems. For example, changes in bacterial microbiomes are implicated in animal performance, in disease development associated with both bacterial and viral origin, and in dysbiosis (disequilibrium in microbial communities) triggered by environmental stressors or diet choice. With the increasing development of novel DNA sequencing technologies, new concepts and cost-effective applications have emerged to better understand the role of microbial communities in the growth and health of farmed vertebrates and invertebrates. Evidence of any of the three main mechanisms that lead to dysbiosis, such as the decrease in diversity, the loss of beneficial bacteria and the expansion of pathogens or potentially harmful microorganisms, can be used as an indicator tool for productivity monitoring/forecasting purposes. This work establishes a methodology and a pipeline for microbiome characterization in three commercial shrimp ponds in Ecuador. Also, we compare the merit of two types of samples (water and sediment) and detect patterns that allow inference on water quality in the ponds and evaluation of productive performance of the shrimp.

Briefly, the samples were filtered to concentrate, remove debris, and capture microbes present. DNA was extracted from the concentrated microbes sequenced to look at the type of bacteria present and the relative amount of each type. This was successful, with clear profiles produced along with some information on pathogen presence. Alpha diversity is a measurement of the microbial diversity of each sample. All sediment samples from the three ponds were very diverse with alpha diversity scores of over 600. Water samples were markedly less diverse with alpha diversity scores generally less than 250. For one of the ponds, alpha diversity was relatively high and over 250. For another pond alpha diversity was less than 100, and a single bacterial species made up over 50% of the bacteria detected in all water samples. In addition, for some water samples potentially pathogenic Vibrio species were identified.

Overall, this information reveals that monitoring the microbiome of production ponds over a grow-out cycle has the real chance to deliver data meaningful for pond management and pond performance. Possible application of this technology/analysis are 1) assessing genotype by environment effects in shrimp performance, 2) understanding of the impact of the microbes in the growth, development, and survival of shrimp, 3) evaluation of the effectiveness of probiotics or diets, and 4) predicting pond performance by evaluation of variations in microbial composition at different timepoints in the production cycle.

PARALLELS AND DIFFERENCES IN [REACTION] TO REASONING FOR SUPPORTING AQUACULTURE EXPANSION: A CASE STUDY IN SEAWEED FARMING VS. MARINE AQUACULTURE

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Expansion of the aquaculture industry hinges on the backing of local residents and other stakeholders capable of withholding necessary social, political, or financial support. Regional and individual variations in level of enthusiasm for marine aquaculture have prompted several public opinion surveys as a way of assessing overall support for, or resistance to, the expansion of aquaculture in the US. These studies generally find that although the majority of the public do not have strong objections to the sector, there is consistently a smaller, but vocal, group that opposes marine aquaculture. A second common finding is a broad lack of awareness of marine aquaculture in the US. Two important unanswered questions concern 1) how malleable public opinion about aquaculture is; and 2) what specific benefits of aquaculture in general and seaweed farming in particular most influence support for the industry.

We will share the outcomes of two surveys conducted by a consulting firm, Prime Group LLC, as part of a series of aquaculture perceptions projects managed by the Aquarium of the Pacific's Seafood for the Future program in Long Beach, California. We will focus particularly on the findings from our before-and-after assessment of how different thematic messages (i.e., environmental, economic, social) resonated across survey participants of different sociodemographic attributes (i.e., income, education, ethnicity, state of residency). We will also speak to how baseline familiarity with marine aquaculture/seaweed farming affects malleability of participant opinion about the sector. Altogether, this work provides critical insight to aquaculture literacy efforts which aim to equip community members with the knowledge needed to engage in decision making processes for marine aquaculture development in their 'ocean neighborhoods'.

COVID 19: CHALLENGES AND OPPORTUNITIES IN THE SEAFOOD MARKET

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The COVID-19 pandemic and the measures to limit the spread of the virus caused a major shock to the world's trade system. As seafood is the most traded food commodity, this indicates a potential of a significant impact. Investigating the impacts is difficult, though, as the relevant data is often published only with a significant delay, with a partial exception for prices. While a number of studies have used different indicators to show significant challenges, these all tend to be focused on specific cases. In this study we will use a few different data sources to show that in aggregate the impact of COVID-19 has been limited in some significant parts of the seafood market.

The FAO fish price index show virtually no significant movement in 2020 or 2021 through August. When disaggregated to farmed and wild fish, there is some indication of a price decline for farmed fish during spring 2020. The largest seafood importer in the world, the U.S., had its largest year-on-year growth in imports this century from 2019 to 2020. While this certainly does not invalidate all the reports of challenges caused by COVID-19 and disrupted logistics, it does suggest that there are ways around those challenges. For producers who can find them, COVID-19 actually seem to have created significant opportunities. This is best illustrated with U.S. retail sales of seafood which increased by 28% from 2019 to 2020.

ARAGONITE SATURATION AS AN INDICATOR FOR OYSTER HABITAT HEALTH IN DELAWARE INLAND BAYS

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Oyster farming in Delaware is a crucial industry, bringing in $300,000 to $500,000 in sales every year. Oysters use calcium carbonate ions in the form of aragonite and calcite to form their shells. Ocean acidification can lead to a decrease in carbonate ions making forming these shells difficult. When aragonite saturation state falls below 3, calcifying organisms become stressed and when it drops below 1, their shells begin to dissolve. Therefore, measuring the aragonite saturation state yields crucial insight into the suitability of habitats to support oyster growth. This project aimed to calculate the aragonite saturation state from seven sites within Delaware Inland Bays to determine their feasibility in supporting the establishment of oyster farms.

Monitoring was conducted biweekly from July to November 2020 and 2021. Temperature, salinity, alkalinity and pH were determined using YSI methods. Using the SeaCarb program package in the R programming language, aragonite saturation state was calculated with the water quality parameters: temperature, salinity, alkalinity, and pH as inputs. In 2020, the aragonite saturation states were under saturated, with the average values of all sites remaining below a saturation state of 3. The highest registered average aragonite saturation value was 1.31 at the Redefer control site and the lowest value was 0.55 from the Bay City control site (Fig. 1). These values do not meet the recommended saturation state for sustainable oyster farming. As salinity is the main factor influencing the aragonite saturation values, oyster variants which are tolerant to low salinity would be recommended for these sites. The 2021 data will expand on aragonite saturation trends in the Bay.

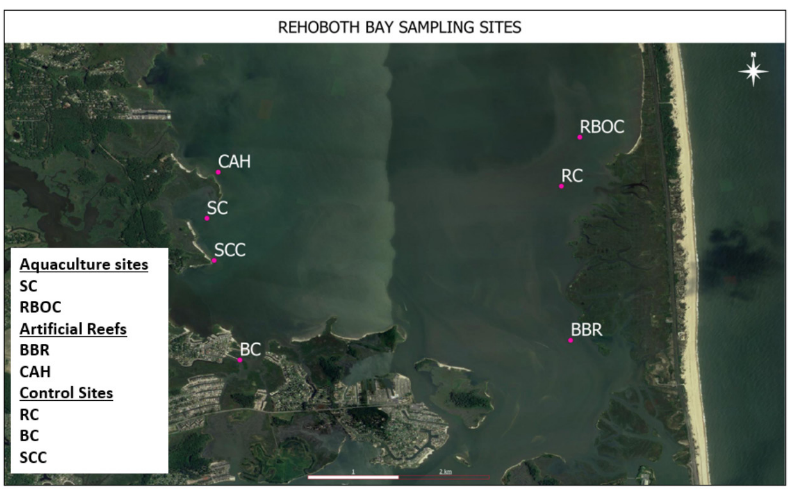

Figure 1. Map showing sampling sites at Rehoboth Bay, Delaware

NAVIGATING THE SOCIAL LICENSE TO OPERATE: AN AREA CASE STUDY

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The social license to operate in public waters is a key barrier to the expansion of sustainable marine aquaculture across the United States. The social license to operate is described as the unwritten social contract between an industry and the broader community. Tools to assist the aquaculture industry navigate social license, stakeholder relations, and addressing riparian landowner concerns have been identified by sea farmers as an urgent need in a recent survey conducted by the Maine Aquaculture Innovation Center. As a part of this project to develop resources for aquaculture farmers in navigating community relations, case studies are being conducted to evaluate best practices for interacting with communities. In this study, we focus on social license to operate in the Damariscotta River area, where a well-established shellfish aquaculture industry is present.

Oyster aquaculture in Maine began in the Damariscotta River in the 1970s, and in 2019 Damariscotta oysters comprised almost 70% of Maine's total aquacultured oyster harvest with a value of over $6.5 million. Today, there are 30 active experimental and standard aquaculture leases on the Damariscotta River for a total of 165.44 acres under culture. We hope to evaluate the social license to operate for aquaculture present in the Damariscotta region using a Q method study.

A media analysis of local and regional newspapers was conducted as well as a review of lease hearing transcripts to develop 25 thematic statements that reflected various viewpoints towards aquaculture in the Damariscotta area. We identified community stakeholders in the Damariscotta River region and asked them to sort the thematic statements onto a Q matrix during an interview. By utilizing a Q method approach, a small number of stakeholders can be interviewed, with responses representing different perspectives on the social license to operate. Factor analysis, along with qualitative information gained from the interviews will be used to identify and describe the most prominent viewpoints in the study group. Information gained from this study will help inform best practices for farmers to use when working with communities to establish aquaculture operations.

GROWTH MODELING OF CHERRY TOMATO (*Solanum Lycopersicon* L.) FERTIGATED WITH BIOFLOC TILAPIA EFFLUENT IN THE DUTCH BUCKET SYSTEM

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Aquaculture effluent is shown to be low in dissolved plant nutrients thus affecting plant growth and productivity. In addition, daily nutrient variability makes it difficult to predict crop yields and negatively affect production planning in aquaponics. Empirical growth models in which growth functions are fitted to experimental data can efficiently predict yield and quality of product. Also, understanding resource use efficiency by assessing physiological responses can assist in proper management schemes. The objective of the current study was to analyze the growth of cherry tomato fertigated with biofloc tilapia effluent using known growth models and evaluate the photosystem efficiency under such conditions. The study was conducted in an experimental natural-lit greenhouse, without supplemental light, at the Auburn University Aquaponics Facility. Cherry tomato (*Solanum Lycopersicon* L. “Favorita”) was raised in 72-cell Styrofoam trays filled with 50% perlite, 50% peat (v/v) mix. Uniformly aged and sized seedlings were transplanted into 11-L rectangular Dutch buckets filled with 100% horticultural grade coarse perlite. The aquaculture effluent was drawn from a biofloc tilapia system using a 1 hp pump. A timer-clock, connected to the pump, was set to come on every 30 minutes and fertigate for 1 minute and was connected to solenoid valves which open when the clock is on. A drip system was used to deliver the fertigation solution to each pot. Single plants per pot were grown with intra-row spacing of 0.46 m (18”) with a total population of 204 plants. Destructive sampling for dry matter growth, and partitioning commenced two weeks after transplanting and proceeded on a weekly basis. Plant tissue nutrient content for macro and micronutrients are being assessed every two weeks. The dry matter progression so far is being fairly described with a logistic growth curve. At week 4 after transplanting, a diurnal assessment of stomata response and chlorophyl fluorescence was done using LI-600 porometer/fluorometer. The trends showed that although stomata conductance showed a bell shape response with the hour of the day and peaked between 10 am and 1 pm, photosystem II efficiency had the opposite response due to higher leaf transpiration. Which indicates that the higher stomata conductance was more correlated with transpiration but not light use due to high mid-day temperatures. It is anticipated that this information would provide insight into establishing a useful predictive model for cherry tomato growth in aquaponics. The results will be used as decision support tool to facilitate effective planning for cherry tomato production in aquaponics.

AQUACULTURE OF PURPLE SEA URCHINS AND GIANT RED SEA CUCUMBERS IN BRTISH COLUMBIA, CANADA

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Sea urchins and sea cucumbers form the basis of lucrative fisheries and an expanding commercial aquaculture industry in many parts of the world. Despite their huge potential and considerable interest, commercial aquaculture of these echinoderms is still in its infancy in North America. Little is known about the early stages (larvae/juveniles) of either the purple sea urchin (*Strongylocentrotus purpuratus*) or the giant red sea cucumber (*Apostichopus californicus*), both candidates for aquaculture in British Columbia, Canada. Increasing larval survival and settlement rates and maximizing post-settlement survival and growth are major challenges in the development of juvenile production technology and profitable aquaculture ventures, but the reliable techniques for hatchery production of echinoderms have been developed for relatively few species. This presentation will focus on various aspects of larval and juvenile production techniques of both the purple sea urchin and giant red sea cucumber. We tested a variety of microalgal diets for larval survival, growth, and settlement. The single-species diet of *Dunaliella tertiolecta* and the mixed-species diet of *D. tertiolecta* and *Isochrysis galbana* supported good growth and survival both for sea urchins and sea cucumbers. The sea cucumber larvae were induced to settle and metamorphose successfully using settlement plates coated with *Spirulina* and the benthic diatom *Amphora salina*.

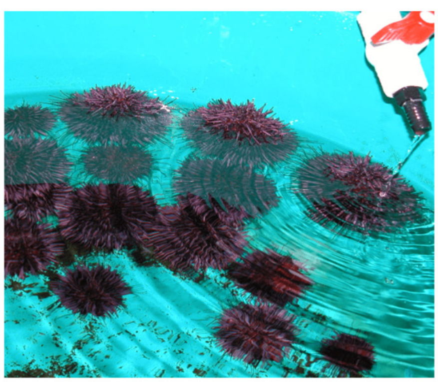

Fig. 1. Broodstock purple sea urchins.

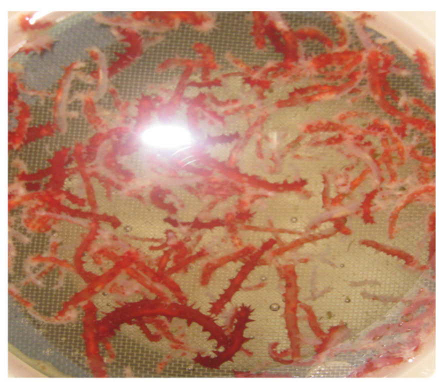

Fig. 2. Juveniles of the giant red sea cucumber.

SEABREAM AND SEABASS FARMING IN OFFSHORE CAGES: LIFE CYCLE ASSESSMENT OF DIFFERENT ITALIAN AQUACULTURE PLANTS

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Aquaculture is more and more considered as a major contributor to the growing demand in worldwide seafood production.

Sustainability has become a key question for aquaculture systems. Aquaculture imply environmental concerns related to the consumption of feed, the emission of nutrients and organic compounds into the water and, sometimes, the consumption of pesticides and antibiotics for pest and disease control.

Even if originally developed for the evaluation of industrial processes, Life Cycle Assessment (LCA) approach has been more and more applied also to the agro-food sector. LCA allows the quantification of the potential environmental impacts related to a production process and/or a product. Consequently, it is the first step for an effective identification of mitigation solutions.

In this study, LCA was applied to assess the environmental impact related to seabream and seabass farming of different off-shore plants located in Central Italy. The selected functional unit (i.e., the reference unit of the study to which all the inputs and outputs should be referred) was 1 ton of fish at the fish farm gate. The system boundary includes the production of feed and other production factors consumed (e.g., fuel, electricity), the rearing operations and all the emissions related to the process (e.g., phosphorous and nitrogen emissions due to the metabolism of the fish during the entire production cycle).

For Gilthead Sea bream and European sea bass, the environmental results related to the environmental issues, as well as for most of the other considered impact categories, showed that aquafeed is the main environmental hotspots. For the Climate Change impact category, aquafeed impact range from 55 to 70% of the total, while for acidification and eutrophication impact categories, the contribution of feed is second only to that of the emissions of N and P compounds. The analysis highlighted a strong relation between aquafeed conversion rate, amount of N and P emitted and, consequently, the impact on acidification and eutrophication.

In a contest where the consumption of fossil carburants must be dramatically reduced, studying new sustainable fish diets is nowadays urgent. Following the example of the SIMTAP system, such new diets should be characterized by limited transportation impact (use of locally produced raw material and diets consumption) and maximizing the use renewable energy and protein sources (e.g., solar power and microalgae).

Acknowledgements
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A REVIEW ON THREE DIFFERENT DIETARY PROBIOTICS AS THE ANTIBIOTIC REPLACERS FOR THE MAJOR AQUACULTURE SPECIES IN THE EAST ASIAN COUNTRIES

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Four feeding trials were conducted to evaluate the effects of dietary probiotics as antibiotic replacers in Japanese eel, *Anguilla japonica*; Nile tilapia, *Oreochromis niloticus*; Whiteleg shrimp, *Litopenaeus vannamei;* and Olive flounder, *paralichthys olivaceus*. In the first experiment, growth performance, non-specific immune responses, immune-related gene expression and cumulative survival rate of Japanese eel fed *Bacillus subtilis* WB60 (0.5×10^7 CFU/g) with mannanoligosaccharide (5g/kg)-BSM diet were significantly higher than those of fish fed control (CON) diet. However, there were no significant differences among fish fed BSM and oxytetracycline (OTC) diet. In the second experiment, growth performances, non-specific immune responses, cumulative survival rate of Nile tilapia fed *B. subtilis* at 1×10^8 (CFU/g)-BS and *Lactococcus lactis* at 1×10^8 (CFU/g)-LL diets were significantly higher than those of fish fed CON diet. However, there were no significant differences among fish fed BS, LL and OTC diets. Immune-related gene expression and enzyme activity of BS and LL diets were significantly higher than those of fish fed CON and OTC diets. In the third experiment, growth performance, non-specific immune responses and cumulative survival rate of whiteleg shrimp fed *B. subtilis* at 1×10^8 (CFU/g)-BS and *L. lactis* at 1×10^8 (CFU/g)-LL diets were significantly higher than those of shrimp fed CON diet. However, there were no significant differences among fish fed BS, LL and OTC diets. Immune-related gene expression and histology of shrimp fed the probiotic diets were significantly improved compared to those of shrimp fed CON and OTC diets. In the fourth experiment, growth performance, non-specific immune responses, immune-related gene expression, cumulative survival rate and enzyme activity of olive flounder fed *B. subtilis* at 1 x 10^8 CFU/g (BS), *L. lactis* at 1×10^8 CFU/g (LL), *E. faecium* at 1 x 10^7 CFU/g (EF7) and *E. faecium* at 1 x 10^8 CFU/g (EF8) were significantly higher than those of fish fed CON diet. However, there were no significant differences among fish fed BS, LL, EF7, EF8 and OTC diets. These results indicated that BSM in Japanese eel; BS and LL in Nile tilapia and Whiteleg Shrimp; BS, EF7 and EF8 in Olive flounder could be ideal synbiotics or probiotics to improve growth performance, immune responses, enzyme activity, disease resistance and gene expression, and to replace antibiotics in those for major aquaculture species in the esst Asian countries Korea, Japan, China, etc.

OPTIMIZING YIELD POTENTIAL AND HARVEST EFFICIENCY OF A SCALABLE COASTAL AND OFFSHORE MARCROALGAL FARM

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Seaweed farming is expanding rapidly in the US and is becoming an important component of our coastal economy. To maintain sustainable growth, both socially and economically, it is essential to 1) optimize the growing potential of permitted lease areas and 2) increase operational efficiency. Maximizing production per unit area will minimize the area required to meet the needs of a growing industry and reduce potential ocean user conflicts and natural impacts. The dense growing structures required to optimize production enable operational efficiencies that are not achievable with traditional farming practices. These efficiencies have the potential to reduce the cost of production, which will allow the industry to enter new markets. As part of the ARPA-E MARINER program an experimental test farm has been deployed over the past two years in Kodiak, Alaska with the goal of increasing yield per unit area and increasing operational efficiencies. During the first growing season 2019-20 the farm had 11,000ft of grow-line in a 1.9-acre footprint. Harvest yielded 59,000lbs at 5.5lbs/ft. The second growing season 2020-21 had 25,000ft of grow-line in a 3.3-acre footprint. The harvest of 68% of the farm yielded 86,600lbs at 4.95lbs/ft. Harvesting system optimization took place with the testing of a purpose-built barge and a commercial fishing boat. The fastest rate of harvest achieved was 15,000lbs/hr with an average harvest speed of 2,800lbs/hr. The test farm will be deployed for a third season with 44,000ft of grow-line in the same 3.3-acre footprint.

Figure 1. Experimental test farm in Kodiak, AK

PERFORMANCE CHARACTERIZATION OF A DIFFUSED AERATION BASIN FOR CARBON DIOXIDE REMOVAL IN RAS

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Fish produce dissolved carbon dioxide (CO_2) as a normal outcome of aerobic metabolism and excrete this gas through the gills into the surrounding water. In recirculating aquaculture systems (RAS) with high stocking densities, CO_2 accumulates to unsafe levels unless some form of carbon dioxide control is used. CO_2 control unit processes are needed to maintain safe levels of CO_2 in the culture tank at high stocking densities. Typical CO_2 removal unit processes include a force-ventilated column with or without packing media, a shallow diffused aeration basin, and a passive ventilation trickling filter. Force-ventilated packed columns can remove 40% to 60% dissolved CO_2 from RAS water when properly designed (Summerfelt et al., 2000). However, pumping is required to transport water to the top of this unit process. Pumps require capital investment, 24/7 operating costs, and routine maintenance. In this configuration it is critical to maximize removal efficiency of CO_2 to minimize the flow that must be pumped. Alternatively, unit processes that remove CO_2 using less energy can be utilized if enough performance criteria are known to properly design them to control CO_2 in the system.

The goal of the project was to evaluate the removal of CO_2 using a low head technology and develop design criteria to optimize such technologies. The project examined a diffused aeration basin that uses diffused air as the stripping gas in a relatively shallow basin. Diffused aeration basins are often used in current RAS designs after the moving bed biofilter. Water gravity flows from the moving bed into the diffused aeration basin and no pumping is required. This study evaluated three different hydraulic retention times (67, 90, and 135 sec), influent CO_2 levels (10, 15, and 25 mg/L), and diffused airflow (G:L of 2 and 5) to characterize CO_2 removal efficiencies in a research-scale aeration basin that was 1.2 m by 0.9 m by 0.9 m deep (1 m^3). The project setup consisted of an influent tank using a low head oxygenator to inject carbon dioxide to achieve the desired dissolved CO_2 level and a diffused aeration test tank with a pump sump for returning treated water to the influent tank. OxyGuard International A/S portable CO_2 sensors monitored the dissolved carbon dioxide in the influent tank and immediately after the diffused aeration test tank. Diffused air for aeration was provided by a regenerative blower and air manifold at the bottom of the test tank. The results of the tests will be reported in this presentation along with recommendations for optimizing the design of diffused aeration basins.

IMPORTANT CONSIDERATIONS FOR PHARMACEUTICAL DEVELOPMENT AND USE IN AQUACULTURE

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To meet the increasing demands for seafood, as well as in maintaining a sustainable source of protein for global populations, the aquaculture industry must expand and develop new resources. As aquaculture farmers races to meet these new demands, the pharmaceutical industry must deliver an ever-increasing portfolio of drugs required to maintain organism health and wellbeing. To date the US Food and Drug Administration has reviewed and approved approximately 16 compounds to combat a multitude of target conditions. Targets are primarily directed at bacteria, fungi, and parasites (external and internal). Other approved drugs are used to mark skeletal and bony structures in vertebrates for identification, and to promote or manage spawning activity. Most drugs are administered through feed or via infusion into the aquatic media. Because aquaculture organisms are primarily developed as food resources for human consumption, their administration, residual tissue concentrations, and monitoring are vital for safety. Equally important are environmental concerns for waste handling and final degradation of excess compounds.

As new drugs are developed and prepared for approval, several important considerations must be evaluated. The type and size of the system, the farmed species, and the target disease prevention are all key factors that will drive the decision to the right application. In a survey of facilities in North America and the North Sea approximately 77% of facilities were designated as flow through (FT) systems, 12% were recirculating aquaculture systems (RAS), and 11 % a combination of FT and RAS. Specifically looking at salmonid hatcheries, seven hatcheries in North America together produced approximately 14.9 million salmon smolts, totaling 1,188 metric tons of fish per year. Once administered, the drugs action is through systemic or extrinsic exposures. Studies are needed to determine the degradation rate and daughter products of pharmaceuticals. For example, some drugs can be excreted through fecal material in a fully active form, while others may exist only in a partially active form. Additionally, fully active drugs may also be present in uneaten food. Either way, laboratory studies must be carried out to fully understand the degradation pathways and exposure potential within FT or RAS systems.

Once degradation is understood facilities must be able to determine how to manage the waste. In both FT and RAS systems fecal material and sludge are the largest waste. Often this can be managed through public treatment systems; however, those that cannot must collect and manage waste. In a survey of facilities, many collected biosolids as a secondary product to sell to land farms. This can introduce certain degradation products or fully active chemicals directly to the environment. Several options for waste management exist for facilities including, drying, containerizing, heating, and filtering.

GENOMIC POPULATION STRUCTURE OF THE EASTERN OYSTER *Crassostrea virginica* IN THE GULF OF MEXICO

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The eastern oyster (*Crassostrea virginica*) is one of the most ecologically and economically valuable species in Texas and the United States. The Texas oyster fishery has experienced an overall decline over the last two decades, and in an effort to revitalize the industry, the state of Texas passed a law in 2019, making commercial aquaculture of oysters legal in state waters for the first time. Previous research has identified three genetic groups in the Gulf of Mexico (hereafter Gulf): a southern population that is only present in Texas, a northern population that extends eastward across the Gulf, and an eastern population in Florida. In Texas, both northern and southern populations naturally co-occur in a transition zone near Aransas Bay. An understanding of fine-scale population structure across the Gulf is needed to inform appropriate management strategies for the aquaculture industry, commercial fishery, and restoration efforts.

Double digest restriction-site associated DNA sequencing was used to genotype 451 individuals collected from Texas to Florida (Fig. 1). Preliminary analysis found similar patterns of genetic structure in the Gulf as previous research, and admixed individuals were identified within all regions. In Texas, re-assessment of the transition zone indicated a slight southern movement towards Corpus Christi Bay. Results of this study suggest long-term monitoring of the northern/southern transition zone may be needed for appropriate mariculture management in Texas.

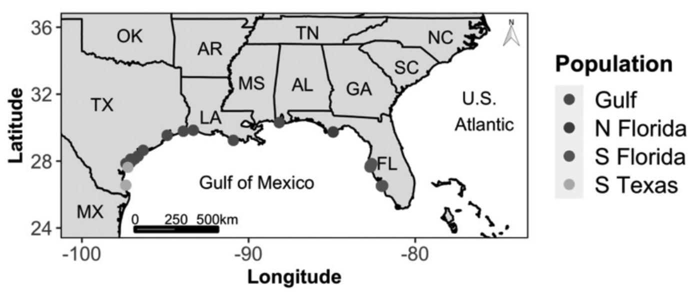

FIGURE 1. Map of sampling locations.

QUANTIFYING FARM-SCALE WATER QUALITY BENEFITS ASSOCIATED WITH EASTERN OYSTER AQUACULTURE AND ACUTE LOW SALINITY EXPOSURE IN THE MID-ATLANTIC

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Nutrient pollution is a global water quality concern for the marine environment. Restoration of natural oyster beds has been one approach to the nutrient pollution problem, and research suggests oyster aquaculture could provide equivalent or greater water quality benefits. However, the scale at which oyster farms provide ecosystem services is not well understood. Furthermore, there is a lack of data concerning site-specific effects of salinity on oyster filtration. This information is important in projecting site-specific impacts of climate change (*i.e.,* an increase in short-term extreme low-salinity events from rainfall events) on oyster filtration.

To address these issues, research was conducted concerning the role of farm and wild eastern oyster (*Crassostrea virginica*) populations in complementing existing nutrient mitigation efforts in the mid-Atlantic under two salinity conditions: contemporary and future salinity scenarios. Specifically, the project aimed to (1) estimate farm-level year-round filtration occurring at three oyster farms, (2) estimate bed-level year-round filtration occurring at one subtidal oyster bed, and (3) quantify the contributions of one oyster farm and the wild oyster bed to improved water quality under the two salinity conditions. Oyster filtration data were collected seasonally between July 2020 and September 2021 at each farm and oyster bed using a flow-through filtration chamber with ambient water. During each experiment oysters representing a range of sizes were placed in the chamber, from which, oyster biodeposits were collected to calculate *in-situ* oyster filtration and clearance rates. Filtration varied seasonally among farms such that warmer temperatures and lower organic content of seston were generally associated with higher filtration rates. Filtration rates generally decreased under low-salinity conditions (Figure 1). These experiments provide a robust dataset of oyster filtration observed under natural conditions and may be used in a broader framework to inform potential oyster aquaculture contributions to nutrient management in the mid-Atlantic.

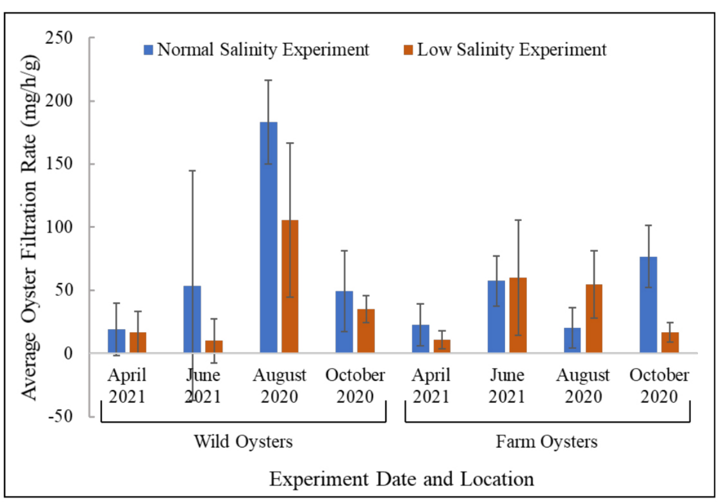

FIGURE 1. Comparison of average oyster filtration rates (standardized to one-gram of dry tissue weight) between wild and farm oysters at two salinity conditions.

A TRANSPOSABLE ELEMENT–EPIGENETICS PERSPECTIVE TO UNDERSTAND ANTIMICROBIAL RESISTANCE (AMR) AND CONTAMINATION BY ENDOCRINE DISRUPTING CHEMICALS (EDCs) LIKE HEAVY METALS, BIOCIDES, GLYPHOSATE, MICROPLASTICS, BIS(2-ETHYLHEXYL) PHTHALATE (DEPH), AND PER- AND POLY-FLUOROALKYL SUBSTANCES (PFAS): ADAPTATION TO GLOBAL 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 neuroendocrine disrupting chemicals (EDCs) in soil, animals and people, and adaptation to global change via horizontal gene transfer and horizontal transfer of transposons.

Heavy metal/biocide 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 in 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. Very little has been published for aquatic species on the interplay of co-selected non-antibiotic factors metals and biocides.

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 can cause cancer and birth defects or other reproductive harm. Exposure to DEHP may increase the risk of cancer and may also harm the male reproductive system. DEHP exposure during pregnancy may affect the development of the child. DEHP is also an antimicrobial from the 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 metals-biocides, microplastics-phthalates, and PFAFs as examples. Urgent transgenerational epigenetic inheritance-One Health research is needed to address them.

A NOVEL ADVANCED OXIDATION TECHNOLOGY THAT RAPIDLY REMOVES GEOSMIN AND MIB FROM WATER AND SIGNIFICANTLY REDUCES ATLANTIC SALMON DEPURATION TIME

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Atlantic salmon (*Salmo salar*) reared in RAS can accumulate off-flavor chemicals (e.g., geosmin and 2-methylborneol, MIB) that reduce fish marketability. A solution to this problem is to hold the fish in separate depuration systems that are flushed with clean water in a single pass or operated with limited water recirculation. During depuration the fish are not fed and can lose significant weight and value.

We tested the effects of a new technology called the Exciton Advanced Oxidation Process® (eAOP®) on the removal of geosmin and MIB from 18 m^3 depuration tanks at a commercial Atlantic salmon RAS operation. eAOP® is a unique technology that works using multiple mechanisms of action including UV photolysis, titanium dioxide photocatalysis, electrolysis, and UV/peroxide AOP. Geosmin and MIB were measured by GC/mass spectroscopy.

In preliminary chemical spiking experiments without makeup water flow, the eAOP® reactor rapidly eliminated all of the geosmin and MIB in the water in 2-3 hrs (Fig. 1). An actual depuration experiment was then conducted to determine the effects of eAOP® on the total purge time. Market-sized (3.8-4 kg) fish from a single harvest were split between control (normal flow-through depuration procedure) and eAOP®-treated 18 m^3 purge tanks. Water and triplicate fish flesh samples were collected from each tank every two days for ten days.

Geosmin levels in both groups followed first order removal kinetics with the following parameters: control tank fish, $k = 0.092$ day^{-1}, $R^2 = 0.91$, half-life = 7.5 days; eAOP®, $k = 0.147$ day^{-1}, $R^2 = 0.95$, half-life = 4.7 d. Thus, geosmin removal from the fish in the eAOP®-treated tank was 60% faster than in the control tank, and the normal 10-day depuration time was reduced to 4-5 days. We conclude that eAOP® can be used to treat purge water to significantly reduce total depuration times while using less water.

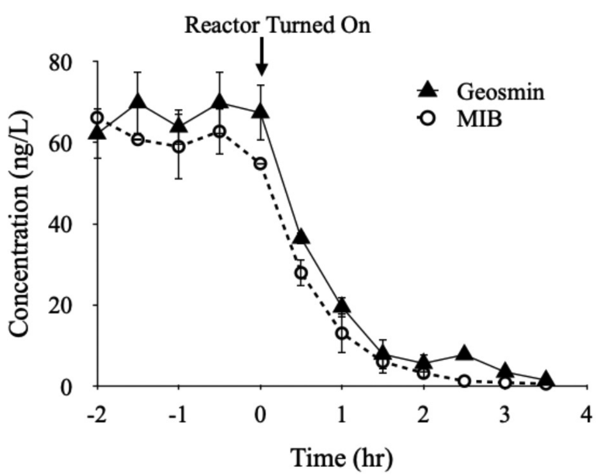

Fig. 1. Geosmin and MIB removal from an 18 m^3 depuration tank using eAOP®.

ICHTHYOTOXICITY OF THE PSP –PRODUCING *Alexandrium catenella* AND DSP-PRODUCING *Dinophysis acuminata*

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Reports of Harmful algal Blooms (HAB) have increased worldwide as a result of enhanced growth and eutrophication as well as improved monitoring programs and climate change. Several species of HAB pause a threat to human health as well as commercial fish and shellfish. Among HAB-forming dinoflagllates of cosmopolitan occurrence, *Alexadrium catenella* produces saxitoxins responsible for Paralytic Shellfish Poisoning (PSP) and allelochemicals of undetermined nature responsible for mass mortalities of aquatic organisms. *Dinophysis acuminata* is another HAB-forming dinoflagellate with a global distribution responsible for Diarrheic Shellfish Poisoning (DSP) which causes economic loss due to closures of shellfish productions.

Alexandrium catenella has been forming recurrent blooms in USA, notably in the Gulf of Maine, Alaska and California. Recent studies have shown that *A. catenella* has been expanding along the West Coast of the USA up to the Arctic region. This species has been associated with contamination of shellfish with PSP, which hampers shellfish aquaculture production, but also with mass mortalities of fish. Blooms of *Dinophysis* and associated toxins are an emerging threat to human health and aquaculture in North America, as documentation of these blooms has increased, notably in USA.

Several studies have dealt with PSP accumulation and detoxification in shellfish. Studies with DSP kinetics in shellfish have been rare due to difficulties in culturing *Dinophysis* species and maintaining large scale cultures for feeding experiments. Studies on the impacts of *Alexanrium* and especially *Dinophysis* on fitness of shellfish are less frequent. In the present study, we report results of the effects of *A. catenella* on a commercial shellfish species using reproduction as a model experimental study. We tested the effects of *A. catenella* on several developmental stages of the Japanese pearl oyster and compared its effects to several other species of the genus *Alexandrium*, producers and non-producers of PSP. We also report our results of the effects of *Dinophysis acuminata* on the reproduction of the Japanese pearl oyster and compare it with other species of *Dinophysis* tested from monoclonal cultures.

Our results show that all tested species have negative effects on reproduction of the shellfish species which warrant further studies of the impacts of uncharacterized allelochemicals of both *Alexandrium* and *Dinophysis* on shellfish as well as fish of commercial importance, especially that the aquaculture sector is being further developed in USA.

PROMOTING CONSERVATION AND MANAGEMENT ACTIONS TO RECOVER ABALONE POPULATIONS IN MEXICO

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Landings of fished abalone (*Haliotis* spp.) have significantly decreased in the last decades and global production has drastically changed from fishing to farming. In particular, multiple stressors related to climate change and overfishing are threatening this resource in California, USA, and Baja California, Mexico. In Mexico, important efforts have been devoted by fishing cooperatives to produce abalone seeds for restocking purposes, but recently most of these seeds are sent to grow-out systems (Fig.1). We are exploring multiple strategies to enhance production, restore natural populations and reduce uncertainty. For example, the establishment of no-take zones to increase wild abalone densities are promising tools, however, we need to promote additional actions such as conservation aquaculture and subsequent restocking efforts. Another potential strategy are adult aggregations through translocations to increase reproductive potential within no-take zones.

We present an array of strategies undertaken across Baja California. In San Jeronimo Island and Puerto Canoas the local fishing cooperatives in collaboration with academic institutions are developing mariculture systems for red and green abalones. We conducted a restocking experiment with 1080 red abalones produced at San Jeronimo Island. We tested two shell lengths, juveniles 33 ± 3 mm, and adults 72 ± 5 mm to compare movement, survival, and predation. Also, at Todos Santos Island we explored the growth of 348 red abalones 51 ± 7 mm, in cages attached to the bottom. We conducted translocations with several abalone species at different no-take zones in Baja. At El Rosario Bay, we translocated 161 green abalones, *H. fulgens*, 25 pink, *H. corrugata*, and 125 black, *H. cracherodii*. At Guadalupe Island, we established 2 no-take zones and translocated 461 green abalone. In this presentation, we will discuss the challenges and opportunities that these strategies face in the path to recover abalones and achieve sustainable production.

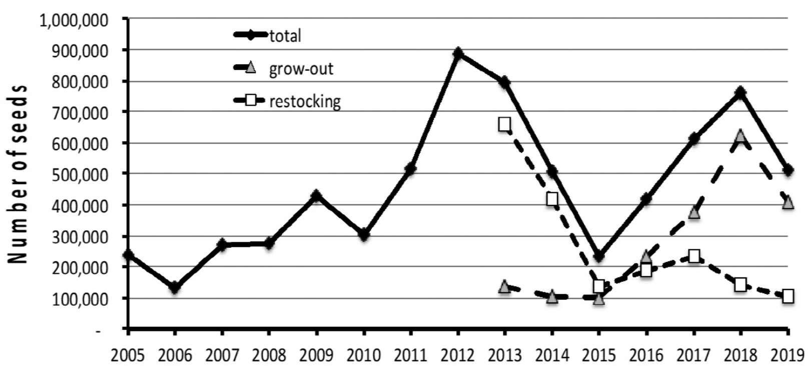

Figure 1. Number of abalone seeds (*H. fulgens* and *H. corrugata*) produced by Mexican cooperatives and used for restocking or grow-out. (Source: Cooperative hatcheries managers)

IMPACT OF CLIMATE CHANGE ON FISHERIES AND AQUACULTURE IN THE DEVELOPING WORLD AND OPPORTUNITIES FOR ADAPTATION

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This review paper reviews the importance of fisheries and aquaculture, with particular reference to poor people in the developing world, and the likely impact of climate change on these activities and on food security. It highlights some practical measures that can be taken to adapt to the expected effects of climate change. These focus in particular on building the capacity of communities to adapt to climate change in ways that allow them to moderate potential damage, to take advantage of new opportunities and to cope with the consequences of climate change, and on enhancing the resilience of communities and the ecosystems on which they depend. The review paper recommends basing interventions as much as possible on local practices and traditions.

SUSTAINABLE AQUAFEED: PRODUCING MICROALGAE WITH ATMOSPHERIC CO_2 AND SOLAR ELECTRICITY

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Global seafood demand (including wild capture and farmed aquaculture) has increased from roughly 100 million tonnes per year to nearly 200 million tonnes per year since 1990. To meet this need, the global farmed aquaculture industry has increased 527% since 1990 [FAO, 2018]. As a result, there is a significant need to supply sustainable feed ingredients for the global aquaculture industry, leading to investigations of using marine microalgae to replace plant-based ingredients (corn, soy, wheat, and potato) and fish-based ingredients (fishmeal and fish oil). Marine microalgae have the potential to reduce the environmental impact of feed production with respect to land use, freshwater consumption, greenhouse gas emissions, and global fishery sustainability. However, conventional microalgae production uses a large amount of fossil-fuel electricity and requires liquefied carbon dioxide that is bubbled into cultivation ponds. Prior analysis indicates that the cost and greenhouse gas impact of marine microalgae can be around \$700/t and 3.7 kg CO_2e/kg, respectively [Beal, 2018], as compared to those for corn/soy, which are roughly \$200/t and 0.4 kg CO_2e/kg (see Table 1). In this study, we compare the conventional model of Beal, 2018 (CONV) with a scenario that replaces fossil-fueled electricity with solar electricity and liquified CO_2 with Direct Air Capture (DAC) of atmospheric CO_2 directly into high-pH cultivation ponds (DAC-SOL), which is a technique that has been developed by Global Algae Innovations (see Figure 1). The DAC-SOL scenario assumes productivity of 18 g/m^2-d as compared to 23 g/m^2-d for CONV [Beal, 2018].

Preliminary results indicate that the DAC-SOL approach can generate algal biomass at roughly half the cost of fishmeal and reduce the GHG impact to be less than terrestrial plant-based ingredients and fish-based ingredients. In addition, using solar power and DAC allows for much greater flexibility in siting algal biomass facilities independent of the power grid or sources of CO_2.

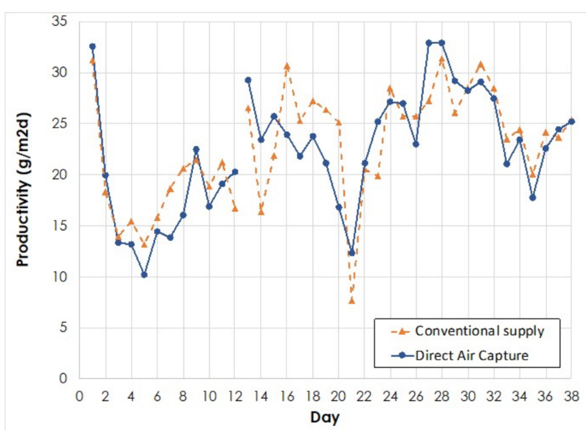

Figure 1. Comparison of flue gas and DAC CO2 supply on productivity for two ponds at GAI facilities in Kauai.

Table 1. Feed Ingredient Comparisons – Preliminary Results

Ingredient	Price ($/t)	GHG (kg CO2e/kg)	Fresh Water (m3/kg)	Arable Land (ha/t)
Corn	$135	0.44	0.17	0.07
Soybean Meal	$296	0.42	0.01	0.44
Fishmeal	$1,483	2.09	0.02	~0
Algae, CONV	$671	3.69	~0	~0
Algae, DAC-SOL	$791	0.35	~0	~0

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AQUACULTURE OPPORTUNITY AREAS: UPDATES

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On May 7, 2020, the White House issued an Executive Order (E.O.) on Promoting American Seafood Competitiveness and Economic Growth (E.O. 13921), which requires the Secretary of Commerce to identify geographic areas containing locations suitable for commercial aquaculture, and complete a National Environmental Policy Act (NEPA) Programmatic Environmental Impact Statement (PEIS) for each area to assess the impact of siting aquaculture facilities there. This includes:

1. Within 1 year of the E.O., identify at least two geographic areas containing locations suitable for commercial aquaculture;
2. Within 2 years of identifying each area, complete a NEPA PEIS for each area to assess the impact of siting aquaculture facilities there;
3. For each of the following 4 years, identify two additional geographic areas containing locations suitable for commercial aquaculture and complete a PEIS for each within 2 years.

These geographic areas will be referred to as Aquaculture Opportunity Areas (AOAs) once the PEIS is complete. The 3-year process to identify and complete a PEIS for each AOA will result in the identification of a geographic area that, through scientific analysis and public engagement, is determined to be environmentally, socially, and economically suitable for aquaculture. The areas identified as AOAs will have characteristics that are expected to be able to support multiple aquaculture farm sites of varying types, but all portions of the AOA may not be appropriate for aquaculture or for all types of aquaculture. NOAA has selected Federal waters within the Gulf of Mexico and off Southern California, south of Point Conception, as the regions where the first two AOAs will be located. By combining 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 will further define those areas.

NOAA will provide updates on our progress towards identifying the first two geographic areas containing locations suitable for commercial aquaculture, planning for the NEPA PEIS for each of those two areas, and the upcoming opportunities for input into the process.

PREVENTION TECHNIQUES FOR SELECTED FRESHWATER PARASITES

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Aquaculture production systems requiring large volumes of water are often reliant on unsecure water supplies to meet high demand. Such sources can be subject to seasonal influxes of pathogens, leading to disease and high mortality. This brief review will provide information on the biology of selected parasites found in North American freshwater aquaculture and techniques that can be utilized to help prevent and manage disease outbreaks.

SUSTAINABLE AND ALTERNATIVE AQUAFEED FROM A MIX OF ALGAL STRAINS: PARTIAL OR FULL REPLACEMENT OF FISH OIL AND/OR FISH MEAL

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It is estimated that the world population would increase to about ten billion by the year 2050. According to WRR (2019), it would require 56% more food and 593 Mha of additional land (2010 baseline) to feed this increasing population. The aquaculture sector is expected to continue to provide animal protein and essential fatty acids. Yet aquaculture depends heavily on fish oil and fish meal that are not sustainable in view of the dwindling total catch of fish from the oceans. It is with this as a backdrop that a subgroup of the Food and Feed for the 21 Century initiative, aimed to study the possibility of using a mix of algal strains to formulate a feed that will totally or partially replace fish oil and fish meal. Unlike other studies that explore single algae strains in limited feed trials, primarily with small species or juvenile fish (Gong et al. 2020, Gong et al. 2019, Peterson and Burr, 2019, Kiron et al. 2016, Sorensen et al. 2016, Sprague et al. 2015, Moroney et al. 2014), we have taken the approach to use the biochemical details of diverse algae strains that are already at modest to large commercial production scale. This includes microalgae produced with heterotrophic fermentation and both micro and macro algae that are autotrophic. The biochemical profiles of amino acids, oils, and bulk calories are introduced to the software Mixit to replace fish meal, fish oil and terrestrial sourced biomass. Preliminary studies are underway to evaluate a formulated feed for salmonid species that reduces both fish ingredients and terrestrial agriculture biomass. In a companion presentation, Beal et al. (2022) show that algae can have a far superior land, water and greenhouse gas impact compared to terrestrial crops. This presentation will highlight some of the findings and discuss future prospects to improve the sustainability of aquafeeds by supplementing or replacing fish meal and fish oil and terrestrial crop components in feed formulations.

Table 1. List of genera of algae under consideration

Algal Genera	Mode of Nutrition	Algal type
Schizochytrium	Heterotrophic	Microalgae
Chlorella	Heterotrophic	Microalgae
Chlorella	Photoautotrophic	Microalgae
Arthrospira	Photoautotrophic	Microalgae
Nannochloropsis	Photoautotrophic	Microalgae
Pheodactylum	Photoautotrophic	Microalgae
Gracilaria	Photoautotrophic	Macroalgae
Ulva	Photoautotrophic	Macroalgae

NOVEL BIOMARKERS FOR SMOLTIFICATION IN ATLANTIC SALMON BASED ON NON-CODING RNAs

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An important phase in the Atlantic salmon life cycle is the smoltification process, which prepares the fish for the parr-smolt transformation. This process is a complex adaptation that consists of several molecular drivers. For instance, in the gills, cortisol and the GH / IGF-1 axis promote the up-regulation of sodium-potassium-ATPase (NaK-ATPase). The NaK-ATPase activity is used in the salmon aquaculture as a smoltification marker, and RT-qPCR for this gene has recently been implemented. However, the NaK-ATPase levels can strongly be altered by hatchery conditions.

This study aimed to explore the putative role of microRNAs during the seawater transfer (SW), and identify novel biomarkers based on non-coding RNAs. For this, Atlantic salmon smolts were exposed to gradual salinity change, and gills samples were used for miRNAs mining by Illumina sequencing. A panel of miRNAs with significant expression changes during the SW transfer were validated through qPCR analysis. For the validation, gills samples were obtained during four weeks before the SW transfer. In addition, NaK-ATPase enzymatic activity was evaluated to corroborate the smolt condition.

Herein, we present six biomarkers candidates to evaluated the smolts conditions by qPCR. A significant NaK-ATPase enzymatic activity levels increase was recorded. Moreover, qPCR analysis showed a significant gradual increase of miR-128, miR-23a-3-5p, and miR-205a-5p during the sampling weeks. On the other hand, miR-205, miR-21a-2-3p, and miR-222 showed a progressive down-regulation during the smoltification (Fig. 1). In addition, the target gene analysis evidenced that ATPase-α subunit and can be regulated by miR-21a-2-3p (Table 1). These findings support novel biomarkers for smoltification in salmon aquaculture.

Funding: ANID-Chile through the Postdoctoral grant FONDECYT (#3190320), and FONDAP (#15110027).

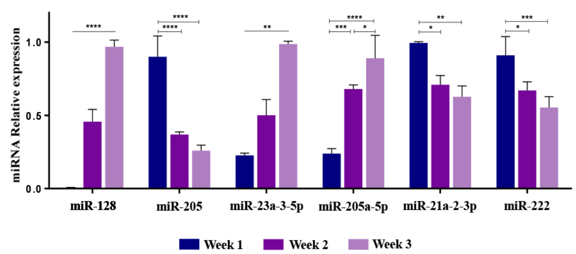

Fig. 1. Transcriptional modulation of miRNAs in gills from Atlantic salmon exposed to gradual changes in salinity

Table 1. miRNAs differentially expressed in gills and their putative target in Atlantic salmon exposed to gradual changes in salinity

miRNA	Delta G	Description
ssa-miR-23a-3-5p	-19.90	PREDICTED: collagen alpha-1(VI) chain-like [Salmo salar]
ssa-miR-205a-5p	-25.50	Fibroblast Growth Factor 2 (FGF2)[Salmo salar]
ssa-miR-21a-2-3p	-17.30	XM_014150738 PREDICTED: Salmo salar sodium/potassium-transporting ATPase subunit alpha-1 (LOC100136390), mRNA.
ssa-miR-222b-5p	-19.20	Transposable element Tc1 transposase [Salmo salar]
ssa-miR-92a-5p	-23.70	PREDICTED: collagen alpha-1(VI) chain-like [Salmo salar]
ssa-miR-205a-5p	-22.20	PREDICTED: ATPase family AAA domain-containing protein 5-like isoform X1 [Salmo salar]
ssa-miR-205a-5p	-18.80	PREDICTED: collagen alpha-2(VI) chain-like isoform X1 [Salmo salar]
ssa-miR-128-1-5p	-22.20	Transposable element Tcb1 transposase [Salmo salar]
ssa-miR-128-1-5p	-19.00	PREDICTED: epidermal growth factor receptor kinase substrate 8-like protein 3 [Salmo salar]

ASSESSING CHALLENGES LIMITING COMMERCIAL VIABILITY OF OFFSHORE AQUACULTURE

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Aquaculture technology for several commercially important species of tropical marine fish has become or is quickly becoming available. Hatcheries are capable of producing juveniles of candidate species for offshore aquaculture such as cobia (*Rachycentron canadum*), hamachi/kampachi (*Seriola rivoliana, S. lalandi/S. dorsalis*), pompanos (*Trachinotus carolinus*), snappers (*Lutjanus guttatus, L. peru and L. campechanus*), yellowtail snapper (*Ocyurus chrysurus*), totoaba (*Totoaba macdonaldi*), red drum (*Sciaenops ocellatus*), mahi (*Coryphaena hippurus*), tripletail (*Lobotes surinamensis*) – among others. Steady supply of high-quality juveniles of certain species is still limited, but it is unlikely that this will remain a bottleneck for industry expansion.

Land-based recirculating aquaculture systems (RAS) and traditional flow-through ponds, raceways and tanks are all viable but limited options. Large scale production required to feed the world in the next decades will have to be produced in the open ocean - where stronger currents and greater depths increase the carrying capacity of the environment. Raising fish in exposed, high-energy areas offshore require advanced technologies demanding high levels of investment and long-term commitment. Hence, fish produced offshore must be sold at high prices to compensate the high capital and operating costs required, limiting their demand in a highly competitive white fish market.

Offshore aquaculture continues to expand the world over, yet the commercial viability of operations remains mostly elusive. Infrastructure and logistics are in place, and market demand is rising. Technology continues to expand rapidly. Tools for site assessment and selection and environmental monitoring have been established. However, as with any relatively new industry, hurdles still must be overcome before commercial viability can be secured. Some issues such as optimizing genetics, nutrition, and diseases control are inherent to all forms of aquaculture - whereas stocking, feeding, chemical treatments, net cleaning, predator avoidance, escapements, biomass estimates and crop management, mortalities collection and harvesting operations are exacerbated in offshore systems. Automation is progressing fast but still needs refinement. Machine learning and artificial intelligence tools are becoming available and being incorporated to perfect systems automation. The development of practical, specialized feeds for all developmental stages of species such as cobia and Seriola remains a challenge. FCRs are still very high, limiting performance and increasing production costs.

We present and discuss these challenges and how the industry is collectively working with researchers to address and resolve issues limiting the technological and commercial viability of offshore aquaculture.

THE POTENTIAL ROLE OF BILE SALTS IN SOYBEAN MEAL-INDUCED ENTERITIS MITIGATION IN RAINBOW TROUT *Oncorhynchus mykiss* FED TWO LEVELS SBM DIETS OVER AN 18 - WEEK FEEDING TRIAL

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The replacement of fish meal (FM) in aquafeeds by alternative sources of protein such as plant ingredients like soybean meal (SBM) has typically encountered a variety of challenges at high levels of inclusion, especially in carnivorous species like rainbow trout (*Oncorhynchus mykiss*). The development of intestinal inflammation, in this context referred to as soybean meal-induced enteritis, is a noteworthy sign of reduced tolerance to SBM, and there is an array of approaches that have been explored to alleviate inflammation. Bile salts (BS) have recently been suggested to have a role in immune signaling regulation, and we have therefore hypothesized their potential as feed supplements towards SBM diets improvement.

The goal of this study was to evaluate the potential mechanisms by which BS might exert a protective effect on reducing inflammation and restore barrier function in rainbow trout over a long-term experimental period. A total of 2,000 fish initially weighing ~ 40.0 ± 1.0 g, were randomly distributed into 20 350-L tanks (100 fish/tank). Five experimental diets (isonitrogenous: 42% crude protein and isolipidic: 20% lipid) including a FM diet (control), a SBM30 diet (30% inclusion level), a SBM40 diet (40% inclusion level) and two SBM-BS supplemented diets (1.5% BS supplemented to each SBM diet) were fed to apparent satiation for 18 weeks. During the trial fish were sampled three times at 6, 12, and 18 weeks.

Feed intake was recorded daily, and fish weight was measured at each sampling point. Samples were collected from the distal intestine for gene expression analyses of the inflammatory markers: TNF-α, NF-κB, IL-8, IL-10; barrier function markers: MLCK, occludin and claudins; as well as the bile brush border transporter TGR5 and basolateral transporter OSTα. Similarly, liver gene expression analysis was done on the bile acid synthesis enzyme. Digesta was taken from proximal and distal intestine 12 hrs. after feeding for bile acid quantification and resorption calculation. Distal intestine and liver samples were also collected for histology analysis.

No significant differences ($p > 0.05$) in weight gain, feed intake and feed conversion ratio (FCR) were observed after 6 weeks, but fish fed the SBM40 diet showed slightly higher weight gain in the BS supplemented diet compared to the non-supplemented one (Fig. 1). Upon completion of the trial, growth performance results will provide a further insight on the possible impact of BS on growth. Gene expression analysis in the distal intestine and liver will help elucidate the molecular mechanism underlying the role of BS in inflammation and SBMIE mitigation.

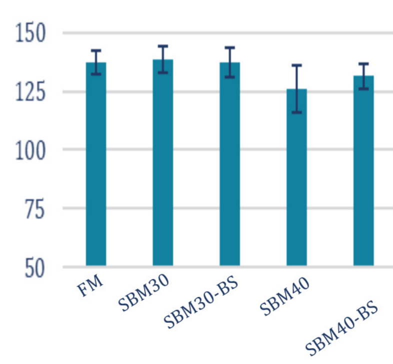

Fig. 1. Weight gain (g) of each dietary group after 6 weeks of feeding.

ASSESSING GROWOUT METHODS FOR U.S. WEST COAST VENUS CLAMS

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Culture of native species provides opportunities for expanding west coast aquaculture, especially since few native bivalves are currently cultured. Further there is interest among growers, regulators and the public in diversifying aquaculture products to include native species and in using suspended cultures to address environmental concerns and help meet growing consumer demand. Assessing ease of growout is generally quicker and easier than assessing hatchery rearing potential, thus we used it is a logical starting point. During summer 2021, we tested suspended growout methods for US West Coast venus clams (*Chione* spp) in a bay and on the open coast (off a pier; Fig. 1) using several substrates including mesh bags, mesh cages, cages with natural sponge and cages with plastic mats (Fig. 2).

Clam mortality rates were higher on the pier than in the bay (Fig. 3) which may have been due in part to the higher energy environment. While the plastic mats and natural sponges may have helped the venus clams orient their bodies, as in their natural benthic habitat, clam mortality rates were higher in the presence of substrate (Fig. 4). Growth rates (length, height wet weight) did not however differ across container types (p≥0.25).

Once complete, this research will inform aquaculture interests of ideal growout methods, including ideal conditions for local clam survival and growth.

Fig. 1. Growout systems tested

Fig. 2. Containers & substrate tested

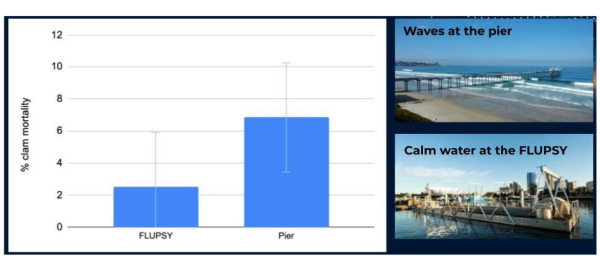

Fig. 3. Clam mortality rates were lower in a bay environment than on the open coast.

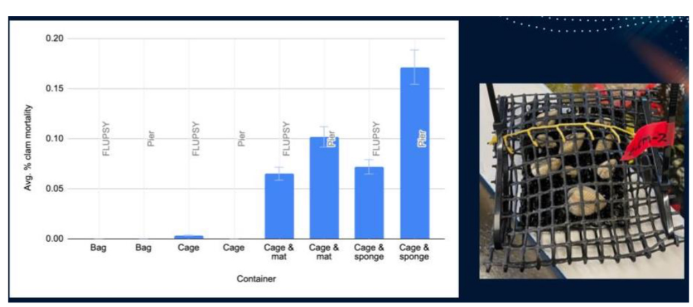

Fig. 4. Clam mortality rates in both open coast and bay systems were lower with an absence of a growout substrate (bags and cages)

DIRECT AND PLEIOTROPIC EFFECTS OF GENE EDITING MYOSTATIN AND *mc4r* GENES IN CHANNEL CATFISH, *Ictalurus punctatus* FOR GROWTH ENHANCEMENT

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The effects of CRISPR/Cas9 knockout of the myostatin (MSTN) gene and *mc*4*r* gene in channel catfish, *Ictalurus punctatus*, was investigated. Growth was generally higher in MSTN mutants when compared to controls at all life stages and in both pond and tank environments. Heterozygous F1 mutants were 218% larger than controls at the stocker stage in ponds. Mean expression level of MSTN was 2.90 times higher in controls than in MSTN F1 progeny (p=0.009). Muscle fiber number was 30-40% higher in mutants compared to controls. When challenged with *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC), MSTN mutants performed equally or better than controls.

Growth was generally higher in MC4R mutants when compared to controls at all life stages and in pond and tank environments. There were no significant differences in body weight between MC4R F1 progeny and controls generated in 2018, although there was a positive relationship between zygosity and growth, with F1 homozygous/bi-allelic mutants reaching market size 30% faster than F1 heterozygotes in earthen ponds (p=0.022), and another set of F1s growing 37.5% faster to stocker size. Some families grew upwards to 70% faster than control full-siblings with 50% better feed conversion efficiency.

Channel catfish have limited ability to synthesize n-3 fatty acids. MC4R mutants had a 94% increase in eicosapentaenoic acid (EPA, C20:5n-3) and a 21% increase in docosahexaenoic acid (DHA, C22:6n-3) compared to non-edited controls.

MC4R edited channel catfish were sterile, and MC4R appears to have a critical role in regulating the HPG axis. Fertility could be restored by applying a combination of luteinizing hormone releasing hormone analogue and human chorionic gonadoptropin.

ISOLATION AND *IN-VITRO* CULTURE OF PRIMARY CELL POPULATION DERIVED FROM WHITE AND BLACK CRAPPIE OVARIAN TISSUE

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Developing methodologies for the isolation and *in vitro* culture of ovarian cells would be a significant breakthrough for germ cell transplantation in fish. Investigations were conducted to develop a protocol for isolating and *in vitro* culture of ovary-derived cells from Black Crappie (*Pomoxis nigromaculatus)* and White Crappie (*P. annularis)*. Ovarian tissues were obtained from one-year-old Black and White Crappies. Five different digestive enzymes: 500U/mL Collagenase type I, 500U/mL Collagenase type IV, 0.05% Trypsin-EDTA, 0.25% Trypsin-EDTA (Gibco), and TrypLE™ Express were evaluated for cell isolation. Isolated cells from the ovarian tissue were next cultured in two different concentrations (10%, 20%) of Fetal Bovine Serum (FBS) in Leibovitz's L-15 growth media. In addition, four incubation temperatures (15, 20, 25, and 30 °C) were evaluated to determine optimal culture temperature for these ovarian cells.

The number of live cells obtained from the 0.25% Trypsin-EDTA and TrypLE™ Express treatments were significantly higher than other treatments. No significant difference in cell growth was observed between the 10% and 20% FBS treatments. Cell growth and division was seen at all incubation temperatures. Cells isolated using 0.25% Trypsin and TrypLE ™ reached 80-90% confluency in 12.5 cm^2 cell culture flask within five days of inoculation in 20, 25, and 30 °C incubation regimes. Whereas at 15°C incubation temperature, the cells took ten days post-inoculation to reach 80-90% confluency. Upon microscopy inspection, cells incubated at 20 and 25 °C appeared morphologically healthier than cells incubated at 30°C, where cell detachment from the substrate and irregular cell shape was observed. Cells were sub-cultured up to passage 2. Based on these findings, we conclude that 0.25% Trypsin and TrypLE™ enzymes are optimal for cell disassociation and isolation, while an incubation temperature of 20-25 °C is favorable for primary cell culture in L-15 media supplemented with either 10 or 20% FBS.

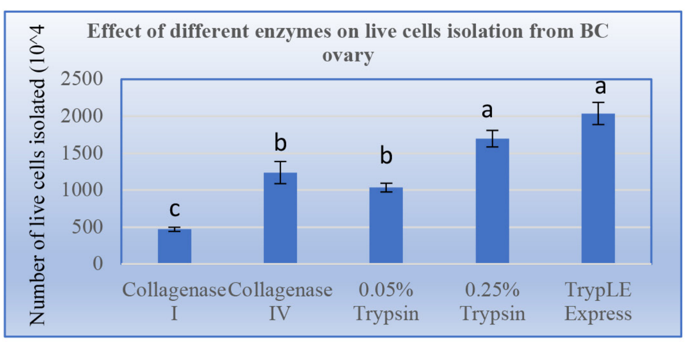

Fig 1: Effect of different enzymes on live cells isolation

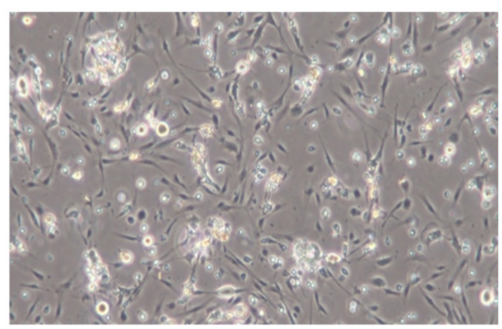

Fig 2: Primary cells 18 h post inoculation

ABERRANT, GONADAL TROPISM OF *Perkinsus marinus* IN THE EASTERN OYSTER *Crassostrea virginica* AND EVIDENCE FOR INTRA-OOCYTIC INFECTION

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Perkinsus marinus is a major molluscan pathogen and the causative agent of "dermo" disease in oysters. Although long present and perhaps a native oyster parasite in Chesapeake Bay, *P. marinus* is still the most significant oyster parasite along the East and Gulf Coasts of the USA, and it infects nearly all market-sized oysters in Virginia waters. Decades of diagnostic work have established clear expectations for how *P. marinus* infections present in oyster hosts. Nonetheless, presentation of *P. marinus* will occasionally diverge from its typical appearance. In particular, two divergent, and undescribed, presentations of *P. marinus* may have implications for the epizootiology and management of the parasite. One has been the atypical presence of the parasite in the gonad of oysters in which the parasite is absent from, or only modestly present in, other organs. The other presentation is infection intracellularly in oyster oocytes themselves. A better characterization of both these unusual *P. marinus* infections was the focus of this research, which addressed the following questions: 1) Are these "alternative" presentations definitively *P. marinus*? 2) Are there sex-based differences in the incidence of these atypical infections? 3) Are there differences in cell sizes and nuclear count of the aberrant gonadal infections as compared to contemporary *P. marinus*? 4) What patterns, if any, in geographical distribution and prevalence can be drawn from these abnormal infections?

To investigate both types of unusual tropisms of *P. marinus*, archived samples in the Virginia Institute of Marine Science Shellfish Pathology Laboratory (VIMS SPL) spanning 2011 to 2021 in which *P. marinus* gonadal tropism was noted during standard diagnostics were reanalyzed. First, a fluorescence in situ hybridization (FISH) assay was used to confirm species identification on representative samples of the noted aberrant gonadal presentation and on representative samples of infected oocytes. Then samples were re-read so infections could be more carefully characterized.

Molecular work confirmed that both unusual infections were *P. marinus* and not some sympatric or new species. Results for the aberrant gonadal infections indicated this type of infection mainly infected males and was geographically widespread with infections found throughout the lower Chesapeake Bay and even down in Florida. Cell size and nuclear count were different from contemporary infections raising questions about potential *P. marinus* strains. Infected oocytes were uncommon but samples did span several years. Both these unusual infections could challenge diagnosticians who rely on "typical" *P. marinus* infections and are not aware of alternative histopathological presentations. So, it is useful that these differing infections have now been described. This work also demonstrates the value in routine monitoring programs, trained experts, and detailed collection and preservation of samples. These types of resources are rare, but vital for managing marine diseases.

METHIONINE RESTRICTION DIMINISHES SKELETAL MUSCLE CELL DIFFERENTIATION THROUGH EPIGENETIC MECHANISMS IN RAINBOW TROUT *Oncorhynchus mykiss*

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For the past several decades, aquaculture has been the fastest growing sector of animal agriculture, with worldwide production of fish and shellfish tripling in volume produced since 1995. This growth has put significant stress of the availability and economic viability of using fishmeal and fish oils, the primary components of aquafeeds. Great strides have been made to decrease the inclusion rate of fishmeal in aquafeeds, while increasing the use of alternative protein sources, like plant proteins, as more sustainable and economical solutions to fishmeal. The most prevalent alternative protein sources are from plant products, which are inherently low in methionine. Therefore, supplementation of methionine, and other essential amino acids, is required for plant-based diets to match the nutritional and amino acid requirements of fish.

When dietary methionine levels are inadequate, nutrient partitioning between fat and protein deposition changes leading to decreases in visceral fat deposition. In addition, IGF-I levels in circulation decline and overall growth is reduced. With the increasing need for more plant-based diets, understanding the role methionine plays in fish growth is paramount. This study focused on the mechanistic effects of methionine on trout muscle cells directly, by using a methionine depletion/replenishment protocol. We hypothesize that methionine, because of its role in cellular methylation pathways, regulates cell differentiation via epigenetic mechanisms. We tested the role of methionine on 1) muscle-specific microRNAs and 2) global DNA methylation. Outcomes were analyzed in trout myogenic cells cultured under three different conditions: presence of methionine for 72h (ctl), absence of methionine for 72h (Meth-), and absence of methionine for 48h followed by 24h of methionine replenishment (Meth-/+).

MicroRNA array analysis revealed three clusters: cluster I corresponds to miRNA upregulated only in Meth-/+ conditions; cluster II corresponds to miRNA downregulated only in Meth-/+ conditions; and cluster III corresponds to miRNAs with high expression in control, low expression in Meth- conditions and intermediate expression after methionine replenishment (Meth-/+). Cluster III aligned with previous data supporting an involvement with differentiation and identified seven miRNAs with muscle-related function (including miR-210 and -133a). Global methylation analysis revealed 348 bases hypo- or hyper-methylated in response to methionine restriction, and 2 methylated sites rescued by methionine replenishment. These data support epigenetic mechanisms regulating decreased muscle growth in response to inadequate dietary methionine intake, and suggest further investigation is needed to optimize supplemental feeding strategies to support efficient and efficacious growth.

NOAA FISHERIES ALASKA AQUACULTURE ACCOMPLISHMENTS

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NOAA Fisheries Alaska has spent the last year building its aquaculture program. Together the Alaska Regional Office and the Alaska Fisheries Science Center strive to support industry and management needs to foster responsible Alaskan farmed shellfish and seaweed.

Sustainable marine aquaculture—also referred to as mariculture in Alaska—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 buffering against ocean acidification, shoreline stabilization, and reducing nitrogen levels in coastal environments. Aquaculture also establishes economic opportunities via the creation of jobs, eco-tourism, and contributions to the seafood sector.

Alaskan aquaculture has room to grow, and efforts are 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 to advance our Joint Alaska Aquaculture Action Plan along the following focal areas:

- Improve existing permitting processes for marine aquaculture in state waters
- Employ genetics to protect natural populations
- Advance understanding of the interactions of aquaculture and the environment
- Improve production efficiency and wellbeing
- Build partnerships to increase research, outreach, and education capacity

As Alaska's marine aquaculture industry continues to grow, NOAA Fisheries Alaska Regional Office and Science Center will play an increasing role in the management, policy, and research that will help build this sustainable food source to complement the region's wild-capture fisheries. Building off our Aquaculture Action Plan and prioritizing efforts based on our agency's strengths, and management and industry needs, will help us focus efforts to best serve Alaska.

CONTROL OF VIBRIOSIS IN PACIFIC OYSTER LARVAE BY A TRANSLATION-BLOCKING PEPTIDE-CONJUGATED PHOSPHORODIAMIDATE MORPHOLINO OLIGOMER TARGETING A *Vibrio coralliilyticus* VIRULENCE FACTOR

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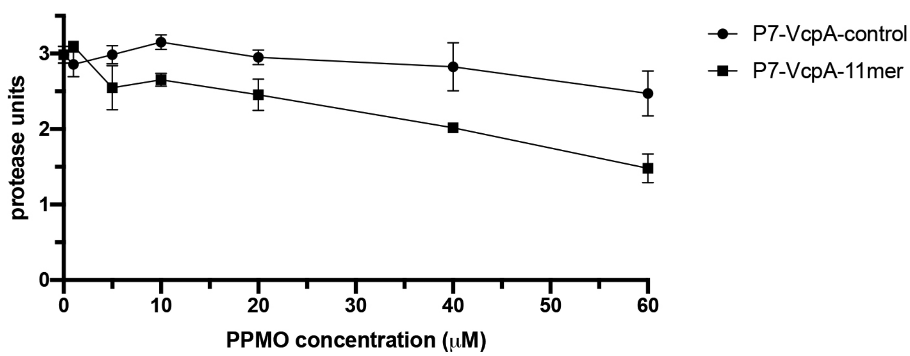
Peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) are synthetic nucleic acid analogs conjugated to cell-penetrating peptides, which enable intracellular delivery and subsequent access to RNA targets. Upon binding to targeted RNAs through Watson-Crick base pairing, PPMOs act through steric blockage to interfere with cellular RNA processing. This technology offers the opportunity to control gene expression within biological systems in a highly targeted manner. Here, we demonstrate the use of a PPMO to block the translation of the *Vibrio coralliilyticus* zinc metalloprotease VcpA, which has been determined to cause disease in larval *Magallana gigas*. This work is intended to provide proof of concept for the use of PPMOs to address vibriosis in oyster aquaculture, an industry-threatening problem for which traditional antibiotics are an unsuitable tool.

Within pure *V. coralliilyticus* cultures propagated in a minimal growth medium, we have determined that an 11-base PPMO targeted to the translation start site on VcpA-encoding messenger RNA has the capacity to reduce proteolytic activity of culture supernatants in a concentration-dependent manner without influencing the viability of treated cultures. Work to examine the effect of PPMO treatment of *V. coralliilyticus* cultures upon the health of *M. gigas* larvae exposed to culture supernatants is ongoing.



Concentration-dependence and specificity of a VcpA-targeting translation-blocking PPMO. VcpA-targeting PPMO (P7-VcpA-11mer) shown with irrelevant control sequence PPMO (P7-VcpA-control). Mean of three replicates shown, error bars represent standard deviation.

GROWTH AND SURVIVAL OF DIPLOID AND TRIPLOID OYSTERS *Crassostrea virginica* GROWN IN FLOATING GEAR IN GEORGIA

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Eastern oyster (*Crassostrea virginica*) aquaculture is expanding rapidly in the United States. Although, oyster aquaculture is extensively studied in other states, the benefits of floating gear and the performance of oysters is lacking in Georgia. We assessed the effect OysterGro™ cages had upon shell height, shell width, shell depth, survival, and condition of diploid and triploid oysters. Diploid and triploid oysters were deployed in floating cages from December 2018 – January 2020 at two sites; an exposed, high fetch site in Wassaw Sound (WS) and a protected, low fetch site in the Skidaway River (SR). Survival and growth were monitored quarterly over the research duration with condition index assessment at the conclusion of the study.

Findings of this research indicated that triploid oysters had ($p<0.001$) greater shell height (89.9 mm $\pm$0.6 SE) than diploid oysters (77.4 mm $\pm$0.6 SE). Shell height differed between sites ($p<0.001$) with oysters in SR larger (91.6 mm $\pm$0.7 SE) than oysters in WS (75.7 mm $\pm$0.5 SE). Survival differed between ploidy in the summer ($p=0.003$) and fall ($p=0.006$) with diploids having higher survival (93.1% and 98.1%) than triploids (84.1% and 93.6%). Site survival differed in summer ($p=0.002$) and fall ($p=0.006$) with WS higher (93.8% and 97.8%) than the SR (84.0% and 93.9%). We did observe a substantial drop on triploid oyster survival in late summer and early fall at SR which was not observed in triploids at WS or in diploid oysters (Figure 1). Oyster condition was highest in diploids.

This study provides first data comparing the growth and survival of diploid and triploid oysters in OysterGro™ system in coastal Georgia. Our results indicate that floating gear is a viable option to grow market-sized oysters for harvest, but that environmental factors at growing location and ploidy can significantly affect seasonal survival and growth of oysters.

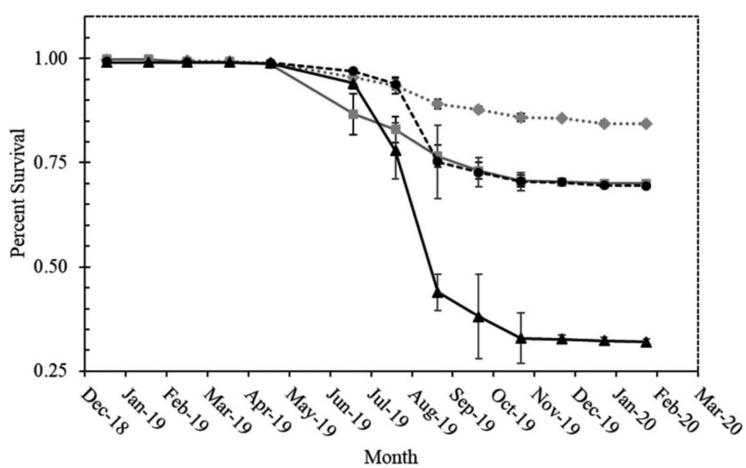

Figure 1. Cumulative survival ($\pm$ SD) of diploid (◆) and triploid (■) oysters in Wassaw Sound and diploid (●) and triploid (▲) oysters in the Skidaway River grown in floating cages from December 2018 – February 2020 in Chatham County, Georgia, U.S.A.

SHOULD WE BE REGULATING AQUAFEED PH?

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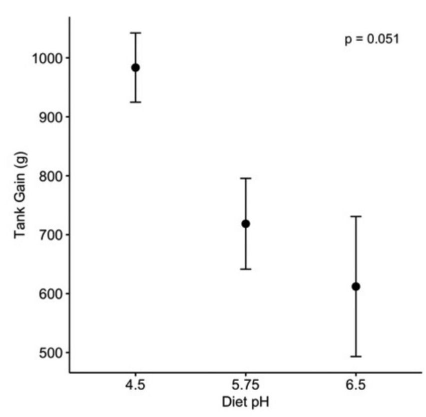
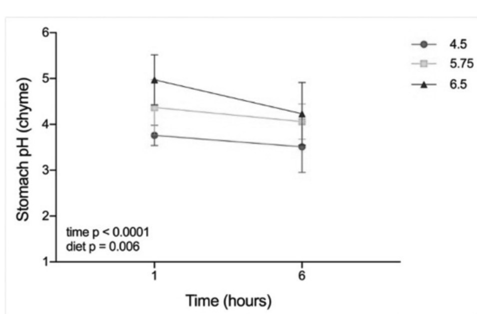
Developing diets that enhance growth and optimize feed efficiency is an essential component to increasing industry sustainability and success. In many fish species, including important aquaculture target species, a decrease in stomach pH occurs after feeding to facilitate digestion. This drop is initiated by parietal cells in the stomach epithelium that secrete protons (H^+) into the stomach lumen and bicarbonate ions (HCO_3^-) into the bloodstream. This process, termed the alkaline tide, as well as the return to acid-base homeostasis may be energy expensive. Modifying the pH of the diet as a means of supplying additional protons could be used to offset the physiological costs associated with digestion. In this study, we assessed the effects of diet pH on growth, feed efficiency, and the physiological and metabolic costs associated with feeding in red drum, *Sciaenops ocellatus.*

Experimental diets were manufactured at pH levels of 4.5, 5.75, and 6.5. Fish (mean initial mass 27.5 g) were stocked into independent 150 gal recirculating aquaculture systems at n=20 fish per tank and duplicate tanks per diet. Tanks were maintained at 25 °C and 25 ppt and fed twice daily to apparent satiation for 12 weeks. At the end of the trial, digestive metabolism (specific dynamic action), gastrointestinal pH, blood acid-base balance, and digestive enzyme activity were measured.

Growth (Fig. 1) and feed consumption were highest at the lowest diet pH tested. Diet pH also affected stomach (Fig. 2) and intestinal pH but not the postprandial flux of HCO_3^- into the blood, which was present after feeding in all three treatment diets. These data suggest that feeding a low pH diet can improve growth efficiency in red drum. Interestingly, similar to what has been shown to occur in rainbow trout, red drum maintain a continuously low stomach pH even in a starved state. Determining the digestive strategy of key aquaculture species and how this relates to optimal feed pH could inform advances in feed formulation, especially for the production of species-specific diets.





USING SIMULATION MODELING OF HIGH-THROUGHPUT EASTERN OYSTER CRYOPRESERVATION TO DEVELOP GERMPLASM REPOSITORIES

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Oyster aquaculture is an integral part of the economy and culture of Louisiana. However, many growers in the region are experiencing oyster mortality events, especially in the summer months. While the exact causes of these events remain unclear, selective breeding programs my offer a solution to the problem of high oyster mortality. However, genetic improvement can prove difficult without the use of tools such as cryopreservation repositories. Repositories have long been used in the beef cattle industry to easily provide breeders with genetic material from all over the country to facilitate rapid genetic improvement. Unfortunately, as of yet, no such cryopreservation and repository systems exist for aquatic species. While high-throughput cryopreservation protocols exist for oysters, protocols alone are not sufficient to foster repository development. To address this issue, this study used time study analysis and simulation modeling to standardize and integrate a high-throughput cryopreservation protocol into a repository development pathway. Nine cryopreservation trials were performed during which each step in the cryopreservation protocol was timed. Time distributions for each step were generated based on time study data. Distributions were then included in a simulation model. Therefore, the model reflected real-world working conditions and could be used to simulate outputs, such as the number of oysters processed (throughput) or cost, in a given about of time based on certain conditions. Conditions included factors such as the number of French straws (the freezing container) frozen per oyster or the number of operators available. Results highlight how altering the number of straws per oyster greatly affects outputs and can be used to inform protocol standards and facilitate cryopreservation and repository integration into oyster aquaculture.

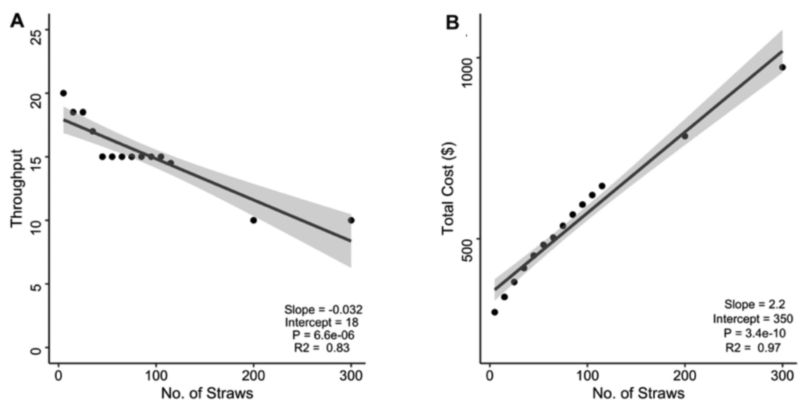

Figure 1. The effect of the number of straws frozen per individual on Throughput (number of oysters processed, A) and Total Cost ($, B) in an 8 hr work day with one operator. The grey line indicates the linear regression trendline, the dots indicate datapoints, and the grey-shaded area indicates the 95% confidence interval.

EFFECT OF PLOIDY AND COHORTS PRODUCED AT TWO HATCHERIES ON THE PHYSIOLOGY OF EASTERN OYSTER, *Crassostrea virginica*

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To supplement traditional oyster farming, alternative oyster aquaculture (AOC) has been promoted in the northern Gulf of Mexico during the last decade. Alternative oyster aquaculture is based on the use of off-bottom grow-out methods, hatchery-produced seeds, and selected seedstocks. Most of the seedstock used in AOC are triploids because of their faster growth. In 2016, oyster farmers in Louisiana and Alabama observed unexpected high mortalities of unknown cause. Triploid mortality was also reported by the aquaculture industry in the Chesapeake Bay. To potentially explain causes of triploid mortality, the physiology of diploid and triploid oysters was compared in this study. Diploid and triploid crosses were spawned at two hatcheries; the Auburn University Shellfish Lab (AU) and the Louisiana Sea Grant Oyster Research Lab (LSU). The diploid cross consisted male and female diploid oysters from Sister Lake. The triploid cross consisted of female diploid oysters from Sister Lake and a male tetraploid line (4MC18 at AU, 4DGNL17 at LSU). Oysters from each hatchery constituted the AU and LSU hatchery cohorts.

Clearance rates, absorption efficiencies, routine (fed) and basal (starved 1 week) oxygen consumption rates, ammonia excretion rates, *P. marinus* infection intensities, valve movement, and mortality rates of oysters from each ploidy and hatchery cohort were measured. Triploid oysters had higher clearance rates, *P. marinus* infection intensities (Figure 1), and mortality than did diploid oysters. Oysters from the AU cohort had higher basal oxygen consumption rates (Figure 2) and ammonia excretion rates than oysters in the LSU cohort. No differences in valve movement or absorption efficiency were found between ploidies or cohorts. Ploidy differences suggest better physiological performance of triploids than diploids based on clearance rate and SFG results. However, based on the physiological factors measured, the cause of triploid mortality remains unclear. Repository storage of cryopreserved genetic material and associated biological information can support further efforts to explain triploid mortality by allowing for future studies on specific crosses and gathering results in a centralized location.

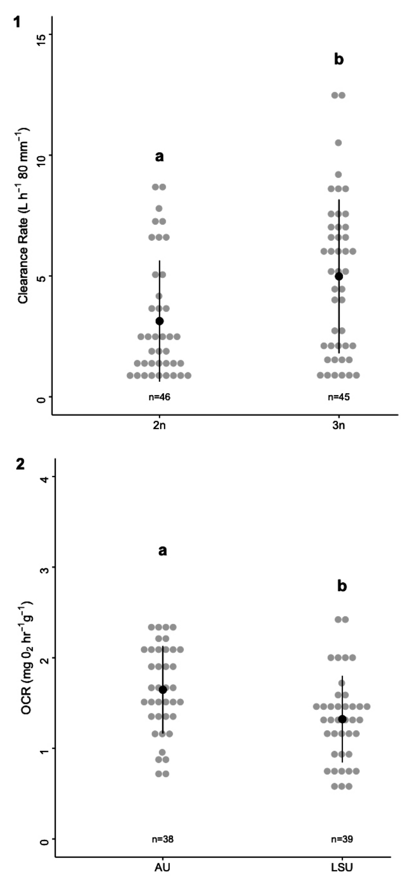

Figure 1. The average clearance rates (standardized by shell height, L h^{-1} 80 mm^{-1}) for diploid and triploid oysters. Figure 2. The average basal oxygen consumption rate (OCR, mg O_2 hr^{-1} g^{-1} of tissue) for AU and LSU oysters. For both plots, grey dots indicate individual observations, black dots indicate means, and black lines indicate standard deviations.

MITIGATION OF THE ACCUMULATION OF OFF-FLAVOURS AND OTHER UNDESIRABLE CHEMICAL COMPOUNDS IN MARINE FINFISH *(Seriola lalandi* and *Argyrosomus japonicus)* PRODUCED IN AN INTENSIVE RECIRCULATING AQUACULTURE SYSTEM THROUGH THE USE OF A NOVEL FEED (AMT) AND FEEDING STRATEGY

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Aqua Management Technologies (AMT) manufactures high-quality feed with highly digestible ingredients specifically designed for use in intensive recirculating aquaculture systems (RAS). AMT feeds come with a recommended rationing programme that avoids feeding to satiation, rather rationing according to species, size, number, water temperature and oxygen content. While this approach maximises food conversion ratios, it also improves water quality in the RAS system significantly.

When AMT feeds are used in combination with Ozone as part of the RAS production system, the accumulation of any chemical residues (heavy metals, pesticides, antibiotics radio-nucleotides, hormones, PAH's etc.) and off-flavours (Geosmin and Methyl-Isoborneol / MIB). Apart from Arsenic (the natural, organic derivative?) being detected, all the other compounds show as 'below detectable limits' in the analysis of flesh samples taken on a routine basis at commercial farming enterprise. (a comprehensive list of the compounds and their measurements will be presented).

As a result of the above, the farm does not use any sort of purging technology or process as part of its production and harvesting technology. Fish are simply starved the day before harvest so that the gut is empty for hygienic processing. They are removed directly from the production tanks, slaughtered, bled and placed into an ice slurry, to be sold fresh 'never frozen' into the high-end retail and food services industry in South Africa.

Market uptake and acceptance has been flawless.

A SUPPLY ANALYSIS OF 20 EMERGING MARINE FINFISH SPECIES FOR COMMERCIAL AQUACULTURE PRODUCTION IN THE UNITED STATES

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While commercial production of marine finfish has grown globally, in the United States there has been little growth in this sector other than for salmon and redfish. The supply of 20 emerging marine finfish (almaco jack, Atlantic cod, black drum, black seabass, California flounder, California yellowtail, cobia, Florida pompano, olive flounder, red drum, sablefish, spotted seatrout, striped bass, tripletail, white seabass, and wolffish) was analyzed. These species were aggregated into the following categories based on the degree of consumer recognition in the U.S.: 1) well-recognized in the U.S. market, 2) well-recognized in regional U.S. markets on the East and Gulf Coast, 3) well-recognized in regional U.S. markets on the West Coast, and 4) largely unknown in U.S. markets.

In 2019, total commercial supply of these 20 species (commercial landings & farmed) was only 81 million pounds; about 23% of the total 2019 production of the U.S. catfish industry. Commercial landings for 17 of the 20 species have declined, potentially offering windows of opportunity for farmed product to capture previous demand for these species. Aquaculture is advantageous for these species because it can supply a consistent and uniform supply to the market regardless of the time of year, something that commercial landings are unable to achieve. Nevertheless, the generally low volumes of current effective demand for these species indicates that farms seeking to raise and sell these fish would be limited to a relatively small scale because of limited demand. Smaller farms typically have higher costs of production and require sales into high-end niche markets to capture premium prices. Successful U.S. farming businesses for these species will attract competition from imported products of the same or similar species. Potential entrepreneurs will need to be prepared to remain competitive with increasing pressure from imported product.

Future work on this project hopes to uncover potential market price points and cost of production for these 20 species. There also needs to be an emphasis on collecting species-specific data on imports of these species for farmers to plan and adjust their business strategies accordingly.

In summary, the low current effective demand for these species will likely mean that farmers will need to initially target upscale, premium-priced markets. An identifiable brand, consistent supply and consumer loyalty will be important keys for success. Partnerships with distributors, restaurants, supermarkets, and wholesalers may be helpful to jointly develop expanded markets and sales over time.

DEVELOPMENT OF ULTRASONOGRAPHY AS A NON-INVASIVE METHOD TO EVALUATE REPRODUCTIVE CONDITION IN RED ABALONE *Haliotis rufescens*

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Abalone (*Haliotis* spp.) reproductive status can be difficult to determine using traditional visual assessments and often rely on lethal histological analyses for conclusive results. However, lethal examinations of endangered species are strictly prohibited. Here, we explored the usefulness of non-invasive ultrasonography as an equivalent means of establishing the reproductive state of abalone compared to histological evaluations. Red abalone sourced from The Cultured Abalone Farm (Goleta, CA) were used to test our methods. We found that ultrasound technology successfully distinguishes between reproductive and digestive tissues allowing for accurate representation of ultrasound index scores. Therefore, we establish that ultrasonography is a successful tool in generating gonad index scores, which can be used to track the development of reproductive tissues over time in both endangered and cultured species of abalone. Monitoring ultrasound gonad index scores over time can be useful for informing when individual abalone have spawned or are ready to be spawned, enhancing production. We recommend the use of non-invasive ultrasonography within the shellfish aquaculture industry to enhance both food production and conservation breeding programs.

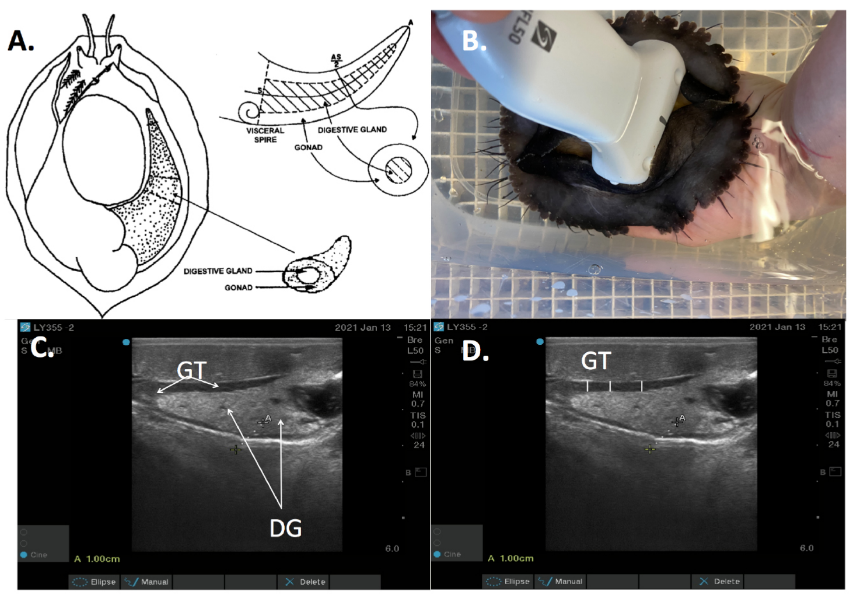

Fig. 1. Diagram of abalone gonad and digestive gland (Rogers-Bennett et. al, 2004)(A). Non-invasive ultrasound exam on cultured red abalone (*Haliotis rufescens*) immersed in seawater (B). Ultrasound visualization of digestive gland (DG) and gonadal tissue (GT) of red abalone (C). Replicate measurements of GT were determined using non-invasive ultrasonography (D). Scale bar (A)=1 cm (A).

SUBLETHAL EFFECTS OF LOW-DOSE EXPOSURE TO IMIDACLOPRID ON THE AMERICAN LOBSTER *Homarus americanus*

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Atlantic salmon farms face the ongoing challenge of managing sea lice infestations due to reduced sensitivity to extensively used chemotherapeutants. Imidacloprid is a neonicotinoid insecticide that has been implemented as a novel chemical to mitigate sea lice infestations on European Atlantic salmon farms. Prior to its consideration for use in the Northwest Atlantic, we tested the effects of these imidacloprid waste-water and dispersal concentrations on the economically valuable nontarget species, the American lobster, *Homarus americanus*. Behavior and hemolymph biochemistry of sub-adult female lobsters were observed following 120-minute exposure to the imidacloprid concentrations 0, 0.3 or 30 ppb. Observations were repeated five days later to evaluate chronic effects. Defensive behaviors were found to be significantly reduced for lobsters exposed to the 30 ppb imidacloprid concentration, and some remained significantly reduced five days after exposure. Interestingly, overall hemolymph endpoints indicative of stress (L-lactate, crustacean hyperglycemic hormone, and total protein) were not significantly different across treatments. This highlights the importance of behavioral endpoint measurement, as limited upstream endpoints may not always capture the full impact downstream. These findings suggest that if salmon farms of the Northwest Atlantic administer imidacloprid as a treatment for sea lice, nearby lobsters may have impaired behaviors of ecological and economic importance.

SEASONAL REPRODUCTIVE CYCLES OF TWO DEVELOPING COMMERCIAL SHELLFISH SPECIES IN MASSACHUSETTS

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The spawning cycles of two potentially valuable commercial shellfish species in Massachusetts were examined to determine timing of gametogenic development. The "southern" surfclam, *Spisula solidissima similis*, is a subspecies of *Spisula solidissima solidissima,* the commercially harvested Atlantic surfclam, and has been experimentally grown in Massachusetts coastal waters with recent success. The intertidal fishery for the razor clam, *Ensis directus*, has become increasingly important in areas of Massachusetts, but little is known of the seasonal reproductive patterns of either species. To address this question in local populations, a sample of 10 clams of each species was examined at monthly intervals over two years. Morphometric data (shell length, weight) was collected before each clam was opened to visually determine gonadal development. A gonad tissue sample was then examined under a microscope to determine the presence/absence of germ cells, and to estimate the developmental stage. Preliminary results suggest that *E. directus* in Cape Cod Bay spawn in May, similar to documented spawning times of populations in northern Europe and Canada and aligning with seasonal water temperature increases. *S. s. similis* appear to spawn in late May-June, later than southern populations but also reflecting the spring warming of water temperatures in the northern latitudinal region. Both species exhibited an additional spawn in September, an occurrence potentially driven by food availability as well as water temperature. While peak spawning typically occurs in the spring, with a warming climate and oceans, and a subsequently longer growing season, fall spawns could become more prevalent in New England species.

LEPTIN METABOLIC FUNCTION AND REGULATION OF OXYGEN CONSUMPTION IN THE TILAPIA, *Oreochromis mossambicus*

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Leptin is a pleiotropic cytokine hormone known to influence numerous physiological processes such as growth, appetite, energy expenditure, stress, reproduction, and immunity in vertebrates. In mammals, leptin is produced by adipose tissue and is thought to work 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 is poorly understood despite leptin's well-conserved anorexigenic actions. 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 prior work in the tilapia (*Oreochromis mossambicus*) determined that recombinant tilapia leptin A, the predominant paralog in fishes, induces hyperglycemia and depletes hepatic glycogen suggesting the hormone induces glycogenolysis. We have also found that hepatic *lepa* and/or circulating hormone levels increase in tilapia in response to various stressors, including fasting and seawater challenge, while others show the hormone rises with hypoxia. 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.

Additionally, in a transcriptomic study of the tilapia pituitary we 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. The hormone also affected hypoxic responsive pathways likely associated with enhanced anaerobic glycolysis. This led us to hypothesize that leptin may act to decrease oxygen consumption by promoting anaerobic glycolysis and suppressing aerobic respiration.

To this end oxygen consumption rates were measured in perifused pituitary RPDs using intermittent flow respirometry. Recombinant tilapia leptin caused an immediate decline in oxygen consumption rate (mO2) that was sustained over the course of leptin exposure. The rate of oxygen consumption was reduced by 12% with leptin treatment. Upon removal of leptin, mO2 slowly recovered to pre-treatment levels. We then evaluated oxygen consumption and mitochondrial function using the Agilent Seahorse platform. Again, we found that leptin caused an immediate 15% reduction of pituitary basal mO2. This reduction was accompanied by decreased mitochondrial ATP production and spare respiratory capacity. Collectively, these results indicate that leptin may suppress cellular respiration or energy expenditure. This may represent a novel function of leptin in facilitating adaptation and survival in the face of physiologic and environmental stressors like hypoxia, infection, and osmotic stress.

THE IMPORTANCE OF PRODUCT ATTRIBUTES FOR FARMED OYSTERS: AN ANALYSIS OF U.S. RESTAURANTS

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A significant share of seafood consumption in the U.S. occurs in restaurants, making knowledge about important product attributes important for producers to succeed in the marketing of their product. U.S. oyster aquaculture is an industry that appears to have successfully used product attributes to attract new consumers and increase consumption. However, there are also indications that attribute preferences vary regionally and that prices vary with attributes, as well as the ability or willingness of producers in different regions to supply various attributes. To understand the importance of attributes and qualities of a premium half shell oyster, we have conducted an analysis of restaurant menus around the U.S. which will be combined with qualitative surveys of restaurant managers and chefs. We randomly selected 358 restaurants that sell raw oysters on the half shell across 18 major U.S. cities to analyze the attributes that are provided on the menu. A hedonic price analysis was conducted to determine the significance of each attribute when determining an oyster's price point. The main region that the oyster was sourced from was found to have the most significant effect on an oyster's price, with the highest priced oysters coming from the West coast and the lowest priced oysters coming from the Gulf coast. Additionally, oyster branding was found to have a significant effect on pricing, while information with respect to the taste of the oyster had a negative effect on the oyster price. Oysters that only had one associated attribute on the menu were seen to fetch lower prices as well.

DISTRIBUTION OF HORSESHOE CRAB EGGS AND OTHER AVIAN PREY AROUND INTERTIDAL OYSTER AQUACULTURE IN DELAWARE BAY

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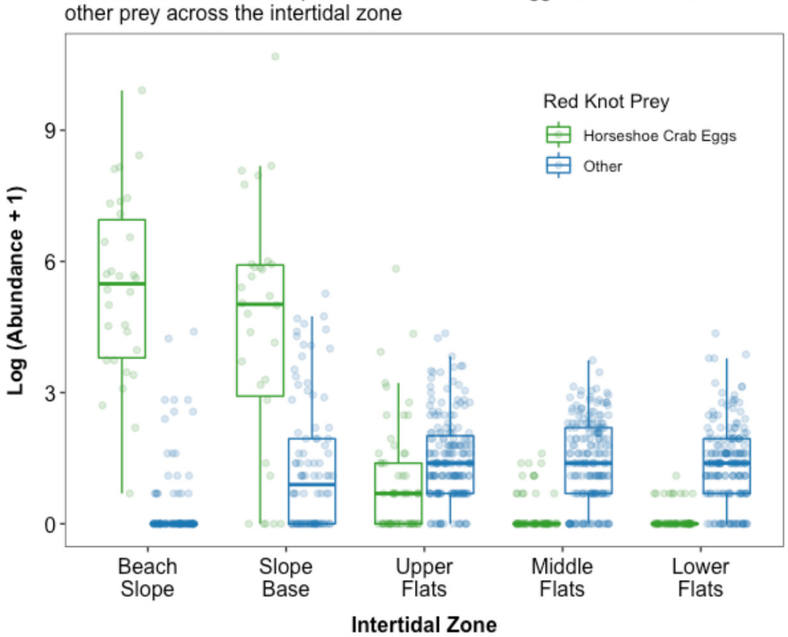
The resurgent eastern oyster (*Crassostrea virginica*) aquaculture industry in Delaware Bay, NJ may influence the relationship between horseshoe crabs (*Limulus polyphemus*) and rufa red knots (*Calidris canutus rufa*). Delaware Bay hosts the world's largest spawning population of horseshoe crabs. Red knots are federally threatened shorebirds that feed on the abundant eggs to fuel their circumpolar migration. The overlap of aquaculture with spawning and foraging habitat may alter this trophic interaction. Research shows that oyster farms do not impact the ability of horseshoe crabs to access spawning beaches; however, it is unknown how they may impact the distribution of eggs or other prey available to the birds. The red knot distribution in Delaware Bay is associated with horseshoe crab egg abundance, and while foraging behavior is not impacted by the presence of farms, red knot abundance is reduced by 2-7% while farms are tended, so the relative abundance and distribution of eggs in relation to oyster farms may significantly impact this shorebird species.

This study examines the effect of oyster farms on the distribution of red knot foraging resources. During the Spring 2021 spawning and migration season, benthic sediment surveys were conducted in four paired plots (90 m x 180 m) across a 3.5 km stretch of tidal flats that contain areas with and without oyster farms in Delaware Bay. Sediment cores determined the relative abundance of surficial eggs and other prey across each plot. Preliminary analysis suggests that the abundance of horseshoe crab eggs was highest on the beach slope and declined with increasing distance from the high tide line (Figure 1). Eggs were scarce farther than 300 ft from the high tide line, where farms were located. Other prey (*e.g.*, bivalves, gastropods, and polychaetes) were more abundant on the tidal flats than horseshoe crab eggs. This preliminary analysis indicates that eggs are not associated with red knot use or avoidance of oyster farms. Furthermore, across the tidal flats and within oyster farms, red knots likely forage primarily on alternative prey. Subsequent paired comparison analyses will examine whether oyster farms affect the distribution and abundance of horseshoe crab eggs or other prey.

FIGURE 1. Standard boxplot of horseshoe crab egg abundance and other prey across the intertidal zone



APPROACHES TO MONITORING AND MANAGING MARINE-LIFE INTERACTIONS WITH OPEN-OCEAN AQUACULTURE FACILITIES: RESULTS OF A WORKSHOP AND ENGINEERING MEASUREMENTS

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To date, protected marine vertebrates have rarely been injured or killed during physical interactions with aquaculture gear. Although the evidence for this observation is compelling when compared with observed effects of fisheries, concerns persist because it is not backed by a substantial body of scientific research or modeling. In 2019, under funding from the NOAA Saltonstall-Kennedy grant program, we conducted a workshop on the science and engineering of interactions between marine vertebrates and open-ocean aquaculture facilities. The workshop focused on evidence of injuries and mortalities; practices adopted by farms to collect observations and minimize interactions; design and engineering of aquaculture gear; and physics and physical models of the behavior of lines and cages.

Evidence presented during the workshop and since supports the rarity of entanglement and injury, amounting to fewer than 25 incidents involving protected species over the last four decades. Biologist participants noted that this count does not include the effects of permitted lethal takes on seals and sea lions after depredation attempts. They also described gaps in effort to collect data. Detailed observations of actual interactions are still very limited and forensic approaches are rarely applied to incidents. There is also substantial granularity in the information available on abundance and movements of species of concern. Farmers, including two successful West Coast finfish operations, described best practices for eliminating gaps, which included ongoing documentation and maintenance procedures. Both farmers and engineers pointed to significant differences between aquaculture and fishing gear, foremost in the tension applied to all types of lines. Engineers described ongoing efforts to model the behavior of aquaculture gear under the influence of waves and currents, and the dynamics of entanglement. To date models of entanglement have focused only on fishing gear.

Line tension is hypothesized as an important factor in takes. It is simplest to study in line-based operations (e.g., bivalves). We made initial physical measurements of a sample of line types used in both aquaculture and fisheries. We included samples of line (10 – 13 mm [3/8” to ½”] nominal diameter) weathered by a macroalgae grower for up to 5 years. Based on measurements of elasticity as tension was applied, weathering made the lines stiffer but less resistant to strain. A preliminary finite element model describing deflection as transverse force was applied (e.g., when an animal contacts a line) showed that even modest tension had a more important effect on line displacement than elasticity. In summary, both the workshop and feasibility measurements emphasized the value of obtaining detailed, direct measurements of marine life interactions with aquaculture gear.

THE ESTIMATION OF RESOURCE USE IN SHRIMP FARMING USING A LIFE CYCLE ANALYSIS (LCA) APPROACH

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Amounts of land, water, energy, and wild fish for feed ingredients used per metric ton (t) of production were estimated for four grow-out intensity levels (extensive, semi-intensive, intensive, and hyper-intensive) of farmed shrimp. The results follow:

	Extensive	Semi-intensive	Intensive	Hyper-intensive
Direct land (ha/t)	1.120	0.295	0.076	0.031
Total land (ha/t)	1.120	0.627	0.374	0.315
Saline water (m^3/t)	24,000	54,300	6,000	1,700
Freshwater (m^3/t)	254	2,980	3,110	3,210
Energy (GJ/t)	14.9	77.3	56.7	67.2
Wild fish use (t/t)	---	0.891	0.646	0.646

Extensive production uses much less freshwater and energy than do the other production methods. Because it does not rely on feed, no wild fish use is incurred in extensive production. Extensive production uses much more direct land (land at the farm level) than does the other methods and especially intensive and hyper-intensive. Extensive shrimp production is nevertheless, not ecologically desirable because farms are located mainly in the intertidal zone. This area contains mangrove and other wetland habitat of importance for its ecological services and high biodiversity. The tradeoff of more energy, more water, and wild fish for less land seems acceptable in shrimp farming.

STATE MARINE AQUACULTURE COORDINATORS NETWORK

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The National Sea Grant Law Center, in partnership with the North Carolina Division of Marine Fisheries and the Florida Department of Agriculture and Consumer Services, recently received funding from the NOAA Sea Grant Program to plan and host an "Interstate Technical and Networking Workshop for State Aquaculture Managers and Extension Personnel." The workshop will provide a networking forum for state aquaculture coordinators and regulators, state extension personnel, and federal regulators. It will also promote efforts to compile regulatory information across state aquaculture programs and help program administrators learn from each other's experiences and challenges on a variety of topics including user conflict issues. Participation in the workshop will be open to representatives from state aquaculture programs in the Mid-Atlantic and Southeast regions (i.e., Virginia to Texas), as well as representatives from federal permitting agencies and individuals from state extension programs (i.e., Land Grant or Sea Grant) in states with aquaculture programming. Goals of the workshop include 1) establish a long-term interstate network of aquaculture professionals (State Marine Aquaculture Coordinators Network - SMACN), 2) enhance communication among states, 3) secure long-term funding for future recurring annual meetings, and 4) expand participating states to a national level.

Originally planned for early 2020, public health concerns and travel restrictions associated with the COVID-19 pandemic delayed the in-person workshop until late spring 2022. During this period, the Project Team hosted a series of webinars to provide networking opportunities, share information on recent developments in state aquaculture programs, and obtain feedback on the desired structure and content of project deliverables from the workshop.

The workshop will also provide an opportunity to discuss long-term goals, including the expansion of future meeting topics, identify network leadership, and finalize next steps for the success of the SMACN. Deliverables from the workshop will include a multi-state inventory of aquaculture regulations, agency contacts, and permitting processes that is usable and can be easily maintained by the SMACN.

TENACIBACULOSIS IN ATLANTIC SALMON (*Salmo salar* L.)

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The aetiological agent of Tenacibaculosis (or yellow mouth) *Tenacibaculum* spp. infect multiple fin fish species globally and is a major pathogen of sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), turbot (Psetta maxima), and Atlantic salmon (*Salmo salar* L.) aquaculture. In Atlantic salmon, the disease and associated mortalities occur both in smolts soon after transfer to sea, and in larger adults. While the disease exhibits variable pathological manifestations, yellow mouth lesions, fin erosion, and skin lesions are characteristic of the disease. In Canada, the disease causes significant losses to the industry and is a priority issue with annual cost of outbreaks for one Canadian company cited as $1.6 million. Moreover, instances of outbreaks in other geographical regions, e.g. Chile, Norway, and Scotland have increased in recent years. At current, there is no commercially available vaccines for use in salmonids and a reliance on therapeutic use of antibiotics has led to a strong need for the industry to establish efficacious alternatives.

Multiple isolates of *T. maritimum*, *T. finnmarkense*, and *T. dicentrarchi*, and one potentially novel species of *Tenacibaculum* originating from Western Canada (BC) were utilised in these studies. Investigations focussed on understanding the virulence, and compound factors that influence disease outcomes, of the different isolates and species. Furthermore, the clinical presentation of the isolates, and understanding how challenge methods can be manipulated for the testing of different technologies and treatments are explored.

Isolates, within and between, species of *Tenacibaculum* have significantly different virulence and clinical presentations. Furthermore, it has been identified that while most environmental, and other compound factors, influence infection and disease outcomes some are unique. The development of these disease models is an important step in the development of technologies to mitigate its impacts on the culture of Atlantic salmon. However, Tenacibaculosis is a complex disease, and often is not exclusive to the presence/ infection of fish by a single species. Therefore, current developments are focussed on multiple species infection models for use in the industry.

WHITE SPOT SYNDROME VIRUS AND *Vibrio parahaemolyticus* CHALLENGE MODEL DEVELOPMENT IN WHITELEG SHRIMP (*Litopenaeus vannamei*)

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White spot syndrome virus and *Vibrio parahaemolyticus* challenge model development in whiteleg shrimp (*Litopenaeus vannamei*)

Shrimp aquaculture is a 38 billion dollar (USD) global industry, with whiteleg shrimp (*Litopenaeus vannamei*) accounting for approximately 80% of production. Due to the intense nature of shrimp aquaculture, disease outbreaks can have major impact. Pathogens of concern include white spot syndrome virus (WSSV), and *Vibrio parahaemolyticus*, the aetiological agent of acute hepatopancreatic necrosis disease (AHPND), both of which have been reported to cause mortality up to 80-100%. No current treatments are available for WSSV, and reduced efficacy of antibiotic treatments for *V. parahaemolyticus* due to development of resistance, together with a negative market opinion on their use, has led the industry to seek novel treatment strategies. To facilitate research into the efficacy of novel disease treatments in whiteleg shrimp, challenge models for WSSV and *V. parahaemolyticus* were developed.
I
n these challenge model development experiments, methods were established to prepare the WSSV inoculum, and bacterial culture growth conditions were optimized for *V. parahaemolyticus*. In addition, the dose-response relationship and the effect of shrimp size on mortality were characterized.

Per os and intraperitoneal injection challenge models for WSSV, and a *per os* challenge model for *V. parahaemolyticus* were established. Although the challenge models were initially developed for challenge of individual shrimp housed in separate tanks, experiments showed that these models translate well to population-based challenges. These allow a larger number of shrimp to be challenged, increasing the statistical robustness of the study. When testing the efficacy of treatments expected to have a moderate protective effect (e.g. a relative percent survival of 20-40% as observed in some functional feed studies), use of a population-based challenge increases the likelihood to detect a significant effect of the treatment

MICROBIOME CHANGES AND RECOVERY IN BIOFILM: TAXONOMIC AND FUNCTIONAL DIVERSITY IN RESPONSE TO CHELATED COPPER TREATMENT

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Chelated copper and copper sulfate are used to prevent parasitic infections and excessive algal growth. Studies have shown that copper treatment can alter the fish skin microbiota by encouraging growth of microorganisms with metal resistance mechanisms and causing an increase in the abundance of opportunistic pathogens. However, none of these studies conducted environmental microbiome analysis or incorporated metagenomic shotgun sequencing to explore functional diversity of the microorganisms. This is an important gap to fill because metal resistance genes (MRGs) and antibiotic resistance genes (ARGs) are often co-selected in many environments including those influenced by agriculture and aquaculture runoff. Copper treatment leading to increases in microorganisms with metal resistance mechanisms may cause increases in microorganisms with antibiotic resistance mechanisms as well. Metal efflux systems can provide cross-resistance to antibiotics since they can also extrude the antibiotics from the cell. In addition, co-selection occurs due to MRGs and ARGs being located next to each other on a mobile genetic element (genetic cross-resistance), which is capable of being transferred between bacteria via horizontal gene transfer. Determining whether this co-selection occurs is significant because increases in ARGs or opportunistic pathogens due to copper treatment can have negative implications for fish health and survival.

The study presented here was an opportunistic sampling of a tank (12,870L) containing twenty Florida pompano (*Trachinotus carolinus)* after a mortality with confirmed *Amyloodinium ocellatum* infection. Triplicate biofilm samples for DNA extraction along with water samples for copper analysis were taken during nine time points before and after chelated copper treatment. Purified DNA extracts were sent to GeneWiz (South Plainfield, NJ) for metagenomic shotgun sequencing (Ilumina HiSeq 2500). DNA sequences were quality filtered, assembled, binned, annotated, and quantified. Taxonomic and functional diversity was analyzed with a focus on exploring the relationships between the abundance of opportunistic pathogens, ARGs, and MRGs in relation to copper concentrations. Water copper concentrations were measured on a Perkin Elmer 8300 inductively coupled plasma optical emission spectrometer.

We hypothesized that: 1) the microbial beta and alpha diversity at lower copper levels will be more statistically similar to the pre-treatment diversity than to the diversity associated with higher levels of copper, 2) there will be a positive correlation between abundances of opportunistic pathogens, ARGs/MRGs, and the copper levels. This study will help establish potential risks associated with copper treatment due to changes in the functional and taxonomic diversity of the biofilm microbiome. Our findings will also inform project design of a more controlled, replicated study to further explore these risks. Understanding the risks associated with copper treatment will allow farmers to take appropriate action to mitigate the effects on fish health and survival.

ANALYZING SEAFOOD SALES, DISTRIBUTION, AND PREFERENCES IN THE NEW NORMAL - FOR MAINE AND BEYOND

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COVID-19 struck seafood producers with a quick and heavy blow. With the vast majority of seafood purchases, by value, essentially wiped off the table with restaurants closing, where were producers to turn? Many became innovative, creating and trying new sales and distribution methods to maintain cash flow and sell their product. Despite the arrival of COVID, their products kept growing, and they needed to be sold.

The Maine Aquaculture Association (MAA), in partnership with FocusMaine, NOAA, and Sea Grant, has interviewed a network of seafood distributors across Maine, New England, and the U.S., and reviewed news and academic articles to analyze the effects of COVID-19 on the seafood supply chain, and how producers reacted. MAA released a guide in the winter of 2021 on aquaculture sales and distribution methods in Maine, highlighting the benefits and drawbacks of both longstanding and new methods.

This will serve as the foundation for the NOAA-Sea Grant funded work in progress with Dr. Kanae Tokunaga, Dr. Caroline Noblet, Dr. Keith Evans, Sam Belknap, Keri Kaczor, Giselle Sillsby, and Allissa Miller-Gonzalez, which will explore, on a broader scale and in much greater detail, the new normal of seafood distribution in the U.S. and updated consumer preferences, using Maine as a case study for how to capitalize on seafood supply chain synergies and consumer tastes to expand Maine's aquaculture and seafood markets.

INCREASING U.S. AQUACULTURE WILL STRENGTHEN OUR ECONOMY, OUR HEALTH AND OUR ENVIRONMENT

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SEAFOOD FOR AMERICA'S ECONOMY – We can do more to strengthen rural America
The U.S. ranks 16th in production of farmed seafood, behind producers in Asia, Europe, South America, Canada and Africa, according to the United Nations' Food and Agriculture Organization (FAO).

The U.S. imports most of the seafood it consumes, and its seafood trade deficit is $14 billion[1] and growing. Half of the fish eaten in the United States come from farms – but not from American farms[2]. American aquaculture (both marine and freshwater) meets only 5-7% of U.S. demand for seafood.

SEAFOOD FOR AMERICA'S HEALTH – We can do more to boost consumption
The U.S. Department of Agriculture (USDA) recommends that Americans consume at least two servings of seafood per week for optimal health. Seafood is a nutrient-rich, relatively low-calorie protein food, and consumption can reduce heart disease risk by up to 36%. According to the USDA, Americans' average seafood intake is far *below* recommendations, with 80-90% not eating enough seafood. As of 2014, Americans are only eating 14.5 lbs. of seafood per year, per person equating to less than half the recommended 8-12 oz. per week.

SEAFOOD FOR AMERICA'S ENVIRONMENT – We can do more to protect our environment
Responsible aquaculture will play a crucial role in reducing impacts to our environment while supplying healthful protein to a growing global population. By 2050, global demand for animal protein may be 80% greater than it is now. Aquaculture is the most efficient means of animal protein production and has a far lower environmental impact than any terrestrial means of meat production.

SOLUTION: A vibrant U.S. aquaculture industry will create American jobs, complement production from wild fisheries, increase sustainable US sources of healthy protein, and reduce the seafood trade deficit. Unfortunately, domestic aquaculture development is constrained by numerous regulatory hurdles, including overlapping jurisdiction of federal, state, regional, county and municipal governments, and the absence of a predictable, affordable and efficient permitting process, particularly in marine environments.

WHAT CAN CONGRESS DO?
- Support legislation that enables and facilitates aquaculture development in federal waters;
- Support actions that clarify regulatory processes and increase interagency coordination to provide efficient, affordable and predictable permitting for aquaculture operations;
- Work closely with the U.S. seafood community, including harvesters, processors, distributors, retailers, and restaurants, to ensure that federal aquaculture legislation achieves the goal of increasing U.S. production of healthful, affordable and sustainable seafood for the benefit of ALL Americans.
  - http://www.nmfs.noaa.gov/aquaculture/aquaculture_in_us.html
  - http://www.nmfs.noaa.gov/aquaculture/faqs/faq_aq_101.html

THE USE OF ACCLIMATION CAGES AND VOLUNTEERS TO HELP RESTORE DEPLETED WHITE SEABASS IN SOUTHERN CALIFORNIA

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Hubbs-SeaWorld Research Institute, in partnership with the Coastal Conservation Association – California, operates a network of pre-release acclimation cages for a white seabass (*Atractoscion nobilis*) replenishment program in California. The acclimation cages are dispersed in embayments or nearshore in the southern California bight and most are operated by volunteers. Growout of seabass is conducted under the direction and authority of the California Department of Fish and Wildlife (DFW) as part of the Ocean Resources Enhancement and Hatchery Program. White seabass are spawned and reared in a marine fish hatchery using intensive recirculating systems for the first four months before they are transported to the volunteer cages. Mandatory DFW health inspections are conducted prior to their delivery and release from these cages. Fish are held in these acclimation cages for two weeks up to seven months in order reach a target size of 20 – 30 cm (8-12”) in length. All fish released from this program have an internal identification tag implanted in their cheek musculature. Fish are normally released at the cage site but some have occasionally been transported by boat to nearshore sites for release. We have demonstrated that these acclimation cages improve post-release survival by two-fold which has eliminated direct releases from the hatchery.

Volunteer growout cage operators are provided with a procedures manual that details their responsibilities including daily husbandry duties. A Growout Coordinator helps to manage pertinent activities among the various cages and serves as a conduit to DFW. Over the course of 30 years, stocking and operational practices have been customized for each facility based on a variety of factors including volunteer support levels, water depth, general water quality, proximity or activity of potential predators, and interactive effects with seasons. Here we report on contemporary practices and outcomes of these acclimation cages.

FIGURE 1. Volunteers stocking hatchery-reared juvenile white seabass into their growout cages.

GROWTH AND SURVIVAL OF LARVAL, JUVENILE, AND ADULT TRIPLOD OYSTERS IN LONG ISLAND WATERS

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Due to the significant growth benefits, triploid technology has become increasingly common in oyster aquaculture accounting for the vast majority of farm-raised oysters in many regions. Although triploids are very common in aquaculture, regions such as Long Island NY have been slower to adopt and implement the technology with anecdotal reports from baymen claiming that the triploids they have previously grown were "frail" or "ugly". The reason for such reports is unknown and may be due to the triploids used originating from distant locations and not optimized for local stressors, diminishing the benefits of the technology. To address this, 9 oyster lines were produced and evaluated, consisting of 3 purebred local diploid lines, 3 hybrid triploid lines (local lines crossed with NEH tetraploids), and 3 hybrid diploid lines (local lines crossed with NEH diploids). Larvae and juvenile resistance to experimental exposure to *Vibrio* pathogens was assessed. Field grow-out experiments were also conducted in 5 separate locations to compare seed growth and survival up to market size. When exposed to *Vibrio* pathogens, larval and juvenile diploids outperformed triploids by 53% and 17% respectively, with no significant difference based on parental genotype. In the field, overall triploid growth was 30% greater compared to diploids. No significant difference was observed in survival when comparing ploidy alone, however significant differences were observed between locations and maternal genotypes. Differences in ploidy survival appear to be most pronounced during early developmental stages but become more dependent on genotype-location interactions as oysters mature.

ENHANCING RESEARCH CAPACITIES FOR ONGOING AND FUTURE MARICULTURE RESEARCH AT THE WADDELL MARICULTURE CENTER IN BLUFFTON, SOUTH CAROLINA

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The South Carolina Department of Natural Resources (SCDNR) has a long history of mariculture research dating back to the early 1980's. Much of that research has focused on aquaculture species with promising commercial potential or of conservation concern, as well as innovative techniques and strategies determined to be practical and cost-effective. During this time, the Waddell Mariculture Center was developed and became the primary location for extensive larval production of important game fish species for the state's saltwater stock enhancement program. Recent and planned remodeling of Waddell's intensive and extensive infrastructure is also paving the way for strong continued stock enhancement and applied aquaculture research programs as well as an increased capacity to collaborate and address additional state and regional aquaculture research priorities.

Marine stock enhancement research in South Carolina has focused on multiple species including red drum (*Sciaenops ocellatus*), spotted seatrout (*Cynoscion nebulosus*), cobia (*Rachycentron canadum*), and striped bass (*Morone saxatilis*), with the most long-standing and current efforts focused on red drum and cobia production. Additionally, new efforts focused on southern flounder (*Paralichthys lethostigma*) culture for stock enhancement have begun. SCDNR staff have outlined an eight year plan to conduct aquaculture, fisheries, and genetic research to ensure a responsible management approach for restocking state waters and have received a substantial contribution of state funds to remodel extensive infrastructure at Waddell and provide yearly support for staff and research costs.

THE PHOENIX BACKYARD GARDEN PROGRAM: HARNESSING URBAN AQUACULTURE (AQUAPONICS) TO MITIGATE FOOD INSECURITY - DIVERSITY, EQUITY AND INCLUSION

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The 2021 city of Phoenix Arizona Backyard Garden Program is funding the installation and comparative study of backyard gardens using aquaponic plant and fish production, raised beds, and other water conserving growing methods as well as training in their use for 90 residents located in food deserts within 4 of its 15 administrative districts called Villages.

It is well documented that regions where the availability of quality food is low (food deserts/food swamps) are often found within underserved communities. This has led to dedicated efforts within Phoenix's planning for sustainable food systems to help mitigate these challenges. A continuation of this long-term effort, the focus of the Backyard Garden Program on food deserts is a unique opportunity to strengthen equity, diversity, and inclusion within the effort to support and expand urban agriculture within the city.

Focusing on the aquaponics component, this review describes some of the initial processes being employed to accomplish this task.

PHOENIX BACKYARD GARDEN PROGRAM: HARNESSING URBAN AQUACULTURE (AQUAPONICS) TO MITIGATE FOOD INSECURITY - IMPLEMENTATION

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The 2021 city of Phoenix Arizona Backyard Garden Program is funding the installation and comparative study of backyard gardens using aquaponic plant and fish production, raised beds, and other water conserving growing methods as well as training in their use for 90 residents located in food deserts within 4 of its 15 administrative districts called Villages.

The 5th largest city in the United States, Phoenix has a centuries old agricultural heritage. Aquaponics however, is a comparatively new method of growing. Thus the development of a local food system based on small-scale aquaponic plant and fish production will require the marshaling of existing often legacy resources, such as feed stores, along with the development of catalytically innovative social, technical and business solutions to create the support network for this farming effort. With a focus on the aquaponics component, this review describes some of the initial processes being employed to accomplish this task.

AQUAPONICS RESEARCH NEEDS IDENITIED BY STAKEHOLDERS AND PRELIMINARY THOUGHTS ON STANDARIZED RESEARCH APPROACHES

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The Aquaponics Association established an open line of communication with members designed to collect and publish research needs of producers. The Association maintains an interactive web site for producers to present and discuss issues, seek help from experienced producers and for companies to make producers aware of their products. Member research needs were solicited twice in the past year via the open forum. This will be an iterative process, conducted twice per year. Members research needs included nutrient databases, dietary inputs, microbial ecology, bacterial preservatives, complete understanding of artificial ecosystems (fungi, bacteria, viruses, etc.), Si/Ca/pH and shelf life of plants, species combinations, feed ingredients in diets, feeding rates and resulting flow of nutrients, comparison of inputs/outputs between aquaponics, hydroponics, and aeroponics, alternative water movement options, renewable energy and intermittent energy usage, and microbial inoculants. The list of needs is not currently prioritized, but can serve as a source of justification for research efforts and a means of connecting stakeholders and research groups. The identified needs encompass all three taxa in a typical aquaponic system; animal, bacteria and plants. However, aquaponic subsystems vary significantly and standardized research approaches appear limited in the short-term, other than on a broad scale.

Much of the variation in systems occurs in the plant subsystem and the approach used to move water and nutrients between subsystems. Effective, efficient grow beds range from deep-water cultures, to thin films, and from constant flows to flood and drain water/nutrient movement. The physical component of plant subsystems ranges from floating rafts to Dutch buckets. Standardization of the plant subsystems in the short-term appears unrealistic as much of this diversity is in response to physical support needs of plants raised in aquaponic systems. Areas that can be standardized include:
• Reporting of feed inputs, genetics/strains/varieties, water chemistry, and growth and production;
Ratios of subsystems;
• Disciplinary specific norms, such as those expected in nutrition, genetics, physiology, and disease research

EVALUATION OF THREE DEVICES TO INDUCE WATER FLOW AND THE EFFECT OF SCREEN MESH SIZE ON FLOW IN FLOATING RACEWAYS

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The rate at which water flows through a floating raceway is critical to its function. Flow rate supports water quality by transporting dissolved oxygen into the raceway and removing waste. Flow rate may vary with design of the water-moving device and the use of screens. Screens ensure fish retention, but also create resistance to flow.

Three Superior Raceway Model 11000 floating raceways were installed in a 0.8 ha pond. Three types of devices were tested: grid airlift (GAL), aerator motor with propeller (AMP), and slow rotational paddlewheel (SRP). Trial 1 was conducted to obtain flow rate and energy consumption for each device pushing water through the raceway without screens in place. Trial 2 was conducted to measure the influence of mesh size (% open) on flow rate for devices tested in trial 1.

Screens (1.2m x 1.8 m) were placed at the inlet and outlet of each raceway. Screen mesh was rated by the percentage of open area: 100% (no screen), 80.8%, 76.4%, 73.8%, and 68% (1/2 inch mesh). Regression analysis of screen mesh and flow rate revealed R^2 values of 0.94, 0.92 for GAL and AMP, respectively.

Trial 1 Water flow in Lpm (Mean ±SE)

Device	Watts	Mean Flow
GAL (1 hp)	1087	11,705 ±576
AMP (0.75 hp)	723	17,553 ±114
SRP	156	6,520 ± 87

Trial 2. Influence of mesh size (% open) on water flow in Lpm (Mean ±SE) for three devices inducing flow in a floating raceway (left).

% open	GAL	AMP
100%	15963 ±280	17,553 ±114
81%	12998 ±178	12,917 ±230
76%	12667 ±41	13,174 ±630
74%	11246 ±543	12,450 ±363
68%	9452 ±33	10,612 ±268

CHARACTERIZATION OF STRESS RESPONSE AND GUT MICROBIOTA IN CULTURED BURBOT *Lota lota maculosa* FOLLOWING FEEDING WITH PLANT-BASED DIETS

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Burbot (*Lota lota maculosa*) are a freshwater cod-like fish species that offer potential as a new commercial foodfish. These fish offer a desirable fillet, demonstrate favorable growth, and can be cultured under similar conditions to rainbow trout. Recently, researchers at the University of Idaho have evaluated dietary formulations for juvenile burbot culture and have found that burbot are tolerant of low-level soybean meal as a fishmeal replacement. In the current trial, an Atlantic cod marine-type diet (fishmeal-based control; REF) and two, 25% fishmeal-replaced diets with soybean meal (SBM) and dried distillers grains with solubles (DDGS) were evaluated. Immediately following a 72-day feeding period, a subset of burbot were subjected to both density and temperature stressors. A high-temperature treatment was set for a water temperature of 20ºC, and the high-density treatment was set at a biomass density of 0.1 kg/L. A control group (no stressors administered) was also included for comparison. At timepoint 0 and 10d post-stress initiation, burbot were sampled for baseline health metrics (n=5 per tank; blood for hematocrit/plasma, liver, and fecal material from the distal intestine). Plasma glucose and lactate analyses were completed via commercial colorimetric assays. In the temperature-stressed burbot, the DDGS group showed lower plasma glucose levels than the REF diet (P=0.007), while the density-stressed burbot fed DDGS displayed lower plasma glucose than both the REF (P=0.001) and SBM (P=0.015) groups. Plasma cortisol samples were analyzed via GC/MS with no differences found across dietary treatment groups (P=0.755), and the experimental stressors did not appear to influence this metric (P=0.501). Post-exposure fecal matter was collected to assess the impact of these rearing stressors on the intestinal microbiota. Microbiota richness, as measured by the number of observed amplicon sequence variants (ASV) was found to be influenced by stressor (P=0.021; decreasing trend with density treatment) but not by diet (P=0.736), and this same stress-related influence was detected for the Shannon (P=0.003) and Simpson (P=0.003) alpha diversity indices. For beta diversity, an unweighted UniFrac analysis also discerned no diet-related differences (P=0.561) but revealed differences in stressed and unstressed fish (P=0.030). *Streptococcus* spp. and *Chryseobacterium* spp. were both found to be differentially abundant in the stressed groups as compared to the non-stressed fish. A better understanding of the burbot stress response will allow for producers to optimize rearing conditions for temperature and stocking densities for this prospective species.

AQUACULTURE IN THE U.S. MIDWEST; ECONOMIC OPPORTUNITY FOR MISSOURI FARMERS?

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U.S. Midwestern farms lack the warm climate (degree-days) needed to compete with pond aquaculture prices. Furthermore, indoor recirculating aquaculture production costs cannot compete directly with commodity seafood prices. In most cases Midwestern farmers will need to produce in intensive/super-intensive, climate-controlled, zero-discharge, recirculating aquaculture systems (RAS). To be profitable, it is imperative to reduce capital and operating costs to a minimum. In an effort to define the most cost-effective indoor RAS, a series of commercial and self-constructed prototype aquaculture raceway systems were installed and evaluated at a private farm in Missouri. Tank construction materials included HDPE liners, polypropylene sheets, and PVC sheets within plywood containment, as opposed to, commercially available, self-supporting glass-coated steel-sheet systems and self-supporting concrete-filled prefabricated PVC forms. Advantages and disadvantages of the various systems are compared, as well as, construction and operational costs.

RAS production costs ranges between 200-600% over pond production cost and commodity fish and shellfish prices. However, RAS costs are 45-65% of typical retail seafood prices. Opportunity in aquaculture exists for Missouri farmers, however, growers will likely need to seek direct sales to consumers capturing profit margin of wholesale/retail chain. Growers will need to bear the holding, processing, advertising, transportation, packaging, time/labor costs needed to market and sell product. Potential aquaculture producers are advised to begin small and focus on markets before addressing technology and investment issues. Issues that should be addressed include, 1) Which marketable species? 2) What product to provide, whole or processed? 3) How much to charge? 4) How to promote/advertise and? 5) Where to sell?

Figure 1; Concreted-filled, prefabricated PVC forms holding 4,000 gallons within climate-controlled, zero-discharge, RAS prototype at a private farm in Missouri.

Figure 2; Farm-gate, break-even, wholesale and retail cost/prices for pond and RAS aquaculture products

TYPE/YIELD	Break-Even (whole)	Farm-gate (whole)	Wholesale (processed)	Retail (processed)
POND				
Catfish (0.32 lb fillet)	0.80-1.00/lb	0.85-1.25/lb	5.00-6.00/lb	8.00-11.00/lb
Shrimp (0.6 lb tails)	1.50-1.90/lb	2.00-3.00/lb	5.00-6.00/lb	5.00-12.00/lb
RAS				
Shrimp (0.6 lb tails)	4.00-8.25/lb			13.00-18.00/lb whole
Bass (0.32 lb fillet)	4.00-6.00/lb	5.00-6.00/lb	15.00-18.00/lb	20.00-28.00/lb

PADDLEWHEEL WATER MIXING IN SPLIT-PONDS USED FOR CATFISH PRODUCTION

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The Partitioned Aquaculture System (PAS) was developed at Clemson University from 1990 to 2009. The technique is one of using slow-moving paddlewheels to provide uniform mixing of fish-pond culture water while cultured fish are held within high density raceways. In this configuration, approximately 5% of system area is used for fish culture with the remaining 95% devoted to water treatment. To take advantage of the enhanced algal treatment capacity of the PAS for application to commercial catfish culture at reduced cost, a simplified version of the PAS entitled, the Split-Pond (SP) was installed at the Warm Water Aquaculture Center in Stoneville Mississippi. The Split-Pond makes use of existing catfish ponds, confining fish at lower densities than in PAS raceway (~20% of total system area). This study was undertaken to compare mixing effectiveness of paddlewheels vs culvert pumps in SPs with, and without, internal levees dividing water flow within the waste treatment zone. Dissolved oxygen and temperature measurements were taken one-ft below the water surface and one-ft above pond bottom using a YSI oxygen meter, suspended from a boat positioned at 30 x 40 ft increments across the five-acre waste treatment zone. Preliminary analysis indicates that SPs containing levees, configured with culvert pumps providing 8,000-gpm water flow, impart mixed conditions to approximately 33% of the volume of the treatment zone, whereas SP treatment zones without levees, configured with paddlewheels providing 10,000 to 15,000-gpm flow, was seen to provide mixing to approximately 50% of the treatment zone water volume.

This study suggests; 1) Widespread split-pond application offer potential for significant expansion of aquaculture production with reduced environmental impact, 2) Split-pond installation cost averages 50% of PAS cost capturing ~ 85% of the production benefit, 3) Performance of split-ponds is dependent on degree of water mixing, 4) Water discharge rates of 15,000 to 20,000 gpm in five-acre split-ponds treatment zones will likely be needed to ensure sufficient mixing to fully utilize pond photosynthetic capacity supporting maximum catfish production.

Figure 1. Seven-Acre Split-Pond without Central Levee in Five-Acre Waste Treatment Zone, Using Paddlewheel to Deliver 10,000 to 15,000 GPM Water Flow.

EDUCATING BEGINNING AQUACULTURE PRODUCERS THROUGH DIFFERENT EDUCATIONAL FORMATS

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Beginning small scale aquaculture producers in the state of Kentucky need up-to-date information concerning production and marketing. Pond-based aquaculture can be an expensive enterprise, specifically in Kentucky where pond construction costs can be three times as expensive as other states due to widespread karst systems and rocky soil conditions. Indoor fish farming is also very capital intensive with most enterprises requiring insulated building with road access, drainage, lighting, and temperature control. These high costs of entry and high levels of risk mean that it is very important for beginning aquaculture producers to have the latest information in order support their enterprise. Although in-person educational programs have been the standard for most extension programs, the pandemic has made alternative educational formats such as online workshops necessary for reaching clientele.

This project looks at what types of educational formats our beginning aquaculture producers prefer for receiving information. A survey of beginning producers in the state was conducted, focusing on the different venues and formats that extension personnel could use to share information with their clientele. Although in-person trainings were preferred by the majority of the respondents, there was a strong indication that online meetings and materials are an important part of educating beginning producers.

PREY SELECTIVITY, EFFECT OF LIGHT INTENSITY ON GROWTH AND SURVIVAL, AND DIEL FEEDING PATTERNS OF REARED YELLOWFIN TUNA *Thunnus albacares* LARVAE

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Extensive research on the early life history of tropical tunas has been developed by the IATTC at the Achotines Laboratory in the Republic of Panama over 30 years. Larval feeding research encompassing prey selectivity, effect of different light levels on growth and survival, and analysis of diel feeding patterns of reared yellowfin tuna (YFT) larvae are described in the present study. Two experiments were conducted providing mixed prey assemblages at different concentrations during the early larval stages to analyze prey selectivity and the onset of piscivory in YFT larvae. Analyses on prey concentration, prey width, larval mouth width and larval growth were conducted. Pearre's C index was calculated to describe selection of major prey items by YFT larvae during development. Results suggest YFT larvae are selective feeders, having different preferences in accordance with size and food concentration, and the early onset of piscivory could be observed and described.

The effect on growth and survival of YFT larvae was analyzed under three different light intensities (low, medium, and high). Light intensity treatments were replicated in multiple tanks, and larval growth, survival and gut content were examined during the first 10 days of feeding. Survival rates were higher in the high light level treatment. Prey consumption during initial days of feeding was highest under the high and medium light levels. However, in the last days of the experiment, the differences in prey consumption among treatments were not statistically significant. These results are consistent with the visual developmental characteristics of YFT larvae.

Lastly, two experiments were conducted to analyze the diel feeding patterns in YFT larvae at first feeding, flexion, and post-flexion stage. The mean number of prey per larval gut and percentage of empty guts were analyzed under diel cycles during the three developmental phases of the larvae. Results suggest that YFT larvae are diurnal feeders but with the ability to hunt and feed in relatively low light conditions.

Feeding habits in the early life stages are a crucial aspect of the feeding ecology and larval nutrition, and a critical process for the early survival of YFT tuna larvae. Hence, information on food habits, preferences, and feeding responses for different environmental cues are an important aspect of the research conducted on early life history of YFT larvae.

DNA DOUBLE-STRAND BREAK REPAIR MACHINERY IN *Penaeus vannamei:* A FOCUS ON THE NON-HOMOLOGOUS END-JOINING PATHWAY

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DNA double-strand breaks (DSBs) are repaired throughout three major pathways that require a specific set of proteins: Non-Homologous End-Joining (NHEJ), Microhomology-Mediated End-Joining (MMEJ), and Homology-Directed Repair (HDR). Such pathways and their proteins have been studied in model organisms, including the fruit fly. Nevertheless, little is known about the DNA repair pathways of DSBs in Crustacea and much less in the penaeids shrimps (Crustacea: Decapoda: Penaeidae). From the joining of oligonucleotides and midgut gland (hepatopancreas) extracts, it has been shown that HDR and MMEJ but not NHEJ occur in Penaeus monodon (Penaeidae).

To know if the NHEJ pathway could come about in *Penaeus vannamei*, we searched the NHEJ-related proteins in transcriptome and proteome databases of *P. vannamei* and other Decapoda species. Expression of NHEJ-related proteins Ku70, Ku80, DNA-PKcs, and DNA ligase 4 (as well as HDR- and MMEJ-related proteins) was assessed in *P. vannamei* gills, midgut gland, hemocytes, and muscle throughout semi-quantitative RT-PCR. DSB repair proteins were found to be expressed in the four tissues, particularly in the gills and midgut gland. Among DSB repair proteins, those related to NHEJ pathway were the most abundant in gills. As far as we know, this is the first report on DSB repair proteins for a decapod. Together, proteomic, transcriptomic, and expression data suggest that the NHEJ pathway occurs in *P. vannamei* and other decapods. The information presented here is relevant for ecotoxicology studies and the designing of gene edition strategies, which have not been developed in *P. vannamei*.

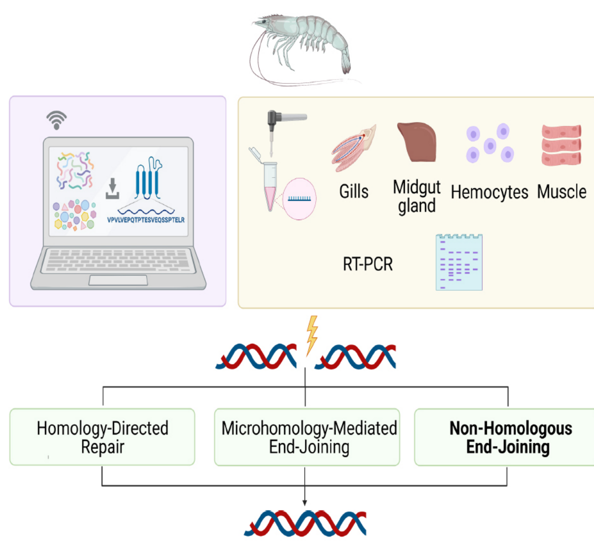

Figure 1. Interrogation of the presence of DSB repair proteins in *P. vannamei*, revission of transcriptomes and proteomes of *P. vannamei* and other crustaceans, and bioinformatic approach complemented by the evaluation of mRNA expression of DSB repair proteins in the midgut gland, gills, hemocytes, and muscle tissues.

FORMULATED ATLANTIC BLUEFIN TUNA DIETS: ADDING SUSTAINABILITY AND PERMANCENCE TO THE GLOBAL TUNA FARMING INDUSTRY

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The tuna ranching industry is a global, high-value aquaculture activity. Current feeding practices are impractical, unsustainable and pose serious ecological risks. The daily feeding of copious quantities of fresh/frozen fish results in extremely high feed conversion rates (~28 : 1). Seasonal availability and quality variation plus the lack of locally sourced baitfish highlight the urgent need for a balanced feed. Objectives for the present experiment were to compare performance and quality indicators of Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) fed herring/mackerel or a compound tuna feed based on sustainable American soy.

A thirteen-week feeding demonstration was conducted in two oceanic net pens moored east of San Pedro del Pinatar, Murcia, Spain (37°49.835' N 000°39.822' W). Each cage held ~45 fish, weighing approximately 99.5 kg (average initial individual weight, evaluated both by expert visual ranking and corroborated by the AQ1's AM 100 fish sizing system). Feed consumption and condition factor were recorded. At harvest, dorsal loins were collected and color, mercury, proximate composition, scombrotoxin levels, oxidative stability index and peroxide values were evaluated in the resulting tuna steaks. In addition, a blind sensory evaluation and a commercial taste assessment were performed on the main sashimi cuts obtained from fish fed either diet.

Steak samples from formula-fed fish had similar lipid contents as those fed baitfish but exhibited improved flesh color, texture and had increased oxidative stability and reduced histamine. Results from sensory and organoleptic evaluations (professional sashimi chefs) indicated that sashimi slices from formula fed fish were similar in flavor but more stable on the counter and superior in color. Importantly, there was a significant reduction in tissue mercury in formula-fed fish as compared to baitfish fed ABFT.

In addition, the formulated diet offered feed management options that are quite compatible with the present operation and equipment available at commercial tuna farms in the Mediterranean Sea. A preliminary economic evaluation (2021 prices) indicated that tuna feed is significantly more cost-effective than imported frozen baitfish. Given increased fishing quotas for ABFT and the severe difficulties in sourcing baitfish experience by Mediterranean tuna farmers, the formulated diet may offer a viable alternative to baitfish feeding. In conclusion, these results indicate that the balanced feed provided suitable nutrition for adult ABFT, enhanced the quality and shelf life of the end product and may enable management strategies to optimize tuna performance, thus increasing farm efficiency and reducing the environmental impact of commercial tuna ranching. This research was supported in part by the United States Soybean Export Council.

SUSTAINABLE BLUEFIN TUNA: AN IDEAL CANDIDATE SPECIES FOR OFFSHORE AQUACULTURE IN THE UNITED STATES. RECENT ADVANCES IN LARVICULTURE

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Most tuna farming operations rely on wild-captured fish for the stocking of net pens and utilize baitfish to feed tuna. The wild Pacific bluefin tuna population has declined due to excessive fishing pressure. San Diego, CA, once was known as the “Tuna Capital of the World”. Following the first tuna cannery startup, the city became a world leader in tuna-related commerce. By the 1960s, San Diego’s third-largest economic enterprise was tuna, superseded only by the U.S. Navy and aerospace, employing over 40,000 San Diegans but now this industry has all but disappeared from Southern California.

Today the sustainable production of farmed bluefin tuna is becoming a reality due to the remarkable advances in hatchery and feeding technologies in recent years. Here we share the recent success on tuna larviculture research and rearing of advance juveniles in RAS systems in San Diego. Fertile bluefin tuna eggs were sourced from collaborators in the Mediterranean Sea. Eggs arrived at the Ichthus Unlimited (IU) hatchery ~30 hours after the spawning (84% survival rate). Water parameters in the transport container were 19°C, 37 ppt and 170% saturation for water temperature, salinity, and dissolved oxygen, respectively. After acclimation to hatchery conditions eggs were stocked at a density of 18 eggs/L in 10, 500-L round tanks with a flow-through system. Flow rates were progressively increased to 0.5 L/min.

Yolk sac absorption was completed 2 days post hatch (dph). At this point the tuna larvae opened the mouth gape and exhibited predatory behavior. Enriched rotifers (*Brachionus plicatilis*, 10/mL) were the only source of exogenous feeding until 14 dph. At 14 dph, *Artemia salina* nauplii (0.5/mL) were provided in addition to rotifers. From 17 to 25 dph bluefin tuna larvae received and *ad libitum* provision of newly hatched red drum (*Sciaenops ocellatus*). Tuna larvae cannibalism was reduced by maintaining a suitable amount of prey at all times combined with frequent sorting-grading. Illumination regimes ranged from continual illumination to natural photoperiod starting 25 dph. Tuna larvae was weaned onto an artificial diet on 25 dph and metamorphosis was completed. At this point fish were transferred into a recirculation system. Throughout the rearing period, average environmental parameters were maintained at 25.6°C, 160% and 33.2 ppt for water temperature, dissolved oxygen saturation and salinity.

The survival rate of bluefin tuna juvenile >200 dph was relatively low at 3% rate and was due in part to an exceedingly low transport temperature which resulted in operculum malformations. Sourcing eggs near the hatchery should significantly increase survival rates. This research was funded in part by the Foundation for Food & Agriculture Research (American Aquaculture Program) and the Illinois Soybean Association.

EFFECT OF MUSSEL DENSITY AND FOOD AVAILABILITY ON SEX RATIO OF *Mytella charruana*

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Mytella charruana is a native mussel species of South and Central America and is currently an invasive species to the coastline of Florida, Georgia, and South Carolina. *M. charruana* has been introduced in the St. John River estuary in Jacksonville, Florida, and other areas by the release of ballast water from cargo and cruise ships. A study done in 2007-2008 in our laboratory showed that *M. charruana* had the potential of displacing local species, including oysters and native mussels, posing both an ecological and economical threat to Florida, Georgia and South Carolina including maintenance of biodiversity of coastal ecosystems, commercial and recreational fishing, eco-tourism, power plants functionality by clogging seawater intakes pipes. In fact, *M. charruana* showed an opportunistic type of gametogenesis, a pelagic larval phase and, for the most part of the year, a female biased sex ratio in the non-native environment. Moreover, the adult individuals had the ability to switch sex in response to food availability. In the condition of starvation, most of the individuals changed sex from female to male, possibly as an energy saving mechanism, since spermatogenesis requires less energy than oogenesis. Another possibility is that the sex reversal, of part of the population to male, is a mechanism to reduce the next generation population size when food resources are limited.

In this study we tested the effect of mussel density on population sex ratio since density can have a direct effect on food availability. Mussels were collected in the St. John River estuary in Jacksonville, Florida, and maintained in aquarium tanks. We tested a density of 25 mussels per 19 L and 50 per 19 L, in conditions of optimal feeding or starvation.

Contrary to what observed in 2007-2008, the population sex ratios at the collection sites were not significantly different from 1:1 throughout the year. The experimental trials showed that the sex ratio was not significantly different from 1:1 in fed conditions at either mussel density. Instead, a male biased sex ratio was observed in the starvation treatment at the lower mussel density, but not at the higher.

Our results suggests that, in conditions of limited food availability, mussel density might be a factor influencing *M. charruana* sex ratio. More studies will be needed to understand why, contrary to what expected, the lower density, and not the higher, triggered sex reversal to male. Moreover, the sex ratio at the collection sites was not significantly different from 1:1, similarly to what observed for most marine mussel species, including *M. charruana* in its native environment. The fact that in 2007-2008 *M. charruana* sex ratio at other collection sites, in Florida and Georgia, was widely variable throughout the year suggests that there might be other factors involved. For example, water quality, which can influence the physiology of the mussels.

DEVELOPMENT OF AN 'EARLY WARNING SYSTEM' FOR THE OSTREID HERPESVIRUS 1 ON THE US WEST COAST

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One of the most significant disease threats to Pacific oyster (*Crassostrea gigas*) culture are pathogenic variants of the Ostreid herpesvirus 1 (OsHV-1). In the 1990's, losses of larval and juvenile oysters caused by OsHV-1 were first described in France, New Zealand, and the US; in the US OsHV-1 has remained in a narrow range in northern California, primarily affecting production of Pacific oysters in Tomales Bay. Since 2008, an economically devastating increase in *C. gigas* mortality has been associated with genetic variants of OsHV-1, the OsHV-1 µvars causing losses in Europe, Australia, New Zealand, and Asia. A novel OsHV-1 microvariant was detected in the US in San Diego Bay, California (OsHV-1 µvar SD) in 2018. Recognizing the risk of regional spread and the commercial impact of the OsHV-1 a multi-state oyster sentinel program, with strong industry collaboration, was initiated in 2020 as an 'early warning system' to monitor the prevalence and pathogenesis OsHV-1 in juvenile Pacific oysters planted at commercial growing grounds in California, Oregon, and Washington. In 2020 and 2021, two sentinel families were created, a hybrid cross ("YxP") previously shown to be susceptible to OsHV-1 microvariants, and a second cross anticipated to be high-yielding and expected to be less tolerant to OsHV-1 infection based on pedigree data ("29.001" in 2020 and "30.001" in 2021). Spat were planted out by industry partners in San Diego Bay (CA), Tomales Bay (CA), Tillamook Bay (OR), Willapa Bay (WA), and Totten Inlet (WA). Industry partners counted spat every two weeks and sent samples to us for analyses, including OsHV-1 presence and viral load. In 2020 sentinel spat at sites in Oregon and Washington demonstrated high survival and tested negative for OsHV-1. Sentinel spat from both crosses planted in San Diego Bay experienced nearly 100% mortality over a four-week period and contained high OsHV-1 viral loads; sequence analysis indicated the genotype to be the OsHV-1 µvar SD. In Tomales Bay during the 2020 field season, each replicate bag typically displayed a single viral load peak. The magnitude of the peak within each replicate bag was correlated with both overall survival at the end of the field season and average shell length at the time of peak viral load. Genotype also affected overall survival, with 29.001 (bred for high survival in Tomales Bay) showing greater tolerance to OsHV-1 compared to the YxP (non-selected control cross). For select samples, 16S microbial community analysis is being used to explore the relationship between oyster survival, OsHV-1 and shifts in microbial communities. Differences between the 2020 and 2021 (currently ongoing) field seasons will be discussed.

DOUBLE-CRESTED CORMORANT *Nannopterum auritum* USE OF CATFISH AQUACULTURE PONDS IN MISSISSIPPI

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Commercial production of catfish (*Ictalurus* spp.) is the largest aquaculture industry in the nation, with most production occurring in Mississippi. Human-wildlife conflict in Mississippi is a contentious issue between producers and fish-eating birds through the consumption of cultured fish. Most notable of these birds is the Double-crested Cormorant (*Nannopterum auritum;* hereafter, cormorant), which can cost producers millions of dollars annually.

Our research focuses on answering various questions related to cormorant behavior relative to catfish aquaculture in Mississippi. We examined historic versus current cormorant activity, pond and fish size preference, cormorant use of catfish ponds versus natural water bodies, roosting behavior, and the influence of regulatory policy on cormorant use of catfish ponds. We used cormorant roosting data and pond survey data from multiple sources collected over the last 20 years to address these questions. Information gained from this research will help reduce cormorant impact on the aquaculture industry and offers insight into cormorant foraging ecology.

Aquaculture production in Mississippi peaked in the early 2000s, but cormorant density on aquaculture ponds has not changed since that time even though aquaculture area has declined by 70% (Figure 1). Similarly, roost counts today are approximately one third of those 20 years ago. We also found cormorants prefer ponds located farther away from trees and farm workshops, larger ponds, and ponds nearer the edge of pond clusters. Specific pond contents influenced cormorant preference, including fish species cultured, pond systems, and fish types. Cormorants favored aquaculture over natural water bodies later in the winter season coinciding with spring migration, indicating a switch toward catfish in preparation for their migration north. Likewise, cormorants showed increased use of roosts with more surrounding aquaculture later in the winter season. Lastly, we found cormorant use of aquaculture relative to natural water bodies was greatest when lethal control was suspended and least when it was allowed, suggesting lethal measures provided by regulatory policies were an effective means of altering cormorant distribution, thereby reducing damage at aquaculture facilities.

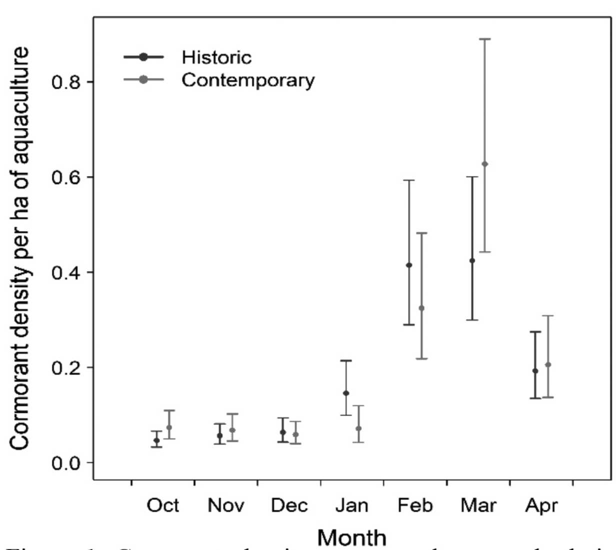

Figure 1. Cormorant density on aquaculture ponds during historic (2001–2004) and contemporary (2015–2018) periods.

SEAWEED FARMING AND CARBON SEQUESTRATION AS A TOOL FOR REDUCING CLIMATE CHANGE IMPACTS: DESIRE OR FACT?

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Seaweed stands create some of the most productive habitats in the world, but their role carbon sequestration and the potential of seaweed farming activities for climate change impacts mitigation in the oceans are not well understood. It has been proven that seaweed farming at large scales can extract nitrogen coastal areas reducing eutrophication in coastal areas. However, carbon pathways are much more complex in the ocean to understand their potential role as a mitigation tool by sequestering carbon. By harvesting the biomass, the transport and process of the biomass will produce again CO_2. Also, seaweeds are not trees and they will not meet the criteria for sequestering carbon for 100s of years. In addition, seaweed produce large quantities of dissolved and particulate organic matter and how matter move with the currents are complex as they may not always allow this matter to reach high depths to be considered sequestered. Finally pieces and whole plants get detached by storms and this biomass will move also into different directions. Whether the amount of Carbon sequestered is higher than the biomass decomposed in the surface producing again CO_2, still requires a deeper understanding. This study analyses known and unknows of the potential mitigation of large-scale seaweed farming on climate change and discuss present limitations. Funding: ANID (CeBiB, FB-0001).

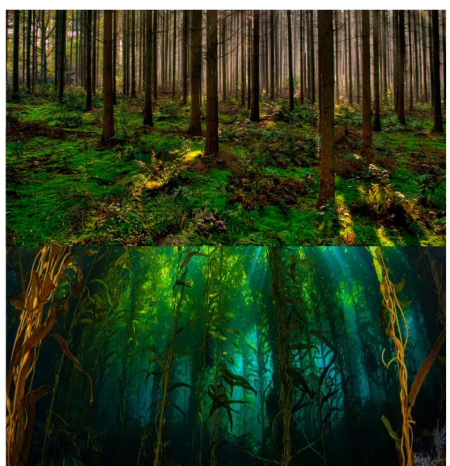

Figure. Kelp and terrestrial forests are not equal from a carbon sequestration viewpoint.

EFFECT OF ENVIRONMENTAL HISTORY ON *Macrocystis pyrifera* RESPONSES TO OCEAN ACIDIFICATION AND WARMING: A PHYSIOLOGICAL AND MOLECULAR APPROACH

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The capacity of seaweeds to adapt and/or acclimate to climate change might differ among distinct populations, depending on their local environmental history and phenotypic plasticity. Kelp forests create some of the most productive habitats in the world, but globally, many populations have been negatively impacted by multiple anthropogenic stressors, including ocean warming (OW). However, it has also been shown that local drivers such as nitrogen (N) availability might modulates their responses to global stressors by enhancing their thermal tolerance. Here, we compare the physiological and molecular responses to ocean acidification (OA) and OW of two populations of the giant kelp *Macrocystis pyrifera* from distinct regimes of CO_2, pH, temperature and N availability (weak vs strong upwelling). We found that juveniles *Macrocystis* sporophyte responses to OW and OA did not differ among populations: elevated temperature reduced growth while OA had no effect on growth and photosynthesis. However, we observed higher growth rates and NO_3^- assimilation, and enhanced expression of metabolic-genes involved in the NO_3^- and CO_2 assimilation in individuals exposed to strong coastal upwelling. Our results suggest that despite no inter-population differences in response to OA and OW, intrinsic differences among populations might be related to their local natural environmental variability driven by coastal upwelling. Financial support: FONDECYT 3170225, CeBiB FB 000-1 (ANID).

THE REGIONAL SHELLFISH SEED BIOSECURITY PROGRAM (RSSBP)

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Shellfish diseases are a concern for shellfish farmers, fishers and resource managers worldwide with many examples of devastating outbreaks and chronic impacts on growth and survival. At the center of the rapidly expanding shellfish aquaculture footprint along the U.S. East Coast is the hatchery production of seed. Interstate seed transfers, crucial for industry growth, are regulated by individual states that typically require testing individual batches of seed for biosecurity. Batch testing expensive, time consuming, and often unnecessarily impedes commerce because shellfish diseases are not contained by nor distributed along state boundaries. All agree that biosecurity is vital, but we can be smarter and more efficient while improving the biosecurity of seed transfers.

The Regional Shellfish Seed Biosecurity Program (RSSBP) was created collaboratively among representatives of the shellfish aquaculture industry, shellfish scientists and pathologists, state regulators and extension personnel. The goal is to use the best available science to minimize risks associated with interstate transfers of bivalve shellfish seed. The RSSBP is comprised of three components accessible via a web portal (www.rssbp.org):

1) A database that aggregates data on shellfish pathogens of concern along the Atlantic and Gulf Coasts of the United States.
2) A set of best management practices (BMPs) for shellfish hatchery biosecurity that includes a system to audit hatcheries and verify compliance for those that choose to participate to reduce batch certification requirements.
3) An Advisory Council representing industry, regulators, researchers and extension personnel with a Pathology Working Group to provide technical guidance and oversight.

Details of the RSSBP are presented on our poster. We invite you to explore and query the database at the website listed above for your own purposes and consider providing feedback for improvements or, if applicable, provide data to increase the spatial and temporal coverage of the database. In winter 2020 and 2021, we piloted a Hatchery Compliance Program as part of the RSSBP and the states of Rhode Island, Massachusetts, Maryland, Virginia, North Carolina and South Carolina have already considered these audits and/or the program in requests for shellfish seed importation.

RSSBP
Regional Shellfish Seed
Biosecurity Program

EXAMINING THE EFFECT OF PHOTOPERIOD ON THE CONDITION INDEX OF *Crassostrea virginica* IN A HATCHERY SETTING

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The eastern oyster (*Crassostrea virginica*) is one of the primary shellfish species cultivated on the eastern coast of the United States. In addition to human consumption, the demand for eastern oysters is on the rise for environmental conservation and habitat restoration projects. To meet this growing demand oyster hatcheries have become more common and efforts are being made to maximize the number of juvenile oysters that can be produced. Manipulating temperature is the most common way that hatcheries are able to produce ripe broodstock for spawning outside of the natural season in an area. Some studies have looked at the combined effects of temperature and photoperiod. However, the individual effects of photoperiod on oyster condition have not been investigated.

This study will compare the effect of different light treatments on condition index in adult oysters. Adult oysters will divided randomly into three groups and held under different light:dark periods: 8:16, 16:8, and a progression from 8:16 to 16:8 hours. All oysters will be maintained in the same conditions of water quality and daily diet ration. Temperature will be maintained at 16°C for the duration of the experiment. The amount of feces produced, as a proxy for feeding activity, will be measured periodically. At multiple time points, oysters from each of the treatments will be sacrificed and their condition index will be analyzed. This poster will discuss the data from the experiments.

PERFORMANCE OF CHANNEL CATFISH *Ictalurus punctatus* FED PLANT-BASED DIETS WITH DIFFERENT CONCENTRATIONS OF INORGANIC IRON (FESO4) OR ORGANIC IRON

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The US catfish industry has been affected by catfish anemia causing massive mortalities and losses to farmers. Without any specific data farmers are using high iron diets (fortified with inorganic iron) to mitigate the problem. However, there is no proof that the high iron diets are mitigating the problem. This study sought to evaluate the current industry practice of using high iron diets to prevent or reverse anemia of unknown causes, and to compare the effects of using inorganic and organic forms of iron in catfish diets. Five plant-based diets (industry standard) supplemented with different concentrations of either FeSO4 or iron methionine (FeM) (i.e., 0, 125, 250 mg Fe/kg) were formulated and extruded as floating pellets for a feeding trial.

The trial was conducted with catfish fingerlings (6.1±0.2 g grams initially). Fifteen fish were stocked in 3 replicate 240-L tanks per diet in a recirculating system. Fish were fed twice daily to satiation for 10 weeks. Weight gain, FCR, Hepatosomatic index, and survival did not differ among diets. The total iron concentration in the blood and intestine were also similar among diets (Table 1). Total liver iron concentration was significantly higher in fish fed diets supplemented with 250 mg Fe/kg in both iron forms than in those fed diets with less iron. Histological analysis of the liver showed that diets supplemented with 250 mg Fe/kg in both iron forms caused significant liver damage compared to the other diet groups. No obvious signs of anemia were observed in fish fed the basal diet. Results indicate that high-iron diets can produce liver damage in catfish fingerlings without reducing growth performance. Other results will be presented at the meeting.

Table 1. Total Iron Concentration (mg/g dry weight) in Channel Catfish Liver, Blood and Intestine.

Diet ID	Liver iron (mg/g dry wt)	Blood iron (mg/g dry wt)	Intestine iron (mg/g dry wt)
250 FeM	0.13[a]	0.011[a]	0.004[a]
250 $FeSO^4$	0.13[a]	0.015[a]	0.137[a]
125 FeM	0.05[b]	0.011[a]	0.003[a]
125 $FeSO^4$	0.04[b]	0.008[a]	0.005[a]
Basal	0.03[b]	0.008[a]	0.001[a]

IMMUNOPROTECTION OF NILE TILAPIA (*Oreochromis niloticus*) AGAINST *Edwardsiella tarda* INFECTION, UGANDA

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Edwardsiella tarda is a gram negative bacterium belonging to the family Enterobacteriaceae, it is the causative agent of edwardsiellosis a disease which is among the major constraints in aquaculture worldwide. It causes mass mortalities of fish that results into high economic losses in both aquaculture and fisheries.

This study aimed at determining the immunoprotection of Nile tilapia against *E. tarda*. A total of 50 fish in five groups were used to determine the LD_{50}, fish were exposed to *E. tarda* intraperitoneally by injection with dilutions $1.5x10^6$ to $1.5x10^9$.

In the second phase, two groups of 20 fish each were vaccinated with formalin-killed *E. tarda* and phosphate buffered saline for control; a booster dose was given two weeks after the first dose. Blood was collected weekly from six fish in each group for serum to determine antibody titer by agglutination in microtiter plates. Two weeks after the booster dose, all fish were challenged with 100μl of 10^8 CFU/ml *E .tarda* (LD_{50}). Fish were monitored for 4 weeks; dead fish were recorded, examined for clinical signs and pathological changes. Bacteriology was done to confirm the presence of the pathogen in freshly dead or moribund fish. Bacterial load in the liver kidney and spleen was determined by drop plate counting from 10-fold serial dilutions of homogenized tissues. LD_{50} of $1.6x10^8$ was determined in this study.

Infected Fish showed signs of skin and fin hemorrhages, ulcers, depigmentation, exophthalmia, erosion and distended abdomen externally. Grayish nodules in the spleen, kidney, congested internal organs, fluid filled intestines, black spots in the liver, mottled liver were observed internally. The lesions were more severe in the non-vaccinated groups. There was high bacterial load in the kidneys than in the spleens and livers. All the sampled dead fish were *E. tarda* positive which was confirmed using API 20E kits. Significantly high antibody titers were found in vaccinated fish and the Relative Percentage Survival was 32.4% indicating relative protection. No significant difference in percentage mortalities was found between groups ($p>0.05$), there was high bacterial load in the kidney than in the liver and spleen and the bacterial load in non-vaccinated fish was highly significant than in vaccinated fish ($p<0.05$). The antibody titers in the vaccinated fish were highly significant than in non-vaccinated ($p<0.05$). Results indicate that formalin-killed cells enhance production of specific antibodies, induce specific immunity and can confer protection to the fish. These results can be used as a baseline for vaccine development after a series of studies on different age groups of fish and doses of different formulations of vaccines under optimized conditions.

"TALKING STORY: HOW HAWAI'I'S MODERN AQUACULTURISTS DISCOVERED A SOLUTION TO THE FUTURE IN THEIR PAST," THE POWER OF STORYTELLING

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Hawaiian fishpond aquaculture is the oldest form of aquaculture on the Pacific Rim. Ancient Hawaiians built a kuapā (rock wall) to encircle the estuary where a freshwater stream meets the ocean. Freshwater lured pua (baby fish), and a mākāhā (sluice gate) allowed them to swim into the pond. They grew fat within the pond: in a few weeks, they couldn't swim out. The existence of more than 480 loko iʻa (loko=pond & iʻa=fish) have been documented.

Fishponds are also cultural icons. In the wake of Western colonization, fishpond numbers have declined, and the remaining few have degraded structurally. Loko iʻa were engineered based on keen observations of earth's natural processes. To operate them, kiaʻi loko (fishpond caretakers) had to intimately know the pond and its organisms. Managing fishponds was an art and a science, and fishponds themselves are culturally and ecologically important. They grow culturally important organisms; they are places where people discover and define themselves. In modern times they are a potential means to restore Hawaiʻi's nearshore ecosystems, re-establish food sovereignty, and develop economic self-sufficiency. A cohort of modern Hawaiian aquaculturists are rediscovering the art of tending loko iʻa. Accompanying are a host of challenges to restoring the ponds and growing fish.

The intent of this project is to reach a local and broad audience: demonstrate the resilience and dedication of the fishpond community, highlight the power of their work, and celebrate Hawaiian culture. Education and effective communication are important tools to gain recognition, garner support, and improve public perceptions. On-site research was done as interviews and informal get-togethers with more than forty members of the loko iʻa community. This research illuminated the debates on the nuances of loko iʻa caretaking, management philosophies, and the diversity of opinions on aquaculture, conservation science, and coastal restoration. The details from the collective interviews will be presented with respect to cultural sensitivities and how modern aquaculture and fishery management can contribute to the restoration of production in loko iʻa.

The University of Hawaiʻi at Mānoa's Center for Oral History retains archived transcripts of interviews. A project blog documents the details of this project. A blog post for the Yale Program on Climate Change Communication (YPCCC) analyzes how climate change is affecting fishponds and impacting their management. The project is reviewed in blog posts for the National Humanities Alliance and the Yale Sustainable Food Program. ECO Magazine and Oceanographic Magazine have published articles from this research. The story of fishponds, their caretakers, and their environment is reaching an expanded audience to better understand the complexities and significance of this work.

UTILIZING REFURBISHED RECIRCULATING AQUACULTURE SYSTEMS (RAS) TO OPTIMIZE SHELLFISH PRODUCTION

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Hatcheries traditionally rely on static culture for shellfish production. This method consists of holding larvae in batch tanks that are drained out two to four times a week. Though a reliable method, static culture is labor intensive, using a lot of resources such as algae and treated ambient water. Additionally, when water quality declines, facilities using batch culture are unable to efficiently reuse water, causing production to stall. As the demand for shellfish production continues to increase, innovative methods for culturing are necessary to support the industry and increase its resilience to environmental disturbances. With its efficient use of space and resources as well as buffering capacity against environmental variation, there is increasing interest in using RAS as a method of shellfish larval production.

A RAS is defined by a closed loop system where water is continuously treated and recycled for an extended period of time without water exchange. In a typical RAS, water from the culture tanks gets directed through a mechanical filter, UV sterilizer, foam fractionator, and biofilter before being directed back to the culture tanks. This method of shellfish culture has been demonstrated experimentally, though is yet to be adopted on a commercial scale.

The Shellfish Aquaculture Innovation Laboratory (SAIL) at the University of Maryland Center for Environmental Science's Horn Point Laboratory recently refurbished three RAS that were previously used for finfish culture. Refurbishments included adding appropriate mechanical filters, biofilters, and charcoal filters. We then assessed the growth and survival of Eastern oyster *Crassostrea virginica* larvae in the RAS in comparison to static culture. Numerous trials were conducted in both 2020 and 2021. In early trials, larvae were clear and would not grow in the RAS, suggesting starvation. Fluorometry assays revealed algae was quickly being removed by RAS filters and limiting larval access to algae. Food availability was subsequently improved by reducing the use of filters from 24/7 down to 2 hours every 5 days. Additional stressors considered were the effects of constant flow on developing larvae. This was assessed by culturing larvae within the RAS under static, constant high-flow, constant-low flow, and semi-continuous treatments. The results from these and other assays will be discussed. The assays at SAIL and other locations demonstrate culturing *Crassostrea* larvae in RAS is certainly possible but refining the culturing process so that it can support commercial-scale production will require more research.

DETERMINING THE OPTIMAL SALINITY CONFORMITY PERIOD FOR HARVESTED, MESOHALINE OYSTERS *Crassostrea virginica* USING RECIRCULATING WET STORAGE SYSTEMS

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As the oyster aquaculture industry continues to grow, small farmers may need novel methods for diversifying their products to secure their niche in a crowded marketplace. One way to create a more unique product is by altering the oyster liquor in wet storage to enhance the products natural merroir.

This project seeks to assess the potential and optimal application of using a modified wet storage system to finish harvested oysters using local water that has been artificially salted. For our study, we tracked the survival, condition index, and osmolality of oysters to determine an optimal range of conditions needed to properly modify the salinity profile of mesohaline oysters with minimal mortality and reduction of shelf life. Oysters were placed in ambient salinities (10 ppt), then exposed to 20 ppt salinity for a range of time periods (0, 3, 6, 9, 12 days) before being exposed to 30 ppt water. Oysters were then held at 30 ppt for 10 days before being placed in cold-storage to monitor shelf-life. This experiment was then repeated over four temperatures.

Our research indicates that shelf life and salinity osmoconformity are dependent on changes in temperature and varying salinity exposure regimes. For each salinity exposure conducted, a resulting decrease in shelf life was observed. Additionally, the shelf life of oysters was reduced further in warmer treatments (Fig. 1). However, the osmoconformity rate increased positively with water temperature (Fig. 2). These data will be used to provide guidance on how shellfish growers may use recirculating wet-storage systems to enhance their product or transfer oysters among water bodies with different salinities for finishing while minimizing mortality during shipment.

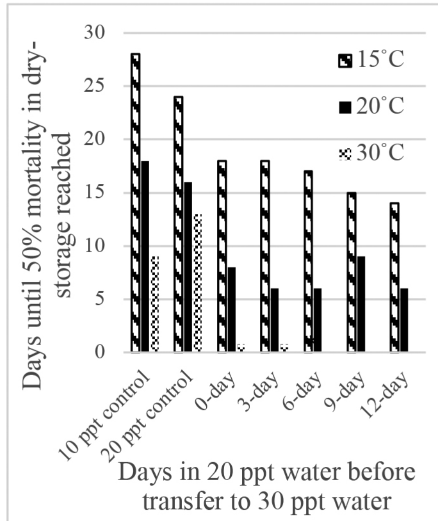

Figure 1: Shelf life of oysters in dry storage after being exposed to 20 ppt intermediate water before being transferred to 30 ppt water.

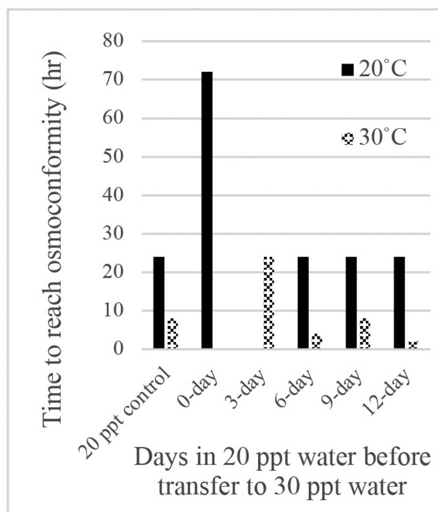

Figure 2: Length of time for oyster hemolymph osmolality to match ambient concentration when exposed to 20 ppt, then 30 ppt water.

OPTIMIZATION OF THE DEPURATION PROCESS FOR OYSTERS: CURRENT KNOWLEDGE AND FUTURE WORK

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Oysters are sustainable high protein food sources. Many experienced seafood consumers prefer eating oysters raw due to the negative perception of processing on the quality and taste of oysters. Raw oysters also offer economic benefits to the seafood industry. North Carolina's Coastal Federation (NCCF) reported North Carolina Eastern oyster mariculture value had increased from 1.67 million to 2.40 million from 2016 to 2017, respectively. While there is growing demand for raw oysters, significant food safety risks for consumers are also present.

Usually found in brackish water systems, oysters are filter feeders that pump in water from the surrounding water column for their nutritional needs. Bacteria in the water are concentrated in an oyster by up to 100 times. The bacterial genus, *Vibrio*, is of main concern in oysters with the most prevalent being *Vibrio parahaemolyticus* and *Vibrio vulnificus*, two potential pathogens. Illness as a result of *V. parahaemolyticus* and *V. vulnificus* infection can cause diarrhea, vomiting, and sometimes death in humans. Annually, approximately 84,000 Americans in the United States are infected after consumption of raw oysters. Unfortunately, recent studies suggest growing *V. parahaemolyticus* and *V. vulnificus* abundance in brackish water systems due to climatic variations raising marine water temperatures.

There exist post-harvest processing techniques that can reduce bacterial contaminants in the oysters, but overall oyster quality and viability is negatively affected. The depuration process, by contrast, utilizes flowing, sanitized seawater and the oyster's own biological filtering ability to purge the oyster of any bacterial contaminants (Figure 1). An effective depuration process should yield a live oyster with reduced contaminants. Studies have demonstrated the benefits of water disinfection treatments on maximizing *Vibrio* reduction in depurating oysters. Previous research shows that depuration parameters like water salinity, temperature, flow rate, and processing time can maximize the reduction of *Vibrio* in oysters. This presentation will present current information on the depuration process and will explore future possibilities in optimizing the process to provide safer oysters for human consumption.

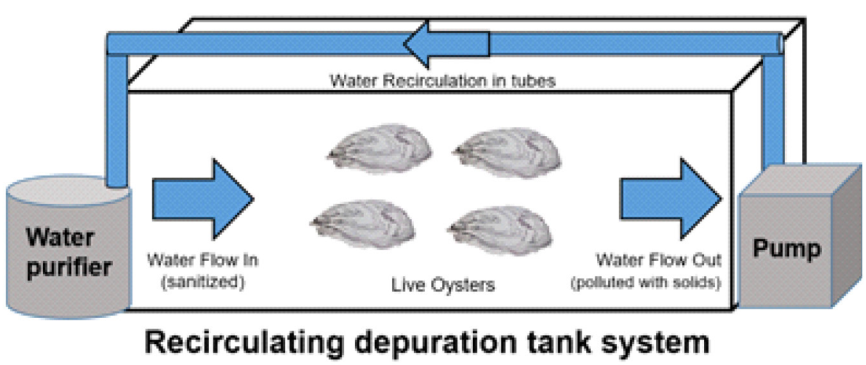

Figure 1. Diagram of a lab-scale recirculating oyster depuration system.

ISOLATION AND CHARACTERIZATION OF OUTER MEMBRANE VESICLES FROM *Moritella viscosa* AND PROTECTION INDUCED IN LUMPFISH (*Cyclopterus lumpus*)

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Outer membrane vesicles (OMVs) are nano-sized proteoliposomes shed from the cell envelope of all Gram-negative species. OMVs are now recognized as a generalized secretion pathway that provides a means to transfer cargo to other bacterial cells and eukaryotic cells. OMVs play an important role in pathogenesis, delivering virulence factors to the host cells, including toxins, adhesins, and immunomodulatory molecules. *Moritella viscosa* strain Gram-negative pathogen causes winter ulcer disease in several marine fish species. However, the levels of mortality in lumpfish have not been fully understood.

First, the aim of this study, we determined the lethal dose (LD_{50}) of *M. viscosa* could infect and produce the pathogenesis of winter ulcer and mortality in lumpfish used to evaluate the efficacy of the vaccine. Lumpfish was injected with *M. viscosa* intraperitoneal (i.p.) with 3.1 x 10^7 colony-forming units (CFU) fish^{-1} had rapid mortality with more 50% of fish mortality and typical clinical signs of ulcer disease after 5 days post-infection.

Secondly, to characterize an OMVs isolated from *M. viscosa* grown in medium iron-rich and iron-limited conditions. OMVs were characterized by transmission electron microscopy and protein analysis. *M. viscosa* OMVs in both conditions are spheres of 39.8–370 nm diameter that contains small RNA and DNA. The main OMVs proteins have a molecular size of 45, 30 and 20 kDa. OMVs isolated from iron-limited condition harbor an additional protein of approximately 60 kDa which is absent in OMVs isolate from bacteria grown under iron-rich conditions. The protein profile of the 60 kDa protein band had an enzyme of Metal-dependent carboxypeptidase, Glucose-6-phosphate isomerase, Glucose-6-phosphate isomerase and transport systems including the peptide ABC transporter, extracellular solute-binding protein, and Oligopeptide transport system, permease protein B.

In summary, our results provide strong that *M. viscosa* could induce the ulcer disease in lumpfish. These OMVs products may partially explain the play key roles in pathogenesis of *M. viscosa*. Further studies will be conducted to characterize OMVs cargo, toxicity, and utility as vaccine candidates.

AQUAPONICS CERTIFICATION: WHAT DOES IT MEAN FOR YOU?

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Today's marketing to consumers has included more and more logos and certification. Some are very recognizable, and others are new and up-coming, others are just confusing. There are several that the aquaponics, aquaculture and agriculture producers can investigate for use on their farm products or in their marketing. This session will cover a few of the certification programs, what they mean, what the consumers think and how you, the producer, might come to the decision to seek certification or not. The applicable certification programs will be discussed along with the pros and cons of each in order to guide you towards a better understanding of certification and marketing.

A MULTISEASONAL *IN SITU* STUDY TO ASSESS PREY CAPTURE EFFICIENCY IN BIVALVES AND ASCIDIANS

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Benthic suspension feeders play an essential role in the trophic dynamics of near-shore waters. By efficiency removing planktonic cells and suspended particles from the water column, they contribute to benthic-pelagic coupling and to top-down control of planktonic communities. However, information regarding the *in situ* capture efficiency of different planktonic cells by common benthic suspension feeders is scarce. To narrow this gap we assessed the capture efficiency of different planktonic groups by four filter-feeding species common in Long Island Sound coastal communities: the blue mussel, *Mytilus edulis*; the ribbed mussel, *Geukensia demissa;* the clubbed tunicate, *Styela clava*; and the vase tunicate, *Ciona intestinalis*. Sampling was performed *in situ* during the summer and fall seasons, using the InEx-VacuSip method. This method allows *in situ* and continuous collection of paired inhaled and exhaled water from the animals. To examine patterns of prey particles captured by the animals, cell abundance in paired-water samples was analyzed by flow cytometry. Phytoplankton populations were grouped into cyanobacteria, large eukaryotes and small eukaryotic algae.

Both mussels and tunicates showed, in most cases, a higher capture efficiency for cyanobacteria compared to the larger "small eukaryotes" *in situ*. This finding could be the result of several physicochemical properties of the cyanobacteria, including possible aggregation of the cells. These potential mechanisms are currently being investigated.

Figure 1 InEx method on Blue mussels.

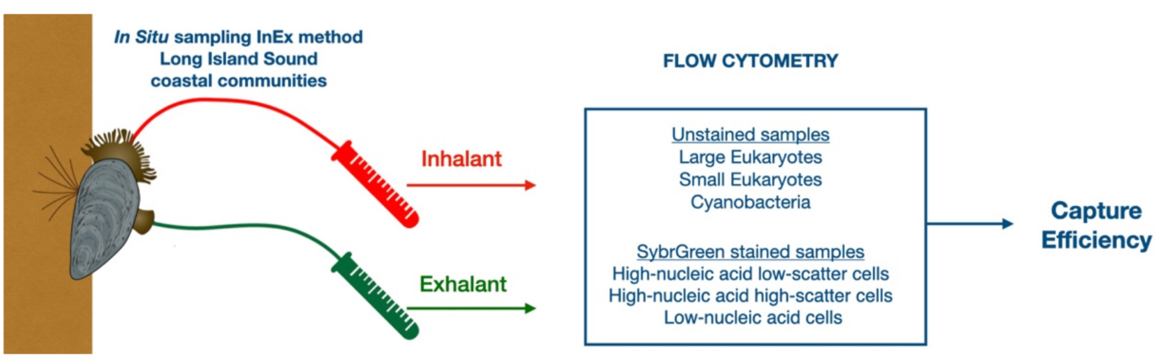

Figure 2 Schematic representation of the methodology applied. Graphic credit: Eric Heupel and Martina Capriotti.

POTENTIAL NEGATIVE IMPACTS OF SUBMARINE GROUNDWATER DISCHARGE ON OYSTERS IN GEORGIA: IMPLICATIONS FOR RESTORATION

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Reef building oysters, *Crassostrea virginica*, provide numerous ecosystem services, including provision of commercially valuable product, improving water quality and clarity, buffering shorelines, and creating complex habitats, among other things. Unfortunately, populations have declined throughout their geographic range, and considerable effort and investment is directed toward managing and restoring reefs. While several well-studied metrics are considered prior to restoration efforts (i.e., substrate, elevation, water quality), one potential factor that has not received much prior attention is submarine groundwater discharge (SGD). SGD may be beneficial for oysters by helping maintain favorable salinity and/or delivering nutrients that stimulate phytoplankton production, although groundwater flux may also deliver contaminants and decrease water quality.

Initial surveys in Oyster Creek, Georgia, suggested a negative, non-linear relationship between SGD and oysters – as SGD increased, oyster density decreased, although there were no patterns with oyster condition index or growth rates in caging experiments. To see if this pattern was consistent in other Georgia estuaries, we sampled 3 additional creeks along the Georgia coast and found similar patterns with density (Figure 1) and condition. Although the SGD in Georgia's coastal creeks is recirculated seawater, it reenters surface waters with low pH (7.34 ± 0.25 mean ± SD), and we hypothesize that this results in recruitment disruption at high flux sites, as our results also suggest negative relationship between recruitment and SGD. However, surveys across the coast suggest that there are additional complexities in this relationship that should be investigated further. Since we observed potentially negative effects of SGD on oyster density and recruitment, we suggest that SGD be considered and potentially incorporated into management plans and restoration siting efforts.

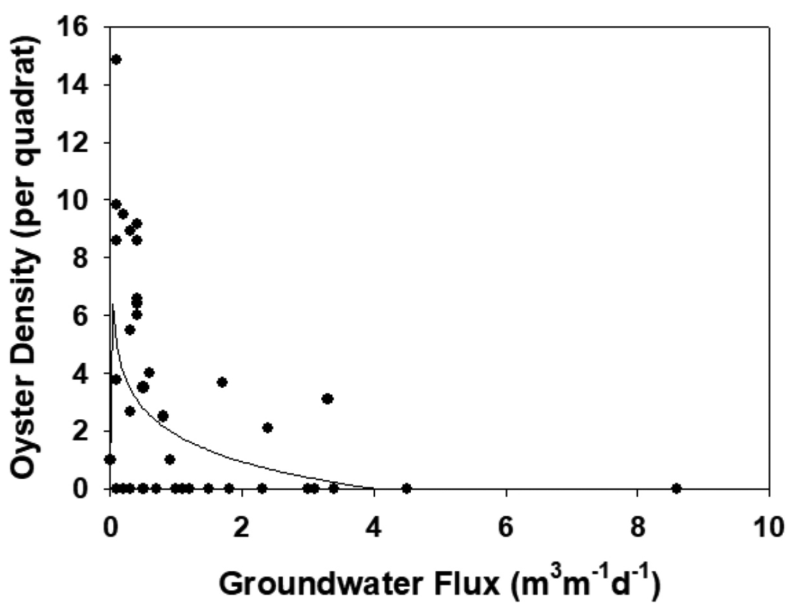

Figure 1: Relationship between groundwater flux ($m^3m^{-1}d^{-1}$) and oyster density in 10 x 10cm quadrats.

MANIPULATING THE AQUACULTURE MICROBIOME

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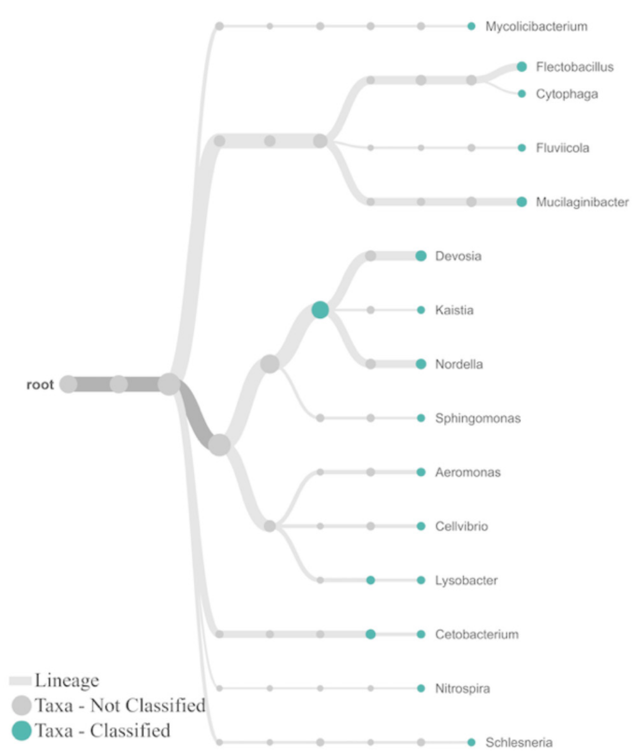
There is considerable interest regarding the composition of the microbiome in aquaculture systems. The objective of this project was to evaluate how the microbiome changes in an aquaponics system with changes in the plant component.

Five aquaponics systems were set up and inoculated with water from an established Koi aquaponics system. Three guppies (*Poecilia reticulata*) were added to each system. Two systems grew Basil, two grew Arugula and one was left as a control. The duration of the project was 8 weeks. For each system 500 mls of water were sampled at the beginning and end of the experiment, and passed through a 0.45 micron filter. Bacterial DNA was extracted using the ZymoBiomics DNA miniprep kit and libraries were prepared using the Oxford Nanopore 16s Barcoding kit. Libraries were sequenced using an Oxford Nanopore MinIon. A preliminary test of the sequencing method was also done by comparing the microbiome of an established Tilapia aquaponics system with the Koi/Goldfish aquaponics system and also bacteria from a 3% and 10% NaCl cabbage lactofermentation experiment.

Results for the plant treatments are still pending, but some interesting results arose from the two preliminary tests. While the 3% NaCl microbiome was predominantly Lactobacillus, the 10% NaCl harbored mostly Staphylococcus and Lactobacillus. The Tilapia system had a variety of bacteria but was dominated by Polynucleobacter whereas the Koi/goldfish was more diverse with the most abundant genus being Devosia and Flectobacillus (see diagram). We are still working to identify the species of nitrogen bacteria in each system.

Clearly the microbiome varies with water chemistry and species composition of the system. Understanding factors that effect the bacterial species of the aquaculture microbiome will help to promote beneficial species and avoid pathogens.



COMPARATIVE STUDY OF SMAST DROP CAMERA SURVEY TECHNIQUES ON ATLANTIC SEA SCALLOP, *Placopecten magellanicus,* BIOMASS AND DENSITY ESTIMATES IN THE GULF OF MAINE

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The University of Massachusetts Dartmouth School for Marine Science and Technology (SMAST) has historically used a centric systematic survey design to assess Atlantic Sea scallop population from Canada to the United States southern Mid-Atlantic, however the argument has been made as to how this design compares to a stratified random design. In 2020 SMAST was funded to examine such differences as part of a two-year research project focused on the Gulf of Maine. In year one we conducted the traditional systematic survey to gather baseline data which was then used to develop strata for sampling in the subsequent year. In year two, we surveyed each of the identified banks (Figure 1) with both sampling techniques simultaneously as to avoid any discrepancies which could results from fishing pressure or seasonality. Strata were derived from 2020 station level density data which was interpolated through hot spot analysis and inverse distance weighted (IDW) analysis in ArcGIS. The number of sampling stations required within each stratum were estimated using methods outlined in Ecological Methodology (Krebs, 2013) where we utilized the coefficient of variation from the mean density of scallops in each identified area from the previous year's survey. When the survey analysis was completed, researchers compared the derived results to determine if there was any significant difference between the sampling techniques.

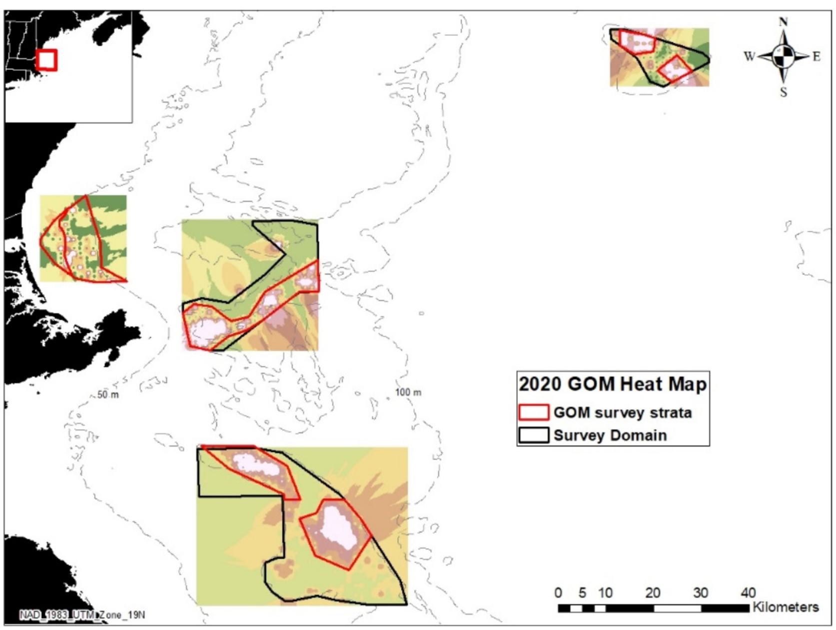

Figure 1. Heat map analysis of 2020 drop camera data in the Gulf of Maine identifying overall high- and low-density areas and overlaid with the derived strata from those data.

SPERM CRYOPRESERVATION OF GREEN ABALONE *Haliotis fulgens* FOR CONSERVATION AQUACULTURE

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Green abalone, *Haliotis fulgens,* is one of the five species along the coasts of Baja California, Mexico, that has become economically important. Nonetheless, nonregulated fishing activities and anthropogenic impacts have contributed to the decline of its populations, increasing the interest in abalone conservation; therefore, habitat restoration and stocking of native species need to be considered.

Cryopreservation of sperm of aquatic species can be helpful in such programs to protect endangered species in natural disasters or accidents that can affect wild populations. Additionally, the aquaculture industry can also benefit from artificial insemination, reducing the risk of disease transmission, creating new lines with favorable traits, conserving stocks, and transporting good quality gametes between farms. Developing a cryopreservation protocol for green abalone would aid in such restoration efforts, providing an alternative breeding option for this aquaculture industry that is not entirely established.

This work aimed to develop cryopreservation protocols for sperm of *H. fulgens*. In this study, three commonly used cryoprotectants (CPAs) were chosen: Dimethyl sulfoxide (DMSO), glycerol, and methanol. Four different CPAs concentrations (5%, 10%, 15%, and 20 %) and four equilibration times (5, 10, 15, and 20 min) were evaluated in a manually controlled cooling Styrofoam chamber.

Thawed sperm quality was evaluated by estimating the percentages of sperm motility and plasma membrane integrity (PMI) using a dual staining technique. Results suggested that 15% DMSO was the best among the CPAs evaluated, resulting in higher motility (48%) and PMI (58%) (Fig. 1).

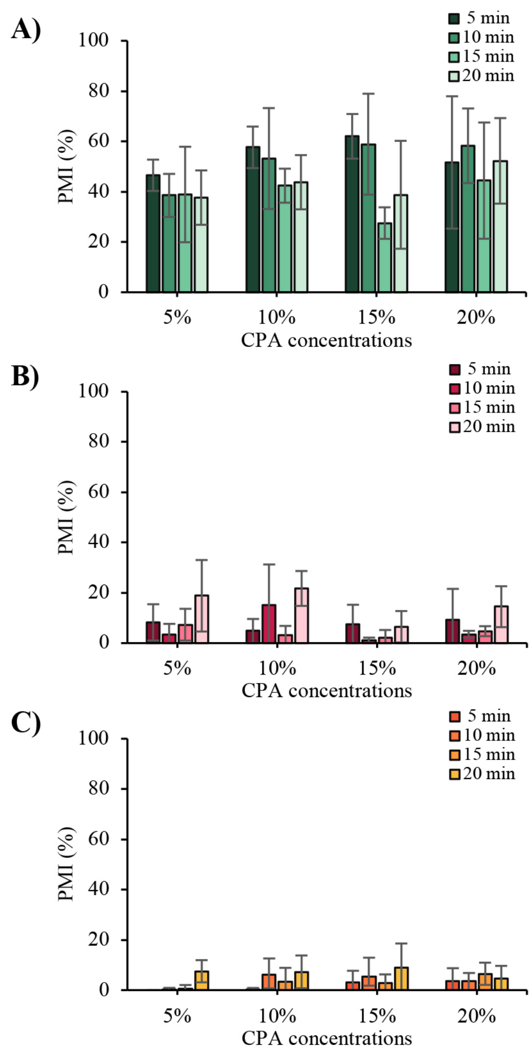

Fig. 1. Percentages of PMI after exposure to A) DMSO, B) Glycerol, and C) Methanol at different times and concentrations.

TRANSCRIPTOME MODULATION OF *SALMO SALAR* IMMUNIZED WITH *Caligus rogercresseyi* VACCINE PROTOTYPE: A HOST-PARASITE INTERACTION

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Caligus rogercresseyi is an ectoparasitic that produces the greatest economic losses in the salmon industry. Therein, vaccine-based control strategies for this sea louse have long been desired. The genome and the transcriptome data reported for *C. rogercresseyi*, allow the identification of putative antigens using the reverse vaccinology approach. This study aimed to evaluate the efficacy of vaccine prototypes during the sea lice infestation and their effects in host-parasite transcriptome modulation.

Atlantic salmon were immunized with recombinant proteins peritrophin, and cathepsin identified from sea louse genome. Four experimental group were vaccinated with different prototypes peritrophin, cathepsin, peritrophin/cathepsin (P/C) combination and PBS as control. Follow 400 UTAs, vaccinated salmons were infested with 35 copepodid per fish. Sea lice attachment were evaluated at 7 and 25 days post infestation (dpi). Samples of head kidney and skin tissues, and *C. rogercresseyi* female were taken for mRNA Illumina sequencing. RNA-seq analysis were performed. Moreover, for contigs differently express GO and KEEG pathway analysis were performed. In addition, the morphometry of adult lice exposed to immunized fish was evaluated.

Fish vaccinated with cathepsin, and P/C showed 57% efficacy, reducing adult lice bunder. Transcriptome analysis indicated a vaccine-dependent gene modulation, both at 7 and 25 dpi. Furthermore, at 7 dpi fish vaccinated with P/C and cathepsin showed an upregulation of genes associated whit metal ion binding, molecular processes energy production comparing with the control group. While at 25 dpi for Atlantic salmon and sea lice, genes associated with ATP binding, calcium ion binding, iron ion binding and zinc ion binding were strongly upregulated. Notably, the morphometric analysis shows difference in shapes between *C. rogercresseyi* exposed to vaccinated fish, with 8.3% of the shape explained by canonical variables 1 and 2. The results suggested that the vaccine prototypes stimulate energetic metabolism in salmon at 7 dpi. In addition, competition for metal ions between the host and the parasite in the infestation was evidenced. Finally, this study uncovered the molecular responses produced during host-parasite interaction in vaccinated fish and provide a new strategy for sea lice control in the salmon industry.

Acknowledgments: This study was funded by FONDAP grant #15110027 and ANID-PCHA/Doctorado Nacional (Grant 2018-21180084).

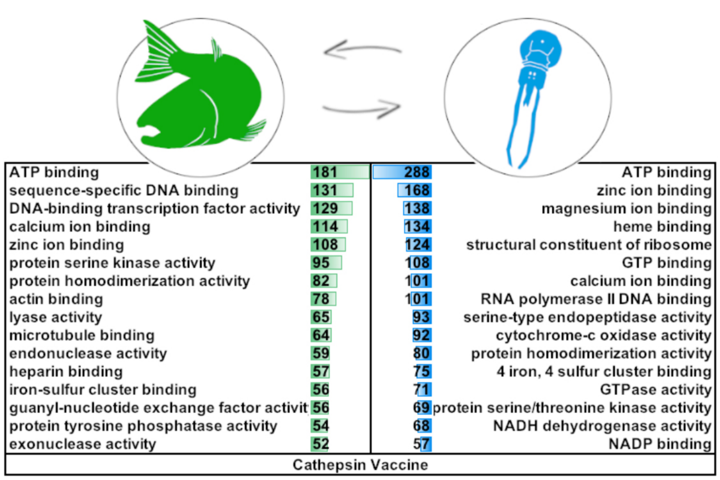

Figure 1. GO enrichment of differentially expressed transcripts of cathepsin vaccinated fish groups and exposed *C. rogercresseyi* female.

USING SONAR TO ASCERTAIN THE EFFECT OF SEAWEED AQUACULTURE FARMS ON FISH DENSITY

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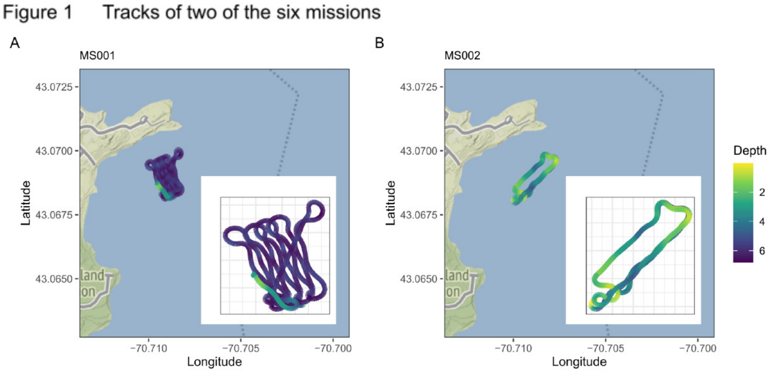
Seaweed culture can provide numerous direct benefits to humans, ranging from meeting the increasing demand for seafood products to biofuel production (DOE ARPA-E program). At the same time, kelp farms themselves are regarded as an environmentally friendly aquaculture form. Their positive environmental effects, however, have still been understudied, particularly the benefits of added structure and biomass to an area. Similar to natural kelp forests, kelp farms and other forms of aquaculture, such as fish and bivalve farms, increase abundance and diversity of wild species due to habitat and food source provisioning. Given this added structure, we predicted that fish will be attracted to kelp farms in higher abundances than surrounding areas. Here we quantify changes in density of wild fish at and away from kelp farms using hydroacoustic surveys in order to determine if aquaculture farms will have a positive effect on wild fish populations.

We used hydroacoustic surveys to quantify fish densities around two different kelp farms, which allowed for continuous surveys throughout and around the kelp farms. Surveys were designed to capture data both within and moving away from the farm in order to get a larger picture of the effect of kelp farms on wild fish abundance. Data were generated by the KelpBot team at Woods Hole Oceanographic Institute over six different autonomous underwater vehicle (AUV) missions on both the east and west coast, two of which are shown in Figure 1. Target strengths, useful in calculating fish density, were paired with spatial data to calculate fish densities via echo counting and echo integration. Additionally, missions were undertaken at different points in the kelp growth cycle to determine if fish densities changed throughout the growing season. This is the first study to quantify increases in fish densities around seaweed farms through hydroacoustic surveys, results which will be important to strong and effective management of ocean farms and local wild fisheries. Our methods demonstrate an efficient and effective means of measuring the impacts of an ocean farm on the movement and aggregation of wild species. Overall, these results help understand the role that kelp aquaculture will play in maintaining healthy ecosystems and biodiversity in the areas they are implemented, possibly contributing to farm placement and design strategies.

Figure 1 Tracks of two of the six missions



THE CHILEAN SALMON INDUSTRY'S SOCIOECONOMIC IMPACTS

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The Chilean salmon aquaculture industry has shown impressive development in terms of production growth. From the mid-1980s, the production has increased to almost 900,000 mt in 2019. The Chilean government conceived of raising artisanal fishers out of poverty by allowing them to catch fish of higher value, an objective that to a large extent has been met. As a result of the salmon farming industry's advent and the economic activity it created, southern Chile experienced a significant transformation. For example, the Los Lagos region was transformed from forestry, agricultural, and livestock to mainly aquacultural.

However, despite having created employment at the local level, the industry is often controversial and not particularly welcome in the localities where it operates. There is a widespread opinion that local communities have remained relatively excluded from the economic benefits associated with the industry. For instance, labor unions complain about unfair treatment, hiring contracts lacking adequate social security protection, lacking social services and on-the-job training, and more. Local communities speak about low corporate social responsibilities and scarce company involvement in community and municipal affairs.

In this paper, we will discuss the economic impacts that the salmon industry delivered in southern Chile. The focus is primarily on socio-economic impacts such as employment (including the massive inclusion of women in the workforce), poverty reduction in rural areas, impact in income levels and income inequality, and the creation of educational and research institutions as well as other public services.

THERMAL AND NUTRITIONAL STRATEGIES AS ALTERNATIVES TO ANTIBIOTICS IN AQUACULTURE

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Introduction

Aquaculture industry is growing at an astounding rate, overtaking capture fishery production and foresighted to represent 60% of global finfish production by 2030. The increasing demand for healthy and less expensive protein has catalysed intensification and favoured an ideal environment for several production-related problems to flourish, impacting the environment, the animals and human health. One of the best-understood risks that aquaculture intensification poses is the heavy use of antibiotics and the concerning consequence of increasing antimicrobial resistance (AMR). On farms, fishes' welfare is compromised by poor health, nutrition, and rearing practices with implications in susceptibility to stress and diseases. As a result, the weakened immune systems increase the possibility of disease outbreaks and lead to prophylactic and therapeutic antibiotic overuse. Managing antibiotic use associated with the prevention and control of aquatic animal diseases is, therefore, necessary to secure the business venture and product brand. Research on alternatives and sustainable management strategies against infection become crucial and imperative to safeguard the different actors of the aquaculture chain. Bearing this in mind, this research introduces environmentally friendly alternatives to boost the fish's immune system. A nutritional strategy including diets supplemented with natural immunostimulants, and a thermal strategy relying on physiological requirements and environmental availability, will be developed to increase fish's fitness towards challenging situations and reduce the use of antibiotics and mitigate their consequences. Overall, such alternatives will boost the welfare of the fish and leverage the industry with forthcoming management protocols to promote a more resilient and sustainable aquaculture -secure future.

Material and Methods

In a first approach, gilthead seabream will be infected with the bacteria *Tenacibaculus Maritimum* to characterize the bacterial infection through Label-free shotgun proteomic (LC-MS/MS) analysis and identify possible disease biomarkers. In this experiment, the fish behavior will be observed through cameras displayed in the tanks and the optimal temperature of non-infected seabream will be assessed in thermal gradient tanks. Furthermore, hematologic, histological, and microbiological analyses will be performed. In the second experiment, seabream will be subjected to infection and allowed to swim along a thermal gradient or being exposed to a constant temperature (obtained previously). Post-infection fish behavior and thermal preference will be evaluated through video recording. At last, seabream will be subjected to a nutritional therapy where will be fed with two experimental diets with natural immunostimulants against a commercial diet. After this, fish will be infected with the bacteria to assess the diet's impact on fish disease behavioral phenotype and proteome. Biochemical parameters of plasma (cortisol, lactate, and glucose) and liver glycogen will be assessed.

Expected outcomes

Dietary supplementation with balanced natural immunostimulants and environmental thermal gradient availability improves welfare and enhance fitness of fish under challenging farming systems. Such strategies may well be promising avenues to tailor aquaculture management protocols to boost aquaculture sustainability and consumer's safety.

Acknowledgements

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COASTAL INTEGRATED MULTI-TROPHIC AQUACULTUER OF STEELHEAD TROUT, BLUSE MUSSELS, AND SUGAR KELP ON A FLOATING PLATFORM

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Recent decline of New Hampshire's iconic groundfish industry has created hardships for local fishermen. The few fishermen that remain are currently contemplating alternative sources of income through shellfish aquaculture, seaweed farming or integrated multi-trophic aquaculture (IMTA). Funded by NOAA Sea Grant, the University of New Hampshire has been developing IMTA approaches for Steelhead trout (*Oncorhynchus mykiss*), Blue mussels (*Mytilus edulis*), and Sugar kelp (*Saccharina latissimi*). The benefit of this culture method is the lower trophic shellfish and seaweed species bio-extract nutrients generated from the fish to reduce Nitrogen input to the environment. They also provide extra income to the farmer and better utilize the 3D farming space of a permitted site. A new winter growout model was demonstrated in 2021 with trout survival of 95%, FCR of 1.2:1 and growth from 150g to 1.85kg in 7 months. Economics of the system are underway and will become available to help fishermen and entrepreneurs adopt this new technology in the Gulf of Maine.

CARBON LIFE CYCLE ANALYSIS OF SEAWEED FARMING – IMPACTS OF DIFFERENT NUTRIENT SOURCES

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The Department of Energy's (DOE) ARPA-E (Advanced Research Projects Agency – Energy) MARINER (Macroalgae Research Inspiring Novel Energy Resources) program, is developing tools to enable the United States to become a global leader in the production of marine biomass. Primary interest is in the potential for large-scale offshore seaweed cultivation to provide a renewable substrate for biofuel production while decreasing atmospheric carbon and ocean acidification. There is a lack of understanding on how different nutrient sources affect carbon uptake and emissions surrounding the seaweed farm. As part of a MARINER project lead by Ocean Era, Inc. we are attempting to estimate the net carbon impact of three different nutrient sources (ambient surface water, deep sea water upwelling, and artificial fertilization) that could be used to cultivate seaweed commercially. To do this, we will examine the carbon emissions related to the different the nutrient sources and the carbon sequestration potential for different seaweed candidate species that could be cultivated on Ocean Era's single point mooring system being launched off of the island of Hawaii.

EFFECT OF LOW DISSOLVED OXYGEN ON THE PATHOGENESIS OF *Edwardsiella piscicida* IN CHANNEL AND HYBRID CATFISH

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Edwardsiella piscicida is a highly pathogenic bacteria with a wide host range impacting global aquaculture. *E. piscicida* is reported to be more virulent to hybrid catfish (♀ channel (*Ictalurus punctatus*) ′ ♂ blue (*I. furcatus*)) than channel catfish. The prevalence of *E. piscicida* in hybrid catfish is particularly concerning since >90% of cases are reported in market-size fish. Reliant and reproducible laboratory infection models are essential while exploring the pathogenicity of these bacterial isolates. So far, only intracoelomic injections of the host have been successful with experimental *E. piscicida*-infections. As new bacterial strains emerge and evolve in response to environmental challenges, their virulence towards the host might alter. Interactions among various environmental triggers and the intensive rearing conditions may catalyze these host-pathogen relations. Low dissolved oxygen is a significant stressor leading to immunosuppression in fish. In this study, the effect of low dissolved oxygen (~ 1.5 ppm) on the pathogenesis of *E. piscicida* was explored in hybrid and channel catfish exposed via immersion. Infected fish displayed hemorrhages, ulcers, and hole in the head (Figure 1A). Histology of tissues from infected fish had lesions consistent with bacterial septicemia in the kidney as well as multifocal inflammatory lesions in the gills (Figure 1B-C). Significant mortality was observed in treatment with pathogen and low dissolved oxygen stress compared to fish exposed to stress or pathogen alone. Also, presence of a stress factor (prolonged hypoxia) was found to augment *E. piscicida*-infection in fish exposed via immersion mimicking natural infections and thereby offering a reproducible laboratory challenge method. Studies on the stress-induced pathogenesis of *E. piscicida* will provide insights on the disease progression mechanisms and management efforts can be designated in the right direction to mitigate the effects of this deadly disease.

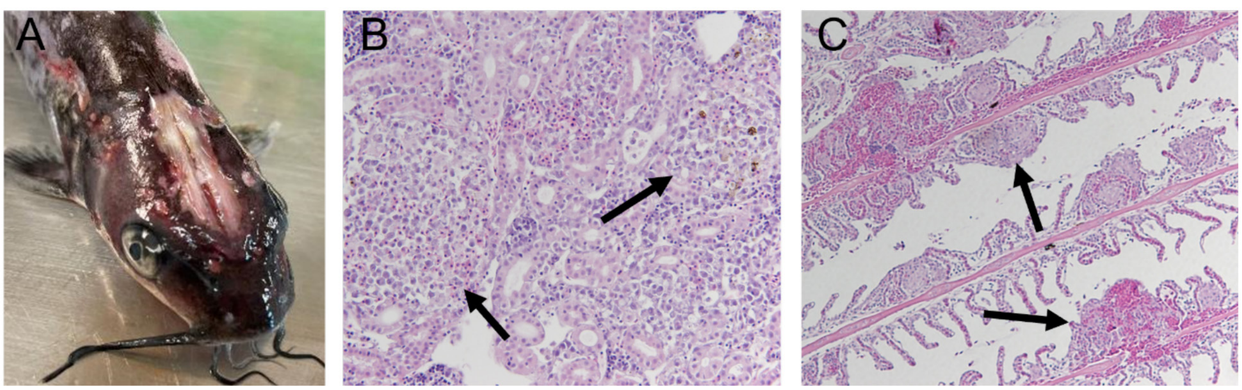

Figure 1A. *Edwardsiella piscicida* infected catfish with ulcers and hole in the head. Histopathological analysis of tissues from the infected catfish showed inflammation in the kidney (B) and multifocal inflammatory lesions in the gills (C).

ECONOMIC RISK OF CATFISH PRODUCTION STRATEGIES

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U.S. catfish farming practices have evolved with the dynamic needs of markets and recent studies showing a general trend of intensification of culture practices. Increased production intensity has resulted in greater economic risk stemming from financial, production, and market sources. This research quantifies and compares the economic risk of different commercial catfish farming practices using data from over 330 commercial ponds from 38 farms. Standardized enterprise budgets for various identified catfish farming strategies were used to perform risk simulations. Economic analysis employing iterative Monte Carlo simulations was performed using Crystal Ball® to develop cumulative probability distributions of breakeven price above total cost (BEP/TC) for six identified catfish production strategies. The analysis identified factors such as fish yield, feed conversion ratio, feed prices, and fish prices as important variables affecting economic risk. Stochastic ranking of commercial catfish farming strategies found intensive production technologies to be stochastically dominant over less intensive production practices. Productivity enhancement measures that target feed management and yields have the potential to improve profitability and reduce risk.

GENOMIC REGIONS ASSOCIATED WITH THERMAL ADAPTATION IN REDBAND TROUT *Oncorhynchus mykiss gairdneri*

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Redband trout (*Oncorhynchus mykiss gairdneri*) is a subspecies of rainbow trout inhabiting interior regions of the pacific northwest. Populations of redband trout thrive in different environments ranging from cold montane forests to hot deserts, making them a good model to study adaptation to climate change. When acclimated in common garden, desert populations have demonstrated higher thermal tolerance and better cardiorespiratory performance than those from montane forests. This is suggesting the phenotypic divergence in adaptive traits has a genetic basis. One challenge in studying the genetic architecture of local adaptation is to achieve high marker density to detect candidate genes in natural populations that often have small blocks of linkage disequilibrium. Recently, we used low coverage whole-genome resequencing to identify genome regions associated with both population differentiation and adaptive traits. Results suggest one region on *O. mykiss* chromosome 4 was consistently the most significant not only among populations, but also in the associated with acute and chronic thermal tolerance, as well as cardiac performance. Thus, the *CERK* (ceramide kinase) gene from this region appears to be a strong candidate gene in thermal adaptation. In particular, the association with cardiac function suggests a key role of *CERK* to influence pathways that lead to enhanced oxygen delivery in ectotherms that experience hypoxic conditions in warming environments. With the advancement of whole-genome resequencing and bioinformatics, genomic signatures of adaptation to climate change can be anticipated to be gradually discovered.

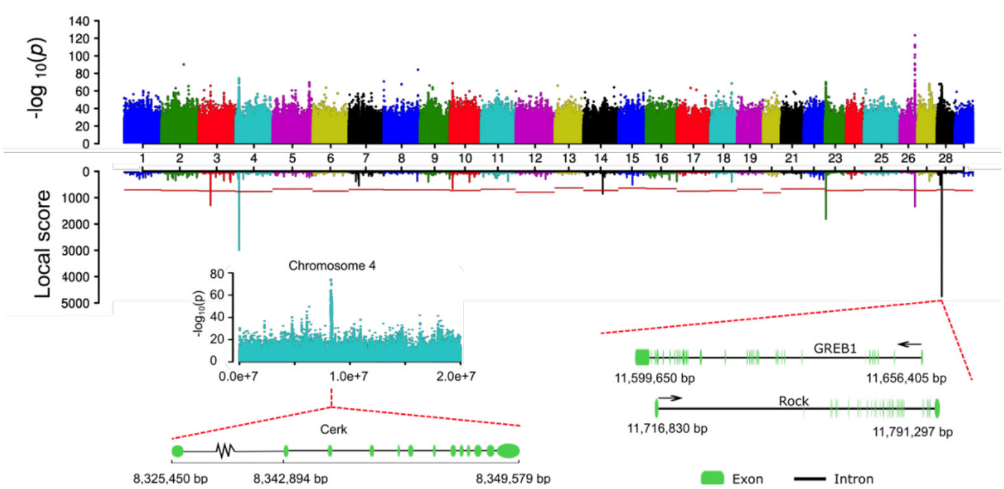

Figure 1 Differentiated genomic regions among redband trout populations from different climates.

ADVANCING FRESHWATER MUSSEL AQUACULTURE PRACTICES USING INNOVATIVE MATERIALS AND METHODS IN THE MID-ATLANTIC, USA

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Freshwater mussels (order Unionida) are a diverse group of bivalve shellfish with over 700 species recognized worldwide and nearly 300 species native to North America. However, freshwater mussels are one of the most imperiled aquatic animal groups, with over 70% of species listed as endangered, threatened, or of special concern. Mussel populations in the Delaware Estuary have similarly experienced decreases in their range, species richness, and overall abundance. Hatchery propagation of mussels is one tool that has been developed to address these issues. While hatchery propagation techniques help to increase mussel production, juvenile mussel grow-out is labor intensive and difficult to scale up to meet conservation needs. Younger and smaller mussels may be transplanted directly into the wild, but older and larger mussels have a better probability of success and are preferred.

Mussel aquaculture typically occurs in a controlled pond with access to floating grow-out systems via floating dock or wading. As infaunal organisms, freshwater mussels require substrate to burrow within. Therefore, juvenile mussels are typically grown in a floating basket with a thin layer of sand and Nitex mesh. Baskets and sand must be cleaned to avoid smothering and maintain adequate flow through the basket. Baskets must be thinned to accommodate greater biomass as mussels grow. Field experiments were conducted to test whether an alternative substrate (chicken grit) and alternative aquaculture gear (floating cages, Fig. 1) can promote faster mussel growth. The two mussel species used for these trials included the Eastern Pondmussel (*Saggitunio nasutus*) and the Alewife Floater (*Utterbackiana implicata*). Early data suggest chicken grit is a useful alternative to sand and provides cost savings on materials and labor due to easy cleaning and recovery. Mussel growth and survival in oyster aquaculture gear without any sediment has also been favorable.

These tests have demonstrated that aquaculture practices and gear developed for marine shellfish can be just as suitable for the culture of freshwater mussels, which will enhance production capacity to meet the growing needs for conservation, restoration and enhancement projects in diverse freshwater ecosystems.

Figure 1. Freshwater mussels in an oyster cage that would have otherwise been grown in multiple floating baskets.

DIETARY IMPACT OF FLORIDA POMPANO (*Trachinotus carolinus*) BROODSTOCK ON EGG AND LARVAE QUALITY AND DEVELOPMENT

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Obtaining consistent and high egg quality is often a challenge that is faced in marine fish broodstock. A maternal diet that contains an optimum amount of lipids and fatty acid composition is essential for successful reproduction and healthy offspring. A high quality broodstock diet can improve egg hatch rates, ensure healthy development, and decrease offspring mortality. The aim of this experiment is to use commercially available broodstock diets to determine broodstock reproductive performance and offspring quality. There will be two dietary treatments tested on four experimental broodstock tanks that contain 4 female and 3 male broodstock Florida Pompano, *Trachinotus carolinus,* each. Two tanks will be fed with a commercial diet (Vitalis, Zeigler) and two tanks will be fed with a Breed M gel diet. A control treatment will be fish fed with cut bait diet (n = 4). The spawning season will last a total of six months where the fish will be induced to spawn three times; once every two months. Egg and larvae samples from each spawn will be collected for histology, biochemistry, and proteomics analysis, where essential fatty acid, amino acid composition, and proteomics will be determined. Larvae samples from each tank will be collected once a day before onset of exogenous feeding and then once every two days until the weaning stage (16-18 dph) when the experiment will end. This study aims to provide a greater understanding and insight into the nutritional dietary needs of broodstock pompano. This will consequently contribute towards a more reliable and steady supply of high-quality seed stock and juvenile pompano with the most optimal traits, further improving its economic viability as a staple aquaculture species.

DETERMINATION OF OPTIMAL ORAL DOSAGE AND WITHDRAWAL TIME OF FLORFENICOL IN NILE TILAPIA *Oreochromis niloticus* REARED AT 25°C

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Antimicrobials misuse in aquaculture can bring more harms than benefits. Since pharmacokinetic information critical for determination of dosage and withdrawal time (WDT) is temperature dependent for aquatic species, prudent use of antimicrobials should include customized optimal dosing regimen taken into consideration the rearing temperature and antimicrobial sensitivity. The current study used a pharmacokinetic-pharmacodynamic (PK-PD) approach to calculate the optimal dosing regimens of florfenicol (FF) in multiple MIC levels when Nile tilapia was reared at 25°C. Then, the optimal dosage targeting MIC of 3 μg/mL was given orally for 5 consecutive days to evaluate tissue depletion and WDT and compared to those of the conventional recommended dosage. The optimal doses determined by PK study (Table 1 and Figure 1) of a single oral 10 mg/kg FF at 25°C were 1.45±0.41, 2.89±0.83, 4.34±1.24 and 5.79±1.65 mg/kg/day for MICs of 1, 2, 3 and 4 μg/mL, respectively. Consequently, a round-up dosage of 5 mg/kg/day and a general dosage of 10 mg/kg/day were comparably studied for their tissue depletion and WDTs. The serum concentrations at 24 h after the last optimal dose were 3.26 μg/mL, which was comparable to the target MIC of 3 μg/mL; whereas, the corresponding serum concentration of the 10 mg/kg/day was 8.06 μg/mL. By using the sum of FF and its major metabolite florfenicol amine (FFA) as the marker residue and a MRL of 1 μg/g, the WDT determined by linear regression analysis following the optimal dosage was 7 days, whereas that of 10 mg/kg/day was 10 days (Figure 1). The current study suggested that overdosing may occur at lower rearing temperature and the general dosage was likely determined at temperatures higher than 25°C. With the established optimal dosing regimen, serum concentrations could be maintained above the target MIC and the WDT be shortened. Further investigations of optimal dosages and WDT at higher rearing temperature are warranted to elucidate the degree of influences temperature has, and might explain the observation of sporadic over- and futile dosing of FF in the field.

TABLE 1. Selected PK parameters (mean ±SD) of 10 mg/kg FF in Nile tilapia following oral administration at 25°C (n=4).

Parameters	
Ka (1/h)	1.13 ± 0.51
$t_{1/2Ka}$ (h)	0.75 ± 0.61
α (1/h)	0.66 ± 0.28
$t_{1/2\alpha}$ (h)	1.05 ± 1.14
β (1/h)	0.043 ± 0.005
$t_{1/2\beta}$ (h)	15.96 ± 2.13
C_{max} (μg/mL)	20.37 ± 5.12
T_{max} (h)	1.65 ± 0.46
AUC(h·μg/mL)	292.2 ± 53.7
Vz/F (L/kg)	0.81 ± 0.08
CL/F (L/kg/h)	0.035 ± 0.006
MRT (h)	20.17 ± 0.79

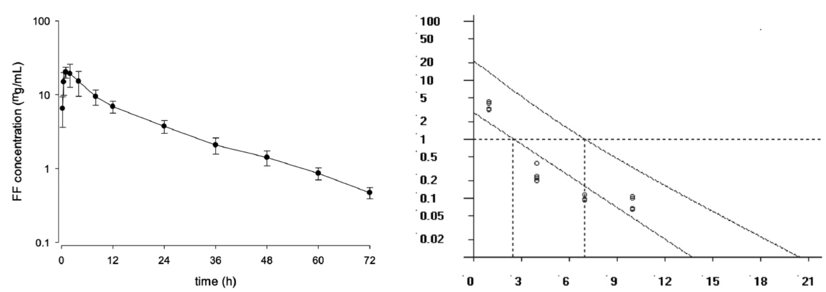

FIGURE 1. (Left) Semi-logarithmic plots of serum concentration-time profile (mean±SD) of 10 mg/kg FF in Nile tilapia following oral administration at 25°C (n=4). (Right) Tissue residue depletion of FF+FFA in the muscle/skin of Nile tilapia following oral administration at the optimal dosage (5 mg/kg/day once daily for 5 days (n=4)).

CHALLENGES TO ANAEROBIC DIGESTION OF WASTE STREAMS FROM LAND-BASED RECIRCULATING AQUACULTURE SYSTEM (RAS) FACILITIES

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Land-based recirculating aquaculture systems (RAS) have led to increased fish production efficiencies through increased stocking densities and have the additional benefit of capturing environmentally harmful concentrated sludge from fecal matter and uneaten feed. In addition to waste sludge production, land-based RAS facilities can also generate unintentional fish mortalities. Furthermore, on-site processing of harvested fish can produce waste offal (e.g., heads, guts, scales, bones) that can be up to 40% of the original fish weight. Anaerobic digestion (AD) is a sustainable and effective means of organic waste treatment in the dairy, swine, and wastewater treatment industries. In the absence of oxygen, microorganisms break down organic matter and produce biogas, a mixture of methane and carbon dioxide, which can then be used for renewable energy production. The AD process also results in the formation of a nutrient-rich liquid fertilizer with a reduced solids content. Research on the waste treatment of RAS sludge is still in its infancy, and AD could be a potential solution to this issue. There has been some research on the anaerobic co-digestion of fish offal mixed with other organic wastes, but the results have been variable. Challenges to operational and process stability still need to be addressed. Sludge from RAS facilities, mortalities, and fish offal, utilized as AD substrates, can significantly influence the operational and process parameters in a digester, such as, alkalinity, total ammonia nitrogen, long-chain fatty acid and volatile fatty acid concentrations, carbon/nitrogen ratio, organic loading rates, hydrogen sulfide concentrations, salinity, and pH. Understanding the impacts of these waste streams on the aforementioned parameters is vital for gauging the feasibility of AD as a potential sustainable waste treatment technique for land-based RAS facilities. This presentation will discuss specific concerns associated with the digestion of these waste streams (sludge, mortalities, offal) from RAS facilities and potential solutions to address these issues.

SUSTAINABLE MARINE AQUAPONICS: EFFECTS OF SHRIMP TO PLANT RATIOS AND C/N RATIOS

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Integrated aquaponic food production systems are capable of producing more food on less land using less water than conventional food systems, and marine systems offer the potential of conserving freshwater resources. However, there have been few evaluations of species combinations or operational parameters in marine aquaponics. The goal of this experiment was evaluation of stocking density ratio of Pacific whiteleg shrimp (*Litopenaeus vannamei*) to three edible halophytes (*Atriplex hortensis*, *Salsola komarovii*, and *Plantago coronopus*) with two C/N ratios in a 3×2 factorial design. There were three stocking density ratios (shrimp : plant), 2:1, 3:1, and 5:1; and two C/N ratios, 12 and 15. The results indicated that stocking density ratio exerted a significant impact on shrimp growth. Shrimp reared in 2:1 and 3:1 treatments had better growth performance. In contrast, plants were affected by both stocking density ratio and C/N ratio. Halophytes grown in stocking density ratios of 3:1 and 5:1 with a C/N ratio of 15 had better growth performance and nutrient content. The concentrations of TAN and NO_2^- were below 0.2 mg/ L throughout the experiment, including the higher stocking density ratio treatments. In conclusion, the stocking density ratio of 3:1 with a C/N ratio of 15 was suggested as the optimal condition for the operation of marine aquaponics in which whiteleg shrimp and the three halophytes are target crops.

OPTIMAL DIETART CURDE PROTEIN FOR SHRIMP AND HALOPHYTES IN MARINE AQUAPONIC BIOFLOC SYSTEMS

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Feed input for the animal subsystem is the primary nutrient input into aquaponic systems, but should also provide adequate nutrition for several bacterial populations as well as a plant crop. Dietary crude protein is one of the most expensive macronutrients in fish diets, and the source of nitrogen (N) for other biotic components. However, N nutrient flows through aquaponic systems is not well understood. The aim of the present study was to investigate the effect of shrimp feed with different protein concentrations (30%, 35%, or 40%) on water quality and the growth performance of Pacific whiteleg shrimp (*Litopenaeus vannamei*) and three edible halophytic plants (*Atriplex hortensis*, *Salsola komarovii*, and *Plantago coronopus*) in biofloc-based marine aquaponics. The experiment was conducted for 12 weeks, the plants were harvested and seedlings transplanted every 4 weeks. Protein content did not influence shrimp growth in the current study, indicating that feeds with lower protein concentrations can be used in biofloc-based marine aquaponic systems. During the early and mid-stages of cultivation, plants grew better when supplied diets with higher protein concentration, whereas no differences were observed for later harvests. Hence, for maximum production, we recommend providing a higher protein concentration feed in the early stages of system start-up, and switching to a lower protein concentration feed in later stages of cultivation.
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EXPLORE THE IMPACT OF DIFFERENT PH LEVELS AND ADDITIONAL ON THE OPERATION OF SUSTAINABLE MARINE AQUAPONICS

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pH is the major issue that concerns all producers in aquaponics, as the main three organisms (aquatic animal, plant, and microbes) have different preferences. While additional C is the potential approach to amend the growing environment and improve shrimp and plant growth, and microbe establishment. Aquaponics under saline conditions has, however, not been studied in detail in regard to the effect of pH and additional C. In this study, we evaluate the impact of pH and additional C on the growth of Pacific whiteleg shrimp and five edible plants (three halophytes and two glycophytes) in marine aquaponic systems using nutrient film technique (NFT). The results suggested that plants grown better in both pH 6.5 treatments; however, additional C improved the growth in pH 7.5 + C treatment and had similar yield to lower pH treatments. The results indicated both pH and additional C had little impact on shrimp growth. This trend was likely due to the greater impact of imbalanced ionic composition on shrimp performance. Therefore, we suggest that RO water is not a suitable water source for shrimp-based aquaponics unless the ionic composition is managed. Yet, more research is needed.

Mytilus californianus AS A POTENTIAL NEW SPECIES FOR AQUACULTURE PRODUCTION: A PROOF-OF-CONCPET AND ESTABLISHED SEED-TO-FARM PIPELINE IN SOUTHERN CALIFORNIA

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Once a major staple of shellfish consumed along the US West Coast from pre-colonial through the late 1900s, depletion of wild stocks of the California mussel (Mytilus californianus, “CM”) and a lack of hatchery-based aquaculture have eliminated market presence of this species. This is despite a historically high demand in seafood outlets, reaching peaks of 500 tons for annual North American landings through the 1980s. In the US, low-trophic aquaculture (e.g., seaweeds and bivalves) present both environmental and social benefit, and untapped economic opportunities for emerging native species via hatchery-based aquaculture. While oysters traditionally make up a large portion of global shellfish aquaculture, mussels production increased from 2010 to 2016 by ~23% for Mytilid and ~35% for Chilean varieties, demonstrating that demand for mussels, specifically, is on the rise. Here, a collaborative team from Holdfast Aquaculture, Santa Barbara Mariculture, and the Nuzhdin lab at the University of Southern California will report on findings from a USDA sponsored Small Business Innovation Research (SBIR) grant surrounding the amenability of CM to hatchery-based aquaculture. Further, we demonstrate a new seed-to-farm pipeline established exclusively in Southern California, filling an important economic niche for area industry. The team will show that there is potential to introduce CM as a new “California-raised” aquaculture product (trademarks pending), similar to New Zealand’s Green Lipped Mussel (GLM) which is valued at ~$200 million. NZ exports ~68 million pounds of GLMs annually, compared to the U.S. which exported 1.5 million pounds and imported 67 million pounds of mussels in 2019.

In our research, the team demonstrated several key milestones for CM hatchery husbandry and spawning, including: 1) mass-spawns occur regularly, 2) spawn induction is possible (protocols developed), 3) potential amenability to Gamete Conditioning System ripening, 4) settlement onto fuzzy rope, 5) preference for fuzzy rope over other substrates, 6) retention during juvenile re-socking, 7) equal- or out-performance of other Mytilid market metrics (e.g. shell strength, Figure 1). Through grants and investments, new aquaculture facilities have been developed at AltaSea in Los Angeles, where Holdfast Aquaculture can produce 30,000-feet of seeded line per year, and Santa Barbara Mariculture is in the process of developing permits for CM out-planting on their 72-acre farm. The team’s genomics studies demonstrate high mutational load for bivalves, which may impact any selective breeding programs developed for CM and other species. Together, the study data suggests that there is a significant opportunity for US West Coast aquaculture in CM, production of this native species through hatchery-based aquaculture is possible, and that CM performance matches or exceeds currently farmed mussel varieties for the region.

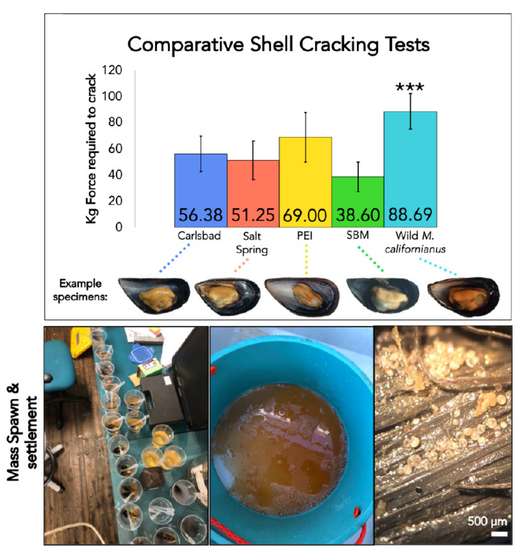

Figure 1. Comparative shell strength tests

THE EFFECT OF DENSITY ON REPRODUCTIVE ACTIVITY IN ATLANTIC SEA SCALLOPS *Placopecten magellanicus*

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The Atlantic sea scallop fishery employs a rotational closed area strategy designed to increase future yield-per-recruit and spawning potential due to fast growth observed by scallops afforded protection from fishing pressure. It is unclear how well the analytical models that underpin this strategy hold under conditions that deviate from long-term averages, such as the high-density recruitment events observed in 2012 in the Nantucket Lightship and 2013 in the Elephant Trunk. The scallops at these sites persisted at high densities and initially exhibited varying degrees of impacted performance. The effect of scallop density on growth, yield, and reproduction was investigated through quarterly sampling trips in 2018 and 2019 with sampling at 21 sites divided among high, medium, and low-density scallop beds. In addition to total catch and length data, 30 scallops were retained at each site to determine meat, viscera, and gonad weights along with sex and reproductive stage. Reproductive effort was quantified as the ratio of gamete production to total production to investigate how this metric differs across population density. Overall, scallop density was an important factor in predicting reproductive effort, with scallops at the extreme densities observed in the Nantucket Lightship exhibiting reduced reproductive effort compared to the traditional densities found in the resource. Reproductive activity also occurred over a shorter window in the Nantucket Lightship, where the percentage of scallops staged as mature or spawning reached 50% during only one of the seven sampling trips (Figure 1). A subset of female scallop gonads collected during sampling is currently being examined to investigate impacts on fecundity, egg size, and egg quantity, further clarifying the effects of density on reproductive success.

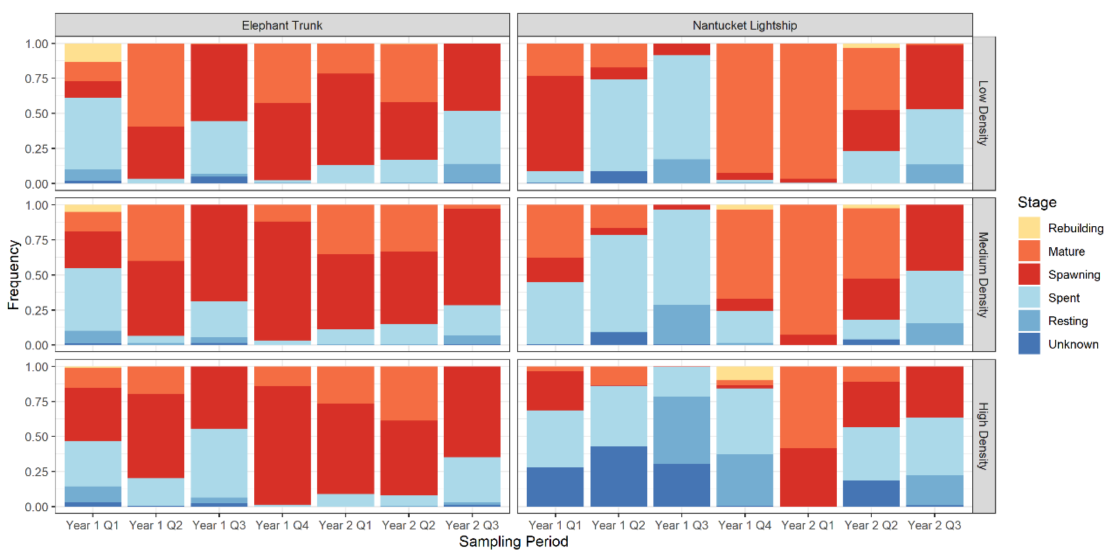

FIGURE 1. Frequency of sea scallops in each reproductive stage across sampling periods, study areas, and scallop density.

REGULATORY COSTS ON ATLANTIC COAST SHELLFISH FARMS

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Regulatory compliance on Pacific Coast shellfish farms has been shown to increase costs of production, have a disproportionate negative effect on small-scale farms, and to constrain growth. A survey was conducted of the shellfish aquaculture industry in twelve Atlantic Coast states (Maine, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Delaware, Maryland, Virginia, North Carolina, South Carolina, and Florida) to assess the on-farm economic effects of regulations on the U.S. Atlantic Coast.

The survey covered several topics related to shellfish farming, including the costs of permits and licenses, costs of manpower for compliance, lost sales and opportunities for expansion, production costs, marketing costs, and other on-farm activities. The survey instrument transitioned from more generic and broad questions, used to introduce a topic, into more detailed questions about specific regulatory challenges and/or costs related to that topic.

Despite disruptions to data collection efforts resulting from the COVID-19 pandemic, seventy-nine farms completed the survey between May 1st, 2019 and August 31st, 2021. Contact lists of known shellfish producers were obtained through the assistance of state Cooperative Extension Specialists, industry associations, list of permit holders, and independent online searches. Regulatory compliance costs were calculated per farm and per state as well as on a percentage of total sales. Lost business opportunities resulting from regulations were identified and quantified where possible. Results were compared with those of the Pacific Coast shellfish producers.

Response rate per Atlantic coast state			
State	Initial contact list (#)	Participants (#)	Response rate (%)
Maine	200	8	4.0%
Massachusetts	416	11	2.6%
Rhode Island	28	6	21.4%
Connecticut	41	3	7.32%
New York	37	6	13.5%
New Jersey	34	5	14.7%
Delaware	No list provided	1	N/A
Maryland	160	10	6.3%
Virginia	340	12	3.5%
North Carolina	39	8	20.5%
South Carolina	10	1	10%
Florida	385	9	2.3%

COMPENSATORY RESPONSE OF THE SOMATOTROPIC AXIS FROM IGFBP-2B GENE EDITING IN RAINBOW TROUT *Oncorhynchus mykiss*

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Insulin-like growth factor (IGF) is recognized as a central regulator of anabolic growth in vertebrates, largely due to its positive effects on muscle and bone growth. However, the majority of IGF-I is not free in circulation; approximately 99% of IGF-I is bound to IGF binding proteins (IGFBP). In addition to extending the half-life of IGF-I in serum, IGFBPs sequester IGF-I to peripheral tissues and can promote or inhibit ligand binding to surface receptors. Characterizing the functions of the IGFBPs is essential to understand the role of these proteins as regulators of IGF signaling. We used gene editing technology to edit IGFBP-2b, the most abundant IGFBP in serum that binds an estimated 80% of free IGF-I. The objective of this study is to determine how components of the IGF/IGFBP system respond to a reduction in serum IGFBP-2b abundance.

Rainbow trout with gene editing-induced reductions in serum IGFBP-2b (Mutant) exhibit similar growth performance compared to fish without IGFBP-2b gene disruption (Control). Editing the IGFBP-2b genes resulted in an 83% decrease in serum IGFBP-2b in mutants. This resulted in a 35% reduction in serum IGF-I (Figure 1c), which was offset by reduced expression of hepatic *igfbp-1a2* and increased muscle *igfr-1a* (Figure 1a,b); these responses suggest that an increased IGF-I signaling capacity offset reductions in serum IGF-I. During feed deprivation, the differential expression of *igfbp* genes supports the attenuation of the growth inhibitory response, likely due to the further reduction in serum IGF-I that alleviated the need for an IGF-inhibitory response. Unique *igfbp* expression patterns occurred during refeeding, suggesting an enhanced IGF-I signaling capacity in controls. Collectively, these findings support that the role of IGFBP-2b is to regulate serum IGF-I concentrations. The compensatory regulation of IGF/IGFBP system genes indicates that adjustments in other IGFBP, both circulating and at the local level, maintain IGF-I signaling at a level appropriate for the nutritional state of the fish.

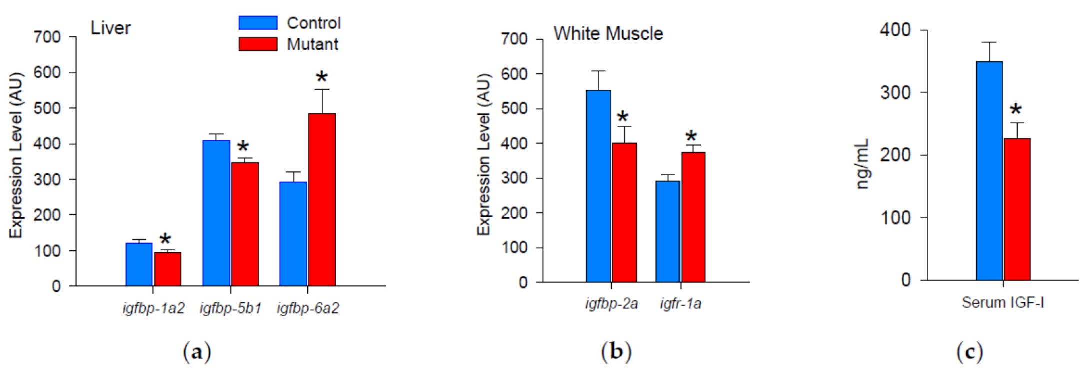

Figure 1. Differential regulation of somatotropic axis components in a) liver, b) white muscle, and c) serum. Asterisks indicate significantly different means, $p < 0.05$.

AQUACULTURE EDUCATION USING A MULTI-PRONG APPROACH

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Aquaculture and aquaponics are valuable tools to teach academic concepts as well as life skills. It is easy to see the integration into STEAM subjects like biology, chemistry, math, physics, and agriculture. However, there are less intuitive opportunities to incorporate aquaculture into other subjects including business, culinary, and sociology. To maximize the usefulness of these forms agriculture as educational tools people need to be informed. Using a multi-prong approach, the Fisheries Learning Center at Auburn University directs efforts at, 1) Training teachers, 2) Engaging students directly in immersive experiences, and 3) Extending its reach through video creation and social media. The Aquaculture Education and More YouTube channel allows for global reach and on-going asynchronous support. Using these methods, it is possible to reach 100,000 people each year.

INTEGRATING AQUAPONICS INTO THE SECONDARY SCHOOL CLASSROOM: BEYOND BIOLOGY AND CHEMISTRY

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Aquaculture has, perhaps more than any other agriculture or academic content area, the potential for interdisciplinary and collaborative instruction. The multifaceted nature of aquaculture provides numerous opportunities and classroom activities to engage students in any number of subject matter areas. Students are confronted with complex problems that allow them to experiment and build their problem solving and cooperative learning skills. These problems help translate the academic principles; they are required to learn, into real-world applications. The hands-on nature of many of these activities helps hold student interest and provide ongoing motivation. Concepts learned can be applied in many other fields and can help better prepare students for higher education. Table 1 indicates other academic areas that relate directly or indirectly to aquaculture.

The connections to biology and chemistry are readily apparent but connections to other disciplines require more effort. Our role as Extension educators and content specialists is to help teachers make and explore these connections. This presentation describes methods to maximize the cross curricular capacity and effectiveness of an aquaculture teaching platform.

Biology	Chemistry	Physics
Math	Economics	Plumbing
Mechanical Systems	Construction	Sales
Marketing	Hydraulics	Language Arts
Business Planning	Finance	Home Economics
Food Sanitation & Safety	Nutrition	Physiology
Morphology	Fish Health	Fish Reproduction
Genetics	Art	History
Sociology	Carpentry	Masonry
Hydroponics	Computer Technology	Public Relations

IMPROVEMENTS IN SEAWEED CULTIVATION AND GROWTH THROUGH UNIQUE MICROSTRUCTURE SUBSTRATES . . . AN APPROACH INSPIRED BY NATURE!

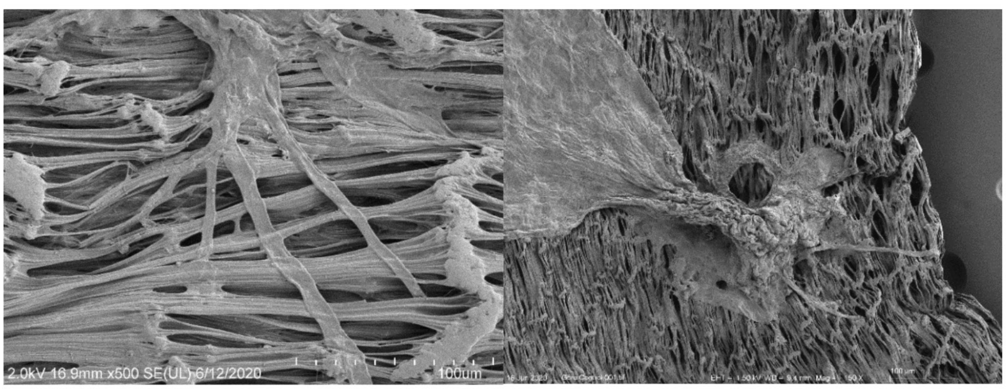
Dr. Norman Clough

W.L. Gore & Associates

W.L. Gore & Associates (the makers of Goretex® waterproof and breathable membranes) are developing a wide range of unique seaweed cultivation substrates. These substrates have microstructures tailored to the attachment and encapsulation of specific seaweed spores and direct seedlings providing large improvements in plant attachment strength, fast/healthy growth and biofouling protection.

Inspired by nature, these "artificial rock" substrates show truly unique plant to microstructure interactions (see examples below) resulting in new cultivation opportunities for both established and difficult to cultivate species e.g. Saccharina Latissima and Palmaria Palmata which will be discussed during the presentation.

Using a variety of characterization techniques, the presentation will also highlight how structural changes in these substrates can be used to control plant attachment strength, specific location of plant attachment, uniformity and spacing of seedling densities.



EXPANDING AQUACULTURE EDUCATION AND TRAINING OPPORTUNITIES IN FLORIDA: SUCCESS STORIES AND LESSONS LEARNED FROM RECENT INITIATIVES

Marcy L. Cockrell*, Katrina Bayliss, Charlie M. Culpepper, III, Portia Sapp, Eric Cassiano, Anita Grove, and Joe Taylor

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Aquaculture can be used as a dynamic teaching tool for a variety of subjects, at many different grade levels and in a broad range of classroom and experiential learning contexts. From classroom lectures to running a fully operational production facility, aquaculture presents unique opportunities for enrichment of both educators' and students' knowledge, skills and professional development. There are, however, many challenges to introducing and implementing aquaculture education, including financial resources and training for educators. In Florida, the Florida Department of Agriculture and Consumer Services (FDACS) Division of Aquaculture, along with project partners at several different institutions, have been working to overcome these barriers and bring aquaculture to Florida educators and students through several different projects and initiatives. In this presentation, we will discuss four different past and ongoing projects with the objective of enhancing aquaculture education and workforce training in the state of Florida. The methods, results, successes (Fig. 1 for example) and lessons learned from each project will be discussed.

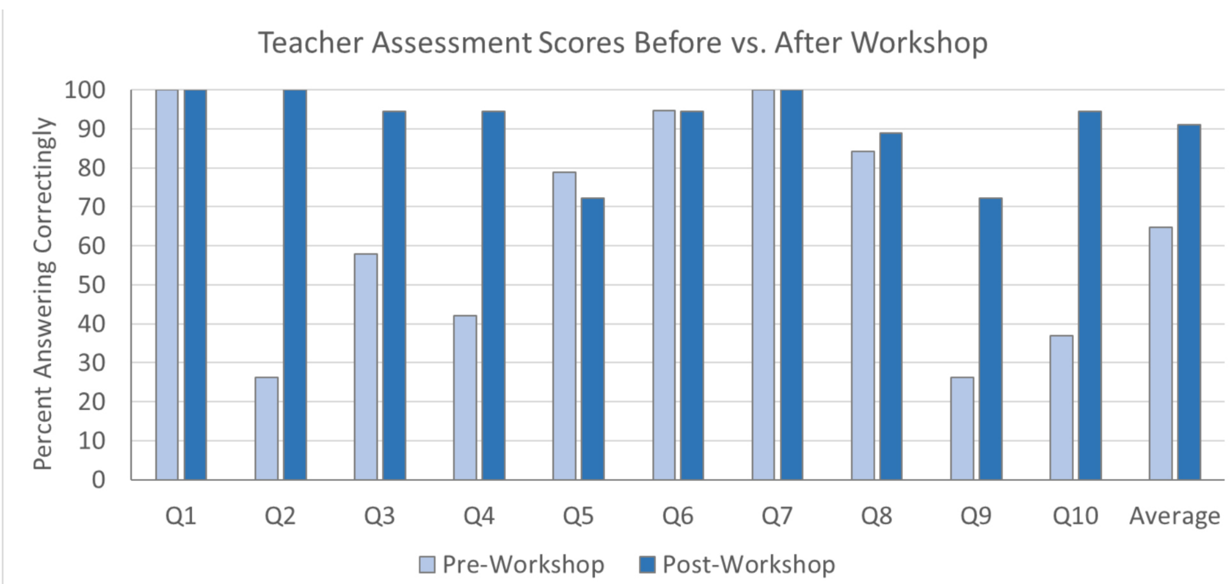

Figure 1. Pre-workshop (light blue) vs. post-workshop (dark blue) knowledge assessment scores from participants during an aquaculture education training workshop. Correct answer scores increased from an average of 65% before the training to 91% after the training.

MARINE SPATIAL PLANNING FOR OFFSHORE AQUACULTURE IN FLORIDA: A FIRST LOOK AT POTENTIAL OPTIONS FOR SITING IN THE GULF OF MEXICO

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Offshore aquaculture for finfish, seaweed and shellfish holds great potential to bolster U.S. domestic aquaculture, domestic seafood supply chains and economies in coastal communities. The ability to find suitable sites for offshore aquaculture, however, remains a significant challenge for anyone looking to start an offshore enterprise. With the goal of providing baseline information for those interested in offshore aquaculture, the Florida Department of Agriculture and Consumer Services (FDACS) Division of Aquaculture partnered with the NOAA National Centers for Coastal Ocean Science Coastal Aquaculture Siting and Sustainability Program to perform a marine spatial planning exercise for offshore aquaculture in Florida state waters. The siting analysis was performed for the Florida Gulf coast from Pensacola to Tampa Bay using 41 discrete spatial data layers categorized as national security, natural and cultural resources, industry and navigation and fishing and aquaculture. The analysis revealed 54,906 total acres (222.2 km^2) across four geographically distinct planning areas that could potentially be suitable for offshore aquaculture (Table 1). This analysis presents a “first look” at potential options for siting offshore aquaculture along the Florida Gulf coast in the future. The methods and major results of the siting analysis, potential next steps and remaining hurdles that exist for permitting offshore aquaculture in Florida will be discussed.

Table 1. Results of the spatial planning analysis to examine suitable areas for offshore aquaculture in Florida state waters of the Gulf of Mexico. POAZ = potential offshore aquaculture zone.

Region	Planning Area	# Suitable POAZs	Total acres in POAZs
Northern	Northwest (Pensacola)	5	18,153
	Northeast (Apalachicola)	2	1,913
Southern	South A (Tampa)	3	8,225
	South B (Sarasota)	24	26,615
	Total	***34***	***54,906***

A PEPTIDIC OOCYTES-SPECIFIC DELIVERY-TOOL DEVELOPMENT FOR GENE SILENCING

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To serve the metabolic needs of the developing embryo, Oviparous animals, such as decapod crustaceans and the prawn *Macrobrachium rosenbergii in* specific, are characterized by an elaborated vitellogenesis process. The process is designated by enhanced production and accumulation of vitellogenin (Vg), the major yolk protein component of the oocyte. Yolk accumulation is commenced by receptor-mediated endocytosis. The specific interaction between Vg and its receptor and the immense accumulation of the Vg in the oocyte during vitellogenesis is utilized herein to develop a sophisticated oocytes-specific delivery tool.

A specific peptide conserved among crustaceans vitellogenin was derived from *M. rosenbergii* Vg, synthesized, and labeled with a fluorescent fluorophore. The fluorophore-labeled Vg-peptide was found to enter into *M. rosenbergii* and *Litopeneous vannamei* oocytes both *in vitro* and *in vivo*. However, a peptide with the same amino acid composition but scrambled order (scVg) could not enter the oocytes. Additionally, *in vitro* and *in vivo* experiments demonstrated that dsRNA electrostatically connected to the Vg-peptide is internalized into *M. rosenbergii* oocyte. *PAX6* dsRNA connected to the Vg-peptide and injected into vitellogenic females was found functional and capable of inducing gene silencing, which led to the interruption of the eye development in the embryos of the treated mothers. More than 90% of the embryos of treated mothers were affected.

The high efficiency of the delivery tool and the fact that it is conserved throughout crustaceans would benefit the fast-growing crustacean aquaculture. Specifically, when a single treated female would produce thousands of affected embryos with the desired trait. The tool could be utilized for silencing aquaculture-relevant genes to develop a sustainable fast-growing and vaccinated population.

EXAMINING THE EFFECTS OF NYLON MICROFIBERS ON THE GUT MICROBIOME OF THE BLUE MUSSEL *Mytilus edulis*

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Microplastics are a ubiquitous emerging contaminant across marine systems. Because of their small size and widespread distribution, microplastics occupy the same size fraction as the food source of many suspension feeders, such as marine bivalves, and ingestion of plastic particles could pose a threat to the health of these organisms. For example, microplastics may act as a vector for anthropogenic chemicals and foreign or pathogenic microbial species. Ingestion of plastic particles with adsorbed compounds or microbial communities could potentially affect the gut microbiome of the host through mechanical blockages or abrasions, leaching of plasticizers or adsorbed chemicals, or direct influence on microbial communities in the gut. In many species, the microbial community of the gut aids in digestion, mediates abiotic stressors, and affects host immunity. Similar functions may be performed in bivalves. Thus, changes in the gut microbiome may have indirect effects on bivalve health.

To test whether microplastics affect the gut microbiome or tissues of the blue mussel, *Mytilus edulis*, animals were exposed to nylon microfibers (length = 500 µm, diameter = 30 µm), *Spartina* spp. particles, or no particle, for 21 days. Mussels were fed a microalgal diet of *Tetraselmis* spp. (concentration in microcosm of 10,000-15,000 cells/mL) and Shellfish Diet® (concentration in microcosm of 5,000 cells/mL). All particles were aged for three days to develop a biofilm. Two experiments were conducted, one with an exposure concentration of approximately 50 particles/L/hr/mussel/day, and one using a concentration of approximately 100 particles/L/hr/mussel/day. *Spartina* spp. particles were of comparable size and aspect ratio to nylon fibers and were used to control for the presence of indigestible particles. Genomic DNA was extracted from gut tissue, nylon, *Spartina* spp., and stock water samples and sequenced using 16S high throughput techniques to determine community taxonomic composition. Data indicate that intrinsic control over gut microbial communities outweighed exposure to the microbial community on nylon fibers (Figure 1). The microbial community on nylon microfibers differed from the community in seawater and on *Spartina* spp. particles, however, gut microbial communities of mussels exposed to microfibers were similar to both mussels exposed to *Spartina* spp. particles and control mussels.

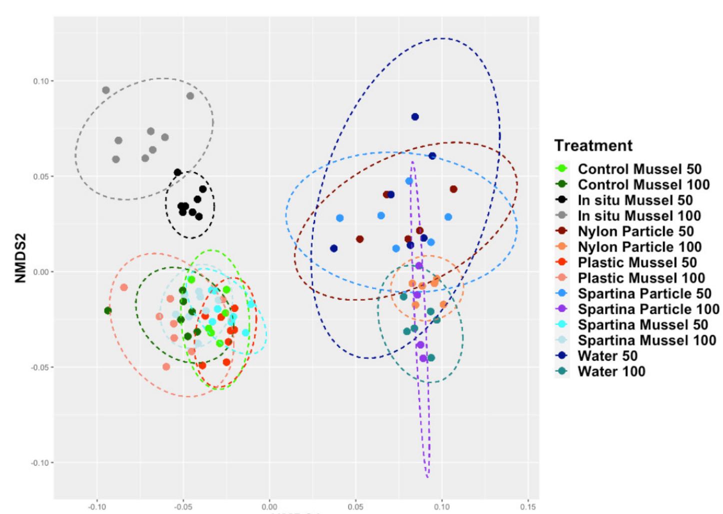

Figure 1. Ordination plot displaying non-metric multidimensional scaling on Bray-Curtis dissimilarities for environmental and experimental mussels, and water and particle samples.

COMPARISON OF NUTRIENT COSTS FROM FISH FEEDS AND INORGANIC FERTILIZERS FOR AQUAPONICS SYSTEMS

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One potential advantage of aquaponic systems is reduced resource consumption compared to separate fish and plant rearing systems, but little has been published on the costs of providing nitrogen and phosphorus nutrients by fish in comparison to the purchase of inorganic fertilizers. The cost of providing nitrogen and phosphorus from six commercial fish feeds was compared to 9 commercial agricultural fertilizers. Waste production from fish was corrected for excretion of urea and the impact of feed wastage. For a typical aquaponic system without mineralization of fecal solids, the soluble nitrogen and phosphorus excretion ranged from 36.9 to 44.0 g/kg feed and 1.4 to 3.7 g/kg feed, respectively. The cost to provide 1 kg of nutrients from feeds ranged from $15-$29/kg for nitrogen and from $115 - $583/kg for phosphorus. Compared to the purchase of these elements from inorganic fertilizers, the feeds were 7-14 times more expensive for nitrogen and 17-88 times more expensive for phosphorus. The feed/fertilizer cost ratio ($FFCR^{feed}$) required to replace the nitrogen and phosphorus in 1 kg of feed ranged from 2-4 times for monoammonium phosphate to 14-17 times for anhydrous ammonia and triple superphosphate.

Based on the specific conditions and assumptions in this work, the economic value of nutrients provided by fish in aquaponic systems has been greatly over-stated.

Table 1. Summary of feed/fertilizer cost ratio ($FFCR^{feed}$) for the six feeds.

Nitrogen fertilizer	Phosphorus fertilizer	$FFCR^{feed}$
Ammonium nitrate	Triple superphosphate	8-10
Ammonium sulfate	Triple superphosphate	5-7
Anhydrous ammonia	Triple superphosphate	14-17
Urea	Triple superphosphate	9-12
Urea ammonium nitrate	Triple superphosphate	9-11
Ammonium phosphate	Ammonium phosphate	2-3
Ammonium phosphate sulfate	Ammonium phosphate sulfate	4-6
Diammonium phosphate	Diammonium phosphate	4-6
Monoammonium phosphate	Monoammonium phosphate	2-4

COMPUTATION OF FEED CONVERSION RATIO (FCR^{PLANT}) AND PLANT-FISH MASS RATIO (PFRM) FOR AQUAPONIC SYSTEMS

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To better document the potential impacts of aquaponics, performance metrics need to be clearly defined and relevant to both economic, environmental, and management objectives. For fish, one of the most important performance parameters is the feed conversion ratio (FCR^{fish}= amount of feed supplied/increase in weight of fish). While much less common, it is also possible to define a FCR for plants (FCR^{plant}= amount of feed supplied/increase in weight of plants). This parameter measures how well the wastes from the fish are converted into plant tissue.

In contrast to the FCR^{fish}, a theoretical value of FCR^{plant} for nitrogen and phosphorus can be estimated from (a) the composition of feed and plants, and (b) the nutrient retention values:

$$FCR_N^{plant} = \left[\frac{\mathrm{DM}^{\mathrm{plant}}}{DM^{feed}}\right]\left[\frac{\mathrm{N}^{\mathrm{plant}}}{\mathrm{N}^{\mathrm{feed}}}\right]\frac{1}{\mathrm{NUE}^{\mathrm{plant}}} \tag{1}$$

$$FCR_P^{plant} = \left[\frac{\mathrm{DM}^{\mathrm{plant}}}{DM^{feed}}\right]\left[\frac{\mathrm{P}^{\mathrm{plant}}}{\mathrm{P}^{\mathrm{feed}}}\right]\frac{1}{\mathrm{PUE}^{\mathrm{plant}}} \tag{2}$$

While it is possible to estimate FCR^{plant} for both nitrogen (Equation 1) and phosphorus (Equation 2), there is only one FCR^{plant} value for the system. The element with the largest FCR^{plant} will be the controlling element for the aquaponic system. The largest FCR^{plant} will result in the smallest FCE^{plant}.

Only 50% of the experimental FCR^{plant} values fell within this theoretical range. This analysis indicates substandard performance of the plant components of many aquaponics systems. This reduced performance may be due to (a) low nutrient and micronutrient concentrations, (b) suboptimal pH and temperatures, and (c) build up of salts. It is strongly recommended that the value be reported in all future research.

NURTURING THE SUCCESSFUL GROWTH AND MATURATION OF A DOMESTIC SEAWEED AQUACULTURE INDUSTRY: IDENTIFYING AND REMOVING BARRIERS AND PROMOTING OPPORTUNITIES

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Seaweed aquaculture is an emerging industry in the United States. Several states are actively cultivating seaweed at the commercial or research/investigative scale, however, several significant barriers exist which prevent the expansion of this new industry to meet its potential. Although efforts to address these barriers are currently underway in individual states, progress is slow due to limited resources and no mechanism facilitating an exchange of information. A comprehensive and collaborative effort was necessary to move this emerging industry forward. With the support of federal funds in 2019, the National Seaweed Hub, a partnership of 10 Sea Grant programs and their diverse stakeholders, was established with the goal to support the successful growth and maturation of a domestic seaweed aquaculture industry through sharing of evidence-based, non-advocate information.

Diverse stakeholder-driven work groups were formed based on four common challenges identified through a national needs assessment conducted in early 2020: Market Opportunities, Post-harvest and Processing Infrastructure, Regulations, and Production Systems. Facilitated by Sea Grant Extension and National Sea Grant Law Center staff, work group participants are developing achievable strategies or work plans addressing barriers they determined to be priorities preventing the expansion of domestic seaweed aquaculture. An update of work group progress and work plan developments will be provided and made available on the Seaweed Hub's website (www.seaweedhub.org).

INHIBITION OF FLAGELLAR ASSEMBLY IN THE BIVALVE PATHOGEN *Vibrio coralliilyticus* DURING COMPETITION WITH THE PROBIONT *Phaeobacter inhibens*

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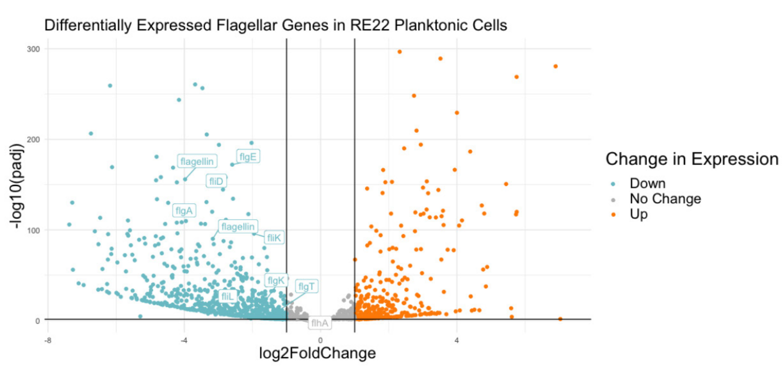
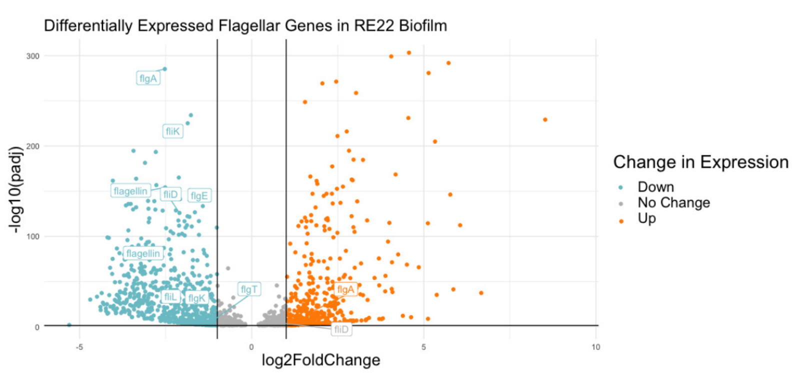
Disease outbreaks in oyster hatcheries can decimate the stock, delay production, and cause supply chain shortages for the expanding oyster aquaculture industry. The daily addition of the probiotic bacterium *Phaeobacter inhibens* S4 (S4) to hatchery tanks has been demonstrated to protect eastern oyster, *Crassostrea virginica,* larvae against bacterial pathogens such as *Vibrio coralliilyticus* RE22 (RE22). Mechanisms of action of S4 against RE22 pathogenicity in oysters are complex, including antibiotic production, formation of thick biofilms, decrease in virulence through quorum quenching, and host immunomodulation. To better understand the ways in which S4 helps protect oyster larvae against vibriosis, a competition assay was performed between S4 and RE22, following which RNA was sequenced and differential gene analysis performed. In addition to downregulation of several virulence factors, analysis of the transcriptomic data uncovered decreased expression of several flagellar protein genes, including flagellin, *flgA, flgE, fliD*, and *fliL,* in RE22 co-cultured with S4. Reduced RE22 motility in the presence of tropodithietic acid (TDA), an antibiotic produced by S4, has been previously reported. However, this transcriptome analysis indicated that this effect of S4 on RE22 motility may also be due to downregulation of disulfide bond family protein genes *dsbA* and *dsbD*. Mutations or deletions of *dsbA* have been linked to decreased virulence and motility in gram-negative bacteria, including other *Vibrio* species. By now including downregulation of flagellar function, this study further highlights the complex ways in which S4 may be able to reduce RE22's devastating effect on oyster larvae, protecting this vital aquaculture commodity.





HYDRAULIC ASSESSMENT OF A D-ENDED TANK PROTOTYPE FOR PELAGIC SPECIES AQUACULTURE USING THE COMPUTATIONAL FLUIDS DYNAMICS METHOD (CFD)

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One of the constraints to developing viable technologies for the cultivation of hydrobiological species is the creation of a culture environment that allows the maintenance of commercially viable production indices. As a result, several authors agree that the water quality in aquaculture will be determined by factors specific to the target species to be cultivated as well as engineering associated with the design of the aquacultural rearing vessel. This study examines and evaluates the hydraulic behavior of seawater in relation to changes in the geometric proportions in the design of D-ended tanks for pelagic species aquaculture in land-based systems. Based on the findings obtained, a prototype D-ended tank for marine fish aquaculture is proposed.

CFD was used to evaluate the hydraulics of two types of aquacultural rearing tanks: a circular central bottom single drain tank (Fig. 1) and a D-ended prototype tank with a central division (Fig. 2). Six depth:diameter ratio and/or width combinations at a constant water volume of 8.4 m^3 were studied, as well as their impact on the response variables of water velocity, water mixing processes, and hydraulic residence time of solid particles. The engineering factors and edge conditions considered in the CFD analysis were based on the domains's spatial discretization using the Finite Element Method (MEF).

In the hydraulic analysis of a given circular tank, no significant changes in the pattern of water circulation were observed as the depth:diameter ratio increased (1:3, 1:5, and 1:10) (Fig. 1). An analysis of different geometries of the D-ended hippodrome tank prototype, on the other hand, allowed inferring that at small diameters, a hydraulic is generated, facilitating the extraction of suspended solids.

Warm colors (Figs. 1 and Fig. 2) indicate high speeds approaching 60 cm * s^{-1}. Speeds in the D-ended tank ranged between 30 and 45 cm * s^{-1}, which is within the range suggested by various authors for self-cleaning purposes (Fig. 2).

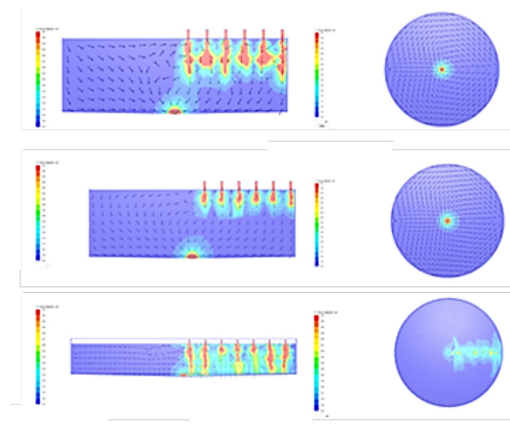

Fig. 1: CFD analysis for tanks with circular geometry

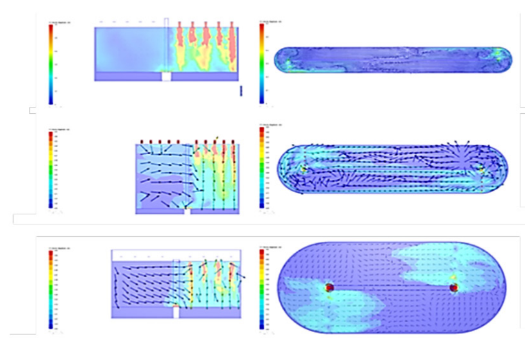

Fig. 2: CFD analysis for tanks with D-ended geometry

NOAA'S OFFICE OF AQUACULTURE SCIENTISTS WILL DISCUSS METHODS AND RESULTS ON THE INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA'S (ICES) WORKSHOP ON PATHWAYS TO CLIMATE-AWARE ADVICE (WKCLIMAD)

Cliff Cosgrove, Michelle Rub

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NOAA's Office of Aquaculture scientists will discuss methods and results on the International Council for the Exploration of the Sea's (ICES) Workshop on Pathways to Climate-Aware Advice (WKCLIMAD). The purpose of the WKCLIMAD was to outline actionable strategies and approaches that can be taken to promote resiliency in fisheries, aquaculture, and ecosystems and scope the next steps for an operational approach, expanding the relevant aspects of climate change that impact management decisions in fisheries, aquaculture and ecosysten.

THE FAO ECOSYSTEM APPROACH TO AQUACULTURE AS AN INTEGRATED PLANNING FRAMEWORK FOR SITING AQUACULTURE IN COMMON PROPERTY AREAS

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What are the keys to success for radical transformation of aquaculture to become a major food source? How do we learn from our failures, evolve, plan better, and invest smarter? Development of aquaculture in common property resources of oceans and lakes in aquaculture's "new geographies" (almost everywhere outside of Asia) involves assessments not only of technological advances to the sector as aquaculture enters a complex social-ecological milieu of existing resource uses, and less-than-fully informed users. Successful aquaculture developments consider not only appropriate technologies, economic viabilities and environmental impacts but ways to advance societies, both rural and urban.

The FAO (2010) Ecosystem Approach to Aquaculture (EAA) is a valuable framework for use by societies worldwide who are questioning aquaculture development in its new geographies. The EAA stands above all aquaculture certification schemes as it is not a binary approval system. Rather, it is a strategy for the integration of aquaculture developments within the wider ecosystem for equity and resilience of interlinked social-ecological systems. The EAA accounts for the complete range of stakeholders, spheres of influences, community and education development, and interlinked processes. Three principles of the EAA are: (1) Aquaculture development and management should take account of the full range of ecosystem functions and services and should not threaten the sustained delivery of these to society, plus advance methods to enhance them, (2) aquaculture should improve human well-being and equity for all relevant stakeholders, and (3) aquaculture should be developed in the context of other sectors, policies, and goals. The tool kit for implementation of an EAA include assessments of: (1) appropriate bioengineering technologies, (2) spatial planning and zoning, (2) carrying capacity, (3) social-ecological capabilities, and (4) governance. The EAA is an especially important planning framework for new aquaculture operations being considered in crowded, common property resources of coastal oceans and lakes in the Large Ocean and Lake Nations of East Africa, the Pacific, and Macaronesia (Azores, Madeira, Canary Islands, Cape Verde). Examples of successful freshwater and marine aquaculture ecosystems developed using an EAA framework and tool kit in both developed and developing economies are discussed for salmonids, carps, tilapias, seaweeds and shellfish. Wider use and adoption of the EAA will advance the growth of sustainable aquaculture developments worldwide and contribute more to the urgent needs for global transformation as contained in the United Nations Sustainable Development Goals (SDGs).

FISH AGGREGATION AT OCEAN AQUACULTURE CAN AUGMENT WILD POPULATIONS AND LOCAL FISHING

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Seafood is an efficient way to provide nutrition for the growing human population in an increasingly resource constrained world. While fisheries management is generally improving around the world, wild seafood catch has remained largely stagnant for several decades and wild populations and biodiversity are increasingly being compromised by climate change and other anthropogenic stressors. Meanwhile, aquaculture is the fastest growing food sector in the world, and ocean spaces are considered the next frontier for aquaculture expansion. As the aquaculture industry continues to develop in marine environments, simultaneously maintaining the health of wild populations and fisheries is critical, both as a backstop for biodiversity and as a food security and livelihood source for millions of people around the world. Given the social and economic importance of wild fisheries, understanding how potentially competing seafood production methods interact will be important to optimize co-management of these closely connected sectors and the persistence of healthy marine ecosystems into the future.

Marine aquaculture impacts wild populations through a myriad of mechanisms, which vary by farm type and farmed species, environment, and wild species characteristics, among other factors. Improved farm level management is decreasing negative impacts of farms through more efficient feeding, replacement of antibiotics and general improved health. Even with optimized farming health, inherent to ocean farms is a certain level of attraction to the area and protection from fishing. Farms attract wild individuals due to novel farm structure in an area that may provide habitat for wild species and excess feed from fish farms, fouling on infrastructure and wastes from cultured product can provide nutritional supplements to wild species. In fact, case studies have documented attraction of wild organisms to farm areas and increased biomass at and around farms. Farming operations often restrict other ocean uses, including wild capture fisheries, either intentionally through directed regulations or by physically obstructing access to fish around the farm.

We used spatially explicit population models to forecast how marine aquaculture might impact wild populations of harvested species and the fisheries they support. Specifically, we explored how farms impact total population abundance and catch, testing the influence of farm design, species movement rates, and direct impacts on fitness. Attraction to farms can increase protections of the farm which benefits population biomass and can improve fishing catches when fisheries are overfished. Our results are intended to inform strategic marine aquaculture design and planning to promote productive marine aquaculture growth, sustainable fisheries, and healthy marine ecosystems simultaneously into the future.

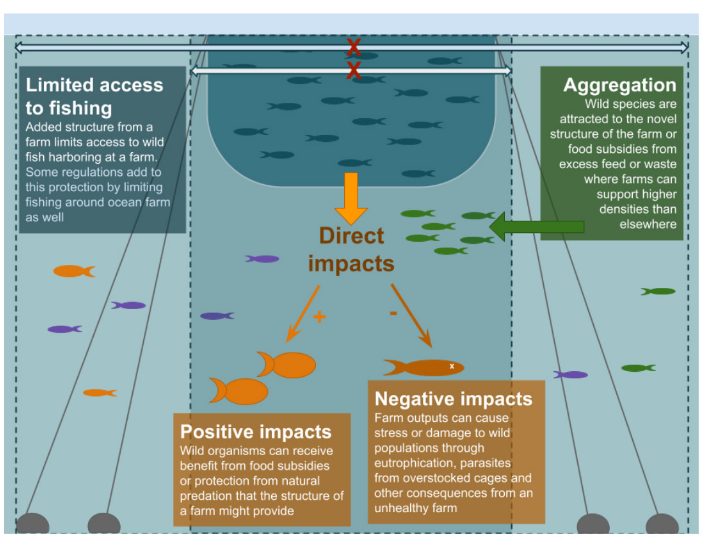

Figure 1: Diagram of marine aquaculture interactions with wild populations and capture fisheries.

GENETIC CHARACTERIZATION OF WILD AND HATCHERY-BRED OLYMPIA OYSTER *Ostrea lurida* SEED USED FOR RESTORATION

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Olympia oysters (*Ostrea lurida*) are the only oyster native to the west coast of the United States and were once abundant in Puget Sound. Intense commercial harvests, habitat loss, and pollution led to collapse of the fishery by 1930. While small remnant populations have persisted in many areas across their historic range, dense core aggregations are extremely rare and thus the focus of several restoration programs across the West Coast. Puget Sound Restoration Fund (PSRF), in collaboration with NOAA and the Washington Department of Fish and Wildlife, produces hatchery-bred juvenile Olympia oyster seed at the Kenneth K. Chew Center for Shellfish Research and Restoration. Juvenile Olympia oyster seed have been out-planted to a number of sites in Puget Sound for restoration.

A primary goal of the shellfish recovery effort is to have minimal negative impacts on existing wild stocks. While hatchery protocols are carefully designed and implemented with the goal of producing seed that mimic the genetic structure of local wild populations, characterizing the genetic makeup of out-planted individuals in relation to local wild populations is extremely important to determine potential impacts on the genetics of the recovering populations. To address this, we are using high-throughput sequencing (RAD-Seq) to produce thousands of genetic markers (single nucleotide polymorphisms (SNPs)) for 3 Olympia oyster populations and their hatchery-reared progeny. Preliminary results, including the development and analysis of SNPs to evaluate various genetic diversity metrics in out-planted seed, will be presented.

IDENTIFICATION OF A NOVEL CALICIVIRUS WITH NUCLEAR LOCALIZATION ASSOCIATED WITH MASS MORTALITIES IN CULTURED WHITELEG SHRIMP (*Penaeus vannamei*)

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As long as the culture of shrimp remains as one of the most profitable sectors in the animal food production industry, the expansion of shrimp culture to new regions and the implementation of innovative culture techniques will continue. This entails movement of shrimp stocks across regions, countries and continents. The transboundary movements of shrimp poses risk of emergence of novel viruses that will restrict shrimp production from time to time, as it has happened in shrimp farming over the past few decades.

Since 2018 unusual mortalities associated with infectious myonecrosis virus (IMNV) have been occurring in several states of Brazil. Initially, we associated these mortalities with a novel strain of IMNV. However, transcriptomic analysis showed the presence of another viral sequence in high abundance.

We characterized the novel virus using a combination of RNAseq and phylogenetic analysis, *in situ* hybridization, PCR, qPCR and histopathology. The full-length genome of the novel virus is ~10.4 kb and consist of +ssRNA genome. The genome encodes for one large open reading frame (ORF) with putative domains for a helicase, RdRp, Calicivirus coat protein, G-patch and Kinase (Figure 1A). Phylogenetic analysis using the RdRp places the novel virus as member of the *Caliciviridae*. Further phylogenetic analysis using the Calicivirus coat protein show that the virus does not belong to any of the know genera of the *Caliciviridae*.

In situ hybridization shows that the virus is localized in the nuclei of the epithelial cells of the hepatopancreas (Figure 1B), stomach and can be observed in the lymphoid organ and muscle tissue. To our knowledge this is the only RNA virus of shrimp to infect the nuclei.

We have provisionally called this virus as "*Penaeus vannamei* calicivirus" (PvCV).

The *Caliciviridae* are well known pathogens causing a wide range of syndromes. Recent additions to the *Caliciviridae* are the genera *Minovirus* and *Salovirus* that infect marine fish. The PvCV will be the most recent addition to the family. Finally, a publication is being prepared to report the diagnostic techniques to help mitigate the spread of the virus and contribute to its control.

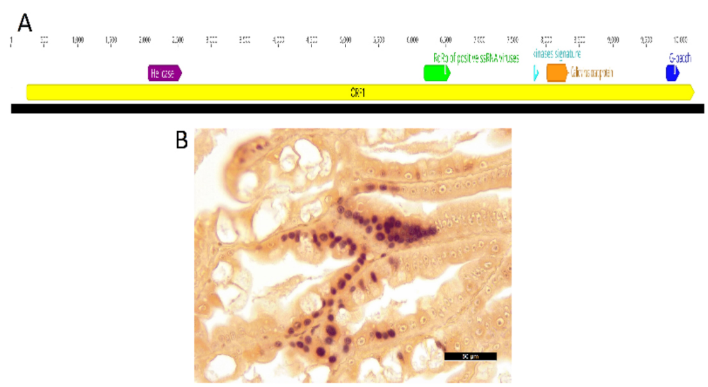

Figure 1. (A) Genome organization of PvCV showing the putative domains for a helicase (purple), RdRp (green), Calicivirus coat protein (orange), G-patch (Blue) and Kinase (light blue). (B) *In situ* hybridization showing a positive signal in the nuclei of the cells of the hepatopancreas.

MAKING SPACE FOR MARICULTURE WHILE LIMITING IMPACTS ON FISHERIES: CRITICAL ISSUES AND OPPORTUNITIES

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There is growing interest and support for expanding mariculture to complement fisheries' seafood production. But neither ocean farming nor fishing can occur just anywhere, and the space needs for both are complex. Thus, integrating new mariculture activity with ongoing fisheries activities requires particular attention to their respective needs for space. Current mariculture development processes and associated mapping efforts fall short.

Through discussions with agency staff, fishermen and mariculturists, we identified information gaps and needs for improving the understanding of space use needs. We found a general lack of knowledge about 1) the diversity of fisheries, 2) interpretation of fisheries data, including recognition of the usefulness and limitations of the data, 3) key fisheries information needed to inform the mariculture development process and ways to obtain and synthesize it, and 4) effective ways to engage fishing communities and integrate information about their space uses and needs in the process. We also identified key issues (Table 1) and potential solutions (Table 2) for addressing them.

Based on our findings, we developed an interactive StoryMap website, *Sharing Ocean Space to Enhance Seafood Production*, which includes: 1) Introduction to the Issue, 2) Current Processes, 3) Mapping Efforts, 4) Mapping Fisheries, 5) Beyond Mapping, 6) Improving the Process and 7) More Info. Using California's Santa Barbara Channel as an example, we illustrate how mapping can be useful and provide information sheets about the limitations and considerations required when using fisheries data. We provide examples and tools for developing fisheries profiles to help build understanding of fishery space needs to better inform mariculture site selection. We also describe the issues and potential solutions and identify additional sources of information. Sharing ocean space can be challenging, but a process based on improved understanding and engagement – a key aim of the website – will facilitate continued and enhanced seafood production.

Table 1. Issues with Current Process
Limited capacity (knowledge, expertise, time, funding)
Reliance on existing spatial data
Limited use of local knowledge
Requirements of the applicants
A lack of understanding of ocean space uses
Limited consideration of scaling and feasibility
Cumulative loss of ocean space for fishing

Table 2. Potential Solutions to the Issues
Improved capacity and communications
Establishment of mariculture & fisheries Liaison
Enhancement of public comment process
Enhanced access to information & expertise
Integration of a proof of concept approach
Consideration of repurposed & multiuse sites

WHERE DO DOUBLE CRESTED CORMORANTS *Phalacrocorax auritus* GO AFTER ROOST HARASSMENT

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The Wildlife Services-National Wildlife Research Center's Mississippi Field Station working in conjunction with Alabama WS operations conducted 49 harassment events on Double-crested cormorant (DCCO) night roosts. Approximately 77,225 DCCO (97 %) were dispersed using a combination of non-lethal and lethal harassment techniques.

We fit a sample of DCCOs with GPS transmitters to monitor their movements relative to night roost harassment. We deployed a total of 26 transmitters from January-February 2020 and November 2020 to February 2021, of which 17 produced more than 1 month of location data. We have gathered data encompassing 25,521 discrete DCCO locations over a total of 2,741 bird days in 2020- 2021.

Six GPS-DCCOs were present at the night roosts during roost harassment events during February-March 2021 and one DCCO was present at the night roost harassed in early December 2020. These seven DCCO used 10 or more nights roosts during the two weeks prior and after roost harassment. We calculated the daily average and maximum distance traveled from the harassed roosts two weeks before and after the harassment event.

After harassment we found that 3 of the 7 DCCOs increased their daily distance from the harassed roost and foraged in different areas, containing other catfish ponds, than those prior to roost harassment. Four out of 7 DCCO did not increase their daily distance after harassment and foraged in the same areas before and after harassment. We plan to deploy 20 additional GPS transmitters from November 2021-February 2022 to increase our sample size and more accurately assess the impacts of night roost harassment on the night roost use, foraging areas, and daily movement.

EFFECTS OF CLIMATE CHANGE ON ATLANTIC SURFCLAM, *spisula solidissima solidissima*, LARVAL BEHAVIOR: SURVIVAL, GROWTH, AND DISPERSAL IMPLICATIONS

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As carbon emissions increase, coastal waters are experiencing climate change impacts including ocean warming (OW) and decreases in pH via ocean acidification (OA). Supporting a multi-million fishery in the western Atlantic, the Atlantic surfclam, *Spisula solidissima solidissima* has demonstrated sensitivities to climate change phenomena; however, potential synergistic effects and behavioral responses relevant to larval transport have yet to be investigated. Specifically, if climate change induces larval behavioral changes, the time and space in the water column that larvae occupy will change, which may affect dispersal patterns. This study used laboratory experiments to measure surfclam larval mortality, growth, swimming behavior and pelagic larval duration (PLD) (i.e. time to settlement) in response to projected OW and OA scenarios using a experimental design that assessed both main and interactive effects. While mortality did not differ between treatments, growth increased under OW and was reduced under OA. While the percent of swimming larvae did not differ between treatments, OW yielded lower swimming speeds. Furthermore, OW but not OA reduced PLD. These results suggest that while projected climate change scenarios may not increase larval mortality, development and behavior relevant to transport may be altered, thereby affecting dispersal patterns and settlement success. Specifically, larvae with a shorter PLD and that swim slower and therefore spend less time in the surface mixed layer may experience reduced dispersal distances. Scaling up these behavioral changes to larval transport patterns may provide insight regarding projected spatial changes in settlement success and recruitment.

EFFICACY OF TWO DISINFECTIANT TECHNOLGIES FOR USE IN POST-HARVETS TREATMENT OF OYSTERS

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It is imperative that aquaculturists can continue to provide consumers with safe seafood as the industry grows and adapts to a changing climate. Oyster aquaculture is particularly vulnerable to the intersection of seafood safety and climate change. Harvested oysters are at risk for carrying enteric bacteria and viruses that can make consumers ill, notably from *Vibrio* spp. bacteria. The prevalence of *Vibrio* spp. is expected to rise with increasing water temperatures. While post-harvest processing approaches currently employed to reduce risk of contaminated oysters are effective in reducing pathogens, they have specific drawbacks for growers, including requiring high levels of capital to gain access to treatment equipment, poor consumer reception of treated products, and death of treated oysters.

As part of my thesis research, I aim to demonstrate efficacy of two new technologies, individually and combined, in removing pathogens from oyster tissues and aquaculture system water leading to a post-harvest treatment that is more effective than traditional depuration, has low oyster mortality and is cost-effective for growers. This will be accomplished through a series of controlled experiments utilizing both technologies. The first technology is a modified photocatalytic oxidation process which produces ozone and other free radicals, which are incorporated into the experimental system water through the air supply. Output from this technology is being quantified using the indigo method to measure dissolved ozone. The second technology creates nanobubbles using magneto-chemistry and is plumbed in-line with the filtration of treatment tanks. The free energy that is generated by this technology will be measured in millivolts using a multimeter.

The first step and focus of this presentation is the calibration of the two disinfection technologies and determination of their respective disinfection potentials in our experimental systems. These measurements will allow for the determination of a base level output for each technology. Once the base output is determined, conditions within the experimental systems will be manipulated (water flow, temperature, salinity, and volume) so that disinfection potential under a variety of conditions can be determined. It is expected that temperature, water flow and system volume will have an effect on the output of the technologies.

IMPROVED FISHERY INDEPENDENT SAMPLING OF GAPER CLAM (*Tresus capax*) POPULATIONS IN OREGON ESTUARIES USING AN ADAPTIVE CLUSTER SAMPLING DESIGN

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The gaper clam (*Tresus capax*) is a common bivalve species in Oregon estuaries which supports a thriving intertidal recreational fishery and a subtidal commercial dive fishery. Management of these fisheries is dependent on the extensive fisheries-independent surveys of gaper clam and other bay clam populations conducted by the Oregon Department of Fish and Wildlife (ODFW). Gaper clams occur in highly aggregated populations and tend to be undersampled in the stratified-random sampling (SRS) design currently used by ODFW. This can lead to inaccurate stock estimates of gaper clam populations and a misrepresentation of the spatial distribution of this species.

To address these issues, a two-part field study was conducted in Yaquina Bay, Oregon during 2020 and 2021. The first part of the study compared the SRS method to two adaptive sampling approaches: Two-Stage Sampling (2S) and Adaptive Cluster Sampling (ACS). The gaper clam populations at the study sites were highly aggregated and spatially discrete. The ACS approach provided the best representation of the spatial distribution of gaper clams at the study site relative to the SRS and 2S sampling methods. The ACS method also had an almost 7-fold decrease in variance relative to the SRS method and the most accurate population biomass estimates. The trade-offs for using the ACS approach are sampling uncertainties and a larger sample size which can create logistical challenges for large sampling programs. One of the issues with the comparison between the SRS and ACS methods was whether the improved variability and accuracy in biomass estimates was due to the larger number of sites sampled in the ACS method rather than the sampling approach.

The second part of the study explicitly tested whether increasing the sample size of the SRS would provide comparable estimates of population biomass to the ACS method. The initial field study was repeated in 2021 using the ACS and SRS sampling designs. The increased sample size of the SRS design did improve the variability of the biomass estimates relative to the first year of the study. However, the SRS method underestimated the mean biomass of the gaper clam population relative to the ACS method. In both studies, the ACS design provided a significant improvement in variability for gaper clam population biomass and consistently captured the spatial extent of the clam populations at the study site.

In summary, ACS decreases variability in population density and biomass estimates that can be used to improve management of gaper clam fisheries. The higher sampling intensity of ACS limits its incorporation into the extensive fisheries-independent sampling used by ODFW but can complement the standard SRS methods with focused ACS sampling on regions of the estuary with known gaper populations and/or fishing effort.

COMPARISON OF SURVIVAL, GROWTH AND PLOIDY OF KOI CARP, TRIPLOID KOI, AND TRIPLOID HYBRIDS OF KOI FEMALE WITH KOI X GOLDFISH MALE

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Hybridization of common carp and goldfish was first investigated by a Japanese scientist, Matsui Yoshiichi in 1918, which inspired numerous lines of research conducted in Japan, China, Russia, Hungary and Poland. First generation of hybrid males were described as sterile. Nina Cherfas in Russia then discovered in 1980 that hybrids of common carp and goldfish are somatic diploids, but produced diploid (2n) gametes. This phenomenon, premeiotic endoreduplication, provides a direct opportunity for implementing sterility in backcross progeny (triploids) and may serve as an ideal candidate for exploring hybrid growth and metabolic (resistance to hypoxia) advantages as well as biocontrol.

We tested 6 males of first generation Koi (female) x *Carassius auratus* (male) hybrid (H) and obtained motile sperm following hormonal stimulation, although sperm density was low and highly variable compared to koi sperm. We then carried out single spawning where eggs were divided to obtain control purebred koi (K), koi subjected to cold shock (1-2^{o}C of 45- or 90-min duration) (CS), and koi female backcross with H males. We confirmed through these experiments, Gomelsky's conclusion that fertile H males do occur, though rarely. Fertile H males produced diploid sperm, and when crossed with K females, the resulting progeny were 100% triploid, characterized by high viability and excellent, uniform growth. CS 45 min fish were all triploids; however, they had very variable individual growth rate, while the CS 90 min group suffered high mortality and consisted of both triploid and diploid individuals.

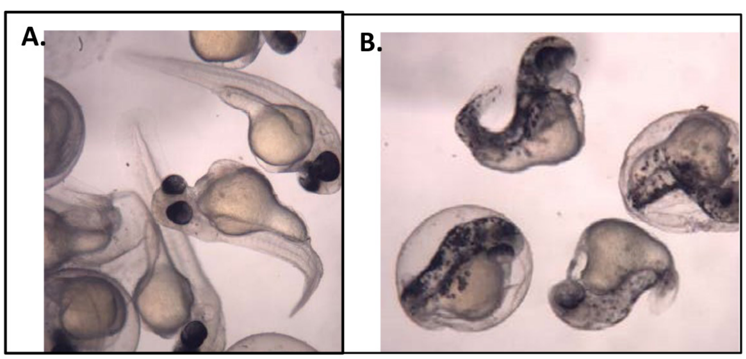

Fig. 1: A) Casper strain B) Brahmaputra R. strain haploids at hatching.

COMPARISON OF GYNOGEN SURVIVAL, GROWTH AND SEX RATIO IN WILD (Brahmaputra River, Bangladesh) AND DOMESTICATED "CASPER" STRAIN OFZEBRAFISH

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Hybridization of zebrafish (*Danio rerio*, female) and common carp (*Cyprinus carpio*) results in the production of non-viable hybrids, and insemination of zebrafish eggs with UV-irradiated sperm of common carp generates haploids (Delomas and Dabrowski, 2016). However, an exception to this can occur where UV-irradiated carp sperm induces spontaneous diploidization of maternal chromosomes (SDM; Delomas & Dabrowski, 2017) without induction of a physical shock. We have used gynogenesis to validate the assumption that the sex determination system in zebrafish is polygenic as the result of domestication (Casper strain) in comparison to wild populations obtained from Bangladesh (Brahmaputra River, "Brahma"). We have carried out insemination of zebrafish eggs from domesticated (Casper) and wild (Brahma) strains with UV- irradiated koi carp sperm and then subjected them to a 41°C heat shock at 13 min post insemination for 2 min duration to induce diploidization. Survival of heat shocked gynogens and non-shocked control (presumed haploids) groups at 24 hours post insemination (26 somite) amounted to 20.1 and 57.9% (Casper) and 21 and 25.4% (Brahma), respectively. Flow cytometry analysis performed upon hatching confirmed 95% and 100% haploidy in non-shocked groups of Brahma and Casper strains, respectively. Haploid Brahma individuals exhibited severe deformities i.e. "haploid syndrome", in comparison to those of Casper (Fig. 1). Flow cytometry analysis confirmed 100% induction of diploidy in heat-shocked Brahma groups, while heat-shocked Casper groups exhibited 96.6% diploid individuals and 3.4% mosaics (n and 2n). Fish will be raised to maturity and sex ratio will be determined.

Dabrowski K., Miller, M.E. 2018. Zebrafish 15: 295-309. Delomas, T.A., Dabrowski, K.2016. Biol. Lett. 12: 20160628.
Delomas, T.A., Dabrowski, K. 2017. LARVI Symposium, Ghent, Belgium

COMMERCIAL PROTEASE DOES NOT IMPROVE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* GROWTH, FEED CONVERSION RATIO, OR SURVIVAL WHEN CULTURED IN LOW SALINITY WATER

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Pacific white shrimp *Litopenaeus vannamei* are raised in low salinity waters in west Alabama. Therefore, methods to improve growth and immune response to improve production and profitability are of interest to commercial producers. These studies examined the use of a protease (AG-175) in two different protein diets for shrimp. The first study evaluated three diets including a 32% crude protein diet with AG-175 added to the mixture and extruded, a 32 % top coated onto extruded feed, and a 32% protein feed in which no AG-175 added. This 6-week study was carried out by stocking 20 shrimp, mean initial weight 0.366 g ± 0.001, in an on-levee tank system with 12 800-L tanks installed adjacent to a shrimp production pond on a commercial farm in west Alabama. The shrimp were offered three different 32% protein feeds and all treatments were quadruplicated. The second study used the identical 32% diets and 28% diets with AG-175 incorporated as mentioned for the 32% (6 diets, 4 replicates). All diets were evaluated regarding shrimp growth, feed conversion, and immunological parameters. These parameters included cell counts, enzymatic activity, and protein content in Pacific white shrimp. In this study fifteen shrimp were stocked, mean initial weight 0.10 g ± 0.0006, into 24 75-L aquaria that were part of a recirculation system consisting of a sand filter, biofilter, and sump (3900 L total system volume) at the Alabama Fish Farming Center. This study lasted six weeks. No significant differences in growth, FCR (Figure 1A,B), or survival occurred for any of the diets tested in these studies. Immunological parameters are being analyzed and will be reported. Based on these preliminary findings, AG-175 does not appear to improve shrimp production in low salinity waters.

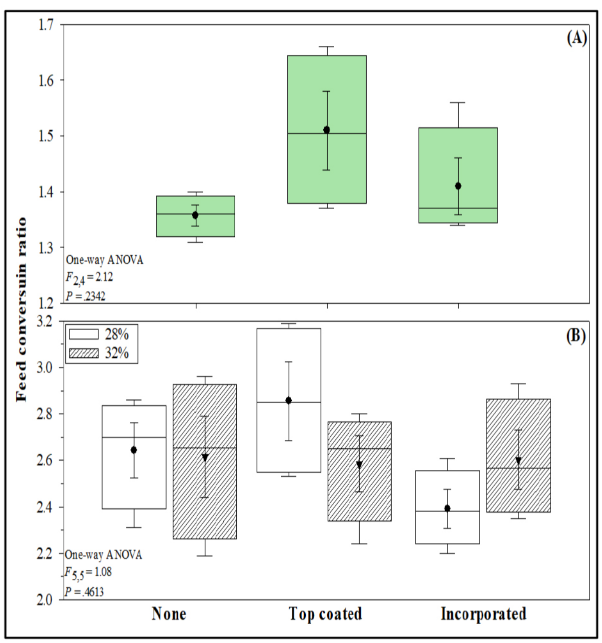

Figure 1. Feed conversion ratios for Pacific white Shrimp fed 32% protein diet (A) and 32% or 28% diet (B) containing 0 or 196 g of AG-175/tonne, either top coated or incorporated into the diet.

REAL TIME WATER QUALITY MONITORING IN BRAZILIAN HYDROELECTRIC RESERVOIRS FOR PREVENTION OF ENVIRONMENTAL RISKS TO CAGE AQUACULTURE

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Cage aquaculture in hydroelectric reservoirs relies on good limnological conditions for efficient production and high-quality products. One of the most relevant limnological characteristics for the water quality at these sites is the water column stratification, which can lead to bottom anoxia and stimulate cyanobacterial blooms, producing toxic metabolites with potential risks for fish farming. In this study the vertical structure of water column in aquaculture areas of the Nova Avanhandava reservoir was studied using a multiparametric probe, which allowed to access real time, high resolution vertical profiles using electronic devices including data on phytoplankton abundance and distribution.

These profiles enabled to evaluate vertical patterns of thermocline, dissolved oxygen, chlorophyll and phycocyanin. Objectives of this work was to understand the occurrence of thermocline associated with bottom anoxia and the vertical distribution of phytoplankton in reservoirs. Three aquaculture areas were monitored from 2014 to 2016, sampling inside each farm, upstream and downstream. Relationship of climate and water outflow upon stratification were also evaluated, as well as the applicability of *in situ* fluorometry for monitoring cyanobacteria. Results showed the predominance of stratification conditions with bottom anoxia in all sampling seasons for at least one sampling site. Maximum values of pigments in subsurface layers were common, indicating underestimation by surface water samplings, traditionally used for the trophic state evaluation of aquatic environments, as chlorophyll maximum frequently was registered in subsurface layers. The stratification strength was variable and we had no evidence that hydrological regime, air temperature, and precipitation influenced on the thermal stratification and bottom anoxia. Thus, the use of real time water monitoring technology proved to be a useful and important tool to follow the changes in the vertical structure of reservoirs. Sites with high oxygen in surface frequently had bottom anoxia extended several meters up in the water column, posing severe risks to cage aquaculture. Assessing vertical distribution of oxygen and phytoplankton lead to proper evaluation of environmental risks for cage aquaculture in large hydroelectrical reservoirs.

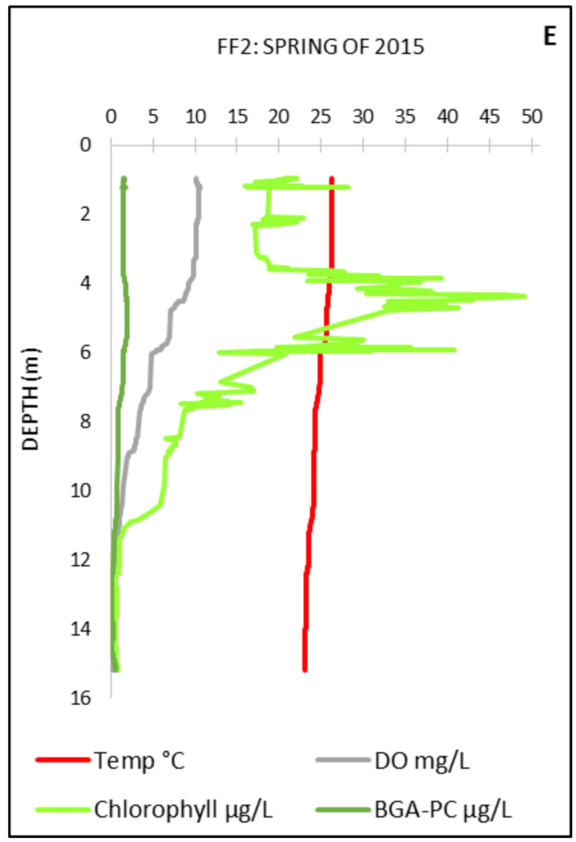

Figure 1: Vertical structure profiles of Temperature (Temp °C), Dissolved Oxygen (DO mg/l), Chlorophyll (µg/L) and Phycocyanin (BGA – PC µg / L).

OFF-FLAVOR OBSERVATIONS: FACTORS INFLUENCING ACCUMULATION IN RAS AND NEW INFORMATION ON GEOSMIN TASTE-TRESHOLD IN ATLANTIC SALMON *Salmo salar* FILLETS

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Off-flavor compounds, geosmin (GSM) and 2-methylisoborneol (MIB), bioaccumulate in Atlantic salmon *Salmo salar* and other species produced in recirculating aquaculture systems (RAS) causing earthy/musty taints that are unacceptable to consumers and an increased likelihood of economic loss due to rejected products. Improved knowledge of environmental dynamics that inhibit off-flavor in land-based systems would help to ensure that edible products are off-flavor-free and could lead to solutions to this critical industry challenge.

Observations from two Atlantic salmon studies recently improved our understanding of variables that could influence off-flavor accumulation in RAS. During these trials, increased GSM and/or MIB production was attempted via discontinued system cleaning, first in eight identical partial reuse systems (PRS; Study 1) and later in six replicate RAS with fluidized sand biofilters (Study 2). Partial reuse systems were pre-disinfected by recycling 200 mg/L hydrogen peroxide, while microbially mature RAS that had been operated for >1 year without shutdown were utilized during the second study. Time-series water samples were collected, at minimum, every other week and shipped overnight to a contract laboratory for analysis. Further, salmon fillets (Study 1) containing 211-231, 305-319, and 432-467 ng/kg GSM were tested against control fillets (43-103 ng/kg GSM) by an untrained taste panel to determine if differences could be detected.

Average waterborne GSM increased from 4.6 ± 0.5 ng/L to 15.5 ± 1.3 ng/L in replicate PRS and reached a maximum level of 20.5 ng/L after one month without cleaning, but MIB was rarely detected. Conversely, GSM and MIB levels measured in replicate RAS did not increase beyond 3 ng/L for two months despite the lack of cleaning. Additional attempts to increase off-flavor levels were largely unsuccessful over the next two months, including discontinued use of an ultraviolet light treating makeup water, increased water flushing, sand transfer from a RAS with higher off-flavor levels, and on/off cycling of pumps to disrupt microbial biofilms in the fluidized sand biofilter. Moreover, sensory analysis showed that participants detected a difference in salmon fillets with 432-476 ng/kg GSM vs. control fillets; however, qualitative remarks did not separate earthy/muddy off-flavor as the direct cause.

These studies indicate that GSM and MIB production may be influenced by microbial maturity, water flushing rate, and/or the presence of a fluidized sand biofilter. This work also shows that PRS are not exempt from off-flavor accumulation. Additional research is needed to understand the microbial dynamics related to these conditions. Although new information was gleaned relative to GSM taste threshold, a trained taste panel is still required to determine if consumers with sensitive palates can detect low-level GSM and MIB in RAS-produced Atlantic salmon.

A FISHERS OPERATED PILOT-SCALE QUEEN CONCH *Aliger gigas* HATCHERY AND NURSERY FACILITY FOR RESTORATION AND SUSTAINABLE SEAFOOD SUPPLY IN PUERTO RICO

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The queen conch, an important fishery species in the Caribbean, has been overfished. The conch 'carrucho' fished in Puerto Rico are consumed locally. With the decline in populations in the Puerto Rico state and federal waters, closed seasons, and disruption of conch habitats from hurricanes, conch is a prime aquaculture candidate species.

The project goal (S-K NOAA Award NA19NMF4270029) is to assist with restoration of the queen conch fishery in Puerto Rico by producing 2,000 conch per year in a fishers-operated pilot-scale hatchery and nursery facility. In June 2021, the conch hatchery and seawater system were completed at the Naguabo Commercial Fishing Association (Fig. 1). The fishers collect up to three small sections of conch egg masses every one to two weeks during their fishing trips. In the hatchery, the egg masses incubate in a recirculating saltwater system. On the fourth day, each egg mass hatches in a 68-L larval tank. The conch veligers (larvae) are cultured for 21-25 days and fed microalgae (*Isocrysis galbana* and *Chaetoceros gracilis*). In July 2021, the first culture of larvae successfully metamorphosed in shallow trays in a recirculating tank system. Detrital seagrass blades were used as the metamorphic cue and the conch were provided with flocculated *C. gracilis* food. Multiple batches of larvae were raised in the Puerto Rico, Naguabo Queen Conch Hatchery from June to November 2021. The recirculating juvenile nursery tank system is designed to culture conch on sand substrate. At this stage the conch will be fed a gel-diet for 12-months prior to release in nearby seagrass beds. This project serves as a model that can be transferred to other fishing communities in Puerto Rico and elsewhere in the Caribbean.

Figure 1. Puerto Rico, Naguabo Queen Conch Hatchery located at the Commercial Fishing Association.

SEA VEGETABLES GROWN IN AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM IN FLORIDA

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Halophyte plants also known as sea vegetables have historically been foraged along many coasts for consumption and are now grown as crops, in places such as the Netherlands, Israel, Hawaii, and South Carolina. Sea vegetables are a desirable, environmentally sustainable crop since they are a carbon sequestering plant, remove excess nutrients from coastal ecosystems or aquaculture systems, can handle extreme conditions, require zero freshwater, and their salt content makes them more resistant to pests and diseases. FAU Harbor Branch has been growing sea vegetables for human consumption in studies since 2014. The plants utilize dissolved nutrients from the production of fish and shrimp in an Integrated Multi-Trophic Aquaculture (IMTA) system. In the past three years we have investigated the growth and nutritional value of three cultured sea vegetables: sea asparagus (*Salicornia bigelovii*), sea purslane (*Sesuvium portulacastrum*), and saltwort (*Batis maritima*) (Fig. 1). The studies are focused on ways to optimize the culture of the species and the trials have included growing these species in different substrates (sand, clay pebbles, and raft) and during three seasons (winter, spring and summer). Results show that the preferred substrate depends on the plant species and all species can be grown year round in central Florida. They are also low in caloric value; fat-free; and provide a natural source of protein, fiber, minerals, vitamins, and iodine. Sea vegetables grown in Florida have the potential to be introduced into the domestic food industry, targeting restaurants as well as households. This will generate a market for this new aquaculture crop with economic and nutritional benefits.

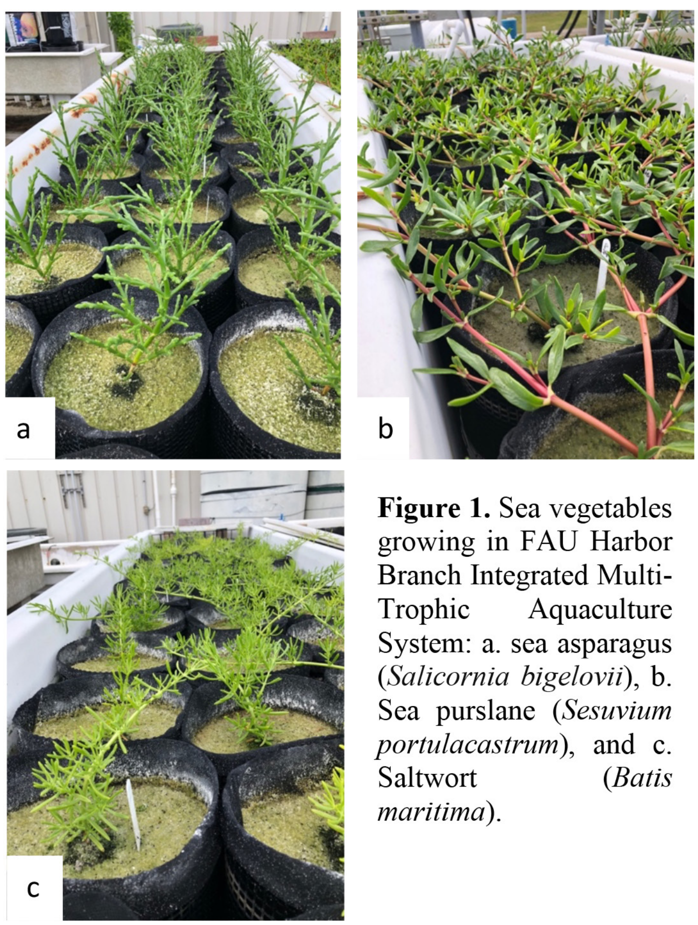

Figure 1. Sea vegetables growing in FAU Harbor Branch Integrated Multi-Trophic Aquaculture System: a. sea asparagus (*Salicornia bigelovii*), b. Sea purslane (*Sesuvium portulacastrum*), and c. Saltwort (*Batis maritima*).

ECONCH: ELEARNING FOR GROWING QUEEN CONCH A*liger gigas*

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The Queen Conch Lab is an aquaculture, conservation, research, and education program at Florida Atlantic University (FAU) Harbor Branch Oceanographic Institute. The program works with partners on community-based queen conch projects across the Caribbean. Culturally important, queen conch are prized for their pink shells and nutritious meat. Communities depend on conch as a source of food and income. As grazers, they play a key ecological role in seagrass habitats. Intense fishing pressure and habitat loss have resulted in declining queen conch populations. In 2020, the Queen Conch Lab established the 'eConch: eLearning for Growing Queen Conch' initiative as a strategy to address requests from Caribbean communities for information on how to grow conch for restoration, conservation, and sustainable seafood purposes. 'eConch' is an interactive online course being developed in partnership with FAU Center for Online and Continuing Education. The syllabus will feature eight modules that include video content, live virtual presentations, activities, and group discussions designed for a broad audience. This concept emerged based on findings from two focused planning sessions with site partners throughout the Caribbean that have signed up to beta test the course in 2022. The success of this initiative will be the implementation of a well-tested eLearning course on the cultivation of queen conch that can be offered to additional participants in the Caribbean. Wide-ranging distribution of this knowledge will benefit the species, the ecosystem, and the communities that depend on the fishery.

Figure 1. Map showing partner sites.

MACROALGAE CULTIVATION MODELING SYSTEM (MACMODS): ASSESSING OFFSHORE MACROALGAL FARMING AS A CONTRIBUTOR TO ENERGY SECURITY AND CARBON SEQUESTRATION

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Offshore macroalgal farming is being assessed for its potential as a biofuel resource and as a strategy for carbon sequestration. Yet, scalability and siting are still under evaluation. We have developed a framework to site and estimate offshore macroalgal harvest potential by simulating farm growth from oceanographic inputs coupled to a dynamic macroalgal growth model. We will demonstrate the utility of this tool for informing farm design and siting through simulations of *Macrocystis pyrifera* cultivation along the California coast, as well as at the global scale to evaluate the potential of seaweed-based CDR.

FROM FIELD TO PLATE: AGRICULTURAL PESTICIDE PRESENCE IN THE GUAYAS ESTUARY (ECUADOR) AND COMMERCIAL MANGROVE CRABS

Andreé De Cock*, Marie Anne Eurie Forio, Niels De Troyer, Isabel Garcia Arevalo, Arne Deknock, Wout Van Echelpoel, Lenin Riascos Flores, Jasmine De Rop, Liesbeth Jacxsens, Pieter Spanoghe, Luis Dominguez Granda, Peter L.M. Goethals

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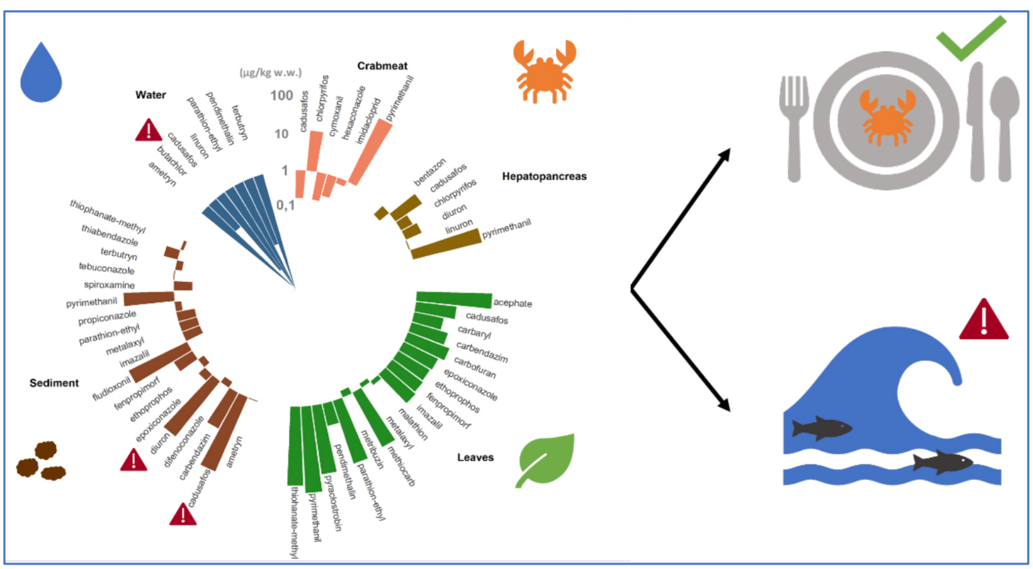
Mangroves are unique coastal ecosystems, located in tropical and subtropical regions. Yet, the functioning of these essential ecosystems is threatened by the presence of pollutants, including pesticides originating from agricultural activities. We investigated pesticide residues in the Guayas estuarine environment, since agricultural activities rapidly increased in the Guayas river basin over the past decades. A multi-residue analysis involving a selection of 88 pesticides was performed on the white meat and the hepatopancreas of the red mangrove crab (*Ucides Occidentalis*) at 15 sampling sites within the Guayas estuary along with water, sediment, and leaves samples.

We found that 35 active compounds were present in the Guayas estuary, of which pyrimethanil was most detected and had the highest concentrations in almost all compartments. Also, cadusafos was present in all studied compartments of the Guayas mangrove system and several prohibited pesticides (including carbendazim, carbofuran, and parathion) were detected. An ecotoxicological and probabilistic consumer risk assessment pointed out that current butachlor, carbendazim, and fludioxonil concentrations can cause adverse effects in aquatic organisms in the long term. Moreover, high potential acute and chronic risks of cadusafos residues on aquatic invertebrates and of diuron on algae in the Guayas wetlands were observed. Still, the exposure results indicated that the health risk for the consumers of the commercial red mangrove crab is low concerning cadusafos, chlorpyrifos, diuron, linuron, and pyrimethanil residues in crab tissues. The findings presented in this research can provide a useful basis for local water managers and environmental conservation groups to act and reduce the usage of pesticides, to avoid threatening aquatic and human health.



FROM MANGROVE TO FORK: METAL PRESENCE IN THE GUAYAS ESTUARY (ECUADOR) AND COMMERCIAL MANGROVE CRABS

Andreé De Cock*, Niels De Troyer, Marie Anne Eurie Forio, Isabel Garcia Arevalo, Wout Van Echelpoel, Liesbeth Jacxsens, Stijn Luca, Gijs Du Laing, Filip Tack, Luis Dominguez Granda, Peter L.M. Goethals

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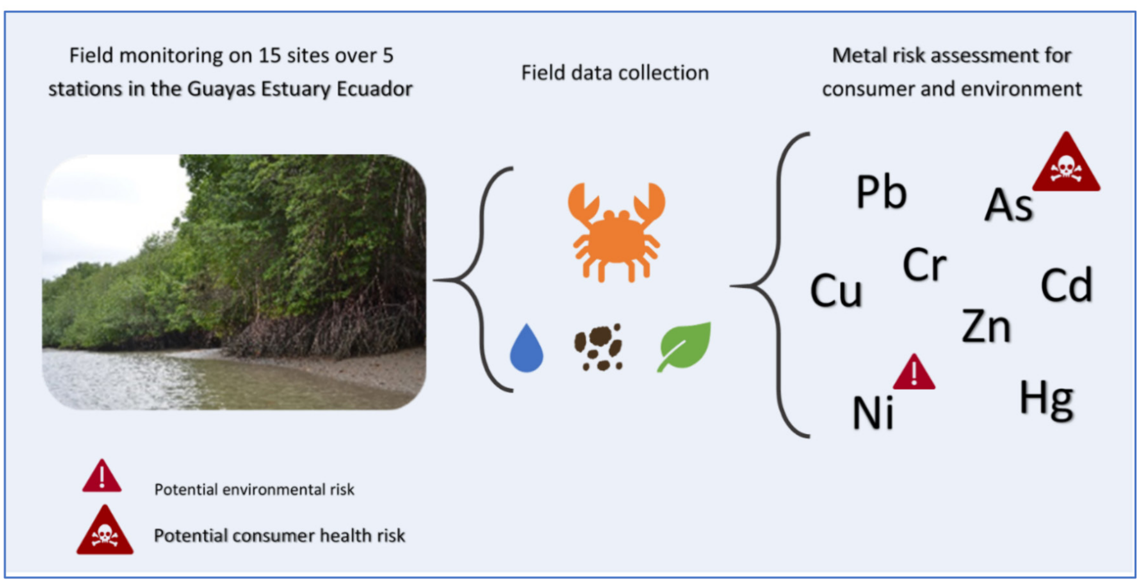
Mangrove wetlands provide essential ecosystem services such as coastal protection and fisheries. Metal pollution due to industrial and agricultural activities represents an issue of growing concern for the Guayas River Basin and related mangroves in Ecuador. Fisheries and the related human consumption of mangrove crabs need scientific support. To protect human health and aid river management, we analyzed several elements in the Guayas Estuary. Zn, Cu, Ni, Cr, As, Pb, Cd, and Hg accumulation were assessed in different compartments of the commercial red mangrove crab *Ucides occidentalis* (hepatopancreas, carapax, and white meat) and the environment (sediment, leaves, and water), sampled at fifteen sites over five stations.

Consistent spatial distribution of metals in the Guayas estuary was found. Nickel levels in the sediment warn for ecological caution. The presence of As in the crabs generated potential concerns on the consumers' health, and a maximum intake of eight crabs per month for adults is advised. The research outcomes are of global importance for at least nine Sustainable Development Goals (SDGs).

The results presented can support raising awareness about the ongoing contamination of food and their related ecosystems and the corresponding consequences for environmental and human health worldwide.



UNDERSTANDING OF THE SHRIMP IMMUNE RESPONSE TO PATHOGENS FROM ITS TRANSCRIPTOME

Marcos De Donato*, Carlos Fajardo, Hectorina Rodulfo, Acacia Alcivar-Warren, Ashutosh Sharma

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According to GLOBEFISH, the global shrimp and prawn trade is estimated at USD 28 billion per year, representing one of the most important sectors in aquaculture industry, creating large number of jobs and being a key factor for economic development in tropical regions. The main challenge facing the sector is the incidence of viral and bacterial pathogens which has caused outbreaks throughout the world with large economic impact in production and profitability. Thus, there is the need to generate more knowledge that could be used to design strategies to prevent or treat infections that have been greatly affecting the shrimp industry. Genomic resources have been developed for *Penaeus vannamei*, *P. chinensis*, *P. japonicus* and *P. monodon*, with BUSCO completeness analysis higher than 93%, estimating the number of genes around 25-30 thousand. However, the high content of repetitive sequences found in shrimp genomes makes it difficult to build highly contiguous assemblies as well as to be able to annotate the genes to their full length. On the other hand, more than 1,500 RNA-Seq data sets have been published to date in the SRA database of NCBI. Most of the RNA-Seq experiments have used muscle, hemocytes, hepatopancreas and gills. Important processes related to the immune system have been identified in studies of penaeid shrimps when challenged by viral and bacterial pathogens, including those related to hemocytes function: autophagy, phagocytosis, antimicrobial peptides (AMPs) production, and the pro-phenoloxidase system. Specific components were identified, such as transglutaminase and lysozyme, alpha 2-macroglobulin, caspase, RAB7, chitinase, mucin, chitin deacetylase, Toll signaling pathway, anti-lipopolysaccharide factors (ALFs), penaeidins, astakine, catalase, peroxinectin, hemocyanin, glutathione peroxidase, glutathione-s-transferase, superoxide dismutase, lectins, β-glucan binding protein (LGBP), scavenger receptors (SRs), and Down syndrome cell adhesion molecule (DSCAM), among others.

ANTIMICROBIAL RESISTANCE (AMR) IN AQUACULTURE FROM A ONE HEALTH APPROACH

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One Health proposes an integrated approach to work collaboratively to study the connection between the health of people, animals, and the environment (https://www.cdc.gov/onehealth/index.html), recognizing the interconnection in the spread of pathogens and their antimicrobial resistance (AMR). Diseases in aquaculture are mainly associated with intensive cultures, poor biosecurity, and impacted environments. Antimicrobials are being used widely for the treatment of diseases, as growth promoters and to eliminate or inhibit the proliferation of pathogenic bacteria to prevent outbreaks. Metaphylaxis (antimicrobial administration to a group of individuals to control of a disease) in aquaculture affects both pathogenic and symbiotic bacteria and it is being discussed in terms of the risk for spreading AMR, due to the emergence, persistence, and transmission of reservoirs of antimicrobial resistance genes (ARGs). AMR bacteria is produced by conditions of the farm and the susceptibility to infections of shrimp due to the stress and usually having low genetic variability. The high levels of human contact with water and animals, the nationally and international shrimp trade, the relative lack of infrastructure for waste treatment, including antimicrobials, which are released directly into the aquatic environment completing the Human-Animal-Environment cycle.

In this scenario, shrimp producers are suffering infections by *Vibrio parahemolyticus* strains carrying a toxigenic plasmid that causes acute hepatopancreatic necrosis disease (AHPND) and generating losses estimated at more than a US$ billion in Asia. While no concrete actions have been taken in shrimp farming worldwide from 1994 to the present, different bacteria (*Vibrios*, *Aeromonas*, *Pseudomonas*, *Escherichia*, *Klebsiela*, *Shigella*, others) are reported carrying different ARGs that allow them to be resistant to all generations of cephalosporins (CEF), glycopeptides (GLY), macrolides (MAC), quinolones (QUIN), polymxyins (POLY), aminoglycosides (AGLY), ansamycins (ANS), carbapenems (CARB), monobactams (MONO), penicillins (PEN), amphenicols (AMPH), lincosamides (LIN), sulphonamides (SUL), tetracyclines (TET), anti-staphylococcal penicillins (APEN), aminocyclitols (ACYC), cyclic polypeptides (CYPOL) and nitrofurantoins (NIT) (Fig. 1). The increase in AMR and the spread of ARGs in bacteria isolated from shrimp farming is of great concern, suggesting the need to implement systematic surveillance systems at the local, regional and national levels, following One Health approach that allow evaluating the true impact of the problem in order to apply prevention actions to support the industry and to lessen the impact on human health.

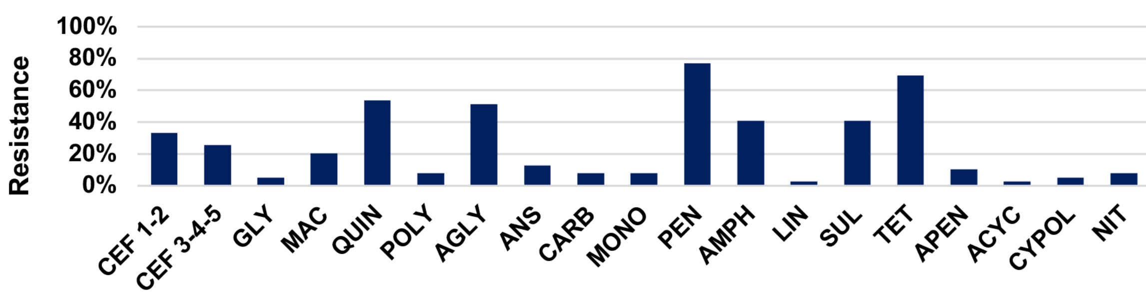

Fig. 1. Antimicrobial resistance in bacteria associated with farmed shrimp (adapted from Thornber et al. 2020, Rev Aquac, 12(2):966-986; doi: 10.1111/raq.12367)

ONE HEALTH EPIGENOMES AND MICROBIOMES: FROM SOIL TO PEOPLE WORKSHOP - RECOGNITION TO STUDENTS AND 'OUTSTANDING ONE HEALTH RESEARCHERS IN AQUACULTURE' AWARDEES

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The ONE HEALTH Epigenomes and Microbiomes: From Soil to People' workshop will address the following topics: reverse transcriptase-related genes and their possible role in the host cell response to transition metal pollution; from mangrove to fork: metal presence in the Guayas estuary (Ecuador) and commercial mangrove crabs; from field to plate: agricultural pesticide presence in the Guayas estuary (Ecuador) and commercial mangrove crabs; a transposable element–epigenetics perspective to understand antimicrobial resistance (AMR) and contamination by endocrine disrupting chemicals (EDCs) like heavy metals, biocides, glyphosate, microplastics, bis(2-ethylhexyl) phthalate (DEPH), and per- and poly-fluoroalkyl substances (PFAS): adaptation to global change; AMR in aquaculture from a ONE HEALTH approach; ONE HEALTH epigenomics, wastewater-based epidemiology and AMR: a role for glyphosate-based herbicides, *Bacillus thuringiensis*, *Vibrio sp*.; metals chelated by glyphosate, organophosphates, disinfectants, and persistent organic pollutants (PCBs, PAHs) in emerging resistant pathogens of public health concern; neutral processes and salinity shape microbial community assembly in mangrove ecosystems along estuary; impact of host genotype on gut and hepatopancreas microbiota of *Litopenaeus vannamei*; a bacteriophage cocktail as an alternative for the control of Vibrio parahaemolyticus responsible for AHPND in *Penaeus vannamei*; the hunt for wild caught probiotics: comparison of microbiomes from 569 vertebrates including 115 fish species; aquaculture at the crossroads of global warming and AMR and the use of bioactive plants and algae as a sustainable alternative; bacteriophage technology, an effective solution to tackle AMR in aquaculture; and the welfare concept – does it apply to shellfish, too?.

Four female scientists will be recognized as 2022 "Outstanding ONE HEALTH Researchers in Aquaculture": Suhua Shi (China), Nitsara Karoonuthaisiri (Thailand), Fuhua Li (China) and Sandra Shumway (USA) by the Foundation for Conservation of Biodiversity (FUCOBI) of Ecuador. Twenty-two students, postdocs, and research associates from twelve countries (Belgium, Chile, China, Ecuador, India, Honduras, Mexico, Nigeria, Philippines, Romania, Thailand, United States) are winners of the '2022 Johnnie Castro Montealegre Travel Awards' of the FUCOBI Foundation to attend the triennial AQUACULTURE 2022 meeting in San Diego, February 28 – March 4, 2022.

THE INTERACTIVE EFFECTS OF DIETARY LIPIDS AND THE USE OF A NUTRITIONAL EMULSIFIER IN JUVENILE NILE Tilapia (*Oreochromis niloticus*)

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The industries' push for alternative feed ingredients and current prices of aquafeed has led nutritionists and researchers to focus towards optimizing feed efficiency. Evaluating lipids and optimizing alternative sources for the use of fish oil revealed that lipids are involved not only in growth, but also in health and development. Vegetable oils, like soybean oil, are commonly applied in current Nile Tilapia feeds as alternative. However, this inclusion has led to decreased growth performance and health concerns including decreased anti-oxidant status. Nutritional emulsifiers traditionally increase the surface area of fat globules by breaking them down to micelles allowing for improved lipid digestion. Although there is sufficient evidence of improved performance and immunity with proper application of nutritional emulsifiers in terrestrial livestock species, the application in aqua remains limited.

The objective of this study was to investigate effects of dietary lipid levels using a commercial nutritional emulsifier (NE; Excential Energy Plus by Orffa additives B.V., the Netherlands) on the growth, feed utilization, immune response and lipid metabolism of juvenile Nile Tilapia. A 5x2 factorial design was implemented with lipid supplement (Soybean oil at 0, 15, 30, 45 and 60 g/kg) and NE supplementation (with or without NE at 0.35 g/kg), creating 10 iso-energetic diets. All dietary treatments were fed in triplicate, 30 fish per replicate (average initial weight of 8.06 g) were randomly stocked in one of the 30 cages and were on feeding treatment for 8 weeks.

Results showed that fish fed diets with NE for 8 weeks had significantly improved weight gain compared with fish fed diets without NE ($p<0.05$), especially for the diet with the lowest lipid content (234% increase) (Table 1). The same results have been observed for the FCR and PER ($p<0.05$) (Table 1).

After 8 weeks, six fish per treatment were randomly chosen to have non-specific immune parameters and antioxidant activities measured. Increasing dietary lipid levels significantly decreased anti-oxidant status and increased liver malondialdehyde, but significantly improved when NE was supplemented ($p<0.05$).

Lipid supplementation was shown to have little effect on the fillet yield, whilst when NE was supplemented in the diet, the fillet content of the fish significantly increased, especially in fish fed the low fat diet ($p<0.05$) (Figure 1).

In summary, dietary NE supplementation has a positive effect on feed digestion and absorption, resulting in improved fish growth, performance, anti-oxidant status and fillet yield. NE supplementation is particularly effective when it is added to diets with low crude fat diets, giving feed formulators the opportunity to decrease fat inclusion in the diet.

Table 1: The weigh gain (WG), specific growth rate (SGR), average daily gain (ADG), feed conversion ratio (FCR) and protein efficiency ratio (PER) of Nile tilapia fed the experimental diets.

	Diet no.									
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Added oil (%)	0		1.5		3		4.5		6	
		NE		NE		NE		NE		NE
WG	10	23.4	8.8	19.5	9	18.9	9.1	17.4	9	13.7
SGR	1.4	2.3	1.2	2.2	1.2	2	1.2	1.9	1.3	1.7
ADG	0.2	0.4	0.1	0.3	0.2	0.3	0.2	0.3	0.2	0.2
FCR	1.9	1.1	2	1.3	1.9	1.3	2	1.4	2	1.6
PER	1.96	4.44	1.72	3.77	1.85	3.6	1.84	3.3	1.76	2.72

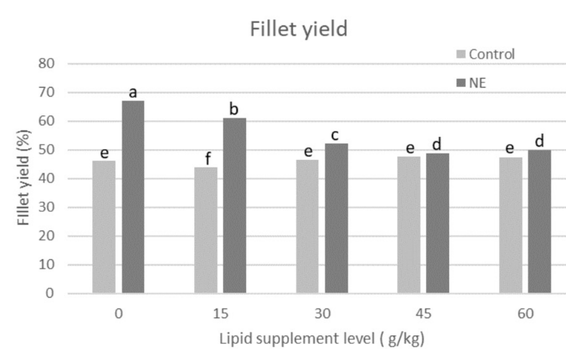

Figure 1: Effect of dietary lipid levels and inclusion of NE on fillet yield.

THE NUTRIENT POLLUTION ASSIMILATION POTENTIAL OF SEAWEED AQUACULTURE IN U.S. WATERS

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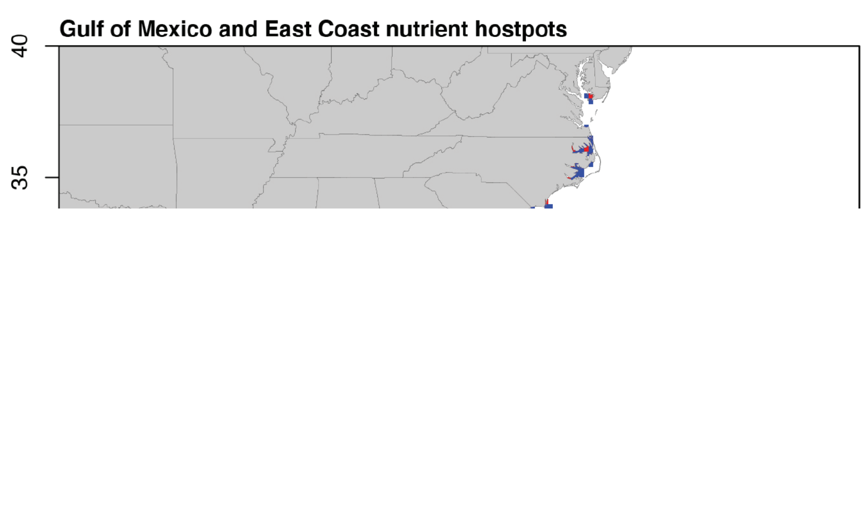
As the United States marine seaweed aquaculture industry develops, there is immense opportunity to site farms in locations that maximize the co-benefits of seaweed aquaculture, including nutrient assimilation. Seaweed aquaculture is capable of removing large quantities of nitrogen and phosphorus from coastal ecosystems, yet seaweed has gained little traction for its potential role in targeted nutrient assimilation. In the U.S., nitrate loading over the past 20 years has been relatively constant at an exceedingly high input level, while phosphate loading has continued to increase[1]. In May 2019, phosphate loads to the Gulf of Mexico were 49% above the long-term average, leading to a historically large dead zone[2]. Current management systems in the U.S. do not fully address the large and growing nutrient pollution problem[3], and the U.S. Environmental Protection Agency is actively seeking new management strategies. Here, we determine the optimal siting locations for seaweed aquaculture with the objective of maximizing the co-benefit of reducing nutrient pollution in the U.S.

Our analysis identifies pollution “hotspots” in coastal waters, where anthropogenic nutrient loading is high compared to natural nutrients, and then assesses seaweed aquaculture suitability in these areas to estimate the assimilation capacity of seaweed farming. Finally, we will corroborate our U.S. wide results with a fine scale case study in the Gulf of Mexico, where we couple a seaweed growth model with highly resolved pollution data. We show that targeted seaweed aquaculture development in key locations, such as the Florida Gulf and much of the Atlantic Coast, can be used to successfully assimilate anthropogenic nutrient pollution. By contrast, naturally high nutrient areas are unlikely to benefit from seaweed aquaculture’s nutrient assimilation services. These findings underscore the opportunity to site seaweed aquaculture to maximize co-benefits and provide important ecosystem services, rather than merely fitting farms into suitable marine space.

1. Mississippi River/ Gulf of Mexico Watershed Nutrient Task Force (2015). 2015 Report to Congress. U.S. Environmental Protection Agency. Washington, D.C.
2. NOAA (2019, June 12). NOAA forecasts very large ‘dead zone’ for Gulf of Mexico. National Oceanic and Atmospheric Administration. https://www.noaa.gov/media-release/noaa-forecasts-very-large-dead-zone-for-gulf-of-mexico
3. Rabotyagov, S. S., Kling, C. L., Gassman, P. W., Rabalais, N. N., & Turner, R. E. (2014). The economics of dead zones: Causes, impacts, policy challenges, and a model of the Gulf of Mexico hypoxic zone. *Review of Environmental Economics and Policy*, 8(1), 58-79. https://doi.org/10.1093/reep/ret024



A GIS-BASED TOOL FOR SPATIAL PLANNING AND MANAGEMENT OF SHELLFISH AQUACULTURE IN NEW JERSEY

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With National Sea Grant support, a team of Rutgers University researchers, in partnership with the New Jersey Department of Environmental Protection is using geospatial tools to weigh and analyze data about conditions affecting shellfish production to develop an interactive tool that can identify areas that are suitable for shellfish aquaculture in New Jersey. Such a tool can be used as a resource to inform planning and policy regarding the diversity of uses of the State's coastal resources, and to identify potential conflicts with other uses of coastal waters. The project is <u>not</u> a comprehensive spatial plan for shellfish aquaculture in New Jersey; rather, it is a data-informed tool that can be used by state and federal agencies and the stakeholder community for aquaculture and coastal management policy, planning and applications for shellfish aquaculture operations.

Data that are incorporated into the interactive tool include:

- Hydrological characteristics;
- Areas not suitable for aquaculture development;
- Areas with physical limitations such as man-made obstructions;
- Climate and environmental data including information regarding current and projected climate or environmental conditions that could affect shellfish production;
- Current shellfish leased grounds ; and
- Social information regarding other coastal resource uses.

The project is complemented by funding from the Pew Charitable Trusts to survey areas for potential shellfish and submerged aquatic vegetation (SAV) restoration, and to map areas of shellfish and submerged aquatic vegetation where data are lacking to inform development of the GIS tool.

ALASKA MARICULTURE INITIATIVE – INDICATORS OF PROGRESS

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In Alaska, mariculture offers tremendous potential to provide resiliency to communities facing future threats from declining fish stocks, ocean acidification and warming, and other changes. NOAA Fisheries is a key partner in helping the mariculture industry grow in an environmentally sound manner, making significant contributions through grant initiatives, engagement in the Mariculture Task Force (MTF), partnership with Alaska Sea Grant, and other collaborations.

In 2014, NOAA provided a grant to the Alaska Fisheries Development Foundation to lead the Alaska Mariculture Initiative (AMI) – a strategy to accelerate the development of mariculture in Alaska. AMI Phase 1 led to the development of the MTF by Administrative Orders under Governor Walker and the adoption of the Alaska Mariculture Development Plan, with the goal to grow a $100 million industry in 20 years. Additional outcomes of this project were two economic analyses projecting the growth trajectory of Alaska's mariculture industry, passage of state legislation allowing for financing to support hatcheries, and submission of 33 new aquatic farm, nursery or hatchery applications.

In 2018, AFDF received another SK grant for phase 2 of the AMI, including the continued facilitation of the MTF, creation of a Five-Year Action Plan, as well as the transition from the MTF to a new nonprofit coordinating body, the Alaska Mariculture Alliance (AMA) and accompanying Mariculture Research and Training Center (MRTC). Phase 2 also led to a new University research position, passage of state legislation to improve the lease process and expand the eligible entities for the Mariculture Revolving Loan Fund, training of 300 prospective mariculture farmers in Alaska, submission of 67 new aquatic farm applications, hiring of two new positions in Alaska Dept. of Natural Resources to process new farm applications and a corresponding reduction in application processing times by over 50%.

In 2021, the MTF provided a Final Report to Governor Dunleavy highlighting accomplishments under the Five-Year Action Plan, and the remaining priority recommendations. NOAA Fisheries aided in the accomplishment of these, including: increased NOAA capacity to support mariculture development; hosting a mariculture workshop and developing a summary report; and facilitating developing an Alaska aquaculture permitting portal to improve permitting efficiencies.

Between 2018 and 2021, over $4 million was invested in mariculture development by federal, state, local, and tribal governments, as well as NGOs, and private companies. However, the latest and most significant funding contribution resulted from the Exxon-Valdez Trust Council recently approving full funding for three Alaska mariculture research projects totaling $32.8 million. The AMA, MRTC, and NOAA Fisheries will continue to be critical partners in advancing mariculture development in Alaska.

ALASKA MARICULTURE INITIATIVE – HOW LOCAL CONTEXT PROVIDES SOCIAL LICENSE

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In 2016, Governor Walker appointed the Alaska Mariculture Task Force – a group of industry and community stakeholders, state agencies, the University and Alaska Sea Grant – to work cooperatively towards the development of the mariculture industry. In 2018, the Task Force completed a statewide comprehensive plan with the vision to develop a viable and sustainable mariculture industry producing shellfish and aquatic plants for the long-term benefit of the economy, environment and communities, and a goal to grow a $100 million industry in 20 years. Since this time, approximately 60 new aquatic farm applications have been submitted, totaling approximately 2,400 acres, new positions have been hired at state agencies, the University and NOAA, new aquatic farm application timelines have been reduced by 50%, over $35 million of new mariculture research has been funded, legislation has been passed to improve regulatory processes, 300 Alaskans have received mariculture training, over $1 million in new loans through the state revolving loan funds have been approved, and many other indications of progress exist towards the $100 million goal.

This progress to date could not have occurred without public support and acceptance (aka social license) for mariculture development. This presentation will outline the strategic decisions during this period and in the future that will help determine whether mariculture development remains on the path of acceptance in Alaska.

METAGENOMIC CHARACTERIZATION OF *Chaetoceros* MICROALGAE

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Microalgae are the support base of the marine food chain and are the natural food of filter feeders. Algae are essential for the production of live food for larval culture. Non-axenic microalgal cultures are commonly used, however, axenic may be required under different biotechnological uses. *Chaetoceros* is one of the most widely used diatom genera in aquaculture, and in this research axenic and non-axenic cultures of three species of microalgae *Chaetoceros calcitrans*, *Chaetoceros muelleri* and *Chaetoceros* sp. We tried to culture in axenic conditions *Chaetoceros* by using a cocktail of antibiotics (ampicillin, neomycin, kanamycin, and streptomycin). In order to determine the axenicity level produced by the antibiotic cocktail since no growth was observed, a metagenomic analysis using next generation sequencing was used with total DNA of the three *Chaetoceros* species. The cultures were harvested in the stationary phase and total the DNA was extracted by means of the commercial protocol DNeasy® Blood and Tissue (QIAGEN®, Valencia, CA). The quantification and evaluation of the quality and integrity of the DNA were carried out, then samples were sent to be sequenced with the MiSeq Benchtop Sequencer platform (Illumina) to the University of Georgia, Georgia, USA. The sequenced reads were assembled using SOAPdenovo software (Li et al., 2009).

Upon assembly completion, a fasta file was generated for further annotation analysis. With the resulting contigs, the NCBI nucleotide (nt) database (https://www.ncbi.nlm.nih.gov/) was blasted to separate bacterial from eukaryote sequences. With the contigs related to bacteria, phyla, genera and species identification was carried out.

The number of blast hits to the nt database corresponding to the Bacteria, Archaea, Eukaryote and Virus Kingdoms of three *Chaetoceros* species for axenic cultures: 47,486, 14, 38,745 and 27 respectively for *C. calcitrans*, 14,105, 13, 23,805 and 35 for *C. muerelli*, and 70,721, 24, 68,816 and 54 for *Chaetoceros* sp. And for non-axenic cultures: 82,849, 9, 13,692 and 52 for *C. calcitrans*, 17,631, 11, 32,962 and 31 for *C. muerelli*, and 48,037, 22, 41,934 and 37 for *Chaetoceros* sp.

The group of cytophaga-favobacter-bacteroides (CFB) filum bacteria, alpha-Proteobacteria and gamma-Proteobacteria were the most abundant in the three species of microalgae. However, regarding axenic cultures in the species *C. calcitrans* and *Chaetoceros* sp., the group of CFB bacteria was more abundant compared to the non-axenic cultures, with the exception of the microalgae species *C. muerelli* in non-axenic cultures it presented more abundance. The response of cultures to antibiotics was species-specific and other specific protocols and antibiotics should be tested for biotechnological uses with axenic cultures.

STOCKING SIZE AND GROWTH RATE ESTIMATES OF ATLANTIC SALMON UNDER DIFFERENT COASTAL TEMPERATURE PROFILES: GUIDING YEAR-ROUND AND SEASONAL SITING UNDER A CHANGING CLIMATE

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There has been a recent trend in Atlantic salmon (*Salmo salar*) aquaculture to stock large post-smolts to reduce fish health risk. While this practice has primarily been implemented to reduce disease potential, it may also enable management strategies to avoid temperature extremes that constrain the growing season in many regions. Depending on local temperature and initial fish size, large post-smolt stocking could enable grow-out to target market weights in less than a year, providing opportunity to circumvent predictable periods of superchill while accounting for heat stress. Such approaches may also need to account for greater uncertainty under climate change.

A process was developed to assess the potential of novel sites for Atlantic salmon grow-out under a changing climate. High resolution empirical temperature profiles were examined at three coastal locations around Nova Scotia, Canada. Functional growth days per season at locations and depths were identified using known temperature thresholds (Fig. 1). The thermal growth coefficient model was applied to calculate the stocking weight required to grow-out salmon, assuming superchill could be circumvented and growth ceased at high temperatures.

Preliminary results suggest that an approximately 1-kg salmon could grow to market size (5 kg) in less than a year in some areas that are typically considered unfavourable for aquaculture due to superchill. Warm water substantially reduced production, with no growth on up to 13 % of simulated stocked days due to heat stress events. The potential for novel sites and implications for stocking and effects of climate change are explored. The R package developed for the analysis can be installed from GitHub and applied to other regions of interest.

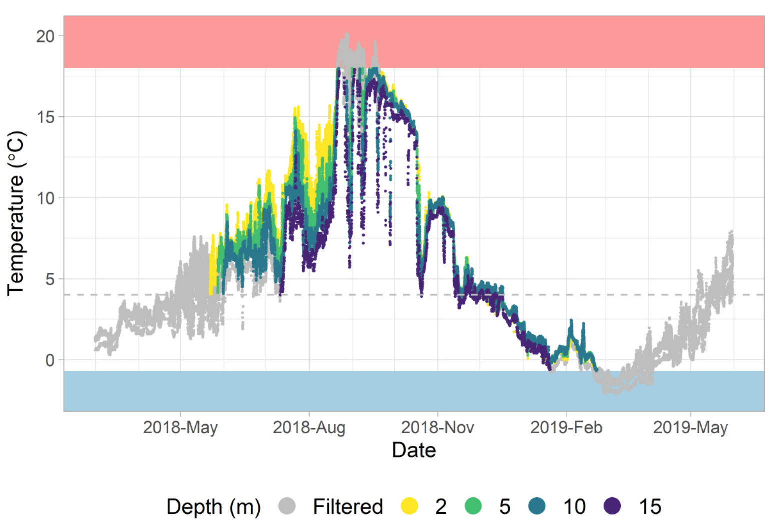

Figure 1: Temperature profile for Beaver Point. The growing season begins when the temperature exceeds 4-degrees (dashed grey line) and does not return below this threshold; the season ends when superchill (blue bar) is observed. No growth is assumed for 24 hours after a heat stress event (red bar). Greyed observations were filtered out of the model based on these rules.

COASTAL OCEAN MONITORING AT BIOLOGICALLY RELAVENT SCALES: SUPPORTING AQUACULTURE RISK MANAGEMENT

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Coastal ocean monitoring is critical for informing aquaculture risk management decisions including site selection, engineering specifications, and carrying capacity. Dedicated nearshore monitoring is required to capture complex coastal dynamics, which cannot be resolved by broad-scale or offshore monitoring. Understanding these coastal dynamics can make the difference between operational success and failure. Long-term data sets are also becoming increasingly valuable to prepare and understand climate change linkages to deleterious events, including unexpected low oxygen mortalities at net pens.

The Centre for Marine Applied Research (CMAR) in Nova Scotia, Canada coordinates an extensive Coastal Monitoring Program to address this fundamental data need. CMAR maintains a network of nearly 70 oceanographic moorings that measure Essential Ocean Variables (e.g., temperature, dissolved oxygen, salinity, sea state, currents), typically within 1 km of the coast. The resulting data products have been used by the aquaculture industry to inform site selection and engineering design and by government regulators to support lease approvals.

To our knowledge, these oceanographic data products are unique in Canadian coastal waters for their static depth profiles, time series length, and high spatial and temporal resolution needed for application to biologically driven systems such as aquaculture. CMAR has prioritized efforts to ensure these data are freely available on several platforms and in multiple formats (e.g., summary reports, processed data) to ensure maximum access and application. Additional coastal stakeholders have begun using CMAR data, and more diverse applications are expected as data sharing initiatives continue.

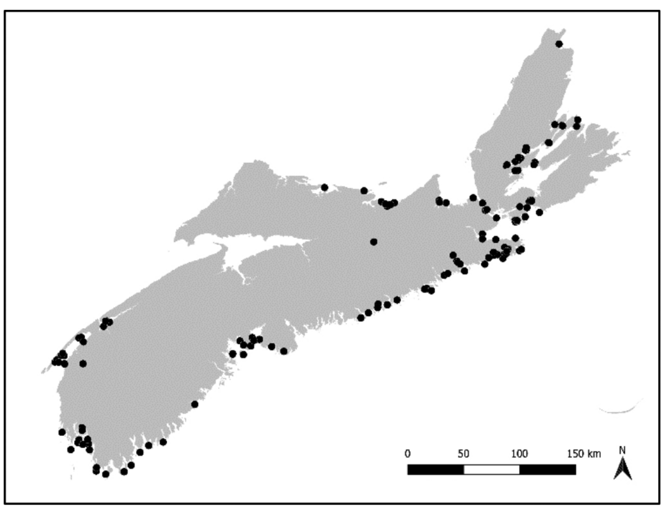

Figure 1: Station locations for autonomous sensors for temperature, dissolved oxygen, and/or salinity data.

UTILIZATION OF ALFALFA NUTRIENT CONCENTRATE IN FEED FOR RAINBOW TROUT, *Oncorhynchus mykiss:* EFFECTS ON PELLET PHYSICAL QUALITY, NUTRIENT UTILIZATION AND METABOLISM OF FISH

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The global expansion of aquaculture has led to increased demand on feed and feed ingredients, which are the main challenges to sustainability in aquaculture. The objective of this study is to investigate the potential of alfalfa nutrient concentrate (ANC) as an ingredient to replace fishmeal in feed for rainbow trout. The ultimate goal is to lay the groundwork for commercialization of value-added products from alfalfa in aquaculture feeds.

Five test diets were formulated (46% protein and 15% lipid) including different levels of ANC (0, 5, 10, 15, and 20%) to replace fishmeal (32% in the control diet) and were processed following a cooking extrusion protocol. Increasing levels of ANC resulted in increased pellet density and sinking pellets when the diet contained 20% ANC. However, increased water stability and durability were observed in response to the increased level of ANC inclusion. The five test diets were used to conduct two feeding trials in flow-through water systems with three replications per diet. The first trial tested feed intake and the apparent digestibility coefficient (ADC) of dietary nutrients. The results showed that ANC inclusion did not significantly impact the feed intake ($P \geq 0.05$) of rainbow trout under the experimental conditions. The ADC of dry matter and protein did not change due to fishmeal replacement, but the ADC of phosphorus was significantly ($P < 0.05$) decreased in fish fed the 20% ANC diet. Another 9-week feeding trial was conducted to determine the effect of test diets on growth, nutrient utilization, metabolism and health of rainbow trout. Fish fed the test diets including 10% to 20% ANC had significantly lower grow rates and a higher feed conversion ratio than the fish fed the diet containing 0% or 5% ANC. Whole body composition and biochemical parameters of plasma were not different due to the different feeds except that the whole-body protein and plasma phosphorus levels decreased in the fish fed the ANC-based diets. Results based on liver metabolomic analysis showed that the 20% ANC diet significantly altered the metabolism of alanine, leucine, valine, and threonine as well as TCA cycle intermediates (succinate, malate, glycerol 3-P) and osmolytes (betaine).

The results of this study demonstrate that ANC did not influence food intake but decreased fish growth rate, which might be due to the impact of ANC on phosphorous digestibility and the alteration of nutrient metabolism under the test conditions. The potential of ANC use as an aquatic feed ingredient may be improved if the impact of phosphorous digestibility can be addressed. This is warrants for future investigation.

Growth performance and phosphorus ADC of rainbow trout fed the test diets for 9 week.

Measurements	ANC-0	ANC-5	ANC-10	ANC-15	ANC-20
FBW	146.2 ± 2.2^{a}	138.4 ± 2.9^{ab}	126.2 ± 2.9^{bc}	119.2 ± 1.0^{cd}	111.8 ± 4.0^{d}
SGR	3.21 ± 0.04^{a}	3.12 ± 0.04^{ab}	2.98 ± 0.02^{bc}	2.89 ± 0.02^{cd}	2.77 ± 0.05^{d}
FCR	0.81 ± 0.01^{a}	0.83 ± 0.01^{a}	0.90 ± 0.02^{b}	0.90 ± 0.02^{b}	0.96 ± 0.01^{c}
Phosphorus ADC	47.3 ± 0.1^{a}	46.8 ± 1.4^{ab}	43.5 ± 0.4^{bc}	42.6 ± 1.3^{c}	41.7 ± 0.2^{c}

Data were presented as mean ± SE, n = 3. Means in the same row sharing different superscript letters are significantly different ($p < 0.05$), as determined by Tukey's HSD test. Initial body weight of fish, 19.3 ± 0.2 g, n=15. ANC: alfalfa nutrient concentrate; ANC-0, ANC-5, ANC-10, ANC-15, and ANC-20 represent the test diets containing 0, 5, 10, 13, or 20% ANC, respectively.
FBW, final body weight (g)
SGR (Specific growth rate) = 100* LN(Final body weight, g/initial body weight, g)/63 days.
FCR (Feed conversion ratio) = (dry feed weight per tank, g) / (total weight gain per tank, g)
ADC of a dietary nutrient = 100*(1 - (Y in feed* nutrient in feces)/(Y in feces* nutrient in feed).

SHELLEVATOR: SCALABLE PLATFORM AND METHOD THAT AUTOMATES AND MOBILIZES SHELLFISH AQUACULTURE

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The patented Shellevator™ invention automates ascent and descent of containerized shellfish through the water column on scalable structures. Two or more forward and aft lift tanks are mounted below a frame with containerized oysters attached to the topside. Compressed air (compressor or air tank) is routed through a primary airline to a manifold that supplies secondary airlines connected to on top at front end of lift tanks. Opening the corresponding valve introduces compressed air and forces water out an exhaust port on bottom back end of the lift tank. Ascent is controlled by sequentially introducing compressed air to completely exhaust water and float forward lift tanks before floating aft tanks. Descent is achieved by completely exhausting air and flooding aft tanks before sinking forward tanks.

The Shellevator™ Express pictured above left is a repurposed 24' pontoon boat. With >3000 Kg lift and>30,000 oyster capacity, the scale is 30-fold larger than manual gear, virtually immovable and hurricane ready when submerged. It can be raised above the sea surface or tilted side to side in a few minutes for <$0.01 using a small 1200-watt compressor automating desiccation, tumbling and harvesting. Shellevator™ Express above right is being towed >30Km/h and can be trailered at highway speeds to avoid harm or quickly relocate to areas for improved growth, quality and safety. A paradigm shift from farming to herding oysters is needed to address the unprecedented challenges of an increasingly uncertain climate.

Figure 1. Shellevator™ in grow position.

Figure 2. Shellevator™ in tow.

DESIGNING OFFSHORE MACROALGAE CULTIVATION STRUCTURES TO PREVENT CATASTROPHIC FAILURE

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Farming seaweed at exposed ocean sites requires a careful balance of costs and risks. In the present case study, a multi-tile kelp cultivation array was designed for an exposed site in the Gulf of Maine. This system employs novel components to minimize animal entanglement concerns (composite lines with large bending radius) and maximize grow line area per site area (modular tiles with low-scope mooring lines and helical anchors). Thus, simulations were used to understand the behavior of the prototype farm prior to deployment and quantify loads on the anchors and structural components. To mitigate the risk of structural failure without incurring excessive capital and operational costs, the system was evaluated using a simulation technique that has been demonstrated to predict mooring loads within 15% of those measured at sea for an exposed kelp farm.

Fifty-year extreme current speeds, significant wave heights and associated peak periods were quantified by incident direction and by month-of-the-year. Simultaneously considering the biomass growth by month of the year and the monthly current and wave extreme values reduced capacity requirements by 48% compared to the conservative assumption of maximum biomass with the maximum significant wave height and current speed. Anchors and structural components were designed to maintaining safety factors in consistent with marine industry standards for both the intact case and damaged conditions, mitigating the risk of catastrophic, cascading anchor-line failure in the array.

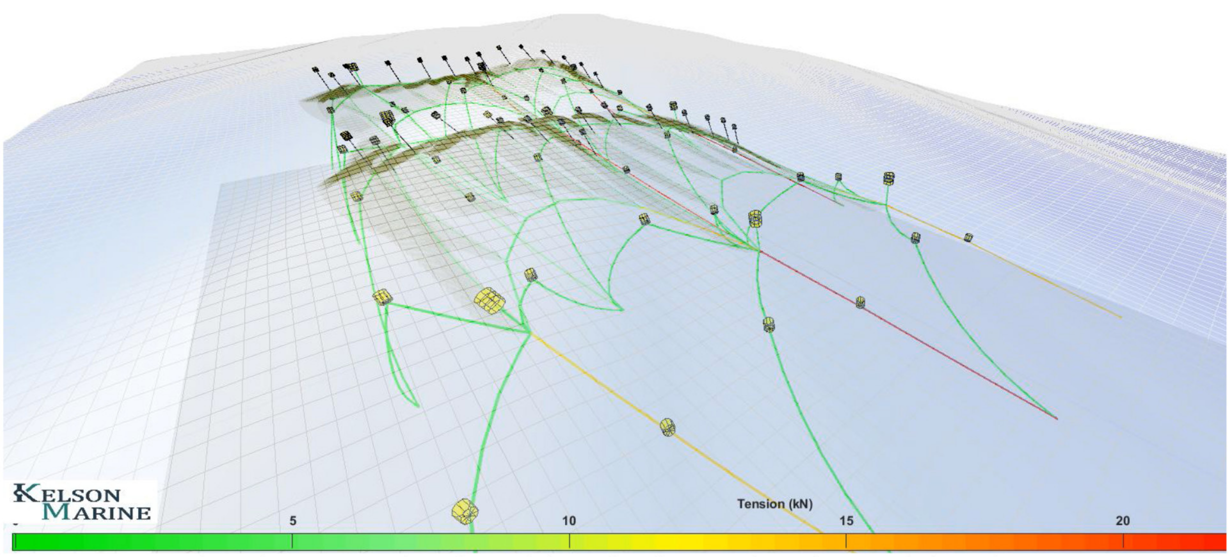

Figure 1. Hydro-/structural analysis of the multi-tile array for cultivating kelp in the open ocean

FLUID DRAG AND INERTIAL FORCES ON GIANT KELP (*Macrocystis pyrifera*) IN CURRENTS AND WAVES DERIVED USING A FULL-SCALE PHYSICAL MODEL

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Giant Kelp (Macrocystis pyrifera) is being investigated for large scale production of biomass for food, feed, fuel, and carbon sequestration. This requires a thorough understanding of the hydrodynamics, including properties of both individual kelp organisms and densely spaced groupings of organisms. The drag (normal and tangential) and added mass of full-scale organisms was evaluated in currents ranging from 0.5 m/s to 5 m/s and oscillations with periods ranging from 3 seconds to 11 seconds. Key variables in the testing included the length of individual fronds, the number of fronds per organism, the spacing between organisms, and the orientation of the aggregate kelp with respect to the current. A full factorial experiment was performed with drag testing at the United States Naval Academy. The kelp models were constructed from synthetic materials to ensure consistency during the test runs. The models were constructed to have the same hydrodynamic properties as live kelp by ensuring that the models' blade geometry, net buoyancy, bending stiffness, and surface area were typical of real kelp. The characteristics of Macrocystis pyrifera vary widely between organisms in the same geographical region as well as in various locations around the planet. An extensive review of the literature was conducted to develop characteristics of a "typical" giant kelp organism. The results are parameterized to allow applicability of these results to a wide range of realistic Macrocystis pyrifera as well as other kelp species. An important goal of this testing was to provide reliable data for representation of Macrocystis pyrifera in numerical models for the design of giant kelp cultivation systems. Results that are presented include physical characteristics of Macrocystis pyrifera, physical characteristics of the kelp models constructed for this study, load cell data and images from the individual runs. Also presented is a detailed description of the procedures used to determine hydrodynamic characteristics of kelp from the collected data and a summary of the hydrodynamic properties.

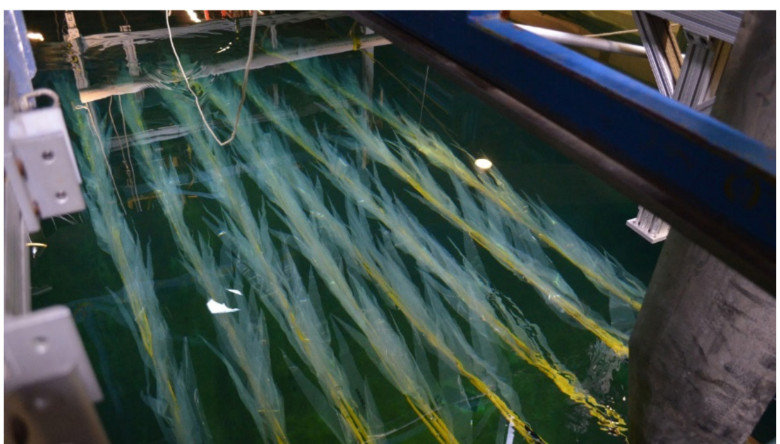

Figure 1. Tow tank image of macrocystis model of 8 thalli each with 5 fronds of 5 m length. Spacing between thalli in the test pictured is 0.3 m

EXPEDITING PATHOGEN DISCOVERY BY COMBINING HISTOPATHOLOGY AND GENOMICS IN SHRIMP AQUACULTURE

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Transformation of shrimp farming from a small fisheries activity to a global industry over the past four decades has been made possible through the development of captive breeding programs that led to the availability of Specific Pathogen Free seed stock, availability of nutritionally optimal formulated feed, and implementation of new measures to improve biosecurity and farm management. Despite much progress achieved to enhance global production, maintaining a steady level of productivity has been difficult due to periodic and unpredictable disease pandemics. Often times diseases have spread beyond the point of origin across countries and even to different continents before the etiologic agent was discovered, and disease diagnostic tools and treatments were developed. To prevent the transboundary movement of diseases and economic losses that result due to disease pandemics, there is a need to identify the causal agent of a newly emerged disease rapidly and develop histopathology and molecular diagnostic tools.

We hypothesized conventional histopathology can be combined with genomics tools to expedite pathogen discovery. Having information on the genomic architecture of a novel pathogen will enable the development of molecular diagnostics to prevent its rapid spread. We demonstrated the feasibility of combining histopathology with next generation sequencing (NGS) to reconstruct the genome of a DNA virus, white spot syndrome virus (WSSV), and an RNA virus, Taura syndrome virus (TSV). Thin sections (5 µm) of paraffin embedded tissue samples were taken from a recent (2017 for WSSV) or old (15 year old for TSV) archived histology blocks at the Aquaculture Pathology Laboratory and examined for pathognomonic lesions of WSSV/ TSV. Upon confirmation of viral infection, DNA (for WSSV)/ RNA (for TSV) were isolated and real-time PCR performed to confirm WSSV/ TSV infection before taking for NGS analysis. High quality sequence data were generated to reconstruct WSSV and TSV genomes. Phylogenetic analysis of TSV genome sequence reconstructed from the archived histology blocks formed clades with genome sequence of homologous isolates deposited to GenBank over decades. This validates the authenticity of TSV genome sequences generated from archived histology blocks.

This study confirms the feasibility of combining conventional histopathology and genomic tools to expedite pathogen discovery and in evolutionary studies. In addition, this demonstrated archived histology blocks could be a treasure trove for pathogen discovery and epidemiology. Conceptually from the time a new disease emerges, conducting the histopathology and NGS analyses to construct the unknown pathogen's genome can be achieved in three months, and developing PCR-based molecular diagnostics can be attained in less than a month. The entire workflow presented here from genome reconstruction to developing molecular diagnostics will undoubtedly enable the industry to prevent the spread of a disease in near real time while shining a light on the epidemiology of diseases of shrimp aquaculture from the past.

COMPARITIVE PATHOGENICITY OF TWO VIRUSES IN CATFISH CULTURE

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Catfish herpesviruses (Ictalurid herpesvirus-1 (IcHV-1)) belonging to the family Alloherpesviridae cause significant mortalities in catfish fry and fingerlings. Channel catfish virus (CCV) causes acute hemorrhagic infection in catfish during the hatchery and nursery phases of catfish culture. Blue catfish alloherpesvirus (BCAHV) is a strain of IcHV-1 isolated from blue catfish fingerlings in 1998. These herpesviruses are reported to be genetically similar (94%). Differential pathogenicity of these two viruses in terms of clinical signs, cytopathic effects (CPEs) in host cell cultures, histopathology, and percentage mortality in different catfish types were studied. Channel, hybrid, and blue catfish fingerlings were experimentally infected with BCAHV and CCV ($10^{3.5}$ $TCID_{50}$ /mL) via immersion. Clinical signs of infection were comparable and included exophthalmia (popeye) and fluid-filled peritoneal cavity (dropsy). The CPEs produced by the two viruses in channel catfish ovary (CCO) cells followed similar morphological alterations (Figure 1). Close resemblance in the clinical signs and CPEs caused by the viruses can be attributed to their genetic similarity. However, the susceptibility of catfish types varied with the virus strain. CCV caused significant mortalities in channel catfish fingerlings followed by hybrid and blue catfish. On the contrary, BCAHV was particularly pathogenic to blue catfish fingerlings and less virulent in channel catfish. Fish less than four months were severely infected by these viruses. The differential pathogenicity of CCV and BCAHV to multiple catfish types demonstrate the host-specificity of herpesviruses.

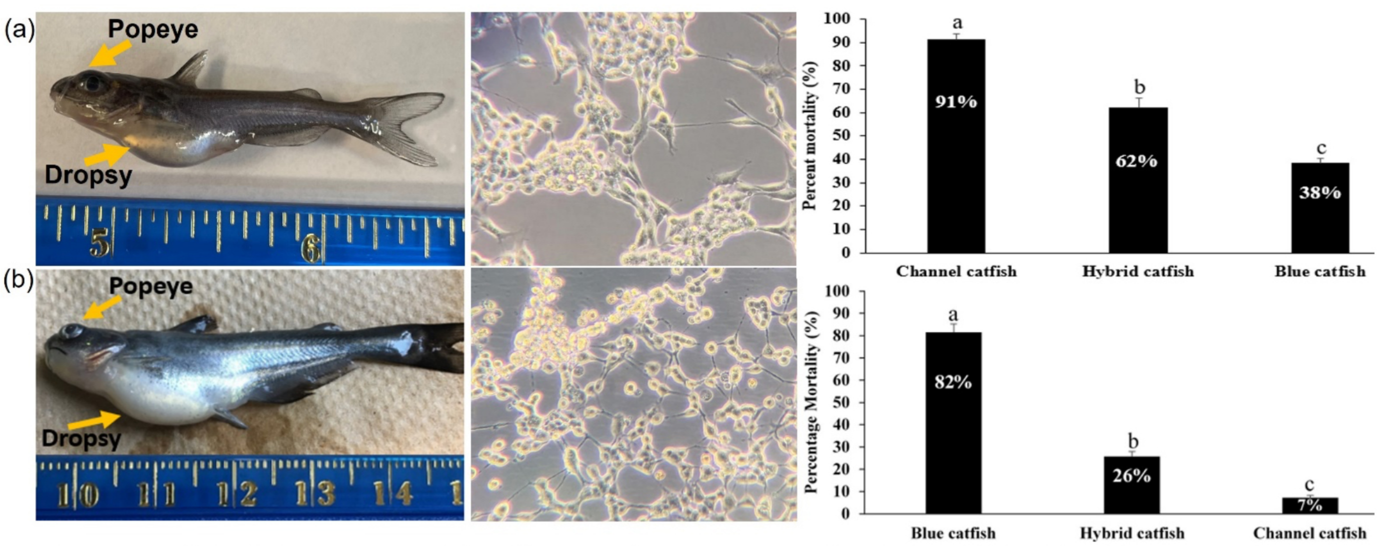

Figure 1. Clinical signs, cytopathic effects, and percent mortality (%) exhibited by (a) CCV and (b) BCAHV in ictalurid catfish.

VERIFICATION OF RIVERS'S POSTULATES FOR BLUE CATFISH HERPESVIRUS

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Herpesviruses are a significant threat during the hatchery and nursery phases of catfish aquaculture. Blue catfish alloherpesvirus (BCAHV) is a novel strain of ictalurid herpesvirus-1 isolated from blue catfish fingerlings. The genome of BCAHV and CCV shares significant similarities. A detailed study on the pathogenesis of BCAHV revealed that it causes substantial mortalities in blue catfish (82%) and hybrid catfish (26%) fingerlings which can drastically affect the viability of farm operations. Thomas Rivers (1937) modified Koch's postulates to include viruses as infectious agents causing diseases. According to Rivers's postulates, the causative relationship between a virus and disease are stated as isolation of virus from the diseased host, cultivation of virus in host cells, proof of filterability, production of comparable disease when the cultivated virus infects naive animals, reisolation of the same virus from the infected animals, and detection of specific immune response to the virus. Validation of theses six postulates are crucial to verify the causation of a disease as a virus. Fulfilment of Rivers's postulates is also essential to formulate pathogen-targeted treatment and management strategies including vaccination. Rivers's postulates were tested and verified for BCAHV infection to confirm the causality of infection in blue catfish (Figure 1). Specific immune responses against BCAHV were observed when the survivors from initial virus exposure were challenged with wild type virus. Hence, BCAHV fulfilled all the criteria to be the primary etiological agent of infection in blue catfish.

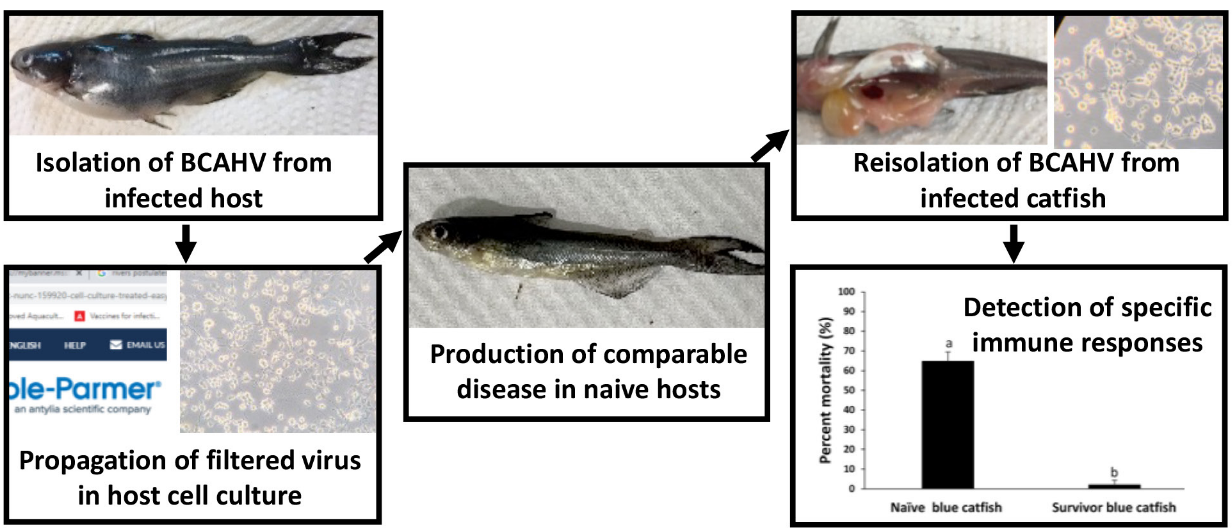

Figure 1. Testing and verification of Rivers's postualtes for the infection in blue catfish fingerlings.

ADDRESSING LARVICULTURE CHALLENGES IN ORNAMENTAL FISHES

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Ornamental fishes represent a lesser-known segment of global aquaculture production. Sales of individual, live, small-bodied organisms, make this commodity somewhat unique in the aquaculture industry. With thousands of species in the freshwater and marine ornamental trade, the diversity of life history strategies and production techniques can be daunting. Consequently, there exists a great need for research that optimizes culture practices for species currently in production, and investigations to define preliminary protocols for species that have yet to be commercialized.

Ornamental fish larvae can be exceedingly fragile and specific environmental and nutritional requirements must be characterized to formulate effective culture protocols. The larviculture phase accounts for the greatest observed mortality throughout the commercial production cycle. Events such as first feeding, swim bladder inflation, flexion, weaning, and metamorphosis represent significant bottlenecks that must be overcome to improve efficiency. A wholistic approach that integrates the culture environment and management strategies with the ontogeny of species specific development processes is prudent to maximize survival and growth of ornamental fish larvae. This presentation will explore approaches used at the University of Florida's Tropical Aquaculture Laboratory to define effective larviculture protocols in marine and freshwater ornamental species.

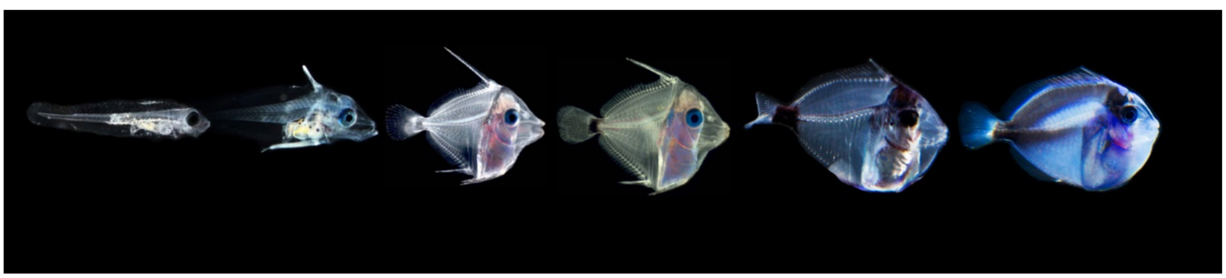

Fig. 1- Larval progression for Pacific blue tang *Paracanthurus hepatus*.

THE GENETIC ARCHITECTURE OF OSHV-1 TOLERANCE IN A PACIFIC OYSTER BREEDING POPULATION

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OsHV-1 currently causes mass mortalities in Pacific oysters in Tomales Bay, California, USA, and a virulent microvariant of this virus has recently been detected in San Diego Bay, California, USA. Selective breeding of oysters for disease tolerance is one of the best mitigation strategies available for reducing the impact of OsHV-1 once it has become established in a bay. Selective breeding conducted by the Molluscan Broodstock Program (MBP), a Pacific oyster breeding program at the Hatfield Marine Science Center (Newport, Oregon, USA), has increased the likelihood of survival to OsHV-1 (Tomales Bay variant) in the MBP oyster breeding population by 21 percentage points. Understanding the causal genes controlling OsHV-1 tolerance can make selection more accurate and increase our understanding of the mechanisms controlling innate immunity in oysters. Conducting a genome-wide association study (GWAS) in the MBP breeding population using SNPs from next-generation sequencing (NGS) data, we found a region on chromosome 8 that was significantly associated with OsHV-1 tolerance. Furthermore, we found that this region is significantly positively correlated with the gene expression levels of two viral innate immunity genes. Family-based QTL analyses replicated the population-based results but also revealed the existence of family-specific genomic regions that are responsible for OsHV-1 tolerance. This suggests that OsHV-1 tolerance in Tomales Bay is controlled by multiple genes possibly active in different innate immune pathways. Selecting for diverse innate immune pathways in oysters can ensure that tolerance to OsHV-1 stays durable against evolution by OsHV-1.

SHALLOW WATER CULTIVATION OF SUGAR KELP *Saccharina latissima*: A NEW OPPORTUNITY TO DIVERSIFY FOR NORTHEAST U.S. OYSTER FARMERS

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Sugar kelp (*Saccharina latissima*) is an emerging mariculture crop in the United States that has generated interest over the past decade for its commercial value and environmental benefits. For oyster farmers, sugar kelp provides a compelling opportunity for crop diversification as it has an opposite growing season from oysters, can be vertically integrated with oysters to provide additive revenue streams, and allows for diversification into non-food markets. Many oyster farms, however, are in near-shore, shallow waters where conventional kelp farming methods cannot be employed, and kelp blades cannot be suspended off the bottom. In this study, we developed and tested methods for shallow-water kelp farming over three kelp growing seasons (2018-2019, 2019-2020, and 2020-2021) at oyster farms located in Moriches Bay (MB; 0.6 m MLW) and Great South Bay (GSB; 1.0-3.0 m MLW) in Long Island, NY, and compared kelp growth and quality in these shallow bays to that obtained at deeper oyster farms in Long Island Sound (LIS; 6.0 m MLW) and the Peconic Estuary (PE; 6.0 m MLW) where water depths were more typical for kelp farming.

Kelp was cultivated along horizontal longlines at all sites from December to May/June, with lines at shallow sites (i.e. <1.2 m MLW) 'staked' a fixed distance (0.3 m) above the bay bottom, and lines at deeper water sites 'suspended' a fixed distance (0.9-1.5 m) below the surface using conventional mooring and buoy systems. The highest crop yields were obtained at the shallowest site in MB using the staked line system, with average line yields of 5.9, 13.4, and 5.6 kg m^{-1} obtained in 2019, 2020, and 2021, respectively (Fig. 1). High yields (5.1 kg m^{-1}) were also obtained in shallow waters in GSB in 2020 using both staked and suspended lines, but ice impacted kelp lines in 2019 and 2021 in GSB highlighting a potential peril of shallow-water kelp farming. Compared to deeper locations, kelp grew faster in the shallow bays, reached a harvestable size one to two months sooner, and cost substantially less to grow in terms of gear and labor. Kelp quality, however, declined earlier in the shallow bays due to fouling, grazing, and blade senescence as water temperatures warmed above ~12 °C, with interannual variability in spring water temperatures impacting the duration of the growing season and crop yields.

Overall, sugar kelp farming along staked lines in shallow waters was found to produce higher crop yields, shorter times to harvest, and have substantially lower costs than suspended line methods in deeper water. This demonstration of shallow water kelp farming reveals opportunities for farmers previously thought implausible and may provide oyster farmers with a means to diversify crops.

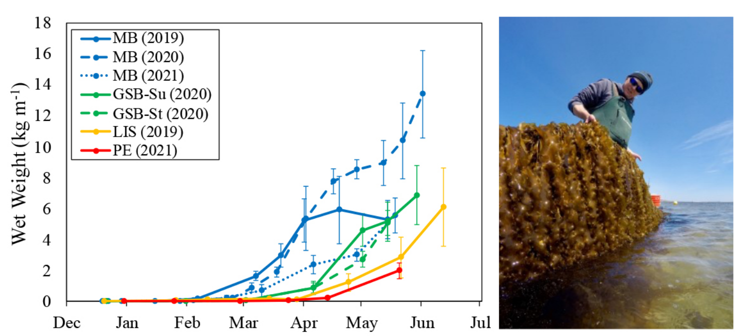

Figure 1. Kelp growth along shallow staked lines in MB.

DEPREDATION IMPACT OF DOUBLE-CRESTED CORMORANTS *Phalacrocorax auritus* ON COMMERCIAL CATFISH PRODUCTION

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The Double-crested Cormorant *Phalacrocorax auritus* is the primary avian predators at catfish *Ictalurus spp.* aquaculture facilities in the U.S. Recent changes in aquaculture practices, regulatory policies, and decreases in overall hectares in the catfish aquaculture industry compelled us to revisit depredation losses. We estimated abundance and distribution of cormorants at their night roosts and assessed diet related to catfish consumption during winters (October-April) 2016-2017 and 2017-2018 (Yr-1 and Yr-2). We used bioenergetics models to estimate catfish consumption by cormorants and developed estimates of economic loss to the industry. Concurrent with these efforts we surveyed producers to estimate bird management costs and overall economic loss.

We flew 25 aerial surveys of cormorant night roosts (Yr-1, n=85/roosts and Yr-2, n=79/roosts) and 24 surveys of catfish ponds (n=1,022/ponds both years) biweekly during both winters. After each survey, night roosts with birds were randomly selected (n=69/roosts) for harvesting cormorants for diet analyses (n=728/cormorants).

Average cormorant foraging days was 212,836/mo., peaking in January of both winters (Figure 1). Catfish and shad *Dorosoma spp.* comprised 33% and 58% of the prey biomass detected, respectively. During Yr-1 and Yr-2 we estimated that cormorants consumed 1.2 and 1.6 million pounds of catfish, respectively and estimated 10.3 and 12 million catfish were eaten.

Catfish farmers surveyed reported a per-acre cost to scare birds of $285 ± $159/acre. The annualized industry-wide value of lost catfish sales revenue to cormorants averaged $47.2 million. The combined total of the annualized costs of scaring birds and the value of fish lost to cormorants averaged $64.7 million/year (range $33.5 M/year to $92.6 M/year).

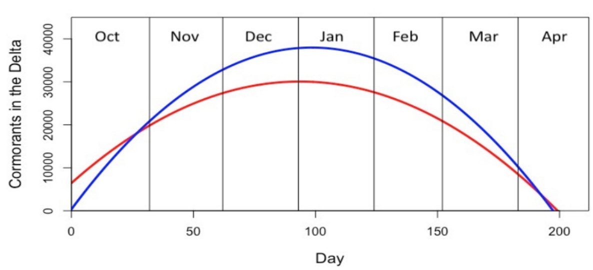

Figure 1. Estimated abundance trend for cormorants residing in the Mississippi Delta during winters 2016-2017 (red line) and 2017-2018 (blue line) calculated from aerial surveys of night roosts.

AQUACULTURE OPPORTUNITY AREAS NATIONAL ENVIRONMENTAL POLICY ACT ENVIRONMENTAL IMPACT STATEMENT STATUS UPDATE

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On May 7, 2020, the White House issued an Executive Order (E.O.) on Promoting American Seafood Competitiveness and Economic Growth (E.O. 13921). The E.O. requires the Secretary of Commerce, in consultation with agencies, officials, and appropriate Regional Fishery Management Councils, and in coordination with appropriate State and tribal governments, to identify geographic areas containing locations suitable for commercial aquaculture and complete a National Environmental Policy Act (NEPA) Programmatic Environmental Impact Statement (PEIS) for each area to assess the impact of siting aquaculture facilities there. Per E.O 13921, each PEIS may include the identification of suitable species for aquaculture in those particular locations, suitable gear for aquaculture in such locations, and suitable reporting requirements for owners and operators of aquaculture facilities in such locations. Each PEIS will analyze the environmental and related social and economic effects of siting aquaculture facilities within a specific area.

The Gulf of Mexico and Southern California Bight were selected as the first regions for focused evaluation. The National Centers for Coastal Ocean Science (NCCOS) then developed two marine spatial planning Atlases that analyze geographic areas that may be suitable for marine aquaculture development in federal waters in these two regions. These Atlases will be one source of information used to help to inform aquaculture planning efforts, including the identification of AOAs and the accompanying PEIS's.

This talk will provide an update on the work that has been accomplished on the PEIS phase of AOA identification, and detail opportunities for public, stakeholder, and industry input.

ENABLING THE SEAWEED REVOLUTION TO ADDRESS THE MAIN CHALLENGES OF OUR GENERATION

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12000 Years ago or so, human beings moved from prehistory to modern history when they stopped being hunters & gatherers to develop agriculture and livestock. Our history has been mostly fueled by land production and today we are still in the stone age when it comes to farming the oceans.

Seaweed has the potential to play a globally significant role in addressing food security, climate change mitigation, and marine biodiversity, as well as contributing to several Sustainable Development Goals (SDGs) that support sustainable job-creation, economic growth, and gender equality.

Seaweed and other algae hold an untapped potential to contribute to food systems. It is a nutritional source of food for humans, by being low in fat and rich in proteins, carbohydrates, minerals, vitamins (B12, A, K) and essential micronutrients (iodine, zinc, iron). Seaweed can be used as feed for aquaculture and land animals, bio-simulants for crops and innovative projects use seaweed extracts to replace single-use plastic as smart food packaging. Seaweed can also be used to support unlimited innovation in medicine or to create new type of fibers more sustainable than coton.

Also seaweed holds a great potential to sequester massively carbon and sink it down in the abyssal sediments. It can also contribute to clean the ocean from agricultural run offs and restore damaged ecosystems in the ocean providing habitat to marine life. Eventually, seaweed is a new source of revenues that supports coastal livelihoods and, based on experiences in Africa, can contribute to gender equality and women empowerment.

Following the launch of the Seaweed Manifesto (www.seaweedmanifesto.com) last year by UN, WWF, FAO, World Bank and many others, followed by a presentation at the 75th UN General Assembly and the launch of the first ever global coalition for seaweed (www.safeseaweedcoalition.org) this year, we are now in a situation to build on that momentum to create a seaweed revolution to support sustainable futures. The potential for seaweed as a Nature Based Solution to Climate Change has been detailed in a UN document (https://seaweedclimatesolution.com/) and has also been voiced at COP 26 in Glasgow as a game changer.

Seaweed, once integrated to other seafood production, has the potential to feed the world of tomorrow while mitigating climate change, restoring ocean biodiversity, mitigating ocean pollution and alleviating poverty in coastal communities .

A SUMMARY OF MARINE FINFISH LARVAL NUTRITION RESEARCH SUPPORTED BY THE WESTERN REGIONAL AQUACULTURE CENTER

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The larval rearing phase in production of aquatic organisms is the most difficult aspect of production once spawning is achieved, especially for new candidate species. Larvae have unique nutritional requirements that are often poorly understood. Nutrition studies on larvae are complicated by ontogeny as it pertains to vision, locomotion, feeding and digestion, immunity, etc. Larvae are fragile and sensitive, and early performance is affected by maternal influences passed down through the egg. Finally, fish larvae are small and can be difficult to observe.

This project was initiated to support marine finfish aquaculture on the West Coast of the United States by developing and applying appropriate larval feeds and feeding techniques that target two selected native marine species, the white seabass (*Atractoscion nobilis*) and the California yellowtail (*Seriola dorsalis*). The objectives of this project were to: 1) establish baseline indices for larval seabass and yellowtail using contemporary culture techniques as a benchmark for evaluating the project's success over time; 2) design, refine and implement methods that allow assessment of larval feed intake and behavior; 3) increase survival and growth of the targeted species during their larval stages through optimization, or nutrient enrichment of cultured live feeds; and 4) increase larval fish survival and growth during weaning from live foods to formulated feeds through the development and/or identification of appropriate formulated microdiets.

Ultimately, this project resulted in refined live feeds feeding practices where unnecessary food items were eliminated (e.g. 1st Instar *Artemia*) or reduced (e.g. rotifers) in days fed. In the case of seabass, the commercial enrichment was modified, which reduced malformation rates significantly. We successfully applied various tracers to live feeds and microdiets in order to assess feed intake, prey selectivity, and leaching rates of nutrients. We measured sinking rates of various complex feed particle types in order to understand availability to the larvae, and pair with nutrient leakage rate information. We demonstrated that taurine supplemented in the rotifer enrichment yielded larger larvae at the end of that feeding stage and that by using liposomes taurine levels in the rotifers could be increased to levels found in copepods. We developed an open formula reference weaning diet that performed similarly to commercial diets and tested several particle types to learn that particle-assisted rotationally agglomerated (PARA) and larval extruded (LEX) diets worked best. Finally, we showed that feed attractants (glycine, betaine, and alanine; GBA) improved the taste of food particles and significantly increased feeding incidence and gut fullness in fish larvae. The commercial attractant ProMega55 yielded the fastest growing and best surviving larvae when compared to control and GBA treatments, and reduced the weaning time by 10 days.

FUSOGENIC REGULATORS, MYOMAKER AND MYOMIXER, ARE ESSENTIAL FOR MYOBLAST FUSION AND FISH MUSCLE GROWTH

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Muscle development and growth require myoblast fusion to form multinucleated muscle fibers. Several fusiogenic regulators have been identified that play vital roles in myoblast fusion in fish embryos. Loss-of-function (LOF) mutations in Jamb, JamC, Myomaker (Mymk), and Myomixer (Mymx) resulted in similar defective myoblast fusion and formation of mononucleated fibers in fish embryos. To investigate their LOF effects on fish muscle growth, we characterized the muscle phenotypes in adult zebrafish carrying single and double genetic mutations in jamb, jamc, mymk and mymx genes. We found that although myoblast fusion was compromised in zebrafish embryos of jamb and jamc single or jamb;jamc double mutants, these mutant fish showed no defect in muscle growth. The mutant fish were able to grow into adults that were indistinguishable from the wild-type sibling. In contrast, the adult mymk, mymx single, and jamb;mymk double mutants exhibited a stronger muscle phenotype compared to the jamb and jamc single and double mutants. The mymk and mymx single and jamb;mymk double mutants were smaller in size and weighed approximately one-third the weight of the wild type (WT) sibling. Single fiber analysis of adult skeletal myofibers revealed that jamb, jamc, or jamb;jamc mutants contained multinucleated myofibers with tens to hundreds of myonuclei per fiber, whereas mymk, mymx single and jamb;mymk double mutants contained mostly fibers with less than 10 nuclei per fiber. Significant intramuscular adipocyte infiltration was found in skeletal muscles of the mymk, mymx and jamb;mymk mutants. Collectively, these studies demonstrate that although Jamb, Jamc, Mymk and Mymx are all involved in myoblast fusion during early myogenesis, they have distinct roles in myoblast fusion during muscle growth. While Jamb and Jamc are dispensable for muscle growth, Mymk and Mymx are essential for myoblast fusion and muscle growth,

XENOGENESIS FOR PRODUCING CATFISH EMBRYOS

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Xenogenesis has been accomplished previously in ictalurid catfish. However, spawning rates and fry production were inadequate for large-scale application to produce channel catfish, *Ictalurus punctatus,* female X blue catfish, *I. furcatus*, male hybrid catfish. A series of experiments were conducted to make stem cell harvest more efficient and productive, and stem cell transfer, colonization and proliferation more effective.

A hydrostatic pressure level of 7,000 psi 5 min after fertilization was needed to produce near 100% triploidy. Application of ROCK I at 50 µM yielded higher viability of spermatogonial stem cells (SSCs) and oogonial stem cells (OSCs) during the isolation phase, and these cells had the highest viability in the first day right after being dissociated from the gonads. The optimal size for collecting blue catfish SSCs in regard to yield/kg was 25 to 39.9 cm. Optimal size for blue catfish OSC collection was 25 to 39.9 cm and 200 to 600 g based on the peak for OSC production in these females. Injecting the stem cells into triploid hosts 4-6 dph resulted in higher colonization and proliferation compared to time periods prior or after that time. Preliminary data indicates that higher transformation and spawning are associated with introducing cells during this time.

In the current study, xenogenic channel catfish and white catfish, *Ameiurus catus*, harboring gametes from channel catfish and blue catfish were utilized to produce pure species and hybrid progeny. By utilizing the protocols above, xenogenic spawning with normal fry production is becoming more frequent. Preliminary results indicate that xenogenic common carp, *Cyprinus carpio*, might be used as blue catfish sperm factories as an alternative approach in the hybrid catfish embryo production technology.

DIRECT AND PLEIOTROPIC EFFECTS OF DESATURASE AND ELONGASE TRANSGENES IN CHANNEL CATFISH, *Ictalurus punctatus* FOR OMEGA-3 FATTY ACID ENHANCEMENT

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F1 transgenic channel catfish, *Ictalurus punctatus*, containing the common carp beta-actin promoter-masu salmon, *Oncorhynchus masou*, Δ5-desaturase like gene (D5D) showed a 33% increase in the relative proportion of n-3 fatty acids coupled with a 15% decrease in n-6 fatty acids and a 17% decrease in n-9 fatty acids when compared to non-transgenic full-siblings ($P < 0.01$). However, while the relative proportion of n-3 fatty acids was achieved, the total amount of fatty acids in the transgenic fish decreased resulting in a reduction of all fatty acids. Insertion of the D5D transgene into channel catfish also had large effects on metabolism, physiology, body composition, and growth of channel catfish. Transgenic channel catfish grew faster, were more disease resistant, and had higher protein percentage than full-sib controls. There were sex effects as performance changes were more dramatic in males. The D5D transgenic channel catfish were also more uniform in their fatty acid composition, growth and other traits.

Masu salmon elongase (*elovl2*) was substituted for D5D in the transgene and transferred using a two-hit by gRNA and two oligos with a targeting plasmid (2H2OP) CRISPR/Cas9 approach. Integration rate of the transgene was high (37.5%) detected and expressed in twelve different tissues of P_1 transgenic fish including relatively high expression in liver and muscle, 13.4 and 9.2 fold-change, respectively. DHA content in the muscle from transgenic fish was 1.62 fold higher than in non-transgenic fish ($p < 0.05$). Additionally, total n-3 PUFAs and omega-6 polyunsaturated fatty acids (n-6 PUFAs) increased to 1.41-fold and 1.50 fold, respectively, suggesting that the β-actin-elovl2 transgene improved biosynthesis of LC-PUFA in channel catfish as a whole. Potential off-target gene editing effects were likely the major factor responsible for morphological deformities detected found in positive and negative siblings compared to sham-injected controls.

The *evol2* gene was also inserted targeting a non-coding region of chromosome 1 or randomly inserted without CRISPR/Cas9. Integration rates were similar in this experiment and in both cases DHA was increased by about 20%. The benefit of the transgene is affected by diet causing genotype-environment interactions.

A variety of approaches were used to knock in 2-3 omega-3 fatty acid biosynthesis genes, masu salmon *elovl2,* rabbitfish *(Siganus canaliculatus)* Δ4 and Δ6 (Δ5) desaturase genes, simultaneously. In some cases (7%), all 3 genes were transferred by microinjecting a cocktail targeting multiple long repeat sequences (LRS) in the genome. For another strategy, 2 or 3 gene tandem arrays driven by a single promoter targeted either LRS or *lh* gene. This technique gave slightly higher and more consistent transformation with no target effect and double the integration with 2 gene constructs compared to 3 gene constructs. The last strategy involved knockin of *C. elegans* Δ12 desaturase and Δ15 desaturase transgenes utilizing mRNA and Cas9/gRNA RNP coupled with homology directed repair, microhomology-mediated end-joining non-homologous end joining with preliminary results indicating possible integration rates as high as 70%.

MOBILE RESPONSIVE CLINICAL FISH HEALTH DATABASE

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A Mobile Responsive Clinical Fish Health Database with associated learning games enables more accurate and timely identification and remediation of fish pathogens by fish health professionals and educates students. The two computer learning games, Fish Detective and SimFish, created in parallel, add to the educational value of this project. The Database contains historical cases from disease diagnostic laboratories and captures current and future clinical records. The Database facilitates data mining for risk assessment and epidemiological studies, including analyses of data from all cases diagnosed by a particular laboratory, by multiple labs within a state, by labs in a multi-state area, and by farmers wanting to track diseases over time on their own farm.

APPLYING MACHINE LEARNING TECHNIQUES TO DEVELOP AND OPTIMIZE LOW-COST SERUM-FREE GROWTH MEDIA FOR SEAFOOD CELL LINES

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The world population is predicted to grow to 10 billion by 2050 that will lead to many food production challenges present significant sustainability and security threats. Cellular agriculture is emerging as an advantageous solution to address these immediate problems by developing sustainable alternative food systems to feed a rapidly growing global population with nutritious and safe food, while protecting our environment and limited resources. Our lab is focused on growing seafood products, specifically fish, that require less resources compared to mammalian cell culture, making them good targets for cultivated meat. However, current cellular agriculture expansion is limited by serum-containing media used to grow cells. The serum component is expensive, unreliably produced, and questionably sourced making it important to create cost-effective and chemically defined serum-free. To our knowledge, there is no published serum-free media formulation specifically designed for long-term growth of food-grade fish cell lines.

Our lab is developing a multi-leveled machine learning protocol to optimize chemically defined serum-free media development for lean fish cell lines with verified growth factors in the least number of possible experimentations. Our first model designed 93 experiments to grow Zebrafish embryonic stem cells with seven component variables at five concentration levels. This model found that traditional growth factors and components used in mammalian serum-free cell culture are not all suitable for fish cells, and that serum remains the most stable component in growth. However, serum was not the most critical component in the initial days of growth compared to more traditionally used growth factors (Fig. 1). Current work optimizing the model with alternative growth factors that may be more crucial for fish cells, such as the essential fatty acids, to continue decreasing serum levels in growth media. Additional cultivated meat related responses will be measured for the best growth medias

Iincluding cryopreservation viability, proliferation potential, differentiation potential, and chromosome stability. Lastly, the most propitious growth medias will be scaled-up in our mini-bioreactor to further validate the growth media formulation. This methodology will create a durable predictive model tool applicable to developing serum-free media for alternative fish cellular agriculture cell lines and increase the capacity of cultivated food on an industrial scale.

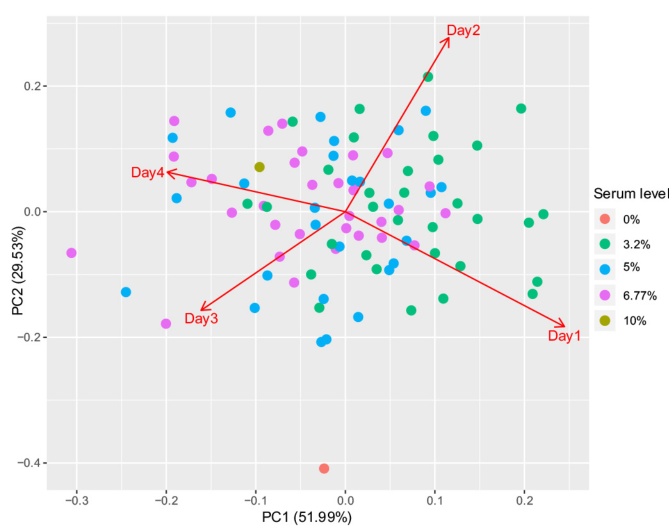

Fig. 1. PCA of relative cell growth rate

EXPLORING THE USE OF AUTOMATED NUTRIENT DELIVERY SYSTEMS (ANDS) FOR AQUAPONIC SYSTEMS

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Due to the high initial investment, aquaponic systems typically rely on low-cost solutions that allow for producers to be profitable at a smaller scale. Examples of these low cost solutions include bell siphons or gravimetric filtration such as radial flow or baffle clarifiers. While these low-cost solutions are invaluable for the success of small-scale producers, commercial producers are limited by the ability of these low-cost solutions. Therefore in order to meet the needs of commercial producers, the incorporation of Automated Nutrient Delivery Systems (ANDS) should be explored.

In brief ANDS are nutrient transport systems that are connected to an automated program that easily allows for precise and real-time control of the transportation of nutrients to and from the hydroponic component. ANDS has been successfully integrated onto media bed aquaponic systems in replacement of a low-cost siphon (Figure 1). While ANDS increases the upfront cost, the benefits to commercial producers is increasing the grow area through the removal of a siphon apparatus, reducing required footprint as the sump volume is reduced, and potentially increasing plant nutrient uptake through the consistent real time control of nutrient delivery. Due to the novelty of integrating ANDS and media bed aquaponic systems there is a lack of peer-reviewed data on the effects of controlling nutrient delivery on a species-specific level throughout the growth cycle of a plant. Additionally there is a lack of peer-reviewed data on the economic benefits of utilizing ANDS and at what scale the incorporation of the novel technology becomes the most economically feasible.

The incorporation of ANDS doesn't stop at media bed systems either. There is also potential for ANDS to be integrated into NFT systems as well as a novel aquaponic system design that incorporates aeroponics as the hydroponic subsystem (Aeroculture) (Figure 2).

FIGURE 1. Display of ANDS combined with a media bed aquaponic system.

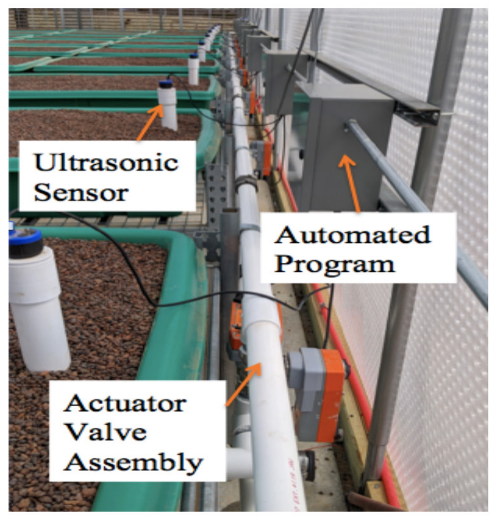
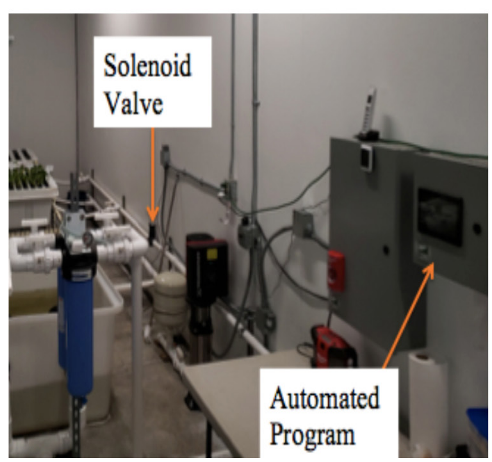


FIGURE 2. Display of ANDS combined with an Aeroculture system.



PHYSICOCHEMICAL PARAMETERS AND FISH FAUNA OF OGBESE RIVER, OWENA AKURE, ONDO STATE, NIGERIA

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Physicochemical properties play essential roles in the maintenance of healthy aquatic ecosystem. The need for water quality monitoring is paramount to safeguard the public health and also to protect the water resource in Nigeria. Hence, this study aimed at assessing the physicochemical parameters and fish composition of Ogbese River in Ogbese, Ondo state, Nigeria. A total of sixteen samples were collected fortnightly, during the months of February to April, 2021. The values obtained for the physicochemical parameters such temperature, alkalinity, pH, total hardness, phosphate, sulphate, DO, BOD, COD and others were 23.56±2.03, 96.82±101.41. 7.47±0.44, 105.25±26.65, 18.39±3.67, 15.66±6.45, 5.18±1.70, 2.56±0.59, 4.00±1.39 respectively. They are all within the range of values recommended by WHO, NIS and FEPA with the exception of dissolved oxygen, phosphate and nitrate. All the assessed parameters were statistically significant ($P < 0.05$) with the exception of hardness and nitrate. Furthermore, a total of two hundred and sixty-two fishes of seven (262) different species were obtained from Ogbese river in Ondo state. The species of fishes caught are; *Clarias gariepinus, Heterobranchus bidorsalis, Oreochromis niloticus, Tilapia zilli, Parachanna obscura, Malapterurus electricus,* and *Hepsetus odoe*. Among the fish species, *Clarias gariepinus* have the highest population abundance (34%) while the least species of fish obtained was *Hepsetus odoe* (2%). In conclusion, Ogbese River is of good quality, fit for domestic and agricultural purposes except for the excessive lead, phosphate, nitrate and dissolved oxygen content. However, further study is recommended on the source and quantification of heavy metals present in Ogbese River for timely intervention to public health advantage.

Table 1: mean and range of physicochemical parameters in four location on Ogbese river

Physicochemical parameters	Late February	Early March	Late March	Early April	Range	Mean ± STD	P Value	WHO	FEPA	NIS
Temperature (°C)	24	24.75	22.50	23.00	20.00-27.00	23.56±2.03	0.00	35-40	<40	40
pH	7.10	7.09	7.98	7.74	6.90-8.30	7.47±0.44	0.00	6.5-8.5	6.9	6.5-8.5
TSS (mg/l)	52.68	42.00	42.85	68.60	31.50-74.70	51.58±12.44	0.00	500	-	500
TDS (mg/l)	98.18	103.35	105.68	100.88	95.50-113.40	102.10±4.86	0.00	500	2000	500
TS (mg/l)	150.88	145.55	148.53	169.48	134.30-181.20	153.60-12.40	0.00	500	-	-
DO (mg/l)	3.63	5.83	5.30	6.00	3.20-8.30	5.18±1.70	0.00	2.0	2.9	-
COD (mg/l)	2.56	3.50	4.75	5.20	1.90-6.20	4.00±1.39	0.01	40	-	-
BOD (mg/l)	2.08	2.88	2.35	2.95	1.12-3.50	2.56±0.59	0.01	10	30-50	-
Hardness (mg/l)	110.95	121.58	120.65	67.85	40.60-151.30	105.25±26.65	0.06	100	-	100
Acidity (mg/l)	1.28	213.28	1.53	45.89	1.20-242.10	65.49±91.48	0.00	-	-	-
Alkalinity (mg/l)	165.43	1.48	217.18	3.23	1.20-250.30	96.82±101.41	0.02	150	-	150
Sulphate (mg/l)	21.28	15.65	19.88	5.84	4.50-22.30	15.66±6.45	0.01	48-200	500-1000	500
Nitrate (mg/l)	6.75	6.73	7.70	16.88	5.20-20.30	9.51±4.80	0.08	1.0	2.0	1.0
Phosphate (mg/l)	19.74	16.03	18.25	19.58	11.30-25.10	18.39±3.67	0.00	10	-	-

SUPPORTING AQUACULTURE INVESTMENT USING A NOVEL GEOSPATIAL AND FINANCIAL PLANNING TOOL

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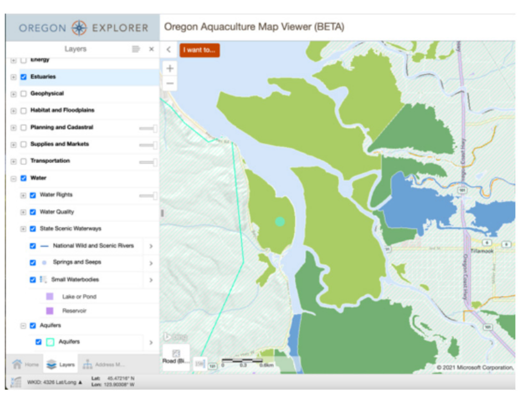
The Oregon Aquaculture Association (OAA), Business Oregon, Oregon State University (OSU), and Oregon Sea Grant (OSG) are finalizing the release of their Oregon Aquaculture Explorer Platform, a unique set of tools designed to advance aquaculture investment in the U.S. Using Oregon as a targeted case, this Platform includes geospatial and financial planning instruments that allow users (e.g., industry, state agencies/planners, investors) to make informed decisions about production systems by exploring spatial resources linked to financial models.

Currently, the tool has been built and peer reviewed for three inland aquaculture species: tilapia and sturgeon in recirculating systems, and hybrid striped bass in ponds. We are now in the process of beta testing and expanding the tool to include coastal and marine systems, as well as additional inland systems. The tool allows the user to select a site (choosing on a map or entering an address), develop an initial resource site report, and then determine likely costs for inputs including energy, water, feed, seed, and labor. Each of these costs can be refined based on the user's estimates. A unique attribute of the platform is directly linking spatial data as inputs into the financial models. The tool allows the user to run multiple scenarios based on alternative production levels, costs, output prices, and resource constraints. Start-up costs, including construction and materials, are also considered. The tool provides a downloadable Excel file for the user to conduct more detailed and site specific analysis.

Although the initial phase of the project has focused on inland species and systems, phase two will focus on marine species and coastal and estuarine aquaculture. With the exception of oyster culture, estuarine and nearshore aquaculture is limited in Oregon. New research will focus on investment opportunities and challenges for novel and emerging systems and species such as red seaweed (dulse) and purple sea urchins.

The goal of this presentation is to provide an introduction to and overview of the Oregon Aquaculture Explorer Platform, highlight how potential users can benefit from the tools, and elicit feedback and conversation around potential refinements, species, and concerns.



THE IMPACT OF TORULA YEAST ON GROWTH PERFORMANCE AND DIGESTIBILITY IN RAINBOW TROUT *Oncorhynchus mykiss*

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Two trials were performed to determine digestibility coefficients for torula yeast in Rainbow trout (*Oncorhynchus mykiss*) and its performance in plant-based or fishmeal-based diets, respectively. For the digestibility trial, six groups of 15 rainbow trout (mean body mass 100 ± 10 g) were placed in 60 L cylindro-conical tanks supplied with well aerated water at a regulated flow rate of 4 L/min. Fish were hand fed one of the 2 diets (Control diet or torula yeast diet) containing Y2O3 (inert marker), twice a day to visual satiation. The torula yeast diet contained 75% of the control diet and 25% torula yeast. The dry matter, protein, energy, and ash apparent digestibility coefficients were 78.6%, 93.8%, 84.8%, and 48.7%, respectively. For the growth trial, Rainbow trout of approximately 44 g initial body weight were distributed into 15 tanks (30 fish per 300-liter tank) and fed a commercial diet twice a day during 10 days for acclimatization to rearing conditions. After this period, the growth trial started, and fish were fed twice a day until apparent satiation with one of the 5 experimental diets. The experiment included three tanks per diet. The total duration of the growth trial was 12 weeks. The diets included a 20% fishmeal reference diet (FM) and a plant-based only reference diet (PB), test diets included 10% torula yeast inclusion replacing either fishmeal (FM SY10) or plant proteins (PB SY10), and a 20% torula yeast inclusion replacing all fishmeal (PB SY20). The plant-based only diets had significantly lower specific growth rate, feed conversion ratio, and daily feed intake compared with the other four diets. No significant differences were observed for hepatosomatic index and viscerosomatic index across treatments. No statistical differences were observed relating to growth performance between any diet containing torula yeast and the fishmeal reference diet. Results indicate that torula yeast is suitable for the replacement of fishmeal in the diets of Rainbow trout in terms of nutrient digestibility and growth performance. Better performance was observed in plant-based diets devoid of fishmeal when torula yeast was included.

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NOT SO SHELLFISH AFTER ALL: HOW NATIVE OYSTERS *Ostrea lurida* MAY AID EELGRASS *Zostera marina* RESTORATION BY NITROGEN FILTRATION

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Eelgrass (*Zostera marina*) is a foundation species in coastal waters that provide vital ecosystem services from habitat provision to trophic support. However, populations have declined globally at alarming rates including within Upper Newport Bay (UNB), CA. A multi-habitat restoration approach with native oysters (*Ostrea lurida)* may be key to promoting more successful eelgrass restoration. Oysters may increase nitrogenous nutrients in sediment porewater for uptake by eelgrass by mediating nitrogen transfer via filter-feeding and depositing nitrogenous waste. Resource managers are concerned about the efficacy of co-restoration with oysters due to potential negative interactions with eelgrass, a protected species. Little research has been conducted to date to address the efficacy of restoring these species together. In summers 2019 and 2021, we collected eelgrass shoots and pore-water samples from three restored sites within UNB, each with eelgrass restored alone versus eelgrass restored adjacent to oysters. We measured leaf and rhizome growth rates, above and below ground dry weight, and pore-water NH_4 and NO_3 concentrations. We analyzed these response metrics relative to distance from and density of oyster beds using co-variogram models. Preliminary results indicated positive associations between oysters and eelgrass at closer distances but only at some sites, with no significant associations at others. Given this generally neutral relationship, project managers should consider restoring both species in combination because each species can return unique ecosystem functions.

ALL CLAMS ON DECK: FLORIDA SEA GRANT'S HARVEST PROGRAM – INTERNSHIPS IN SUPPORT OF FLORIDA'S AQUACULTURE INDUSTRY

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In 2020, Florida Sea Grant (FSG) initiated an aquaculture training program, HARVEST (Helping Aquaculture Reap Value and Enhance Student Training), offering Florida university and college students part-time internships with aquaculture businesses. The internships helped fill a variety of needs expressed by Florida aquaculture businesses to improve their efficiency, public outreach, productivity, and sustainability. Students worked as employees of the University of Florida and partnered with an aquaculture business and a FSG agent, specialist, or affiliate. FSG mentors were responsible for establishing the student-business partnership, providing training where warranted, and reporting program results. The participating aquaculture business was responsible for training and co-mentoring the student in their aquaculture practice, providing a safe and hospitable working environment, and agreeing to diversity, equity, and inclusion (DEI) principles to create a safe working environment for women and minorities. The vision of the program was to foster a strong and productive working relationship among FSG, businesses, and students. The HARVEST program provided internship salaries and additional funding for supplies to participating businesses in support of the interns' work. The interns' experiences ranged from on-farm production work, maintenance of equipment, storm preparation, developing outreach and communications materials related to offshore aquaculture, social media marketing in support of an aquaculture business, and assistance with scientific projects related to improving aquaculture production (for example, evaluating the performance of selectively bred diploid and triploid oysters, and assessing the viability of wild oyster spat collection for aquaculture). Since inception, 7 interns supported 7 businesses, resulting in a $100,000 benefit (defraying employee and supplies costs) for these industry members. The HARVEST program exposed students to the aquaculture industry, provided financial and workforce support for participating businesses during a particularly difficult period due to COVID, and the framework for a continuing program that will train a future coastal workforce, increasing the competitiveness of the Florida aquaculture industry.

PERCEPTIONS OF AQUACULTURE: CURRENT TRENDS AMONG U.S. CONSUMERS

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Despite associated health benefits, few consumers eat the amount of seafood recommended by the Dietary Guidelines for Americans. Increased intake is warranted, yet greater demand necessitates a sustainable supply. Aquaculture can provide healthy, sustainable seafood, yet consumers may prefer wild over farmed selections, limiting the U.S. aquaculture industry and public health initiatives. Our objective was to evaluate U.S. consumers' perceptions of aquaculture products and production methods in order to identify potential barriers and opportunities toward increasing seafood intake.

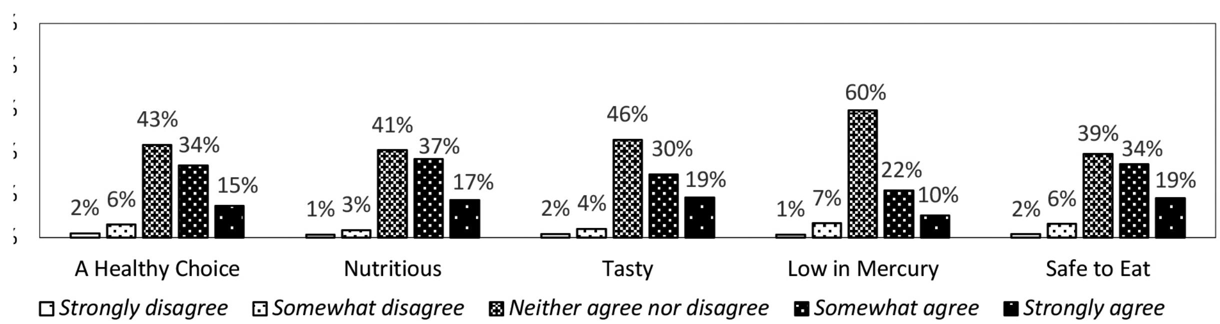
A nationally representative survey of 1,200 U.S. adults conducted in June 2021 assessed knowledge of and familiarity with aquaculture products and production processes. Consumers have low average familiarity as rated via 5-point scale (1=not familiar at all, 5=extremely familiar) with aquaculture products (2.25±1.040) and, especially, processes (1.80±0.999). Women were less familiar with aquaculture products; men were more likely to be moderately familiar (X^2 (12, n=1200) = 45.240, $p<0.001$). Income below $30k was associated with being "not familiar at all" with aquaculture products (X^2(12, n=1081)=46.471, $p<0.001$) and processes (X^2(12, n=1081)=21.905, $p=0.039$). Familiarity with aquaculture products or processes was not associated with age, education level, or living in a coastal area. More than half, and as many as 98%, of consumers failed to recognize that each of seven listed seafood selections are commonly farmed; less than 1% of respondents correctly recognized all seven. Fewer consumers misidentified five wild seafood types as available via aquaculture (Table 1).

Table 1. Percent of consumers identifying seafood types as available via aquaculture.

Salmon	46%
Shrimp	38%
Tilapia	34%
Oysters	30%
Clams	20%
Scallops	17%
Barramundi	2%
Tuna	16%
Crab	13%
Flounder	8%
Mahi	5%
Swordfish	3%

Consumers tend to agree more than disagree that aquaculture products are healthy, nutritious, tasty, low in mercury, and safe to eat, but many neither agree nor disagree with these statements. This, coupled with low familiarity with and knowledge of aquaculture products and processes indicates potential for effective educational efforts to shape consumer perceptions and increase the purchase and consumption of aquaculture products. Increased aquaculture product intake may have important implications for public health, seafood sustainability, and expanded market opportunity for the aquaculture industry.

Figure 1: Consumer agreement with the statement, "Aquaculture products are…"



EFFECT OF DIFFERENT MICROALGAL FEEDING ON SURVIVAL OF ISOLATED COPEPOD *Acartia clausi* FROM MARMARA SEA

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Live prey utilization is very important in larval rearing of cultured marine fish species. First live prey is Rotifer (*Brachionus plicatilis*) following *Artemia* sp. and microdiets, respectively. However, in many cases, most of mortality occurs during weaning period due to incomplete of fully developed gut and lack of digestive enzymes of larvae.

Copepods are natural live prey of fish larvae in marine food-web. Nowadays, several copepod species are being investigated for improving their culture performance. Microalgae are most important feeds for copepod cultures. Selection of microalgae species are crucial for copepod feeding due to the feeding habits and food selection of copepods. In this study, *Acartia clausi* individuals were isolated from Marmara Sea and sub-cultured with different microalgae diets *Chlorella vulgaris, Rhodomonas salina, Thalassiosira pseudonana, Diacronema vlkanium* under laboratory conditions during 15 days.

The strains of *Chlorella vulgaris*, *Rhodomonas salina*, *Thalassiosira pseudonana* and *Diacronema vlkanium* were obtained by Culture Collection of Algae and Protozoa (CCAP) culture collection. *Acartia clausi* fed four microalgae diets; *Chlorella vulgaris* (CV) + *Rhodomonas salina* (RS) (diet CV+RS), *Thalassiosira pseudonana* (TP) + *Rhodomonas salina* (RS) (diet TP+RS), *Diacronema vlkanium* (DV) + *Thalassiosira pseudonana* (TP) (diet DV+TP) and solely *Thalassiosira pseudonana* (TP).

At the end of the study, copepods fed *Rhodomonas salina* and *Chlorella vulgaris* (1:1) mixture resulted higher survival rate ($p<0.05$)(Fig.1). Beside, copepods fed solely *Thalassiosira pseudonana* resulted lower survival among groups. Proximate and fatty acid composition were also studied. Further studies are needed for other microalgae and copepod feeding interaction, feed acceptance and growth of selected copepods.

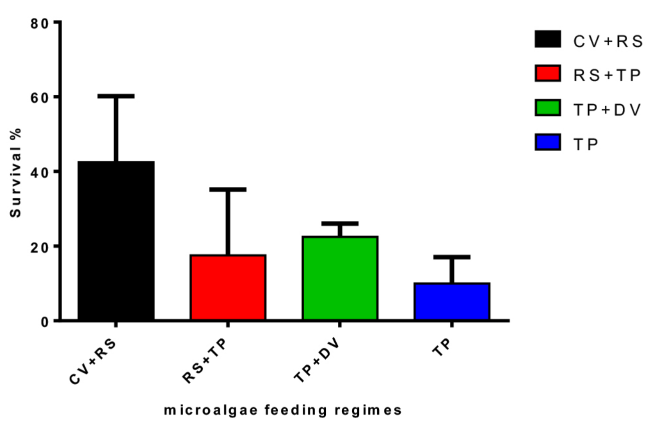

Fig.1. Survival rate of *Acartia clausi* fed different microalgal diets

EDUCATIONAL SUPPLY LINES FOR DEVELOPING AN AQUACULTURE WORKFORCE

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An educated workforce for aquaculture requires many steps in the educational process, but in areas such as the Midwest where aquaculture is less common, we also need to create a mindset for aquaculture. Aquatic farming, like any farming is labor intensive, so the first step is to find individuals who want to farm. Current trends suggest young people in general are not interested in taking up farming, and aquaculture also does not seem to attract large numbers of youth. Where we do see a lot of interest is in aquaponics. Aquaponics mixes traditional aquaculture with hydroponics and may be the best path forward to have an educated workforce. In developing a workforce, we need to move aquaponics from the backyard and onto campus. We also need to elevate the study from being considered a hobby interest to that of an integrated science requiring an understanding of STEM at the university level. However, it is difficult to convince universities to introduce new curricula without demonstrating a market need.

We assessed the level of aquaculture existing in high schools and mapped the locations of the schools as well as extension services and successful aquaculture businesses. www.ncrac-yea.org

We also incentivized interest from high school students through a competition called the Aquaculture Challenge. Our first round of the competition revealed that students quickly surpassed their teachers' knowledge of aquaculture, so we developed and hosted workshops for the teachers to better prepare them to mentor their students.

We will share the insights gained from our grant activities, as well as efforts to instill a campus culture of aquaculture at Lake Superior State University. The project started with aquaponics about ten years ago, but has advanced quickly in the past two years. The students have established an official aquaculture club, maintain a hoophouse and several aquaponics demonstration systems, and are now a student subunit of the USAS.

We propose aquaponics as an educational model to inspire youth to learn about aquaculture. Aquaponics requires a STEM education which provides the basic skills and knowledge needed for entering the aquaculture industry. As more youth are attracted to the aquaculture industry, we are confident that the educational supply lines will expand.

THE EFFECTS OF TEMPERATURE AND BODY SIZE ON THE METABOLISM AND HYPOXIA TOLERANCE OF WHITE ABALONE (*Haliotis sorenseni)* AND RED ABALONE (*H. rufescens*)

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In 2001, white abalone, *Haliotis sorenseni,* became the first marine invertebrate to be listed under the U.S. Endangered Species Act. Allee effects due primarily to overharvesting and disease resulted in individuals being too far apart for successful fertilization. Despite the fishery closure in 1996, *H. sorenseni* still shows no signs of recovery. Today, the White Abalone Recovery Consortium (WARC), a group that includes federal and state agencies, aquaculture organizations, academic institutions, and public aquaria, is working to recover this endangered species back from the brink of extinction.

To better understand the metabolic demands and hypoxia tolerance of the endangered white abalone, *H. sorenseni*, aspects of metabolic physiology were measured via closed respirometry techniques, in comparison with red abalone, *Haliotis rufescens*. The relationship of mean oxygen consumption rate of both *H. sorenseni* (n = 29) and *H. rufescens* (n = 29) to environmental dissolved oxygen level showed a logarithmic best-fit function. This curve shape reveals that abalone are slightly oxy-conforming at high dissolved oxygen levels and become increasingly conforming at lower oxygen saturations. Hypoxia tolerance, as estimated by determining the P_{90}, $P_{75,}$ P_{50}, and P_{25} (values in which oxygen consumption was 90%, 75%, 50%, and 25% of resting metabolic rate), of *H. sorenseni* was similar to that of *H. rufescens*. There were, however, higher levels of variability in P_{90}, $P_{75,}$ P_{50}, and P_{25} for *H. sorenseni* in comparison to *H. rufescens,* suggesting potential fitness differences between *H. sorenseni* cohorts spawned in captivity. Additionally, there was no significant relationship between abalone length (mm) (a useful metric of abalone size used both in the field and laboratories) and P_{50}. Although no differences between species were detected with hypoxia tolerance, *H. sorenseni* had significantly lower resting metabolic rates (RMR) than *H. rufescens* for the entire overlapping the size range tested (total mass 7.7 to 103.3 g). Higher temperatures had a significant effect on P_{90}, $P_{75,}$ P_{50}, and P_{25} for *H. sorenseni,* revealing the potential compounding effects of high temperatures and low oxygen on the species. These metabolic data can help inform outplanting procedures of the endangered *H. sorenseni* such as determining favorable environmental dissolved oxygen and temperatures for outplanting site selection, as well as selecting fit captive bred abalone for outplanting.

CAN RECREATIONAL ANGLERS HELP TO STOP THE SPREAD OF AQUATIC INVASIVE SPECIES (AIS)?

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Invasive species are perceived to be a major threat to the biodiversity of the ecosystems by disrupting the balance of native ecosystems, causing great economic losses. To stop the spread of aquatic invasive species (AIS), management plans to maintain healthy ecosystems are necessary and the behavior of recreational water users, such as recreational anglers, is important as their travel between different recreational sites unconsciously provide convenience to aquatic species transferring to another ecosystem. However, an environmentally responsible behavior of recreational anglers is highly likely to reduce the occurrence of AIS and avoid a great economic cost in the future to repair the environment. This paper investigates the economic values of recreational fishing sites according to different environmental qualities, testing whether leisure purposes, AIS knowledge, environmental awareness and attitudes, etc. are highly relevant to the recreational anglers' behavior to prevent the spread of AIS. A contingent valuation method is applied to ask respondents their attitudes to AIS, and their willingness to visit the recreational fishing sites. This study provides important policy implications on establishing a sustainable management plan to protect the ecosystem, using the power of recreational anglers in preventing the spread of AIS.

IN-POND RACEWAY SYSTEM PRODUCTION TRIALS GROWING STOCKER AND FOODSIZE HYBRID CATFISH PLUS TILAPIA

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In Pond Raceway systems consist of floating or fixed floor rectangular raceways (RW) assembled or placed into existing earthen ponds. This case study evaluated the performance and economics of producing foodsize hybrid Catfish and stocker sized fingerlings (Channel Catfish *Ictalurus punctatus* ♀ x Blue Catfish, *I. furcatus* ♂) in In-Pond Raceway Systems (IPRS) and tilapia (*Oreochromis niloticus*) grown in cages, placed into four 0.4 ha ponds. Growout raceways in ponds 1 and 2 were 63 m^3, and 45 m^3 in ponds 3 and 4. Each pond had one (14 m^3) stocker unit raceway plus a 36 m^3 tilapia cage placed into ponds 2 and 4. Each pond had 5.0 HP of aeration that maintained adequate DO levels. Catfish were fed a 32% CP commercial diet twice a day; tilapia were allowed only to graze the phytoplankton flow generated by the growout and stocker IPRS units. The combined production from growout (186 days) and stocker (142 days) resulted in 19,712 kg/ha in pond 1, 19,302 kg/ha in pond 2, 19,426 kg/ha in pond 3, and 16,555 kg/ha in pond 4. The same number of tilapia were stocked into the two cages and resulted in yields of 2,167 kg/ha (pond 2) and 2,160 kg/ha (pond 4); when combined with catfish production resulted in total gross production of 21,469 kg/ha in pond 2 and 18,715 kg/ha in pond 4. Foodsize average harvest weights ranged from 670 to 894 g, with survival rates ranging from 86 to 98 %, and having weight gain per day (WGD) of 1.74 to 3.22 g/day. Raceway stocker unit achieved harvest weights ranging from 173 g to 186 g, with survival rates ranging from 75 to 90% and having WGD from 1.02 to 1.37 g. In general, efficient FCRs were achieved in all growout and stocker hybrid Catfish IPRS units. Hybrid Catfish raised in IPRS demonstrated excellent fish inventory control, promoted uniform hybrid Catfish production, with 90 to 95% of the foodfish harvested in the preferred premium size range. Production strategies for inclusion of co-cultured tilapia along with the catfish IPRS systems were achieved with little investment and operating costs, resulting in overall positive net returns. Ponds housing IPRS catfish units plus a tilapia cage had reduced investment payback periods, increased net present value and higher internal rates of return (Table 1).

Table 1. Financial measures of profitability for producing foodsize and stocker hybrid Catfish, Channel Catfish *Ictalurus punctatus* ♀ x Blue Catfish, *I. furcatus* ♂), in In-Pond Raceway Systems (IPRS), and tilapia (*Oreochromis niloticus*) raised in cages (Ponds 2 and 4 only) placed immediately downstream of hybrid Catfish IPRS production cells in 0.4 ha ponds, U.S.$, 2019.

Item	Pond 1	Pond 2	Pond 3	Pond 4
Financial net return, U.S.$	-5,679	9,562	-4,090	2,298
Investment cost, U.S.$	39,996	40,796	21,196	21,996
Payback period, year	-7.0	4.2	-2.5	4.9
Net present value, U.S.$	-78,691	29,444	-38,823	7,024
Internal rate of return, %	-	16.5	-	20.7

*Using a discount rate of 5%.

EARLY EXPERIMENTS IN UNDERSTANDING THE ROLE AND OPTIMISATION OF THE MICROBIOME IN MOLLUSCAN LARVAL DEVELOPMENT

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Oysters are the most heavily cultivated bivalve mollusc globally, contributing significantly to food security. As sessile aquatic organisms, oysters have a close relationship with environmental microbiomes, but the impact of this on culture of commercial species such as the Pacific oyster (*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*) is under-studied. This project will study if and how the microbiome can influence development and survival of oyster larvae and in turn, efficiency of hatchery seedstock production.

This project aims to understand the impact of microbes on culture systems, including the characterization of the European flat oyster microbiome throughout a natural spawning event, and the microbiome of *C. gigas* and *O. edulis* throughout hatchery production. Sample collection will be carried out in a number of UK and US based hatcheries alongside recording of physiochemical parameters. Analysis of hatchery environmental samples will provide insights into sources of microbial contamination, a major limiting factor for hatchery output efficiency.

Metabarcoding analysis will be completed using Oxford Nanopore Technologies to identify the bacterial species/genotypes present, and their relative abundances within the microbiome. This broad-scale data will then enable hypothesis-driven manipulation experiments to investigate how the microbiome can be modified to increase hatchery output. Developing our knowledge of the oyster microbiome through these studies builds towards the long term goals of improving hatchery efficiency via disease mitigation and increased larval survival rates. Here we present the outline for this work and a selection of preliminary results.

PERCEPTION OF SEA GRAN'S EXTENSION EDUCATORS REGARDING THE CULTIVATED SEAFOOD PRODUCTS

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Cellular agriculture, the production of seafood products from cells (e.g., muscle and fat cells) using cell culture techniques, has been proposed as a novel approach to complement the conventional seafood industry (i.e., aquaculture and capture fisheries). The cellular agriculture industry is still in its infancy. To build a successful enterprise, the industry should address the technological challenges as well as the social and ethical dimensions of cultivated seafood production to ensure consumer acceptance of these products. The extension educational process is a vital component of any agricultural development process, which may include transferring a particular technology or communicating specific information to help the stakeholders form sound opinions and make sound decisions about the agricultural production systems. Therefore, this study aimed to study the perception of Sea Grant Extension educators regarding the consumption and production of alternative seafood products. With the intention of developing an educational extension and outreach program related to cellular agriculture, we designed a survey to study the Sea Grant Extension Educators' perception regarding cultivated seafood products. In this survey, a series of questions (Figures 1 and 2) were asked to understand the perception and any knowledge gaps of Extension educators concerning the cultivated seafood. Out of 117 respondents, 49 were extension agents, 36 were Extension specialists, and 22 had other extension roles. 57 % of the respondent had concerns about the consumption and production of cell-based seafood products. Safety, sensory attributions, and environmental impacts were the highest-ranked concerns.

This study's outcome is imperative for developing an Extension program to work with extension educators as agents of change for consumers' perception of novel cultivated seafood products.

Figure 2: Do you have any concerns about the production of cultivated seafood?

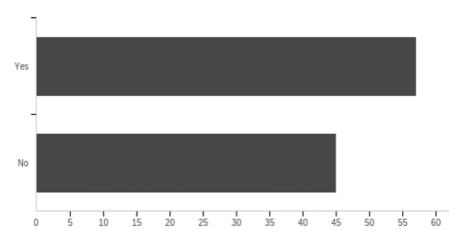
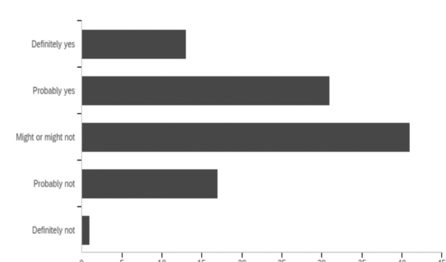


Figure 3: Do you think your stakeholders would like to know more about cultivated seafood?



EFFECTS OF MASS MEDIA ON AQUACULTURE DEMAND. THE CASE OF GILTHEAD SEABREAM IN THE SPANISH MARKET

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The influence of mass media on aquaculture demand is tested in the case of the Spanish gilthead seabream (Sparus Aurata) retail market. This influence was tested by the inclusion of an index measuring the intensity and content of the published news into the market demand function. The index covers a set of scores informing about different attributes of the news published in the five most read National newspapers between 2013 and 2017. The attributes where selected from the literature on previous research on the topic and by using a qualitative Delphi procedure. Once defined, they were scored and recorded in monthly series.

Besides the number of news published, the level of the relation with aquaculture and the directionality of the message (positive or negative) were the most consistent as well as strongly related among them. A principal component analysis was applied with these three series resulting in a single index explaining 66% of the total variance.

The media index and seabream retail prices are included in a function as explanatory variable of seabream quantities sold at the Spanish retail in the observed period. Data on prices and quantities are available at the Food Consumption Panel published by the Spanish Ministry of Agriculture and Fisheries. These two series were found to be non-stationary.

Results from the Johansen test inform about two cointegrating vectors. Both price and the media index were found to be exogenous causing changes in demanded quantities. The effects of price are more significant than those of the media index.

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THE IMPLEMENTATION OF AUTOMATIC FEEDERS IN SEA BASS GROW-OUT FARMS: AN ECONOMIC EVALUATION

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European sea bass (*Dicentrarchus labrax*) is an economically important cultured fish species along the Mediterranean coast, being the EU is one of the largest producers of sea bass in the world (STECF, 2021). It is generally accepted that the highest recurring cost in grow-out aquaculture comes from feeding. Manufactured feed often represents between the 50 and 70 percent of operating costs, being one of the key components of any cage aquaculture operation so that appropriate feed management techniques and/or improvements in feeding may contribute to the appropriate utilization of feed without increasing the cost of production. According to Hasan and New (2013), better management can reduce the feed cost to the extent of 15-20%. The intensive salmon farming, for example, has benefited from huge improvements in feed efficiency and the reduction of operating costs using palletized diets along with modern feeding technology (Blyth and Dodd, 2002). Hence, adopting specific measures to limit the magnitude and incidence of feeding in fish farming will lead to improvements in production efficiency.

The aim of this work is to evaluate the economic impact of the implementation of automatic feeders in a typical sea bass grow-out farm producing 450-g fish. For it, we have used a deterministic static model to simulate the annual income statement of three farms according to different annual production capacities (a micro, small, and medium-large farm) and carry out partial budget and investment appraisal analyses. Model parameters were set up using data obtained from ten European facilities located in the Mediterranean Sea and validated for experts in the field. The impact on the farm's operational factors assumed for this analysis is showed in Table 1, whereas our results are presented in Tables 2 and 3.

Blyth P.J. and Dodd R.A. (2002). *An economic assessment of current practice and methods to improve feed management of caged finfish in several SE Asia regions*. SunAqua Pty. Ltd., Queensland, Australia.
Hasan M.R. and New M.B. (2013). *On-farm feeding and feed management in aquaculture*. FAO Fisheries and Aquaculture Technical Paper No. 583, Food and Agriculture Organization of the United Nations, Rome.
STECF (2021). *The EU Aquaculture Sector – Economic report 2020 (STECF-20-12)*. Luxembourg: Publications Office of the European Union. https://doi.org/10.2760/441510

Table 1. Impact of the measure on operational factors (% of variation over baseline values)

Farm size	Annual production capacity	AGR	FCR	Labor cost	Energy cost	Investment in capital
Micro farm	180 tons	+0.5	-0.5	-20	+2	0.3
Small farm	540 tons	+1	-1	-20	+5	3.6
Large farm	2,250 tons	+5	-5	-20	+10	21.0

AGR = Average Growth Rate. FCR = Feed Conversion Ratio.
Note: The proportion of feed technicians in the farm is estimated around a 40% of the total employees and we have assumed a 50% of cost reduction of these employees with the implementation of automatic feeders.

Table 2. Economic impact on a sea bass grow-out farm implementing automatic feeders

Concept	Unit	Micro farm	Small farm	Large farm
Net operating profit (baseline value)	€/year	-30,348	547,298	3,521,630
Increase in the net operating profit	€/year	36,461	71,938	750,629
Average operating cost (baseline value)	€/kg	5.99	4.67	4.06
Reduction in the average operating cost	€/kg	-0.22	-0.14	-0.27

Table 3. Economic evaluation on a sea bass grow-out farm implementing automatic feeders

Concept	Unit	Micro farm	Small farm	Large farm
Feed technology*	-	Feed blower	Flexi blower	Centralized
Investment in capital (*I*)	€	5,000	140,000	1,800,000
Annual cash-flow variation (*CF*)	€/year	37,005	81,740	877,692
Net Present Value (*NPV*)	€	142,752 €	186,364 €	1,704,369 €
Benefit-cost ratio (*BC*)	ratio	114.0	5.3	3.8

*All technologies include remote control with cameras and software.
Notes: Investment horizon (n) was fixed at 5 years and annual discount rate (k) at 8%. Investment prices were obtained at December 2020.

PROFITABILITY FACTORS IN THE EUROPEAN AQUACULTURE INDUSTRY: ANALYSIS OF COUNTRY DIFFERENCES

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Aquaculture is a worldwide industry that plays an increasingly important role in global food production. In Europe, the aquaculture sector is diverse, encompassing traditional, artisanal and family small businesses to multinational marine farming companies, which accounts for about 20% of fish production and directly employs roughly 80,000 people between full and part-time jobs (EU, 2016).

Nevertheless, despite the growing importance and high priority assigned by the EU policy makers to the development of aquaculture, so far little attention has been given to analyze the economic performance of the sector (Guillen et al., 2015). In the last two decades the entire sector has faced increased market competition, falling/stable prices and rising production costs what have affected the profitability of this sector. Nevertheless, the economic performance of firms presents significant differences that would be necessary to analyze to know which factors are relevant to obtain the best results.

The aim of this work is to analyze which factors can explain differences in firms' profitability in the European aquaculture industry. A sample of firms of the aquaculture industry from Croatia, Greece, Norway, Spain, and Sweden were identified in the Amadeus database and annual economic data of those firms from 2009 to 2013 was collected and averaged to carry out our analysis. Main descriptive statistics of the sample are showed in Table 1. With this sample, we regressed the following model by each country employing the OLS methodology:

Our findings are presented in Table 2. The results support that the firms' profitability is significantly explained by the operating margins. However, efficiency is not significant in the case of Greek and Spanish firms so that there is room for improvement in the European aquaculture industry to improve this factor. On the other hand, the strategies of product differentiation with product certifications and diversification through vertical integration are both positively related to the firms' profitability of Norwegian and Spanish firms.

EU (2016). *Facts and figures on the Common Fisheries Policy. Basic statistical data*. Luxembourg: Publications Office of the European Union. doi:10.2771/830693

Guillen J., Natale F. and Fernandez Polanco J.M. (2015). Estimating the economic performance of the EU aquaculture sector. *Aquaculture International* 23(6): 1387-1400.

Table 1. Main descriptive statistics

Variable	Croatian firms		Greek firms		Norwegian firms		Spanish firms		Swedish firms	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Y	–2.42	10.04	–1.89	7.23	5.53	16.94	–2.17	8.52	0.06	19.41
X_1	–0.85	19.68	–0.72	14.18	6.62	24.81	–4.49	19.43	1.47	25.42
X_2	69.22	86.53	56.56	21.11	71.83	52.91	67.24	100.71	76.24	50.74
X_3	28.68	31.67	29.54	26.85	36.32	28.67	41.73	36.62	45.70	28.47
X_4	0.02	0.14	0.05	0.22	0.09	0.29	0.03	0.16	0.02	0.13
X_5	0.15	0.36	0.18	0.38	0.06	0.24	0.42	0.50	0.11	0.32

SD = Standard Deviation.

Table 2. Regression results

Explicative variable		**Dependent variable:** ROA (%)			
	Croatian firms	*Greek firms*	*Norwegian firms*	*Spanish firms*	*Swedish firms*
Intercept	–8.1644***	–3.2463*	–7.0632***	–4.5966***	–14.6598***
X_1 = Operating margin (%)	0.2050**	0.3290***	0.4485***	0.2288***	0.4962***
X_2 = Efficiency (%)	0.0322**	0.0194	0.0689***	0.0069	0.0862**
X_3 = Solvency (%)	0.1142**	0.0140	0.1218***	0.0460***	0.1460**
X_4 = Product certification (dummy)	–2.6430	–0.3668	0.4833	3.5939***	11.0695
X_5 = Vertical integration (dummy)	3.1705	0.6029	3.3441**	2.3020**	5.0238
Number of observations	54	80	329	180	62
R^2	0.3560	0.4576	0.6776	0.3677	0.6607
F-test	2.59**	7.51***	60.82***	25.60***	10.00***

Note: OLS estimators with White's robust significance intervals have been used to estimate and test parameters of the model. *** $P < 0.01$. ** $P < 0.05$. * $P < 0.10$.

INVESTIGATION OF MITOTIC SHOCKS FOR INDUCING TETRAPLOIDY IN ZEBRAFISH AND PEARL DANIO UNDER VARIED CONDITIONS

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Zebrafish (*Danio rerio*) is a popular model species for human disease, but also aquaculture. A series of experiments was conducted to assess the success of applying mitotic physical shocks, heat and pressure, to zebrafish and pearl danio (*D. albolineatus*) with the aim of producing viable tetraploids.

Six trials addressed the effect of post-fertilization and incubation temperature at 28 °C (high) and two trials were conducted at 22.3 °C (low). Heat shocks of 41.4 °C and pressure shocks of 7000 PSI were applied for a duration of 2 min at various times (minutes) post activation (mpa). Shocks were applied between 2 and 60 mpa for trials with high fertilization temperatures and between 23 and 83 mpa for trials with low fertilization temperatures. Samples of eggs were collected and preserved in a solution of 5% acetic acid and Ringer's solution corresponding to the initiation of each shock attempted to assess the overall developmental stage of embryos. Fertilization, 24-hour, and hatch percentages were recorded for each treatment. At 4 days post fertilization (dpf) larvae were sampled for flow cytometry to assess ploidy. Survivors of groups which were identified as possessing ploidy deviating from diploid in 4 dpf samples were reared until fish were large enough for fin clips to be collected for reassessment of ploidy via flow cytometry.

Fluorocytometric analysis identified that tetraploids, mosaics, and triploids were produced by application of these physical shocks for both species. Heat shocks initiated between 48 and 56 mpa at high incubation temperature or between 72.5 and 83 mpa at low temperature, resulted in the highest proportion of tetraploid zebrafish identified from 4 dpf larvae. However, these shocked groups also suffered from decreasing rates of survival to hatching as the time to shock initiation increased. Samples of eggs taken during at the initiation time of each physical shock revealed that embryos were all at the 2-cell stage with a small portion beginning to transition to 4-cell stage. Only diploids were produced for pearl danio which were incubated at low temperature. No tetraploids were identified from fish which survived to 21dpf.

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \epsilon_{it}$$

COMPARING PERFORMANCE OF COUPLED AND DECOUPLED BRACKISH WATER AQUAPONICS SYSTEMS USING KALE WINTERBOR F1 HYBRID *Brassica oleracea* AND PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Water reuse is a focus of inland brackish water recirculating aquaculture systems (RAS) due to the cost of salt and challenges with saline effluent discharge. Aquaponics systems are designed to minimize water discharge and utilize plant growth as a means of nutrient removal from system water, notably nitrate that builds up over time in RAS. Brackish water aquaponics research has been limited and mainly focused on halophytic plants. Marketing halophytic species can be a challenge, especially for inland locations. Kale is marketable and we have established its salt tolerance up to 20 ppt. salinity. This trial analyzed coupled (C) and decoupled (DC) aquaponics systems at 15 ppt. salinity with an 8-week growout of Winterbor kale and Pacific white shrimp.

Replicated treatments in UVI-based systems (1718 L) were salted using a least-cost salt mix and homogenized with reused shrimp water to attain an initial nitrate concentration of 100 mg/L. Shrimp were stocked at 300 m^{-3} and fed a commercial growout diet (35% protein). Kale seeds were germinated in fresh water and acclimated to salt over a 15-day period; each system was stocked with 66 kale plants. Weekly plant growth metrics (height, number of leaves) were measured on a fixed random selection to include 10% of the plants per system. Plant metrics measured on selected plants at harvest included height, number of leaves, chlorophyll content index (CCI), leaf area index (LAI), wet and dry weights. Total plant wet weights (biomass, roots) from each system were also measured. Water quality parameters measured twice daily included temperature, dissolved oxygen, pH, salinity and conductivity. Total ammonia-nitrogen, nitrite and nitrate were measured thrice weekly; phosphate and potassium were measured weekly and iron was measured twice weekly with additions of DTPA 10% chelated iron throughout the trial to maintain levels at 3 mg/L.

DC systems had significantly higher pH than C systems; C systems had significantly higher Fe levels than DC systems (58.6 g Fe added to C systems, 65.7 g added to DC). There were no significant differences in any other water quality parameter measured. Higher nitrite spikes in DC systems resulted in significantly less total feed input than in C systems, which showed more stable water quality overall. Coupled systems reduced nitrate levels by 19.1% and DC systems by 6.3%. Overall, daily nitrogen uptake per plant was 7.5 mg in C systems and 2.7 mg in DC systems. Plants in C systems outperformed those in DC systems in all metrics except CCI and root to shoot ratio, with significantly higher wet and dry weights, height and system wet weights (roots and shoots). Plant survival in C systems was 95.5%, DC systems was 92.4%. Future research will involve engineering the UVI design to better suit shrimp production and biofloc technology, and modification of DC system protocols to improve plant production.

2021 GLOBAL REVIEW OF TILAPIA PRODUCTION AND MARKETS

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On a global scale, tilapia production has continued a steady increase in recent years up until 2020. Due to the global covid pandemic and subsequent recession, production dropped severely in China and subsequently in other major producing countries. This was in part caused by hatchery and farm workers not being available to work due to lockdowns and transportation restrictions and in part by farmers and processors not risking production or processing with unknown demand. In fact, consumption dropped rapidly in many countries where tilapia was popular in the restaurant trade. Demand for live and fresh whole fish and fresh fillets dropped globally, starting with Chinese New Year and then dropping around the world as restaurants, cruise ships, and schools stopped purchases of fresh and frozen fish of all types.

However, it must be noted that even though tilapia has become an international commodity with millions of tons of fish traded internationally, most tilapia consumption still occurs in the domestic markets of producing countries. Often, tilapia farmers and their neighbors are the single biggest consumers. This floor of demand held up as producers shifted sales into local markets consuming tilapia in place of imports no longer available.

As the pandemic continued, the major importing countries reported increasing grocery sales of fresh and frozen fillets to be prepared for home consumption. The mild flavor and smell of tilapia may have encouraged more consumers to select tilapia over fish with a reputation for leaving strong “cooked fish smells”. For the US markets, this led to recoveries in the imports of fresh fillets from Latin America and frozen fillets from Asian producers. Imported tilapia from China to the US was still constricted due to the imposition of trade tariffs, and large increases in transport costs and delays. Chinese tilapia was somewhat replaced by imports from Indonesia and Latin America into US markets.

India had been showing a rapid increase in tilapia production prior to 2020, but this was stalled in 2020 as both domestic demand and export markets decreased. Ecuador was reported to have reduced tilapia production, switching some production from tilapia back to shrimp. Egypt reported stable production with increasing production from the huge Egyptian-Chinese joint venture replacing some decreased production from smaller farms due to the pandemic. Brazil reported several new tilapia ventures supported by some of Brazil’s agri-business giants but as none had come on-line by 2021, we expect that production probably decreased due to the severity of the covid pandemic in Brazil.

Overall, the best estimates suggest that global production may have decreased slightly in 2020 but was essentially stable. There was severe market disruption in the early months of 2020 but recovery in the second half of the year. The impacts during 2021 are unclear as new restrictions related to the delta and other variants of the covid virus are still being felt globally, but demand in the US was increasing for imports and domestically produced tilapia.

EFFECTS OF DIFFERENT IRON COMPOUNDS AND AQUEOUS IRON CONCENTRATIONS ON KALE WINTERBOR PERFORMANCE IN BRACKISH WATER HYDROPONICS

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Utilizing aquaponics may allow brackish water aquaculture producers remediate wastewater while producing a marketable product. Kale (*Brassica oleracea*) has been shown to tolerate brackish water up to 15 ppt salinity, albeit with reduced growth and harvest weight. Many plants have established ranges of required nutrients for growth but in brackish water such ranges are unknown and uptake rates are likely impacted by the high levels of sodium present in the water. Some nutrients influence sodium tolerance, particulary transition metals like iron, zinc, and manganese. Increasing the levels of these nutrients may allow for increased plant production in brackish water. Selecting a form of each nutrient that is bio-available to the plant in brackish water conditions is also critical. This presentation covers two trials, one testing different iron containing compounds and the other differnet iron concentrations on kale performance in 15ppt brackish water.

The first study examined the effectiveness of three iron containing compounds on kale performance, along with an iron containing foliar spray made from fermeted dandilion (*Taraxacum officinale*) leaves. The iron compounds used were EDTA, iron sulfate (FeSu), and iron citrate (FeCi) and were added to each tank to reach 3mg/l total iron. Twenty four 18L tanks were assigned in triplicate to 8 treatments, 4 without a foliar spray: NoFe (No aqueous iron), EDTA, FeCi, FeSu; and 4 with a foliar spray: NoFe/FS, EDTA/FS, FeCi/FS, FeSu/FS. All tanks contained four plants on a raft, were acclimated to the target salinity of 15ppt using a low cost salt mixture, and fertilized using a hydroponics fertilizer made without iron. The second study examined the effectivness of elevated levels of iron on kale performance. Identical systems as the first study were used with 12 tanks, 4 treatments, and 3 replicates each. The treatments were 0mg/l, 3mg/l, 6mg/l, and 9mg/l, representing the aqueous iron (EDTA) concentation. Water quality parameters (Temperature, O_2, pH, Salinity, Total Ammonia Nitrogen, Nitrite, Nitrate, and Iron) were measured weekly in both studies. Harvest took place after 28 days in both studies, at which time plant performance metrics were measured (Height, weight, roots weight, CCI, etc.).

Results from the first experiment showed no significant differences in kale performance between the different iron compounds, however average kale harvest metrics were all slightly higher in the EDTA treatment. The foliar spray showed modest improvements when combined with aqueous iron citrate, but no difference in the other treatments. EDTA tanks maintained a higher and more consistant level of aqueous iron compared to iron sulfate and iron citrate. Results from the second study showed that iron levels of 3mg/l or higher were acceptable for kale at 15ppt salinity and only minor increases in average kale growth were shown at 6mg/l of aqueous iron. Further research will investigate other forms of chelated iron and their effects on kale performance, along with additions of other elements.

CAN YOU EAT THEM TO SAVE THEM? FARMING THE WEST COAST NATIVE OYSTER *Ostrea lurida* FOR BOTH MARKET AND RESTORATIVE VALUES IN TOMALES BAY, CA

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Olympia oysters *(Ostrea lurida)* were once abundant as a foundation species in the bays and estuaries of the North American West Coast. Archeological remains demonstrate they were also an important wild food for Native Americans, and they were widely harvested as a food resource for waves of immigrants arriving during and after the Gold Rush era. Commercialized harvest combined with extensive habitat degradation led to the decline of *O. lurida* in most California estuaries. This decline, in concert with the 1920's introduction of larger, faster growing Japanese oysters (*Crassostrea gigas)* also led to the virtual disappearance of native oysters from the farms and oyster bars of the West Coast.

While a handful of shellfish growers in the Salish Sea region continue to grow Olympia oysters for market and restoration purposes, in California the commercial farming of *O. lurida* has all but disappeared. In 2018 Hog Island Oyster Co. began experimenting with the culture of native oysters for Bay Area oyster bars and in support of local restoration research. This presentation will give an overview of the Hog Island project, discuss commercial opportunities and challenges, and explore the potential of native oyster farming to enrich larval abundance and recruitment in nearby wild populations.

STREAMLINING CHALLENGES ASSOCIATED WITH POST-HARVEST: EFFORTS OF THE SOUTHERN NEW ENGLAND SUGAR KELP COOPERATIVE

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In Southern New England, the coastal waters are ideal for growing native sugar kelp. Kelp is rich in vitamins, minerals, and omega-3s and is a source of sustainable food. When cultivated locally it brings economic benefits to the region and helps preserve working waterfronts. The short growing season and the small farm sizes (10 acres or less) limits the volume the farmers are able produce in a single season. In addition, Southern New England also lacks processing and transportation infrastructure necessary for seaweed farmers to access and establish outlets for kelp beyond its fresh (raw) form. Finally, the general awareness from the public about why and how kelp should be prepared provides sales and marketing challenges for farmers. The Sugar Kelp Cooperative is a model for seaweed farmers to streamline post-harvest activities for sales and marketing, and deliveries introducing consistent safety standards to the market while making the delivery channels more efficient. Cooperative farmers can also aggregate biomass to entertain institutional sales into the sales pipeline as well as push for a marketplace for the ecosystem services provided by the farmers.

OPPORTUNITY BETWEEN THE TURBINES: A WILLINGNESS-TO-PAY EXPERIMENT REGARDING CO-LOCATION ACTIVITIES WITH OFFSHORE WIND

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With an increasing number of maritime industries competing for space off Virginia's coastline, integrated solutions for marine areas may offer a way to limit conflict and maximize productivity. Countries across the world are researching the different ways in which the space between offshore wind (OSW) turbines can be utilized to provide economic and environmental benefits. As Virginia constructs the first OSW farm in US Federal waters, there are new opportunities for co-location (coupling multiple maritime activities in one area) that could benefit the Virginia economy. Using data from a choice experiment and random utility modeling, this research quantifies Virginia public preferences for various co-location options within the lease area of the Coastal Virginia Offshore Wind (CVOW) farm. These estimates can be utilized as a comparison measure against implementation and management costs of each activity. The experimental design of this study serves as a template for other OSW installments around the world.

Three co-location activities were included in this study based on specific characteristics associated with the CVOW project and presently available technologies: (1) a seaweed aquaculture farm, (2) a non-harvested seaweed forest, and (3) a designated research area. The CVOW lease area hosts regular activity including, recreational and commercial fishing, boating, and charter operations. Permitting public access to ocean space between the turbines is considered the status quo (SQ) operations throughout this experiment.

During the survey, respondents are provided with the option to willingly accept an annual tax for the implementation of various combinations of co-location techniques based on the economic and environmental implications associated with each activity or stick with the SQ of "unlimited public access". A web-based choice experiment was disseminated to a representative sample of the Virginia public in winter of 2021. It was deduced from the econometric examination that the average Virginian household is willing to pay nearly $40 annually for a combination that includes all co-location activities. The second highest WTP amount is associated with a combination of both seaweed aquaculture and a non-harvested seaweed forest at more than $31 per 1,000 acres.

WTP data is helpful information for energy providers, researchers, aquaculturists, and policymakers because understanding public predilection through estimating WTP engages the public, which can aid in circumventing public resistance during implementation. Additionally, this level of insight can be valuable to the local and global economy by potentially stimulating healthy competition in research and technological innovation surrounding the possibilities of co-location with the OSW and offshore aquaculture industries.

HEMOCYTE MORPHOLOGY AND THE EXPRESSION OF PCNA AND P53 IN HARD CLAMS *Mercenaria mercenaria* WITH HEMOCYTIC NEOPLASIA

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Hemocytic neoplasia (HN) is a contagious, leukemic-like disease prevalent in several species of marine bivalves. HN is believed to be transmitted via water when neoplastic cells are expelled from infected individuals and absorbed by naïve ones of the same species. As the disease progresses, neoplastic cells proliferate in the hemolymph and replace normal cells throughout the vascular system, ultimately leading to loss of organ function and death of the organism. HN is currently threatening populations of hard clams, *Mercenaria mercenaria*, primarily in Wellfleet, Massachusetts, USA, resulting in ongoing mortality throughout the warm months of the year. Throughout the summer and fall of 2021, clams were sampled from this area and were maintained in holding tanks for hemocyte evaluations.

Staining methods used to compare the morphology of hemocytes to that of neoplastic cells from naïve and HN-infected clams were Protocol, Giemsa, and Periodic Acid-Schiff (PAS). The proliferative behavior of neoplastic hemocytes was assessed by immunohistochemical and immunocytochemical detection of PCNA, a cofactor protein of DNA polymerase delta that is essential for replication. A commercial antibody is used in the histological staining of sections to detect PCNA. The expression pattern of this protein in neoplastic cells provides insight into the progression of the disease. Attempts were made to identify members of the p53 gene family in hard clams and develop primers for reverse transcription qPCR analysis. p53 is an important tumor-suppressing protein which has been linked with HN in other bivalve species. The levels of expression of p53 will be quantified and compared between naïve and infected individuals.

Each of these assessment methods will contribute to a detailed cytological description of neoplastic hemocytes, as well as p53 abundance, in normal and neoplastic hard clams, and will provide information for comparison to other infected bivalve species.

GENOMIC STUDY OF GABA RECEPTOR LIGAND BINDING SITES OF THE BIVALVE MOLLUSC *Crassostrea virginica*

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GABA (γ-aminobutyric acid) an inhibitory neurotransmitter in molluscs and other animals has not been well studied in bivalves. In humans, impairment of GABA neurotransmission can cause epilepsy. In the bivalve mollusc *Crassostrea virginica*, as well as other bivalves, serotonin is an excitatory neurotransmitter that increases beating rates of gill lateral cell cilia. Previously our lab demonstrated in *C. virginica* serotonin's increase of cilia beating rates is blocked by applying GABA to the visceral or cerebral ganglia. Additionally, bicuculline methchloride, a GABAa receptor antagonist, blocked the effects of GABA. By using HPLC we previously detected GABA in low ng amounts in cerebral and visceral ganglia of *C. virginica*. Our immunofluorescence studies showed the presence of GABA neurons in cerebral and visceral ganglia; and that some serotonin neurons had GABA receptors on their soma. Recently the genome of *C. virginica* and other bivalves has begun to be mapped. By conducting BLAST searches of the NCBI (National Center for Biotechnology Information) database using DNA and protein sequences of *C. virginica* and other invertebrate and mammalian species we found matches for GABAa and GABAb receptor genes on *C. virginica* chromosomes 3 and 5, respectively. Various invertebrates had Percent Identity above 60%, while humans and mice had Percent Identity of about 40% to 50% for GABAa and GABAb. We hypothesize that the ligand binding sites (LBS) for GABAa and GABAb receptors in *C. virginica* are evolutionarily conserved and will closely match those of other animals. To study this, we conducted searches of the NCBI database for GABAa and GABAb LBS of *C. virginica* and compared them to other animals. We found GABAb LBS contained 4 amino acids (N, L, A, Y) in positions 34, 122, 123, 267 that were highly conserved in LBS of other bivalves, gastropods, insects, mice, rats and humans. GABAa LBS for *C. virginica* has not yet been identified. We did find LBS of other animals contained 2 amino acids (L, Y) that were highly conserved among the animals in which it has been identified. The *C. virginica* GABAa receptor does contain L and Y amino acids. The study complements our earlier physiology and cell biology studies demonstrating the presence and a function for GABA in *C. virginica* and shows the genome of *C. virginica* contains genes to produce GABA receptor LBS that are similar to those of other animals. This new information is valuable as it shows the simple nervous system of *C. virginica* can be used to conduct studies on GABA neurotransmission.

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NITRITE INDUCES CHANGES IN THE CHANNEL CATFISH NOSE AND GUT MICROBIOME

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Nitrite, a common pollutant from nitrogenous waste in fish culture, compromises fish nasal and gut microbiomes, increasing susceptibility to nasal pathogens. Also, nitrite is linked to an increase in mortality by *Edwardsiella ictaluri* infections in catfish intensive farming. The nose and gut are the two main portals for *E. ictaluri* that causes enteric septicemia of catfish (ESC) by *Edwardsiella ictaluri* infections. Thus, we hypothesize that nitrite compromises the catfish nasal and gut microbiomes increasing susceptibility to nasal pathogen infections.

To test our hypothesis, Channel catfish (*Ictalurus punctatus*) were exposed to 0mM (control), 0.2 mM and 2mM concentrations of nitrite. Catfish were kept for 30 days in 500 L tanks per treatment, with three replicate tanks per treatment containing nine fish per tank. Exposure to 2mM sodium nitrite was 90% lethal within 24 hours of exposure. A subsample of fish from the 0mM and 0.2mM treatments were obtained at day 0, 5 and 30. The surviving fish from 2mM treatment were sampled after 24 hours. All fish were sampled for nose, gut, muscle, gill, brain, blood, liver and kidney for either biochemical, microbial or histological analysis. Fish exposed to 0.2 mM and 2mM accumulated nitrites in several tissues with values higher from those in water. Fish from the 2 mM exhibited a marked methemoglobinemia due to nitrite-induced oxidation of hemoglobin. Histological analysis showed an oxidative damage in all organs of individuals exposed to 2 mM and a moderate effect in the nose, gill and gut of fish from the 0.2 mM treatment. Nose and gut microbiomes will be sequenced using whole genome shotgun sequencing to determine the impacts of nitrite on the microbiome. It is expected to see a degeneration of the olfactory and gut mucosa, coupled with a decreased nasal and digestive microbiome.

Our long term goal of this study is to develop nasal probiotics that can decrease the negative impacts from nitrite which would lead to better survival from infections. Future experiments will include identifying potential nasal and gut probiotic strains with protective effect against *E. ictaluri* and testing the efficacy in catfish chronically exposed to nitrite. Additionally, loss of olfactory function and bacteria detection will be screened by electro-olfactograms. Combining microbiological, electrophysiological, neurological and clinical approaches, our experiments will reveal nasal mucosa as a target of fish wellbeing. The outcomes of this proposal will produce novel probiotic treatments and will describe delivery pathways for bacterial infections in fish.

BUILDING CAPACITY OF ATLANTIC SALMON *Salmo salar* RAS PRODUCTION IN THE U.S. USING AN INDUSTRY DRIVEN STAKEHOLDER NETWORK (RAS-N): ENGAGEMENT AND EXTENSION EFFORTS

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NOAA Sea Grant has funded a national collaborative project consisting of research institutions, industry, and government agencies across the US. Efforts of the Recirculating Aquaculture Salmon Network (RAS-N) are supported by research, education, extension, and industry partners from the Mid-Atlantic, Great Lakes and Northeast regions of the U.S. (WA). The overarching goal of RAS-N is to build capacity for Atlantic salmon RAS in the U.S by addressing the barriers and needs of industry for successful growth, stability, and economic feasibility. In the first year, a holistic hub was established with formal founding partners from across each region. A main objective of the RAS-N hub is to gather stakeholder input which includes guidance, concerns, ideas and other input regarding industry needs, thoughts on extension, outreach approaches, workforce development, optimal use of available federal/state funding, and other topics.

The first RAS-N workshop was hosted by the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility, which provided initial stakeholder input on industry barriers and needs. In the first year (2019-2020), working groups were formed to focus on priority areas and a concept paper was developed to outline current and future research concerning technical and biological barriers as well as non-technical needs (economics, workforce development, etc). These barriers and needs were the focus of the most recent workshop hosted by the University of Maryland Institute of Marine and Environmental Technology. A RAS-N Priorities survey was also designed to formally prioritize industry needs. The survey was first shared with in-network stakeholder partners and affiliates and initial results were shared with members of the RAS-N Internal Steering Committee and the Research Working Group. The survey was then successfully extended to industry stakeholders outside of the network, both nationally and internationally.

This presentation will highlight RAS-N workshops, its efforts to build and expand capacity-building, results from the RAS-N Priorities Survey, remaining RAS-N activities, including development of a road map, and the foundation that RAS-N played in continuing Extension and Engagement with Atlantic salmon RAS industries in our next major USDA project: Sustainable Aquaculture Systems Supporting Atlantic Salmon (SAS^2).

A HIGH FLYER SENSOR FOR MARINE MAMMAL ENTANGLEMENT DETECTION AND NOTIFICATION

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With the potential of the United States expanding into the Exclusive Economic Zone, an increase risk exists where large marine mammals (i.e. whales) will encounter aquaculture farms. To create a situation that minimizes the risk, a combination of approaches will be necessary. The approaches include; (1) marine spatial planning efforts to site farms with low frequency whale passage; (2) deterring whales from entering the area where gear is deployed; (3) designing farms that will shed off the whale if interaction occurs; and (4) the installation of sensors that can detect and notify response personnel during a potential entanglement event.

With support from the World Wildlife Fund, a sensor system was designed, built and tested with the capability of measuring large mooring system deflections and communicating the information to a shoreside station. The design process first included examining datasets from physical and numerical model tests that described the response of a typical kelp line system to a simplified impact event. The results showed that this particular aquaculture configuration was more sensitive to deflections rather than component tensions. This assessment is consistent with observations of whales with fishing gear where entanglement movements are characterized by translations and rotations. Therefore, the instrumentation package was designed around an inertial measurement unit (IMU) having 9 degrees-of-freedom (DOF). The DOFs include a 3-axis accelerometer, to obtain movements in the x-, y- and z- directions (e.g. deflections), a 3-axis gyroscope to determine the corresponding rotations around the x-, y- and z- axes (e.g. spinning), and a 3-axis magnetometer for sensing the orientation of the 3 axes to the Earth's magnetic field. The IMU is connected to a datalogger that can be configured for cellular, iridium or radio communications. A GPS unit is also connected to the datalogger to determine the location of the sensor. The system's high flyer design serves as a gateway between in air communication and subsurface sensing. The instrument suite was built and attached to mooring gear for a series of impact tests with human powered surface vessels (i.e. students paddling canoes). The instrument successfully detected impact and transmitted the data. The next iteration of system design and ocean testing with aquaculture gear is now in the planning process.

IMPROVING RAINBOW TROUT *Oncorhynchus mykiss* GROWTH PERFORMANCE THROUGH OPTIMIZATION OF DIET-EPIGENETIC INTERACTIONS

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The US imports approximately 75% of domestically consumed seafood, so increasing seafood production through aquaculture is critical for reducing this deficit. Expanding aquaculture depends in part on efficient broodstock management, including appropriate broodstock nutrition. Maternal nutrition can affect growth in the next generation through inherited epigenetic modifications like DNA and histone methylations that persist in the offspring. Therefore, identifying broodstock nutritional strategies that exploit these mechanisms and improve offspring growth performance will increase production capacity. Here we combine genetic selection and nutri-epigenetics to improve performance.

Recently, we demonstrated that maternal dietary supplementation with choline and methionine in fillet-yield selected lines results in increase growth performance in offspring beginning at 360 days post hatch. To better understand the interaction between maternal nutrition and genetic selection on offspring performance we focused on the role of maternal choline intake on trout currently used within the industry – disease resistance selected rainbow trout. Since 2005, the National Center for Cool and Cold Water Aquaculture has maintained, within the odd-year NCCCWA breeding program, 5 disease-resistance selected lines: 1) resistance to *Flavobacterium psychrophilum* (Fp-R), 2) resistance to *F. psychrophilum* and *F. columnaris* (Fp/Fc-R), 3) randomly mated controls (Fp-C), 4 and 5) two susceptible lines (Fp-S and Fc-S). For this work, the disease-resistant selected lines were utilized. Our objectives were to 1) identify how choline levels in rainbow trout broodstock diets affect growth and fillet yield in the next generation and 2) characterize how the choline concentration in the rainbow trout egg affects fry performance. Within each objective, treatment effects on the epigenome and transcriptome were analyzed to characterize mechanisms that respond to choline and establish links between epigenetic modifications in the genome and the phenotype of the offspring.

Interestingly, within the disease-resistant selected trout, maternal dietary intake of choline had no effect on offspring growth performance, which disagrees with previous data in fillet-yield selected trout lines maintained by NCCCWA where offspring growth was increased after 360 days post hatch. However, global gene expression (RNAseq) and global methylation (RRBS) analyses demonstrate that maternal dietary choline intake affects key metabolic, stress, and hypoxia-related pathways. These data indicate that stress and hypoxia tolerance are likely performance outcomes positively affected in offspring by maternal choline intake.

BROODSTOCK DIETS CAN ALTER EGG FATTY ACID COMPOSITION AND EGG QUALITY IN SOUTHERN FLOUNDER, *Paralichthys lethostigma*

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Egg and larval quality can be influenced by broodstock diet even when larvae are reared under uniform conditions and fed a high-quality diet. A study was conducted to examine diet-egg dynamics in Southern Flounder (*Paralichthys lethostigma*), particularly with respect to fatty acids. Questions addressed included: (1) Which fatty acids in eggs can be altered by manipulating broodstock diet? (2) Can dietary manipulations of egg fatty acid composition affect egg quality? (3) What is the temporal window for egg composition to equilibrate with broodstock diet changes?

Two populations of Southern flounder broodstock were fed a common diet of shrimp, squid, and mackerel (2:1:1 by wet weight). One population was given a year-round nutritional supplement (Mazuri Gel); the other received the supplement 1 month before, and throughout spawning. Two other populations of flounder were fed a common diet of shrimp and sardines (1:1 by wet weight) and then switched to either shrimp only or sardines only 16 weeks before spawning started. After hormone injection, adults from each diet treatment were strip-spawned, eggs were incubated under uniform conditions, and egg quality (fecundity, viability, energy density, hatching rate, hatching length) was assessed. Fatty acid composition (% total fatty acids) of broodstock diets and eggs were analyzed by gas chromatography.

Almost all of the 27 fatty acids measured varied between eggs from one diet and another; only 2 (20:2n-6 and 20:3n-6) did not differ significantly among all four broodstock diet treatments. Four fatty acids in eggs (16:1n-7, 16:2n-4, 16:3n-4, 20:4n-6) were especially sensitive to differences in these broodstock diets, as they were significantly different for all diet treatments. Among the five egg quality measurements, only length at hatching exhibited significant differences among diet treatments. Larvae from eggs produced by adults fed shrimp only (2.71 mm $\pm$ 0.27 mm) were significantly larger than all other diet groups, and larvae from the diet of shrimp, squid, and mackerel with year-round nutrient supplementation (2.30 mm $\pm$ 0.19 mm) were significantly smaller than all other diet groups.

Comparing eggs from the two broodstock populations that received the nutritional supplement, nine fatty acids did not differ despite the difference in timing of diet supplementation. All of these were minor constituents of the supplement (< 4% of total fatty acids). Proportions of 12 fatty acids (16:0, 16:1n-7, 17:0, 16:3 n-4, 18:1 n-7, 18:2 n-6, 18:3 n-6, 18:3 n-4, 18:3n-3, 20:4n-6, 20:5n-3, 22:5n-3) were significantly higher in eggs produced on the year-round supplemented diet, indicating that equilibration of eggs to broodstock diet takes more than 1 month. Combining results of the diet change to shrimp only or sardines only with data from a prior study that used the same diets but different timing of the diet change (0-8 weeks before spawning), showed that equilibration of egg fatty acid composition with southern flounder broodstock diet takes 10 to 16 weeks.

NORWEGIAN SEAFOOD EXPORT – THE IMPACT OF QUALITY AND MARKET SHARE ON UNIT VALUE

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Norway is the world´s second largest seafood exporter, and the leading exporter of farmed salmon. A large number of firms export seafood to 145 different markets. When excluding oil and gas, seafood accounts for about 1/4 of the total Norwegian export, with aquaculture products accounting for 70 % of the value. Consequently, this industry's performance in competitive export markets is important for the Norwegian economy. In this paper, we utilize highly disaggregated custom data to investigate the impact of quality and market share, respectively, on exporters achieved unit value in export markets.

In the trade literature, distance is used to explain differences in achieved export prices between markets (countries). Exporters typically upgrade quality and charge higher prices in more distant markets as this may serve to reduce the importance of transportation costs. However, distance and trade costs may also contribute to reduce the number of trading firms and as such weaken the competition in the market. In empirical work, quality is hard to measure and separate from other components that effect prices.

In this paper, we construct an index variable for quality related to Norwegian seafood export. To identify high quality exporters of a specific product, we utilize observed variation in unit value among exporters in different markets. An exporter that obtain an above average unit value in most markets, will typically achieve a high index value for quality. We include the quality variable in a standard gravity model where we, *i.a.*, control for distance, as well as the exporter's market share to a specific importer-market combination. For different seafood product, the results will indicate the impact of quality and market share, respectively, on achieved unit value, when controlling for normal gravity variables. We are particularly interested in documenting differences between aquaculture and traditional fisheries.

GUT MICROBIOTA COMPARISON OF PACIFIC WHITE SHRIMP PRODUCED FROM PLANT AND ANIMAL-BASED FEEDS

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Litopenaeus vannamei also known as the Pacific white shrimp, is the most common shrimp species cultured in-land around the world. *L. vannamei* is native to the Pacific coastlines ranging from the state of Sonora, Mexico, to the northern parts of Peru, where water temperatures are >20°C year-round. These shrimps can reach a maximum length of 23 cm and weight up to 45 g in the wild. Because this species of shrimp can reach very large sizes in a short period of time, it has a high production value for fisheries and aquaculture farms. *L. vannamei* production in 2014 was between 180,000 and 200,000 tons, and it is expected to increase by 10% in 2030. The main production challenge for *L vannamei* inland-aquaculture is the risk for disease outbreak. In recent years, there has been a considerable increase in disease outbreaks and early mortality syndrome in Asia, U.S.A., Mexico, and Central and South America. The feed used in most aquaculture operations is mainly fishmeal. The high protein present in the feed can easily make water parameters such as levels of ammonium, nitrite, and nitrate uncontrollable. Animal maintained under such conditions are stressed and could negatively affect their growth, immunity, and tissue integrity. A cost and growth effective feed replacement can be plant-based such as cottonseed meal, which in previous experiments has demonstrated to result in similar growth capacity for shrimp as commercially available feed, while having high survival rate and better water parameter management when compared to the fishmeal-based feed. An aspect of shrimp growth that is not regularly taken into consideration is the *L. vannamei's* gut microbiota, which constitutes of the microorganisms found in the stomach, hepatopancreas and intestines. Throughout the life of *L. vannamei* crustaceans, the gut microbiota provides metabolic benefits, supports hormonal functions, stimulates the immune system, and has been associated with higher product weight and survivability. In the present study, feeds with different animal-to-plant ratios were utilized in a growth experiment using *L. vannamei*. After 30 days of the assigned feed consumption, the guts of shrimp under four different diets were extracted to analyze microbial diversity using Illumina 16S-amplicon sequencing technology and bioinformatics. Results demonstrated differences in gut microbiota composition between shrimp fed a 100% plant-based diet and the other three diets containing animal ingredients. In all the diets that had animal products the dominant phyla in decreasing order of abundance were Actinobacteria, Proteobacteria and Bacteroidetes, whereas the dominant phyla in the plant-based feed were Proteobacteria, Actinobacteria and Bacteroidetes. These results demonstrate how the abundance of dominant bacteria phyla can fluctuate in response to changes in diet, and that the feed containing animal ingredients greatly influences the presence of specific bacterial consortium. In conclusion, this study enhances the understanding we currently have on the shrimp's gut microbiota and their relationship to feed sources in an effort to benefit the aquaculture industry. Further studies are needed to understand how this change in gut microbiota affects the survivability and overall health of the shrimp.

THE MANGROVE EPIGENOME (MangroveENCODE) PROJECT OF THE FUCOBI FOUNDATION OF ECUADOR: A ONE HEALTH APPROACH TO CONSERVATION OF HEALTHY MANGROVES AND WETLANDS, TO PRODUCE HEALTHY SHELLFISH AND FISH, TO PROTECT PUBLIC HEALTH LONG-TERM

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Programa “UNA SALUD / ONE HEALTH Epigenomics and Microbiomes:
Somos lo que comemos / We are what we eat”
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The long-term goal of the MangroveENCODE project of the FUCOBI Foundation of Ecuador is to help conserve healthy mangroves and tidal wetlands ecosystems through education and research, by (1) promoting replanting of new trees to address both the community needs and the underlying ecological causes of mangrove degradation; (2) understanding mangrove resilience to climate change by studying their genomes and epigenomes, including transposable elements (TEs), and (3) educate about wastewater-based epidemiology and association of antimicrobial resistance (AMR) and contaminants of concerns (COCs) in emerging resistant pathogens of public health concern. The short-term goal is to examine the interactions between greenhouse gas fluxes and carbon sequestration (blue carbon) and microbial communities (microbiome) with AMR and COCs such as endocrine disrupting chemicals (EDCs) like heavy metals, biocides, pesticides, glyphosate-based herbicides, microbial transgene *Bacillus thuringiensis*, *Vibrio* sp., metals chelated by glyphosate, bisphenol A (BPA), microplastics, bis(2-ethylhexyl) phthalate (DEPH), and per- and poly-fluoroalkyl substances (PFAS), organophosphates, COVID-19 disinfectants, and persistent organic pollutants (PCBs, PAHs) in mangrove sediment, considering adaptation to climate change and environmental degradation-related health issues.

Baseline information is being obtained for future studies to test mechanism-driven hypotheses to examine the transgenerational epigenetic inheritance mechanisms involved in the interactions of CO2 with EDCs and the microbiome of mangrove sediment/agricultural soil, using ecological, toxicological, ‘omics’ technologies, and computational tools. Preliminary results will be presented about (a) in-depth review of the scientific literature using NCBI databases about mangroves genomes and transcriptomes, genetic variation, AMR, EDCs, TEs like Gypsy LTR retrotransposons and epigenetic components [DNA and histone methylation, non-coding RNAs (miRNA, siRNA, lncRNA)] involved in salt and temperature stress adaptation of mangroves to global change; (b) protocols to collect 1-meter sediment cores for CO2 and EDCs analyses; and (c) metal concentrations in mangrove sediment, shellfish and people from estuaries of Ecuador.

TRANSCRIPTOMIC AND PHYSIOLOGICAL EFFECTS OF DIETARY PREBIOTICS CHITOSAN, INULIN, AND Β-GLUCAN ON JUVENILE *Totoaba macdonaldi*

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Dietary prebiotics such as 0.5% chitosan, 1% inulin and 0.1% β-glucan have improved the growth, digestion and immune response of different fish species under culture conditions; however, their effects in totoaba juveniles are unknown. In this study, the effects of such prebiotics were evaluated in juvenile *T. macdonaldi* including measurements of immune response (i.e., leucocyte and respiratory burst in blood); digestibility and digestive enzymes (i.e., trypsin, amylase, and lipase), and RNA-seq transcriptomics of the liver.

Fish fed the chitosan added diet showed the highest respiratory burst, immunoglobulin gene expression, and the lowest lipase activity. Differentially expressed genes analysis resulted in significantly higher expression of proteolysis, digestion, and lipid hydroxylation genes (DEGs, Fig 1). Fish fed the inulin diet showed the highest diet apparent digestibility coefficient, trypsin and lipase activities, and the highest expression of trypsin-like and quimiotrypsin-like genes in the liver. In fish fed the β-glucan diet, DEGs were detected mainly in the category of adaptive immune response (Table 1), with downregulation of immunoglobulins and upregulation of genes of the complement system.

Fish fed the chitosan diet showed the highest number of DEGs, while those fed the β-glucan diet the lowest.

Figure 1. Number of DEGs in the liver of *T. macdonaldi* juveniles in each prebiotic diet against the control diet.

Table 1. Functional enrichment of gene ontology terms for DEGs in each prebiotic diet

GO ID	Term	P-value
Inulin		
GO:0007586	digestion	0
GO:0098968	neurotransmitter receptor transport postsynaptic membrane to endosome	1.5E-5
β-glucan		
GO:0002250	adaptive immune response	0.0019
GO:0046886	positive regulation of hormone biosynthetic process	0.0019
Chitosan		
GO:0007586	digestion	0
GO:0006508	proteolysis	0
GO:0002933	lipid hydroxylation	6.0E-6

IMPACT OF HOST GENOTYPE ON GUT AND HEPATOPANCREAS MICROBIOTA OF PACIFIC WHITELEG SHRIMP *Litopenaeus vannamei*

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Crustaceans are the second main aquaculture product farmed worldwide, being the white leg shrimp (*Litopenaeus vannamei*), the most valuable species. Despite its successful production, shrimp may be the aquaculture species most impacted by the disease. There is growing evidence that the structure and composition of its microbiota is an essential factor for its health condition. Nevertheless, the relationship between the host genotype of *L. vannamei* shrimp populations with its microbiota is unexplored. To determine this relationship, we used 16S rRNA amplicon sequencing to characterize the gut and hepatopancreas microbiota and genotypification with Illumina Infinium ShrimpLD-24 v1.0 of two populations of *L. vannamei* shrimps collected from three different ponds of a shrimp hatchery in México.

The organ and genotype were the main factors shaping the shrimp microbiota. With the principal coordinate analysis (PCoA) using Unweighted UniFrac distances, we observed a clear separation between samples tagged by organ (**Fig. 1A**) and by genetic lines (**Fig. 1B**). Additionally, ANOSIM analysis showed that organ was the most important factor shaping the microbial structure, followed by the genetic line.

A genetic line drove microbiota richness and diversity. We found differences between Gen 1 and Gen 2 groups; this can be observed with the Chao1 index (**Fig. 1C**) and the Shannon index (**Fig. 1D**), respectively.

Genetic line influences the abundance of probiotics. Probiotics were increased in healthy as compared to diseased samples, in the same way, there was a significant enrichment of probiotic species in Gen1 compared to Gen2, independently of the organ (**Fig. 1E)**, which implies a possible selection of probiotics from the genetics of the host that could impact in its health status.

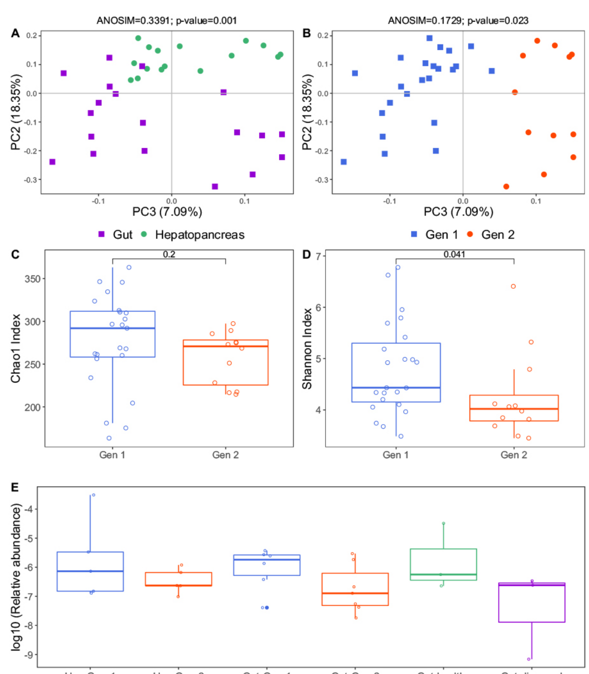

Fig. 1: PCoA unweighted UniFrac contrasting between A) organs and B) genetic lines. C) Chao1 richness and D) Shannon diversity between genetics lines. E) Abundance of probiotics species in the different groups (organ and genetic line) compared against healthy and diseased gut samples.

CELL-BASED ANTIGENS PROSPECTION THROUGH TRANSCRIPTOME ANALYSIS UNCOVERS VACCINE CANDIDATES IN ATLANTIC SALMON AGAINST SEA LICE

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The development of vaccines against sea lice in the salmon farming production is a complex, expensive and long process for the commercial validation. Hence, transcriptomic studies in sea lice have provided valuable information to understand the molecular mechanisms involved in the host-parasite interaction. However, the bottleneck is the in vivo testing of several candidate recombinant proteins, dosage and also polyvalent formulation strategies. This study explored a cell-based platform to prospect antigens as candidate vaccines against sea lice. The experimental model used is the Atlantic salmon Salmo salar and the sea louse Caligus rogercresseyi. Herein, secretome/excretory-related proteins (SEPs) were identified in C. rogercresseyi, cloned and expressed in Escherichia coli. Then, SHK-1 cell line were stimulated with candidate recombinant proteins and evaluated through RNA-seq analysis. The experiment was conducted with 25, 50, and 100 ng/mL of SEPs, and a combination of them during 24 hours. Cell exposure to the SEPs showed significant cell damage comparing with the control group exposed to BSA. Significant changes in the transcriptome profiles of SHK-1 were found in cell groups exposed to SEPs compared to the control groups composed by BSA and LPS. Furthermore, RNA-seq and qPCR analyses determined that Cathepsin has the highest impact on the immune-related genes. The findings archived by the in vitro model was used to formulate and test a candidate vaccine, showing a 44% and 57% of efficacy at 7 and 25 dpi, respectively. This study provides a novel approach to develop sea lice vaccines, improving the antigens identification process and their selection for commercial validation.

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A CALL TO IMPLEMENT THE SEAFOOD COMPETITIVENESS EXECUTIVE ORDER OF 2020

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In May of 2020, the President signed an Executive Order promoting American seafood competitiveness and economic growth. This Executive Order was intended to propel the United States forward as a seafood superpower by strengthening the American economy; improving the competitiveness of American industry; ensuring food security; providing environmentally safe and sustainable seafood; supporting American workers; and ensuring coordinated and transparent federal actions. Specifically, the Executive Order called for the expansion of sustainable U.S. seafood production through:

- More efficient and predictable aquaculture permitting
- Cutting-edge research and development
- Regulatory reform to maximize commercial fishing
- Enforcement of common-sense restrictions on seafood imports that do not meet American standards

The presentation will review the background scientific, trade, and economic data behind this EO and review progress to date. Notably incomplete are actions to release a Seafood Trade Strategy, where aquaculture could have played a significant role, and Section 8 of the EO which directed NOAA to post for public benefit a guidance document describing federal regulatory requirements and agencies. Posting this resource would provide significant assurances to the public that federal regulations are adequate and that recreational and commercial fishing communities' voices are heard. Conclusions will be made that fully implementing the EO will help meet the Administration's objectives for sustainable and equitable economic growth.

TO WHAT EXTENT DO 'HIGH' WATER TEMPERATURES AFFECT THE PHYSIOLOGY, HEALTH AND WELFARE OF ATLANTIC SALMON?

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Increases in average water temperatures and decreases in water oxygen levels (hypoxia), and more frequent and extreme warming events (i.e., heat waves), are predicted to occur with climate change. Thus, there is an urgent need to understand the effects of prolonged and short-term warming on the physiology of cultured fishes, including Atlantic salmon (*Salmo salar*). This is particularly true with regard to Tasmania and Atlantic Canada where water temperatures have approached/exceeded 20°C, in combination with hypoxia, and negative effects on production and fish health (including large-scale mortalities) have been reported. However, with respect to the latter, it is unclear what role temperature and hypoxia played, and how the effects of these environmental challenges on Atlantic salmon can be minimized.

Over the past few years, we have used a multilevel (e.g., epigenetic, genomic, biochemical, whole animal, biologging) approach and several experimental paradigms to understand how high temperatures alone, and when combined with moderate hypoxia (60-70% air sat.), impact salmon production characteristics and key aspects related to this species' cage-site culture under realistic temperature scenarios (i.e., using an IT_{MAX} test; a 1°C increase week^{-1} from 10°C).

In this presentation, I will show that while stress gene expression in salmon begins to be affected at 16°C, and feeding decreases dramatically as temperatures approach 20°C, there is little/no evidence that this temperature, even when prolonged or combined with moderate hypoxia, results in mortalities. The salmon's capacity to mount an innate immune response is not compromised at these temperatures and plasma cortisol levels (indicative of a secondary stress response) do not increase until 21-22°C. Fish in sea-cages do not avoid surface temperatures up to 19-20°C, and show no signs of stress (i.e., abnormally high heart rates or arrhythmias). Finally, in lab-based experiments, mortalities only begin when the fish reached 21°C, and even at 23°C mortalities were only ~ 30%.

Through this research, we have also been able to identify epigenetic and genomic markers of temperature and hypoxia tolerance in salmon, and have identified populations/families of salmon that have critical thermal maximum (CT_{MAX}) and IT_{MAX} values of ~28°C and 25°C, respectively. This should allow us to develop genetic and other markers for use in selecting fish that are more tolerant of these conditions, and for evaluating fish health. However, our data also provide convincing evidence that such efforts must be combined with those to mitigate other stressors/factors that negatively impact the health and welfare of salmon. To this end, I will briefly introduce a 'schematic model' (a work in progress) on how biotic and abiotic challenges may interact to cause large-scale ('mass') mortalities of salmon at sea-cage operations.

ABIOTIC AND BIOTIC CHALLENGES FACING SALMON AQUACULTURE IN THE ERA OF CLIMATE CHANGE: UNDERSTANDING AND MANAGING INTERACTIVE EFFECTS THAT RESULT IN MORTALITY

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Climate change is resulting in increases in average water temperatures and decreases in water oxygen levels (hypoxia), and more frequent and extreme temperature events (i.e., heat waves and 'cold-shocks'). Given that salmon are predominantly reared in coastal marine sea-cages, this has led to a significant amount of research on the effects of these two environmental challenges on the production, health and welfare of this species. However, recent research suggests that high temperatures (i.e., up to 20ºC) and moderately low oxygen levels (e.g., down to 60-70% air saturation), by themselves, are unlikely to result in significant cage-site mortalities.

This was the impetus for us to take a comprehensive look at the abiotic and biotic factors that impact salmon at aquaculture cage-sites, their potential interaction with regard to causing mortalities, and management practices that might alleviate/mitigate, or exacerbate, the loss of fish under culture conditions. The model we have developed has many points of intersection/interaction, and includes factors such as algal blooms, nutrition, environmental variables, pathogens, the presence of 'nuisance' species (e.g., jellyfish), and management practices and interventions.

In this talk, I will provide some background information on the causes of salmon cage-site mortalities, and introduce an interactive model that details the interdependencies and causal relationships between the above and other factors. It is hoped that this model will: 1) improve our understanding of factors that influence salmon welfare and mortalities; 2) prove useful in mitigating these issues going forward; and 3) assist the industry and the research community in selecting directions/priorities for future R&D targeted at making salmon aquaculture more sustainable and profitable. In the future, we aim to transform this interactive model into a dynamic systems model that will predict the interaction between abiotic and biotic factors over time, and forewarn producers of conditions/events that might result in the loss of fish.

ESTIMATION OF LINKAGE DISEQUILIBRIUM AND EFFECTIVE POPULATION SIZE IN A PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) POPULATION USING A NOVEL 50K SNP GENOTYPING ARRAY

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The inclusion of genomic information is expected to accelerate the genetic gain over time in comparison to traditional pedigree-based methods in shrimp breeding. The decay of linkage disequilibrium (LD) between single nucleotide polymorphisms (SNPs) is an important measure to evaluate the feasibility of implementing genomic selection (GS) and genome-wide association studies (GWAS). Our aims were to show the first application of a 50K SNP to estimate the LD and the effective population size (N_e) of a breeding population of Pacific white shrimp.

A total of 96 animals (40 sires and 56 dams) from an Ecuadorian breeding population were genotyped using the novel Illumina AquaArray HD (50K) vannamei®. A quality control of genotypes was performed consisting of four exclusion criteria: call-rate < 0.8 for SNPs and samples, Hardy-Weinberg equilibrium (p-value < 10^{-5}) and minor allele frequency (MAF) with different threshold values, forming three quality control sets (QC): < 0.10 (QC1), < 0.05 (QC2) and < 0.01 (QC3). The LD was estimated using the r^2 measure and the N_e was estimated using a method that takes into account LD and recombination rate. After QC, 34,425, 39,091 and 42,789 SNPs were retained for QC1, QC2 and QC3, respectively, validating the high informativeness of this SNP array to this particular shrimp population. LD decayed rapidly in the first 30Kb of distance between markers from 0.20 to 0.07 and then decreased to 0.02 in the long-range distance (Figure 1). These results suggest a relatively recent incorporation of animals from different populations in the broodstock. N_e size reduction was more significant in the last 300 generations with approximately 62% of total reduction in this period (Figure 2). However, the contemporary N_e estimated was close to 86 showing that despite mass selection applied in this population, the effective population size is at an acceptable level. In conclusion, the level of LD estimated suggests that GS and GWAS are feasible in shrimp by using this SNP array.

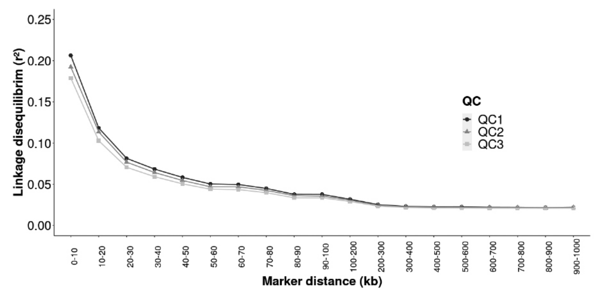

Figure 1. Estimated average LD decay (r^2) over different classes of distance between SNPs using different genotype quality controls (QC).

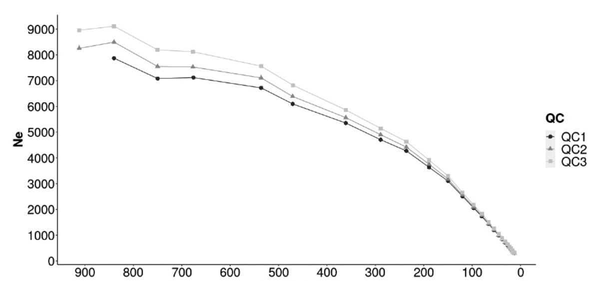

Figure 2. Effective population size (N_e) estimated using different genotype quality controls (QC).

PERFORMANCE OF THE NATIVE CICLHID *Mayaheros urophthalmus* AFTER TWO GENERATIONS OF FAMILY SELECTION

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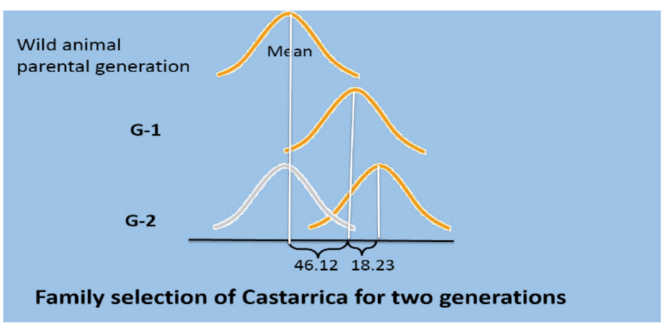
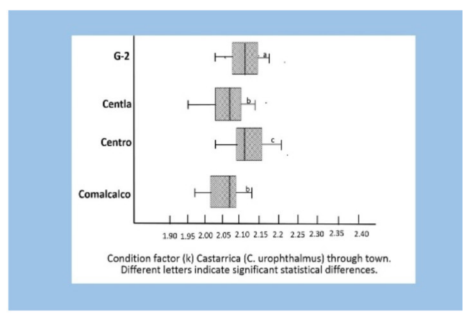
Selection constitutes one of the means by which a breeder can select genetic traits of a population by electing the organisms that will be utilized in the reproductive processes. We stablished a family selection program for the native cichlid *Cichlasoma urophthalmus,* with the objective of obtaining a genetically improved population. Twenty-two families were used, starting from wild fish obtained from three different localities (Comalcalco, Centro y Centla) in the State of Tabasco, Mexico. The descendants from each family and a corresponding control group were stocked in three floating cages (2x1x1.2 m). In the first phase, fish were stocked at a density of 300 fish per m^{-3} and grew-up to first selection. Afterwards, fish were stocked at a density of 20 fish per m^{-3} until second selection. In every selection the best performing fish from a cage (10%) were selected. The best results were obtained from the Comalcalco population locality averaging 32.7 ± 0.12 g in the first selection and 231.0 ± 0.10 g in the second. The gain obtained was 11.61% and 46.12% in the 1st and 2nd selection respectively. For the second generation a rotational breeding plan was used with the best performing 24 families from the Comalcalco population. To evaluate growth, we used the same methods and criteria applied for the first generation. Results from the second generation indicate that the fish averaged 54.0 ± 0.13 g in the first selection and 285.3 ± 0.08 g in the second. Average gain was 8.4% and 18%, respectively. Genetic selection in this native species constitutes a step stone for regional aquaculture.





CO-CULTURE OF *Pacific dulse* AND PURPLE SEA URCHINS: IN SEARCH OF A SELF-SUSTAINING SEA URCHIN FATTENING SYSTEM

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The purple sea urchin *Strongylocentrotus purpuratus* is overabundant in California, turning many once-productive kelp forests into low productivity urchin barrens. Resource managers and stakeholders alike, are concerned with large reductions of kelp and are investigating ways to recovery kelp ecosystem including urchin removal. While a significant urchin fishery currently operates in California, increased fishing effort to remove these overabundant urchins is not currently commercially viable due to the unmarketable condition of the urchin gonads, being that they are primarily too small to justify commercial capture. However, capture and subsequent culture of wild urchins in an aquaculture setting can enhance gonad quality and offers a possible solution to economically incentivize their removal and restore kelp ecosystems. We investigated co-culture of *S. purpuratus* and Pacific dulse, *Devaleraea mollis*, in an integrated multi-trophic aquaculture (IMTA) system to maximize efficiencies by allowing urchin metabolites to enhance seaweed growth which also serves as the urchin's feed. We predicted that we could cultivate a self-sustaining co-culture (constant seaweed biomass) with urchins and dulse in the same tank using seaweed tumble culture, reducing infrastructure footprint, husbandry effort and feed associated costs. This study aimed to identify the optimal urchin stocking density for such a system. We stocked 1350 urchins in 10 (986 L) conical bottom tumble culture tanks of increasing densities of urchins (0, 0.453, 0.599, 0.789, 1.046, 1.271, 1.354, 1.489, 1.802, 1.954 kg/m^2) with a fixed starting density of 3.79kg/m^2 dulse, for four consecutive two-week trials after which seaweed biomass was measured. Increasing urchin densities did not affect urchin mortalities for any of the trials and the mean number of mortalities per tank was low (0.94 +/- 1.60). Increasing urchin densities did affect mean total dulse growth (kg) and mean daily dulse growth rates (kg/day^{-1}) in a negative, linear fashion ($p<0.001$). Our results demonstrate how urchin can be co-cultured with dulse, without increased mortality, for up to urchin densities of at least 1.954 kg/m^2, and positive dulse growth can occur in urchin densities up to 0.789 kg/m^2 equating to 100 urchins per tank for the study. While the study shows the technical feasibility of this urchin fattening system it remains to be determined if the potential benefits of this IMTA design outperform a more conventional system reliant on exogenous supply of feed an likely higher urchin stocking densities.

DIVERSITY IN GLOBAL FISHERIES AND AQUACULTURE

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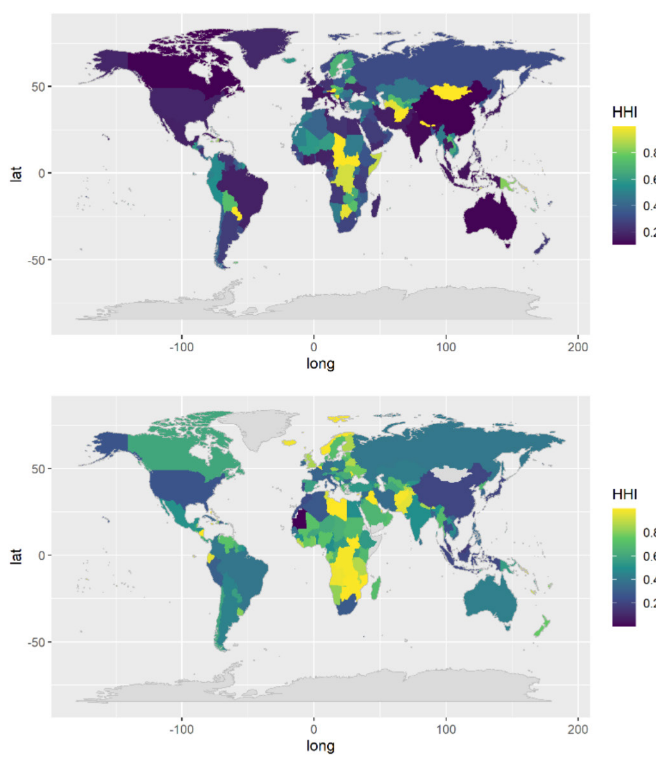
Capture fisheries and aquaculture provide nearly equal shares of total seafood supply, but the composition of species in fisheries landings and aquaculture is very different. Understanding these differences are important to increasing the capacity of seafood to sustainably meet the nutritional needs of a growing population.

We use country-level fisheries and aquaculture production data from the Food and Agriculture Organization of the United Nations to calculate a Herfindahl-Hirschman index, a quantitative measure of species diversity. Linear regression analyses were conducted to examine drivers of heterogeneity in the species composition of fisheries and aquaculture production. Predictor variables included biophysical, governance, social and economic indicators, such as sea surface temperature, human population, and government effectiveness.

We find a larger average Herfindahl index for aquaculture compared to fisheries production indicating that the nearly equivalent amount of seafood produced by aquaculture is significantly less diverse and concentrated within fewer species groups. This implies that aquaculture has surpassed the productivity constraints of natural fisheries resources and can achieve larger within-species production scales. This has important implications for producing an overall greater supply of seafood and maintaining seafood accessibility among vulnerable populations.

Linear regression analyses indicate that *Population* and *Government effectiveness* are positively related to species diversity in fisheries whereas biophysical variables are of greater importance to species diversity in aquaculture. Implications on the role of blue foods in future food security will be discussed.

Figure 1. Species diversity in (a) fisheries production and (b) aquaculture production. Note: Values near 0 indicate presence of many species groups and values near 1 indicate presence of few species group.



EVALUATING VARIOUS NUTRITIONAL ENRICHMENTS OF ROTIFERS *Branchionus* sp.

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The use of rotifers as live food for larval fish continues to be an important part of larviculture of various fish species including southern flounder. However, the nutritional composition of rotifers is generally considered inferior to other live foods such as copepods. The present study evaluated the application of four different types of nutritional supplements to rotifers *(Branchionus plicatilis and Brachionus manjavacas)* including probiotics, prebiotics, vitamin C, and taurine to determine how they may influence rotifer production and microbial composition, as well as nutrient concentrations. Separate trials were conducted to evaluate changes in the production and microbial composition of rotifers exposed to the probiotics Bactocell® and Aquablend (produced by BIO-CAT) and the prebiotic GroBiotic®-A (International Ingredient Corporation) added to the culture water during 4-day production cycles compared to the control with no supplement. GroBiotic®-A in two separate trials showed increased rotifer production. Denaturing gel gradient electrophoresis (DGGE) analysis demonstrated rotifers cultured with the aforementioned additives had altered microbiota composition compared to the control as noted for the GroBiotic-A treatment in Fig. 1.

Vitamin C supplementation was evaluated in two separate trials in which *Brachionus manjavacas* was enriched with various concentrations of ascorbic acid in the culture media. Results showed that rotifers exposed to 4 g of ascorbic acid/liter contained the highest concentration of ascorbic acid averaging 2,308 µg/mg rotifer compared to the treatment without ascorbic acid exposure averaging 360 µg/mg rotifer. Optimization of rotifer production required adjustment of pH in the culture media. Two separate trials also examined the effects of taurine enrichment of rotifers. Taurine was supplemented at either 0, 1, or 2 g/liter and blended with Amplifeed™ Replete after which samples were collected at 1, 2, 4, 8, and 24 h post feeding. Amino acid analysis indicated that taurine supplementation resulted in a dose-dependent increase in taurine concentrations of rotifers with 2 g/liter yielding the highest concentration of taurine which was maintained at 1.13 g taurine/100 g samples at 4 h after feeding.

Details from these trials will be presented to demonstrate that the nutritional and microbial composition of rotifers can be effectively altered to improve their nutritional value to larval fish.

IDENTIFICATION OF GENES INVOLVED IN GERM CELL DEVELOPMENT IN THE PACIFIC OYSTER (*Crassostrea gigas*) USING SINGLE-CELL RNA SEQUENCING

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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 formation and development. The power of this biotechnological approach has been realized recently in some finfish species, where suppression of the germ-cell specific gene, dead end(dnd), results in fish with no detectable germ cells. Given the recent advances in finfishes, the shellfish industry is now poised to adopt these technological advances. Unfortunately, the development of this technology is hampered by the lack of knowledge of the genes essential for primordial germ cell (PGC) specification in bivalves. To overcome this challenge, we are using single-cell RNA-Seq to identify genes involved in PGC specification. This cutting-edge approach allows us to sequence the genes expressed in individual cells and is particularly suited to identify PGC markers in bivalves, as germ cell precursors represent only a small number of cells at these early developmental stages. To date, we have transcriptionally profiled more than 25,000 cells in early Pacific oyster embryos (first cell divisions through blastula stage). Using a germ cell marker gene, vasa, we are able to track cells associated with PGC formation and identify genes that are uniquely expressed in the germ cell lineage. These candidate genes will be used in experiments designed to temporarily block their expression in embryos and evaluate the effects on reproductive development.

IMMUNE SYSTEM GENE EXPRESSION AND ANTIOXIDANT RESPONSES IN *Litopenaeus vannamei* (Boone, 1931) BROODSTOCK MALES FED VITAMIN E (DL-a-TOCOPHEROL-ACETATE)-SUPPLEMENTED DIETS

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The addition of vitamin supplementation to a diet could control melanization syndrome of the gonads. A formulated feed with high nutritional quality served as a basal diet (149 mg/kg) and treatment diets were prepared with 3 vitamin E levels: 894 mg/kg (diet A), 1639 mg/kg (diet B), and 2384 mg/kg (diet C). Three 500 L tanks were set up for each treatment, and seven individuals were set in each tank. Sperm quantity and quality were classified as normal, abnormal, or dead. The cholesterol, triglycerides, glucose, total protein and antioxidant enzyme activity from hemolymph were quantified. The differential expression of immune system genes and genes coding for antioxidant enzymes in the reproductive system was determined. Sperm quantity and quality were higher in the shrimp fed diet A. The addition of vitamin E to diet A, led to a decrease of cholesterol and triglycerides. Lipid peroxidation and differential immune system gene expression were also lower in diet A, which suggests that this diet contributes to a good physiological status, where immune system activation and the genes that code for antioxidant enzymes are not necessary. However, with the increased vitamin E in diets B and C, the triglyceride content and lipid peroxidation in hemolymph increased. Males fed diets B and C presented a high activity of antioxidant enzymes and a low level of sperm, which suggested a state of oxidative stress. Therefore, basal diet A supplemented with 894 mg/kg vitamin E is recommended for the cultivation of broodstock males of *L. vannamei*.

PHYSIOLOGICAL STATUS OF *Farfantepenaeus brasiliensis* LATREILLE (1817) BROODSTOCK MALES FED VITAMIN C-SUPPLEMENTED DIETS

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Controlling oxidative stress in shrimps under culture conditions is of vital importance to have males with good physiological status. The use of diets supplemented with vitamins are widely used for this purpose. The physiological status of *F. brasiliensis* Latreille (1817) broodstock males fed with vitamin C supplemented diets was determined. A formulated Diet was used as a control and three Diets were prepared with three vitamin C levels: 894 mg/kg (Diet A), 1639 mg/kg (Diet B), 2384 mg/kg (Diet C). Triglycerides, cholesterol, glucose, and prophenoloxidase quantities; type of hemocytes; lipid peroxidation; and total proteins in the hemolymph were determined. In addition, the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) were quantified. Differentially expressed genes of the immune system, namely those of α-2 macroglobulin (α2M), peneidin (Pen), hemocyanin (Hc), prophenol oxidase (ProFo), and the genes encoding antioxidant enzymes SOD, CAT, and Gpx were evaluated. Sperm quantity and quality were recorded. In males fed Diet C, the lowest cholesterol values and triglycerides obtained in the hemolymph were not significantly different from those fed the control Diet ($P < 0.05$). Shrimps fed Diet C showed the highest amount of hyaline hemocytes, the lowest CAT enzyme activity, and the highest Gpx activity. The immune system genes only showed differences between α2M and Pen; sperm quantity and quality were not affected by diet. The results indicated that Diet C (2384 mg/kg) was effective for the maintenance of *F. brasiliensis* broodstock males because it controlled cholesterol and triglyceride levels; there was less CAT activity and less activation of the immune system mediated by hemocytes.

ENVIRONMENTAL CUES FOR SPAT SETTLEMENT IN FLORIDA BAY SCALLOPS

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Synchronous spawning of the core population of bay scallops (*Argopecten irradians*) in Florida was described by early studies of this species, and may be critical to population dynamics, but later research has shown that in some of the Florida subpopulations, settlement of spat is nearly continuous. Settlement rates of bay scallops were monitored seasonally from 1992 through 1999 then almost continuously until 2017 in the open gulf and most of the enclosed bays along the west coast of Florida, after which the focus of the program switched to collection of wild harvest of juveniles for restoration. The principal methodology was deployment of artificial spat collectors (½-bushel citrus bags) for 6-8 weeks. Analysis of the long-term data set is still ongoing.

Spat settlement rate was analyzed with generalized linear mixed models using a negative binomial distribution (spat data is zero-inflated) in R software. Primary environmental drivers for settlement models included temperature (T), salinity (S), chlorophyll (when available), turbidity and red tide (*Karenia brevis*) concentration. Statistical models also included variations of these terms including non-linear factors (T^2 and S^2), the change in temperature during deployment (ΔT and ΔT^2), and the change in temperature or salinity during the month preceding deployment (as an estimate of spawning cues; ΔST, ΔST^2, ΔSS, ΔSS^2). The dependent factor in models was the number of spat settling to the collector. Deployment duration was included in all models.

Temperature, salinity, and temperature changes during presumed spawning period were all important contributors to the model. Temperature drops of -10 °C or more during the fall are predicted to produce large settlement events, presumably by serving as a strong spawning cue to the broodstock, either directly or as the waters warm after the passage of cold fronts. Temperature increases in the spring precede smaller settlement peaks. Climate change could result in the loss of the coldest winter temperatures which could reduce synchrony in bay scallop spawning during the fall but increased intensity of weather systems could offset those changes. Frequency and intensity of red tide can both repress settlement, but red tides are infrequent in the regions where the core population persists. Intrusion of red tide to the core region, either increased frequency or intensity, could be devastating to the long-term viability of bay scallops in Florida.

THERE ARE MULTIPLE MARKETS FOR MARINE SNAILS HARVESTED IN FLORIDA

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Commercially harvested saltwater snails are reported in two broad categories in Florida, commercial (largely intended as food or bait) and marine life (largely for aquariums). Some landings data are reported in both state and federal databases and some (primarily marine life) are only reported in state databases. Smaller snails like *Nassarius*, *Marginella*, *Turbo* (Fig. 1) and *Lithopoma,* are marketed as to the live aquarium trade either as grazers or prey for other aquarium organisms. Some, such as *Melongena*, may be reported as marine life but may also be landed as bait for trap fisheries. Still others, such as *Triplofusus*, *Cypraea*, *Cassis* and *Cymatium,* are likely being harvested at least in part for the collectable shells.

Landings of marine life snails such as the *Turbo* and *Lithopoma* snails (both Turbinidae), can exceed one million individuals harvested per year but are commonly reported from only a few Florida counties. Preliminary randomized surveys to assess abundance and distribution in the Florida Keys (Monroe and Miami-Dade Counties), where harvest is high, found that *Lithopoma* star snails were widespread (present at 40% of stations) while *Turbo* spp. were found at only 5% of stations. This finding suggests non-random distribution of *Turbo*, which may indicate a higher susceptibility to potential over-exploitation of limited stocks. While currently abundant, these small herbivorous snails with individual unit prices of up to several U.S. dollars may present a potential market for niche aquaculture.

On-line searches reveal almost endless shells for sale where asking prices for exceptional shells such as tritons, helmet shells and very large Florida horse conch can reach hundreds of U.S. dollars. Even juvenile horse conch shells (Fig. 1) are listed and have asking prices of $5-10 or more. Some sales occur through commercial sites but many are also offered for sale by individual harvesters. Current rules allow harvest of live shells using a recreational fishing permit, but there is no mechanism for reporting such landings by recreational fishers. The sale of shells that recreational fishers have live-harvested is not technically legal in Florida. Unfortunately, collection of empty shells is not regulated, and enforcing the rules for sale of shells collected live by recreational fishers would present enforcement challenges.

Figure 1. *Turbo castanea* (left), *Triplofusus giganteus* juvenile (top) and *Melongena corona* (bottom).

MUNICIPAL STORMWATER PONDS AS LOW-MAINTENANCE, HIGH-IMPACT GROW-OUT SITES FOR JUVENILE FRESHWATER MUSSELS IN THE DELAWARE RIVER BASIN, USA

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Freshwater mussels are filter-feeding bivalves that occupy diverse freshwater environments. In the United States, mussel assemblages are declining in range, abundance, and species richness. Restoration can involve reintroduction of mussels to historical ranges. Reintroduction success (i.e. survival, persistence) depends on both habitat suitability and animal size. Juvenile mussel survival in nature is thought to be low but advances in hatchery spawning and pond rearing have significantly increased survival. Pond rearing can raise hatchery-spawned mussels to sizes appropriate for release. Ponds with appropriate water quality parameters provide the opportunity to grow mussels without external nutrient input and with minimal maintenance, vastly minimizing effort and resources required when compared to hatchery systems.

Freshwater mussels of two species native to the Delaware River Basin, *Utterbackiana implicata* and *Sagittunio nasutus*, have exhibited comparable survivorship and growth rates in large reservoirs, municipal stormwater ponds, and ponds located at botanical gardens. The tolerance of these species to a range of land use and nutrient inflows allows for many pond options in which to grow mussels, providing potential opportunities to partner with companies, governments, and private landowners.

Particulate matter composition (i.e. seston) is of particular interest for selecting an appropriate pond for grow-out. Seston composition of several municipally owned stormwater ponds (Table 1) shows a positive correlation between a high concentration of organic material in seston and mussel growth. In systems that experience problematic quantities of nutrients, pond grow-out of freshwater mussels may provide the ecosystem service of water quality uplift, filtering and transforming those nutrients. This may be of particular interest in municipal stormwater ponds which experience high nutrient inflows from residential lawns and stormwater runoff. Grow-out trials using mussels in stormwater ponds were initiated in 2019 and will be expanded upon through 2023 to refine our understanding of pond carrying capacity and the efficacy of novel aquaculture techniques.

Table 1. Seston composition in relation to growth rates of *Utterbackiana implicata* in stormwater ponds in New Castle County, Delaware.

Pond	Total Suspended Solids (mg/L)	Particulate Organic Matter (mg/L)	Percent Particulate Organic Matter	Growth Rate (mm/day)
Talley Day	23	16	70%	0.07
Rockwood	18	8	47%	0.06
Papermill	8	4	52%	0.04
Airport	7	5	68%	0.03
Winterthur	4	3	73%	0.02

UNDERSTANDING THE STATE-LEVEL SEASCAPE OF MARICULTURE PRODUCTION AND POLICY IN THE UNITED STATES

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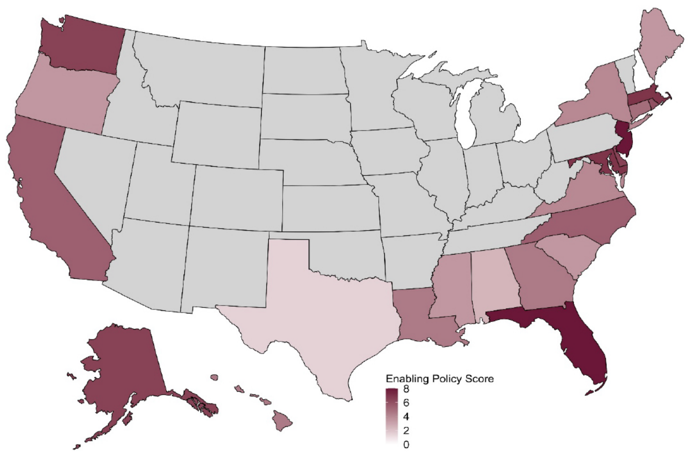
Marine aquaculture (mariculture) currently comprises a relatively small fraction of domestic seafood production in the United States, but it is widely seen as having significant potential for expansion. Most existing mariculture in the U.S. takes place in state waters under state-level jurisdiction, and yet much of the current dialogue on sustainable marine aquaculture development is targeted at the federal level. State-level mariculture management is diverse, resulting in a patchwork of regulating and enabling policy along with uneven reporting of mariculture production. Guiding sustainable development of this industry requires a much better understanding of its current status - including more consistent value and production data and a richer understanding of state-level policy.

We embarked on two parallel but complimentary projects focused on 1) U.S. state-level mariculture policy and 2) U.S. state-level mariculture production data. For the policy work , we compiled information for 16 aquaculture and mariculture policy attributes, including legislation, regulations, and management characteristics, particularly those that could enable mariculture development. We also calculated an overarching enabling policy score for each state based on these characteristics (Fig. 1). We found considerable variation in the way that states manage mariculture development, indicating that there are a suite of approaches that may be influential in enabling sustainable development. With regards to mariculture production data, we aggregated publicly available data from USDA and NOAA along with data solicited from state agencies, in order to synthesize and compare value, volume, and species data for each state across time. We found strong evidence that mariculture is playing an increasingly important role in coastal states, but also uncovered many data gaps and inconsistencies in current data collection sources. We suggest that a standardized digital reporting system and annual data collection would allow for improved strategic aquaculture planning, research, and management. These two complementary projects each provide first of their kind syntheses of key information that is essential for managing a sustainable industry into the future.

FIGURE 1. Geographic pattern of enabling policy scores for state-level mariculture development



THE CONTRIBUTION OF MARINE HEATWAVES TO 'TRIPLOID MORTALITY' DURING COMMERCIAL PACIFIC OYSTER PRODUCTION

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Pacific oysters (*Crassostrea gigas*) are an important global aquaculture species, accounting for the majority of hatchery-reared and farmed shellfish in Washington State. Domestic oyster production accelerated in the 1980's after the adoption of hatchery techniques that induce triploidy, resulting in the production of oyster seed with an extra chromosome set. When compared to diploids (2n), triploid oysters (3n) typically display reproductive impairment, allowing for shifts in energy allocation that enable faster growth and year-round marketability by avoiding the harvest of 'spawny' oysters during summer months. While the enhanced marketability of triploid oysters is irrefutable, increasingly frequent reports from both international and domestic shellfish farmers suggest that triploid oysters can experience reduced survivorship ('triploid mortality') with respect to diploids growing at the same grow-out site, particularly during the summer months.

To investigate the potential cause(s) of reduced survivorship across ploidy, we employed a series of laboratory experiments within a commercial hatchery setting that compared the physiological and transcriptional response of diploid and triploid pacific oysters to a simulated marine heatwave. Treatment conditions simulated those experienced by intertidal organisms on the Pacific Coast during mid-July as a result of the marine heatwave NEP21A. Adult oysters were subjected to elevated seawater temperature (30°C) and/or desiccation stress (aerial exposure for 4h at 44°C) and subsequently monitored for up to 1-month. Significant differences were observed in the metabolic rate, feeding behavior, and gene expression of diploid and triploid oysters following exposure to elevated temperature and desiccation stress in succession, resulting in significantly higher rates of triploid mortality when compared with diploids. However, exposure to elevated seawater temperatures alone was not sufficient to illicit mass die-offs in either ploidy, suggesting that if environmental factors contribute to 'triploid mortality' events observed in oyster aquaculture, exposure to multiple stressors in combination may be required.

EFFECT OF SEASONAL FLUCTUATIONS IN PHYSICOCHEMICAL PARAMETERS ON FISH DIVERSITY

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Kuttanad is one of the Ramsar sites in India popular for paddy cultivation below sea level and fish cultivation. Most people rely on these two practices for livelihood. So seasonal fluctuations in physicochemical parameters such as Dissolved Oxygen, Biochemical Oxygen Demand, Temperature, pH, Salinity, Total Dissolved Solids, Electrical Conductivity, Chloride, Phosphate, and Nitrate will influence the fish diversity thereby impact the economic stability of the society. Kuttanad comprises both fresh water and brackish water ecosystem. The western side of the Kuttanad ecosystem ends up with the Arabian Sea. So five major rivers flowing through Kuttanad from east-west direction joins with Vembanad estuary which opens into the Arabian Sea. Proximity to the sea and geographical elevation causes seasonal saline intrusion into the inland water bodies. It will change the physicochemical properties and decreases the fish diversity seasonally.

There are three main seasons, pre-monsoon (March to May), monsoon (June to November), and post-monsoon (December to February) showing different diversity, in which monsoon season shows a high diversity followed by post monsoon and pre monsoon. Saline intrusion happening in post monsoon and pre monsoon seasons, changes the physicochemical parameters which are significantly correlated ($p<0.01$ and $p<0.05$) with fish diversity. Diversity indices such as Shannon Weinner, Simpson's diversity, Hill's number, Alpha diversity, and Margalef's index were calculated for the study. The study was conducted over 3 years as a part of Ph.D. research.

Among the 63 identified species 30 species significantly correlated with DO, 19 species with BOD, 9 species with chloride, 19 species with phosphate, 31 species with nitrate, 31 species with pH, 34 species with EC, 33 species with TDS, 14 species with salinity, and 19 species with temperature. Out of the 63 species, 2 species *Clarias gariepinus* and *Oreochromis mossambicus* are invasive alien species. Saline intrusion during post monsoon season declines primary productivity or phytoplankton diversity thereby zooplankton diversity and finally ends up with low fish diversity.

Recent studies investigate the impact of arctic ice melting due to global warming causes surface cyclonic circulation in the Arabian Sea which enhances the moisture convergence, eventually leads to extreme extended monsoon rain in Kerala. So the impact of global warming on the fish diversity of Kerala has to be studied immediately.

EFFECTS OF TEMPERATURE ON METABOLISM, SWIMMING PERFORMANCE, AND BLOOD-OXYGEN AFFINITY *Ictalurus* spp.

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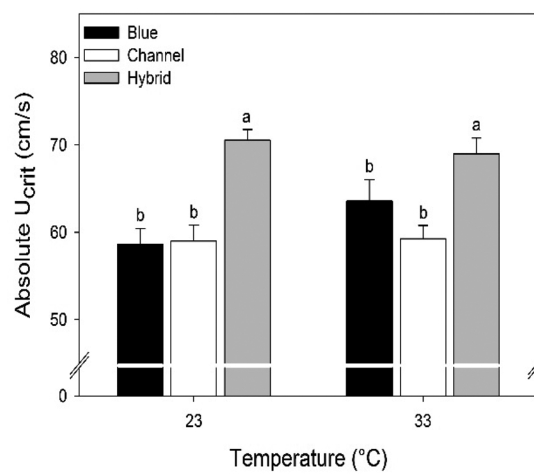
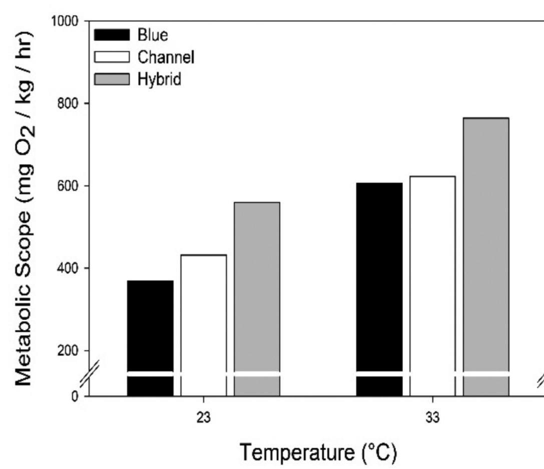
Water temperature is a key physiological regulator of ectothermic organisms. Temperature is closely related to aerobic capacity and blood-oxygen affinity. Because little is known about the aerobic capacity and blood-oxygen affinity of juvenile channel (*Ictalurus punctatus*), blue *(I. furcatus*) and hybrid catfish (*I. punctatus* X *I. furcatus*) related to high seasonal temperatures, the effects of water temperature on standard and active metabolic rates, swimming performance, and blood-oxygen affinity were evaluated.

Fish were acclimated to either 23 or 33°C in separate recirculating aquaculture systems. Standard metabolic rate (MO_{2min}) was measured using intermittent respirometry and active metabolic rate (MO_{2max}) was measured using a swim flume. Metabolic scope was calculated at both temperatures for all three fish types. Blood samples were analyzed for blood-oxygen equilibrium fitting curves, P50 and Bohr coefficients.

At 23°C, hybrid catfish had the highest metabolic scope at 559.5 mg O_2/kg/hr, followed by channel (431.5 mg O_2/kg/hr), and blue (369.1 mg O_2/kg/hr) catfish. At 33°C, hybrid catfish had the highest metabolic scope at 764.0 mg O_2/kg/hr, followed by channel (622.4 mg O_2/kg/hr), and blue (606.8 mg O_2/kg/hr) catfish. Additionally, hybrid catfish had the highest critical swimming velocity at both temperatures. Channel catfish had the highest P50 at both temperatures, followed by hybrid and blue catfish. Hybrid catfish had the highest Bohr coefficients at both temperatures, followed by channel and blue catfish. These findings suggest that hybrid catfish are a better performing fish at warm temperatures, in terms of aerobic capacity and blood-oxygen affinity, than channel and blue catfish. These findings provide an improved understanding of the aerobic capacity and blood-oxygen affinity of Ictalurid catfishes.





CHARACTERISATION OF RESISTANCE AGAINST INFECTIOUS SALMON ANAEMIA IN ATLANTIC SALMON

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Infectious Salmon Anaemia Virus (ISAV) causes a notifiable disease in Atlantic salmon that represents a major problem for salmon breeders and producers worldwide. Current prevention and therapeutic methods are not fully effective, and therefore selective breeding to produce ISAV-resistant strains of Atlantic salmon (*Salmo salar*) is a high priority for the industry. Genomic selection and potentially genome editing can be applied to enhance host resistance. Both approaches can benefit from increased knowledge on the genetic and functional mechanisms of resistance to ISAV. Here we have combined used bulk and single-cell RNA sequencing in *in vitro* and *in vivo* models to study Atlantic salmon immune response against ISAV.

First, we studied the transcriptomic changes in response to ISAV in four different tissues (heart, gills, head kidney and spleen) at three timepoints (pre-challenge, 7 and 14 dpi), finding a clear but unique response to ISAV in each tissue. Comparisons between four resistant and four susceptible fish per timepoint, selected based on their genomic breeding values estimated from a disease challenge in 1,353 fish from the same population, revealed a large number of differentially expressed genes in the head kidney, while the differences in the other tissues were small.

To better understand the host-response interaction at the cellular level, and evaluate the importance of potential candidate resistant genes, we investigated the Atlantic salmon response to ISAV at the single cell level using an *in vitro* model (SHK-1 cells, Atlantic salmon head kidney 1). Cells were challenged with ISAV and single-cell sequenced at 0, 24, 48 and 92h post-infection. Early infection (24h) did not significantly alter the transcriptome of Atlantic salmon cells, however cells from 48h and 96h samples clustered clearly apart whether they were infected or not, suggesting paracrine signalling. At 48 and 96h infected cells significantly up-regulated several genes related to ubiquitination. To further investigate this process we performed immunoprecipitation of ubiquitinated proteins in SHK-1 cells, finding an increase of ubiquitination at 24 and 48h after ISAV infection. Mass spectrometry of the ubiquitin-proteome revealed key host immune mechanisms regulated by ubiquitination in response to ISAV. These results improve our understanding of host response to ISAV in salmon and highlight potential target to improve host resistance.

MORE SAFETY DATA FOR USE OF AQUI-S® 20E (10% EUGENOL) AS A SEDATIVE FOR MARINE FINFISH

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Tricaine (MS-222) is the only drug currently approved by the US Food and Drug Administration (FDA) for fish sedation. Cornell University researchers have teamed up with the USFWS Aquatic Animal Drug Approval Partnership (AADAP) to generate data to support approval of a new fish sedative by FDA's Center for Veterinary Medicine (CVM). Our overall goal is to increase the number of safe and effective drugs that can be used by the aquaculture industry. We have conducted a series of studies under a FDA concurred protocol (AQS20E-18-SEA-TAS.2b) to evaluate the safety of AQUI-S®20E (10% eugenol) to sedate marine fish to the handleable stage of anesthesia in saltwater. This project has generated margin of safety data to support a New Animal Drug Application to the FDA to approve the use of AQUI-S®20E for this indication in marine finfish. FDA-CVM depends on stakeholders such as fish health researchers, drug sponsors, AADAP, and the Drug Approval Working Group (DAWG) to increase the number of safe and effective drugs that can be used by the public and private aquaculture groups. The DAWG is comprised of members from state resource agencies, as well as from USFWS, NOAA, and USGS. Since the late 1990s, AADAP has contributed to virtually every new fish medication approved by the FDA. Cornell University's Aquatic Animal Health Program also brings a long history of conducting research on fish therapeutants.

With support from USDA-NIFA and the FDA MUMS grants we have continued our efforts to generate data for safe and effective therapeutic applications of animal drugs for minor species (such as fish). The ultimate outcome of these studies will be the FDA approval of a fish anesthetic that requires no withdrawal period, i.e., an immediate-release anesthetic. We have completed our three consecutive target animal safety studies with AQUI-S® 20E and determined there was an adequate margin of safety when juvenile Striped Bass *Morone saxatilis*, Florida Pompano *Trachinotus carolinus*, and Yellow Clownfish *Amphiprion clarkii* were sedated with a dose of 400 mg/L or 600 mg/L AQUI-S® 20E. The statistical results from survival, fish health, and behavior qualitative comparisons will be presented.

THE CONNECTICUT SHELLFISH INITIATIVE: A FIVE-YEAR PROGRESS REPORT

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The Connecticut Shellfish Initiative is a multifaceted effort to grow commercial and recreational shellfisheries, revitalize natural shellfish beds and increase public awareness about the economic, environmental and cultural importance of bivalve shellfish. A stakeholder-based process that included industry, regulatory agencies, environmental advocacy groups, academia, coastal community residents and others produced the *Connecticut Shellfish Initiative Vision Plan (2018)* https://shellfish.uconn.edu/wp-content/uploads/sites/62/2016/10/execsumm.pdf. The vision statement, goals, recommended actions and performance measures comprise the plan. The State Department of Agriculture, Bureau of Aquaculture and Connecticut Sea Grant share the responsibility of tracking progress.

Over the last five years, numerous individuals and organizations have used the *Vision Plan* to justify research, outreach, management and policy projects and proposals. Their collective work has resulted in accomplishments and impacts including but not limited to the following:

- Research to place environmental and economic values on harvested shellfish
- Research to better understand aspects of climate change on cultured oysters
- Research on shell substrate and broodstock planting strategies on natural oyster beds
- Research to document the economic importance of the recreational shellfish sector
- Research to understand eating habits and how to get more seafood into residents' diets
- Regulatory guidance document for marine aquaculture
- Regulatory guidance on direct marketing of shellfish
- Planning and regulatory guidance for shellfish restoration
- Sanitation guidance for shell recycling and facilitation of shell recycling partnerships
- Independent review of state aquaculture laws
- Improved GIS mapping capacity
- Increased staffing at state regulatory office
- New and retained shellfish businesses
- Restored natural shellfish bed acreage

Another key benefit of the CSI is the strong relationships formed or improved through the process of discussing important issues, finding commonalities and working together to propose innovation solutions. The CSI also provided a framework for rapid relief for the shellfish aquaculture sector following the losses associated with COVID-19. The value of the relationships among stakeholders cannot be overstated.

EXPLORING CONNECTICUT'S SHELL RECOVERY, RECYCLING AND RESTORATION EFFORTS

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Oyster shells are key building blocks for the beds and shell is at times in short supply. In 2019, over 31 million oysters were harvested in Connecticut (CT Department of Agriculture). The vast majority of the oysters are sold for the half shell market, with little of that shell ever reclaimed as the state lacks a formal shell recycling program. The food service sector and the seafood consuming public lack a mechanism and economic incentives to reclaim the shell, divert it from landfills and return it to the beds where it is crucially needed to enhance recruitment, maintain ecosystem function and support commercial shellfish production. However, state agriculture officials have recognized shellfish restoration and shell recycling as a priority. In July 2021, Governor Ned Lamont signed Public Act 21-24, "An Act Concerning Connecticut's Shellfish Restoration Program, the Connecticut Seafood Council and the Taxation of Certain Underwater Farmlands." This law expands the Department of Agriculture's Shellfish Fund Program to allow the purchase of oyster shells, mature oysters and other materials, as well as contracting the use of vessels to conduct restoration work. In addition, the Department can receive private, state, or federal grants or direct funding to conduct shell recycling and shellfish restoration programs.

Capitalizing on this opportunity, we are exploring cost-effective means to recover and recycle shell, and to increase the quantity of this important substrate returned to the water.

ADVANCING SOUTHERN NEW ENGLAND SHELLFISH AQUACULTURE THROUGH AN ENGAGED PUBLIC AND NEXT GENERATION SUPPORT TOOLS

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Wild shellfish harvest and cultivation are centuries-old occupations in southern New England. Hundreds of small businesses grow oysters and clams on farms that range in size from a few to hundreds of acres. These farms occur on private underwater leases located within the public trust, meaning that shellfish farmers typically only have the rights to the bottom, the gear and the shellfish they are cultivating. State shellfish initiatives intended to grow aquaculture have been established, and in fact, over the past decade there has been a rapid expansion of submerged and floating gear along the southern New England coast. Many of the new farms are situated near shore and in close proximity to high value properties. Aquaculture has become increasingly visible along our coasts, and that has resulted in increased public attention, concern and scrutiny.

Previous outreach efforts focused primarily on assisting prospective producers with site and species selection and business management, and public outreach was aimed at highlighting the benefits of shellfish aquaculture, recreational shellfish harvest and local seafood consumption. With rising interest in aquaculture development, there has been increased effort to better understand perspectives of coastal community residents and leaders. This project explores public concerns and tradeoffs for shellfish aquaculture and the role the media plays in aquaculture messaging, and aims to use the results of that social science research to engage these audiences with new information, tools and outreach programming.

FLOATING SOLAR-POWERED AERATION SYSTEM FOR AQUACULTURE, WATER QUALITY IMPROVEMENT AND EXCESS ELECTRICITY PRODUCTION

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The research team is developing an off gird floating solar aeration system coupled with energy storage that improves water quality and protects underwater organisms and habitats. The particular target of the technology is for commercial-scale aquaculture farming, where supplemental dissolved oxygen is needed to overcome the dissolved oxygen consumed through the breakdown of wastes that accumulate through concentrated animal feeding.

As the utility-scale solar reaches Levelized cost of electricity (LCOE) of \$0.028-\$0.041/kWh, a floating photovoltaic (FPV) powered aeration system could be a cost-effective option. One of the major benefits of the technology is that as the water bodies help cool down the solar panel, higher electricity production can be achieved. This makes the technology's LCOE (Figure 1) competitive with a ground-mounted photovoltaic system (GMPV).

The pilot technology has floating solar panels, energy storage, a paddle wheel aeration system, and fish cages. The technology is expected to provide continuous aeration by incorporating energy storage to address the intermittent nature of solar power and produce excess electricity, which could be supplied to power other needs. Its performance and reliability are monitored and evaluated at the Texas A&M aquaculture research facility (Figure 2). The critical parameters under evaluation include technology's electricity production for aeration and aquaculture's critical parameters, including water temperature, stocking density, and feed. Once the technology is soon perfected, it is expected to revolutionize the application of floating solar aeration for aquaculture, water quality improvement, and excess electricity production, for that matter.

Figure 1: LOCE Cost Breakdown on GMPV and FPV

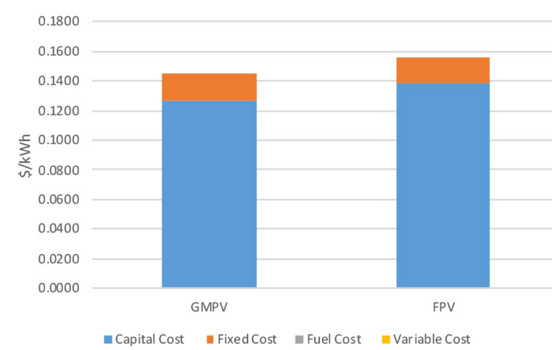

Figure 2: Pilot Floating Solar Aeration System at Texas A&M AgriLife Aquaculture Research and Training Facility

LARVAL PRODUCTION OF THE AMERICAN OYSTER *Crassostrea virginica* IN A RECIRCULATING, ARTIFICIAL SEA WATER HATCHERY

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Seed production of the American Oyster, *Crassostrea virginica*, is a vital aspect of the off-bottom oyster culture industry. The availability of oyster larvae and seed has proven to be a limiting factor for farm grow-out operations in the Gulf of Mexico. A recirculating, artificial sea water hatchery could mitigate inconsistent larval and seed production experienced in flow-through hatcheries due to inconsistent and sub-optimal water quality parameters related to storm events, freshwater incursions, and other factors.

American Oysters were spawned at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center's recirculating, artificial seawater hatchery using temperature induction to produce diploid offspring. Larval performance was evaluated using percent hatch and percent survival to harvest. Percent hatch was calculated for each spawn attempt using the estimated total number of eggs fertilized and the estimated Day 2 larval counts. Percent survival to harvest was calculated using estimated Day 2 larval counts and harvested larval counts. All broods resulting in Day 2 larvae were used to calculate percent survival to harvest, including broods that had zero harvested pediveligers (PV). Throughout the 2018 to 2021 hatchery seasons, a total of 75 (26, 27, 11, and 11 per year, respectively) spawns were attempted resulting in annual harvests of 9.94 x 10^6, 74.70 x 10^6, 33.34 x 10^6, and 175.31 x 10^6 PVs for years 2018-2021, respectively. Improvements were achieved in the percent of broods making it to harvest, percent hatch, and percent survival to harvest. The percent of broods making it to harvest in 2018 was 11.5 to 90.9% in 2021. In 2018 and 2021, percent hatch improved from 40.26 to 43.36%, and the average percent survival to harvest by larval brood improved from 3.96 to 21.11%. Improvements in larval performance are attributed to system modifications, aging of ASW, and changes in the SOPs for larval culture. Major modifications and changes include the addition of a third filtration stage and increased filtration capacity removing greater amounts of nitrogenous waste and solids. Additionally, the inventory volume of artificial seawater was doubled from 2018 to 2021. The current average larval performance in TCMAC's recirculating, artificial sea water system is comparable to averages in traditional, flow-through, natural sea water hatcheries.

ROLE OF RIBOFLAVIN BIOSYNTHETIC PATHWAY PARALOGS, AND RIBOFLAVIN TRANSPORTER (*ribN*) IN *Aeromonas salmonicida* VIRULENCE IN LUMPFISH (*Cyclopterus lumpus*)

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Aeromonas salmonicida is a Gram-negative pathogen of fresh and marine water fish and the etiological agent of furunculosis. Most bacterial pathogens can either synthesize riboflavin *de novo* or scavenge riboflavin from the host tissues through high-affinity transporters. Biologically active flavins from riboflavin (vitamin B2) are essential for intracellular redox reactions and extracellular bacterial physiology. Riboflavin supply pathways of *A. salmonicida* have not been studied.

First, we *in-silico* and experimentally characterized the riboflavin provision pathways in *A. salmonicida*. In this pathogen, the riboflavin biosynthetic pathway (RBP) coexists with the transporter *ribN*. Transcriptional orchestration of riboflavin supply genes by RT-PCR revealed that the main RBP operon consists of *ribD*, *ribE1*, *ribBA,* and *ribH* together with genes *nrdR* and *nusB*, which are involved in functions that are not directly related to riboflavin biosynthesis. *ribE1* and bifunctional *ribBA* from the main RBP operon have duplicated paralogs outside the main operon; *ribE2* (*ribE1*), *ribA,* and *ribB* (*ribBA*). Regulation analysis using qPCR showed that the *ribB* transcriptional unit, conserving a putative FMN riboswitch, is negatively regulated by riboflavin.

Secondly, to study the role of paralogs found in Riboflavin Biosynthetic Pathway (RBP) and riboflavin transporter (*ribN*) in *A. salmonicida* in lumpfish (*Cyclopterus lumpus*), mutants of *ribE1*, *ribE2*, *ribBA*, *ribA*, *ribB*, *ribN*, and *ribA-ribE1* were constructed and characterized. Groups of 60 fish were intraperitoneally injected with 0.1 ml (10^4 CFU/dose) of the respective *A. salmonicida* wild type and mutant strains. Tissue samples were collected at different time points to determine bacterial colonization. Mortality was recorded until 30 days post-infection (dpi). Surviving fish were challenged with 10^3 CFU/dose (10 LD_{50}) of *A. salmonicida* wild type. All fish died within 10 dpi from the Δ*ribE2*, Δ*ribBA*, *ribN*, and wild type infected groups, whereas 100% of the fish infected with Δ*ribE1*, Δ*ribA*, Δ*ribB*, and *ribA-ribE1* survived. After the challenge, fully attenuated mutants; Δ*ribE1*, Δ*ribA*, Δ*ribB*, and *ribA-ribE1* conferred protection with a low relative percentage of survival (10-20%).

In summary, *ribE1*, *ribA*, and *ribB* play an essential role in *A. salmonicida* virulence during host colonization. This study was funded by Canada-First, Ocean Frontier Institute, and NSERC-Discovery (RGPIN-2018-05942).

THE HALO EFFECT: USING SEAWEEDS TO PROTECT AQUACULTURED BIVALVES AGAINST OCEAN ACIDIFICATION AND HARMFUL ALGAL BLOOMS

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Ocean acidification (OA) and harmful algal blooms (HABs) are two significant threats for bivalves in coastal zones. While the open ocean will acidify over the coming decades, a multitude of coastal processes including eutrophication, upwelling, and river discharge can cause ephemeral and seasonal bouts of coastal ocean acidification that can create levels of pH and pCO_2 that are not expected in open waters until late next century. In parallel, the impacts of HABs on aquaculture have been expansive in recent decades and can range from causing slowed growth and mortality to contaminating bivalves with biotoxins and thereby restricting sale of product. While there have been decades of study regarding the mitigation of HABs and OA, approaches investigated to date have had negative consequences for bivalves and/or ecosystems, work at temporal or spatial scales irrelevant to bivalve aquaculture, or have simply been ineffective. One exception has been the aquaculture of seaweeds. During the past decade, our group has documented the ability of temperate seaweeds including sugar kelp (*Saccharina latissima*), Irish Moss (*Chondrus crispus*), *Gracilaria tikvahiae, Porphyra* spp., and *Ulva* spp., to mitigate both ocean acidification and HABs.

Regarding ocean acidification, faster growing seaweeds were found to be most impactful in altering carbonate chemistry to the benefit of bivalves. Laboratory studies with S. *latissima* and *Ulva* spp. grown at aquaculture densities (0.1 - 3 g L^{-1}) have documented their ability to rapidly increase the pH, alkalinity, and saturation state of calcium carbonate (Ω) while lowing pCO_2 concentrations, primarily due to photosynthetic activity and secondarily due to nitrate assimilation. While experimentally elevated levels of pCO_2 and lreduced Ω significantly ($p<0.05$) reduced the growth rates of commonly cultured bivalves (hard clams, *Mercenaria mercenaria*; eastern oysters, *Crassostrea virginica*; bay scallops, *Argopecten irradians*, and blue mussels, *Mytilus edulis*), the co-culture of these bivalves with *Ulva* spp. (all bivalves) or *S. latissima* (*C. virginica, M. edulis, M. mercenaria*) under the same CO_2 delivery rates 'rescued' the bivalves from OA, yielding growth rates identical to control conditions. Beyond the lab, deployment of *C. virginica* with sugar kelp on an oyster farm experiencing acidification resulted in growth rates that were significantly faster than oysters grown in the same location without kelp. These results suggest that that the aquaculture of macroalgae in acidified environments can serve as a refuge for calcifying bivalves that may otherwise be negatively impacted by OA.

Regarding HABs, all seaweeds were shown to significantly reduce the densities of different HABs primarily due to the release of allelochemicals by seaweeds and secondarily due to nutrient removal and/or pH elevation. Of greatest significance has been the ability of S. *latissima* and *Ulva* spp. to cause lysis of the saxitoxin-producing dinoflagellate, *Alexandrium catenella*, and in turn, reduce the accumulation of saxitoxin in co-cultured blue mussels to levels below the USFDA closure limit. Collectively, these studies demonstrate that the co-culture of bivalves with seaweeds can create a 'halo effect' around bivalves to mitigate the harmful effects of OA and HABs.

OPPORTUNITIES FOR FISH PRODUCTS EXPORT TO CHINA: AN ANALYSIS OF CONSUMER PREFERENCES

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2019 world fish & seafood sales amounted to 107.4 million tons. While the share of North America in world fish and seafood sales is only 1.84%, the Asia-Pacific region is the world's largest area for consumption of fish and seafood with 77.5% of sales (as of the end of 2019). The leading sales position in the region belongs to China with 53% of all sales.

While the United States supplies 8% (or 0.32 million tons) of all imported fish to China, Russia is the largest supplier of fish raw materials to China with 27% or 1.1 million tons of fish and seafood products.

According to a survey of Chinese consumers conducted in 2020-2021, the main fish consumers in China are young adults aged 25-35 with a family monthly income over $3000. This population inhabits level 1-2 cities and consumes fish 1-3 times a week. The safety of the product is their most important concern followed be the price, and then the taste of the product. According to consumer surveys, about 40% choose crab meat, 38% fish, and the rest is divided between shellfish and caviar. About 60% prefer to buy at discounts.

The United States fish ranks second in preference for fish and seafood among Chinese consumers, and follows fish and seafood from South Asia. Almost 19% of the respondents reported the USA fish as their first preference. Fish from aquaculture is inferior in consumer preferences to wild fish due to the large amount of aquaculture in China. Consumers aged 25-40 in China tend to consume more processed fish and seafood.

A TALE OF TWO (OR MORE) BACTERIA: APPLYING KNOWLEDGE ON VIBRIO – PROBIONT INTERACTIONS TO MANAGE BACTERIAL DISEASES IN BIVALVE HATCHERIES

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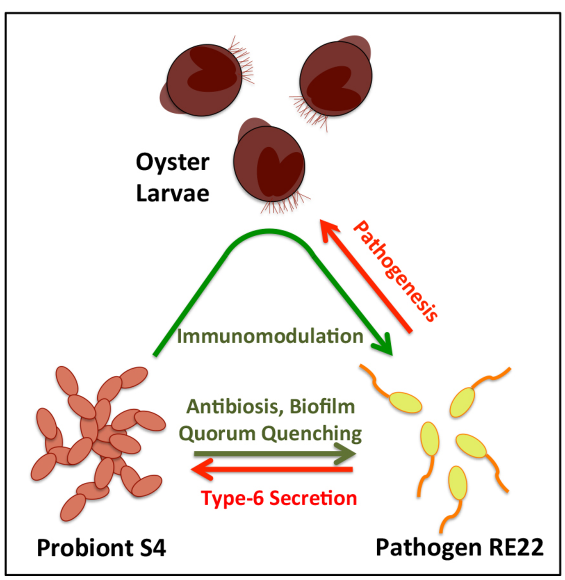
The recent expansion in the aquaculture of bivalves (oysters, scallops, clams) has been constrained by the impact of infectious diseases on different stages of production. At the hatchery stage, bacterial diseases such as vibriosis can lead to rapid and significant losses of larvae. The goal of our research is to apply knowledge on microbial-microbial interactions to manage the impact of bacterial diseases in shellfish hatcheries, using the bacterial pathogen *Vibrio coralliilyticus* RE22 (RE22) and the marine bacterium and probiont candidate *Phaeobacter inhibens* S4 (S4) as a model system.

Pretreatment of eastern oyster, *Crassostrea virginica,* larvae with probiont S4 leads to significantly increased larval survival after challenge with pathogen RE22 as compared to non-treated larvae. Genome sequencing of pathogen RE22 and probiont S4 have led to the identification of putative virulence factors in RE22 and mechanisms of action of S4 against RE22. Targeted gene knock-out experiments in RE22 showed that one of the two copies of the Type 6 Secretion System (T6SS) identified in the genome of RE22 targets mainly the larval host, while the other copy targets mainly other bacteria. The mechanisms of action of probiont S4 are complex, including, but not limited to, antibiosis, biofilm formation, and quorum quenching. Gene expression patterns of RE22 and S4 in competition suggest that S4 can reduce expression of a variety of virulence factors in RE22, including T6SS and motility. Moreover, treatment of oyster larvae with probiont S4 leads to immunostimulation, while treatment of larvae with pathogen RE22 leads to immunosuppression. A liquid formulation of probiont S4 has been developed for commercial use in shellfish hatcheries. Daily delivery of probiont S4 to *C. virginica* larvae in the hatchery led to changes in the bacterial communities in the larvae, including changes in the composition, but not relative abundance, of Vibrionaceae.

Our research contributes to better understanding of the mechanisms of virulence of pathogen RE22, as well as pathogen-probiont interactions *in vitro* and *in vivo*. The ultimate goal is to optimize probiotic use in shellfish hatcheries.



ALASKA'S MACROALGAE MARICULTURE INDUSTRY

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This presentation will give background on the seaweed mariculture industry in Alaska; report on the strength, weaknesses, opportunities, and threats to this nascent industry; and conclude with the types of projects to mitigate our weaknesses and threats, and to take advantage of our strengths and opportunities.

The mariculture industry in Alaska is currently small, yet poised to grow. As of October 2021, this included 39 aquatic farms permitted to grow kelp and 5 hatcheries. Seaweeds, including sugar kelp (*Saccharina latissima*), bull kelp (*Nereocystis luetkeana*), and ribbon kelp (*Alaria marginata*), are increasing in production with over 231,000 lbs harvested in 2021 (Alaska Department of Fish and Game, 2021). There is support across the state to grow the industry. The Alaska Mariculture Development Plan set a goal to grow this industry to $100 million in 20 years (Alaska Mariculture Task Force, 2018). Included in this growth is the predicted expansion of the cultivation of seaweed in Alaska's waters.

Mariculture opportunities are tailor-made for Alaska and Alaskans. Alaska's rich and productive waters are well suited for growing both shellfish and seaweed (finfish farming is illegal), Alaskans are well versed in marine-based activities, and Alaska's commercial fishing infrastructure (boats and processing) can serve to jointly support the mariculture industry.

The future structure of Alaska's seaweed industry is, for the most part, yet to be defined. The history and scale of Alaska's seafood industry has spawned a strong academic support system housed at the University of Alaska, primarily through the Alaska Sea Grant Marine Advisory Program (MAP). Alaska Sea Grant is a recognized leader in Alaska's aquaculture and mariculture industries and has been a major driving force for shellfish and seaweed mariculture development. Now, as seaweed comes into focus for the state's seafood industry, the MAP seeks to empower farmers and other small businesses in developing an industry that's accessible to Alaska's coastal community residents, offers economic promise to those willing to work for it, offers opportunities for value-added processing and marketing within the state, and can grow to compete globally.

THE EFFECTS OF TWO WATER TEMPERATURE REGIMES ON ATLANTIC SALMON *Salmo salar* GROWTH PERFORMANCE AND MATURATION IN FRESHWATER RECIRCULATING AQUACULTURE SYSTEMS

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Precocious maturation of Atlantic salmon (*Salmo salar*) continues to be a source of economic loss for farmers due to the associated decreased growth performance, poor feed conversion efficiency, and reduced product quality (e.g., pale fillets), among other things. Given the natural life history of the species, and the numerous factors (photoperiod, temperature, fish size, growth rate, nutritional status, genetics, etc.) that can influence the onset of puberty, early sexual maturation can be difficult to prevent. This problem has been particularly prevalent in land-based, closed containment recirculation aquaculture system (RAS) facilities raising Atlantic salmon; reported early maturation has ranged from 2% to 100% in surveyed RAS facilities. With the increasing investment in land-based salmon farm projects over the past several years, the issue of early maturation will likely be of major economic significance for land-based salmon producers unless reliable preventive methods are developed.

Well-defined, RAS-specific water temperature thresholds that maintain high fish growth performance while minimizing early maturation are needed to accurately forecast bioplans, operational costs, and gains from reduced maturation downgrades. Accordingly, the objective of this research was to compare growth performance and maturation status of diploid, mixed-sex Atlantic salmon grown to ~1.3 kg in land-based RAS at a standard production temperature of 14 °C or at a cooler temperature of 12 °C. Survival, final weight, condition factor, feed consumption, and feed conversion ratio were similar between temperature treatments while thermal growth coefficient was significantly higher at 12 °C. Maturation prevalence and gonadosomatic index of immature fish was significantly higher at 14 °C. Overall, lower water temperature reduced prevalence of maturation in RAS while maintaining similar production performance; however, more than 20% of salmon cultured at 12 °C matured indicating even lower temperature, additional manipulations to the RAS environment, or use of all-female stocks are needed to optimize Atlantic salmon growout in RAS for reduced maturation.

EVALUATING FRESHWATER FLUIDIZED SAND BIOFILTER PERFORMANCE FOLLOWING EXPOSURE TO LOW-DOSE PERACETIC ACID TREATMENTS

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Applying disease or pathogen control treatments in recirculating aquaculture systems (RAS) can be challenging due to the potential impacts of the administered chemical agent(s) on biofilter performance. Fish farmers utilizing RAS would benefit greatly from possessing more treatment options that are both efficacious towards the intended microbial target(s) and relatively safe in terms of maintaining biofilter function for sufficient nitrification of accumulating ammonia. One particular chemical, peracetic acid (PAA), has shown promise outside the United States for use in controlling disease and pathogen counts in commercial RAS; however, PAA's use in the U.S. aquaculture is currently restricted to materials surface disinfection when food fish are not present. Before approval of PAA as a bona fide aquacultural therapeutant is considered, baseline research is required to assess, among other things, the impact on nitrification as RAS biofilters are exposed to PAA.

To address this knowledge gap, we exposed replicated experimental-scale freshwater fluidized sand biofilters to 4-hour exposures of semi-continuous low-dose PAA at three separate concentrations, and monitored PAA concentration over time to assess peak concentrations achieved as well as chemical decay following cessation of treatments. Following PAA exposure, we monitored all biofilters for a period of two weeks and assessed total ammonia nitrogen (TAN) removal efficiencies, influent and effluent nitrite- and nitrate-nitrogen concentrations, and bacterial biomass. At the time of abstract submission, the study is ongoing, with anticipated final data collection in November 2021. Final PAA dose-response impacts on RAS biofilter performance, as well as recommendations for PAA use in RAS based on study results, will be presented at Aquaculture America 2022.

BIVALVE BODYGUARDS: EELGRASS *Zostera marina* MEADOWS BUFFER HIGH ENERGY BOAT WAKES AND REDUCE DEGRADATION TO OLYMPIA OYSTER *Ostrea lurida* BEDS CONSTRUCTED FOR A LIVING SHORELINES PROJECT

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Significant development in Southern California coastal areas has altered marine habitat that would normally buffer wave energy, leaving shorelines vulnerable to erosion. Living Shorelines projects seek to address this problem by restoring native habitat with the goal of rebuilding ecosystems in a way that provides ecosystem services such as shoreline stabilization. Foundation species such as oysters and eelgrass are increasingly used in these projects because they provision habitat and reduce erosion. Native Olympia oyster beds and eelgrass beds were restored at four sites in Upper Newport Bay in 2016 as part of the Upper Newport Bay Living Shorelines Project to explore the possible combined benefits of restoring two foundation species. All sites have seen differential success since initial restoration in terms of oyster density and bed growth. Human activity such as trampling and wave energy has been previously linked to mussel and oyster bed degradation, so to understand the impacts of human activity on restored oyster beds 30-minute human use surveys were conducted at all sites and all instances of human activity including recreation, fisher activity, boating, and the size of wakes produced by each passing boat were documented. Water level loggers were also deployed to directly measure boat wake energy with and without Living Shorelines plots.

Preliminary analyses show evidence of reduced wave energy by Living Shorelines plots and a trend towards reduced oyster density with increased frequency of large boat wakes. Early evidence also shows that the negative impacts of boat wake energy on oyster density are reduced when oysters are restored upshore of restored eelgrass. This suggests that eelgrass may protect oyster beds from damaging wave energy created by passing boats. Teasing out the ecosystem services provided by these restored habitats can help design future restorations as well as inform our understanding of the ecology of Living Shoreline species.

Figure 1. Degraded bed at high-energy site (left) and consolidated bed at low-energy site (right).

ASSESSMENT THE OXIDATIVE STRESS AND BIO-TRANSFORMATION ENZYMATIC EFFECTS OF 24HR GLYPHOSATE STRESS EXPOSURE ON WHITELEG SHRIMP *Litopenaeus vannamei* (BOONE, 1931)

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In this study, the low level of exposure was performed to assess the oxidative stress and biotransformation of the glyphosate on Whiteleg shrimp *Litopenaeus vannamei*. The concentrations of herbicide as following concentrations as Control(+),10ppm, 20ppm, 30ppm, 40ppm and 50ppm (Part per million concentration at 24hr durations (all are triplicate) . The samples were collected every 6hr time interval. Naturally, the aquatic organisms having self-defense mechanism 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 to overcome the pollution risk. At the same time most possible the releasing of reactive oxygen species (ROS) rate is increased during the defense mechanisms occurred in the cell because of rapid oxidation, reduction, hydroxylation, dealkylation reactions . In the present activity of phase I reduction of 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 *L. vannamei* at different concentrations of glyphosate. The inducibility and activity of phase I reaction of three CYP450 isoforms (benzyloxyresorufin - O-dealkylase [BROD], ethoxyresorufin-O-dealkylase [EROD] and methoxyresorufin-O-dealkylase [MROD]) enzymes were measured in the hepatic S9 fraction treated with glyphosate at different concentrations in the laboratory condition within 24hr duration. The levels of BROD (CYP2B6), MROD (CYP1A2) and EROD (CYP1A1) in the tissues of *L. vannamei* were measured using Multimode reader. All the MFO enzymes exhibited a hierarchical dose-dependent activity in response to glyphosate concentrations. The level of superoxide dismutase (SOD), catalase (CAT) and Glutathone reductase (GR) in all the treatments decreased in a dose- and time-dependent manner except for the concentration 40-50ppm. Finally conclusion of these experiments revealed that 10-30ppm concentrations of glyphosate toward the normal condition when compared to other higher concentrations of 40ppm to 50ppm. The elevation level of increased both oxidative and biotransformation enzymes subsequently became normal at low level concentrations.

ASSESSMENT OF THE OXIDATIVE STRESS AND BIO-TRANSFORMATION ENZYMATIC EFFECTS OF GLYPHOSATE EXPOSURE ON THE PACIFIC WHITELEG SHRIMP *Penaeus (Litopenaeus) vannamei*

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Naturally, aquatic organisms have self-defense mechanisms that protect them from the actions of endocrine disrupting chemicals (EDCs) such as the herbicide and antimicrobial Glyphosate (GLY). The changes in expression levels of different types of enzymes could be used as biomarkers of GLY exposure such as hepatopancreatic cytochrome P450-associated enzyme activity. It is also possible that the rate of release of reactive oxygen species (ROS) increased during GLY exposure, and the defense mechanisms occurred in the cell because of rapid oxidation, reduction, hydroxylation and dealkylation reactions. 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, Tamilnadu. The 41% GLY was used to prepare 100% stock and serially diluted to different concentrations (10ppm, 20ppm, 30ppm, 40ppm, and 50ppm). The effect of Glyphosate exposure was examined to assess the oxidative stress and biotransformation of *P. vannamei* post larval stage (PL12–PL15) collected from a commercial farm near Pattukkottai, Thanjavur District, Tamilnadu, 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): control, 10ppm, 20ppm, 30ppm, 40ppm, and 50ppm GLY, for 24hours. 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.

The levels of antioxidant [superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)] and biotransformation enzymes (CYP450 isoform-EROD, MROD, BROD and GST) increased at 6hr and 12hr in the 10ppm-30ppm GLY groups, their activity decreased afterwards. In the 40ppm-50ppm GLY groups, the antioxidant and biotransformation enzymes increased up to 24hr.

Results suggested that (a) antioxidant enzymes were elevated at early stages of exposure in 10-30ppm groups, (b) GR, SOD and CAT play a crucial role against ROS and neutralized by them, (c) enzymatic biotransformation is essential to eliminate GLY, and (d) the cellular enzyme defense mechanism protect shrimp at certain levels of exposure.

A NATURE-BASED SOLUTIONS APPROACH TO SHRIMP AQUACULTURE EFFLUENT MANAGEMENT

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Expansion of the shrimp aquaculture sector has resulted in the destruction and degradation of mangrove ecosystems across much of Asia and South America. Although the rate of mangrove deforestation has largely decreased over the last several decades, the deficit of ecosystem services from lost and fragmented forests remains, leaving communities vulnerable to coastal climate risks. While habitat conversion was the primary environmental concern for the mostly extensive shrimp aquaculture sector in previous decades, the shift to more intensive production creates new environmental risks (Figure 1). Intensification increases the nutrient loads in effluent from aquaculture production, which amplifies the potential for untreated effluent to degrade surrounding water bodies and ecosystem. Conventional gray infrastructure, such as settling ponds and mechanical filtration, are most commonly used to treat effluent before discharging into the environment, but these systems provide few ecosystem services beyond their water quality benefits. Here, we compare the spatial requirements, farm-level economics, and ecosystem services of conventional and nature-based effluent treatment options to understand their viability, costs, and co-benefits.

Constructed mangrove wetlands emerged as the green-gray engineering approach with the most potential to reduce negative impacts from aquaculture, while also realizing environmental co-benefits at competitive capital and operational expenditure costs. By combining both engineered design of conventional treatments, with the environmental benefits and further removal of nutrients of NbS options, a green-gray engineering combination provides ecosystem services in addition to water purification attributes. We propose that effluent treatment can be designed in a way that improves water quality, while conferring increased coastal climatic resilience that benefits both the farmer and the environment.

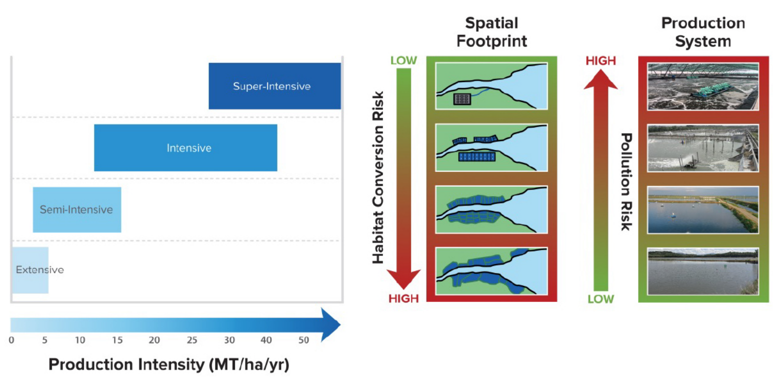

Figure 1. The spectrum of production intensities, expressed as yield in metric tons (MT) per ha per year, associated with four categories of shrimp aquaculture production. Habitat conversion risk is relative to the spatial footprint required by each production category. Pollution risk is relative to the amounts of effluent discharged from production systems.

COMMERCIAL-SCALE SEAWEED FARMING SYSTEMS

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Seaweed farming is the fastest-growing aquaculture sector and over the last decade has become an increasingly important sector of our coastal economy. Significant growth has occurred in the farming of kelps in New England and in Alaska. The markets are primarily aimed at food products but larger opportunities exist for use as chemical feedstocks, animal feed, fertilizer, and energy production. In order to compete with agricultural sources in these markets, the scale of the seaweed farming sector must grow and the costs of production must be reduced. Needed steps include increasing the spatial efficiency of farming systems, the adoption of more robust systems able to exploit less protected waters, and the introduction of mechanized harvest methods.

In New England where smaller farms dominate, single lines are suspended horizontally below the surface, held in place by anchors at each end and suitable buoys. Farms can utilize multiples of these single-line systems but to avoid entanglement with each other and resulting loss of crop, wide spacing is needed, limiting the production potential of a lease area.

This presentation will report on multi-line farm systems developed under several projects funded by the Department of Energy ARPA-E Mariner program. These systems range from 5 to 110 lines across and line spacing as close as 2.5’ (0.76m). The methods of farm design, construction, and deployment will be discussed as well as the key role of innovative components in the success of these systems deployed in New England, Alaska, and the Caribbean.

LAND-BASED PRODUCTION OF HIGH-VALUE SEAWEEDS IN CALIFORNIA: PROS AND CONS OF PRODUCING FRESH SEAWEEDS IN THE COVID ERA

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Monterey Bay Seaweeds is a land-based seaweed farm in California that produces 4-7 species for the US live culinary seaweed market. We work in partnership with San Jose State University to grow our seaweeds using proprietary tumble-culture methods with flowing seawater (450 gpm), producing up to 15 tons of fresh seaweed annually (25 tons/acre/year) for restaurants, food services, and cosmetics/nutraceutical companies. Given disruptions to the restaurant market due to COVID-19, Monterey Bay Seaweeds has begun research and development on mid-value high volume applications that highlight the high protein content and nutritional value of our focal species dulse (*Devaleraea mollis*). The utility of land-based seaweed farms to meet the increasing demand for culinary seaweed products will require the development of high-volume high-value applications that extend beyond direct consumption in high-end restaurants.

HIGHS AND LOWS: EXAMINING HATCHERY YIELDS AND CRASHES AT HORN POINT LABORATORY WITH PRODUCTION ANALYSIS

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Shellfish hatcheries have become an increasingly important component of aquaculture production in the United States. Although the industry has been advancing technologically over time to stabilize production and supply, detailed production analysis has rarely been reported. To help fill the data gap and initiate a broader discussion on production trends, we report on long-term production trends (2011-2020) at Horn Point Laboratory's oyster hatchery, which included persistent production failure during the 2019 season. During the 2019 season, larval assays were conducted to determine drivers of production failure; however, no clear culprits were identified. Production metrics of interest where production yield (millions eyed-larvae produced) and production rate (days to reach the eyed larval stage). A variety of factors stretching across the entire production process (i.e. broodstock selection, conditioning, spawning, larval culture) were considered as possible predictors of production metrics. Furthermore, water quality in the Choptank River, the source of water for the hatchery, were used as input during analysis. Machine learning was used to train models of production yield and hindcast the specific conditions when the hatchery's production was most efficient. We identified several important factors that predicted production yield and production rate. Many of these factors the hatchery staff can manipulate or represent a stage in the production process, which improved, could lead to greater production efficiency. Collectively, we conclude that more research, data sharing, and cross-institution collaboration are needed to understand production variability within and among shellfish hatcheries to maintain high levels of consistent shellfish aquaculture production.

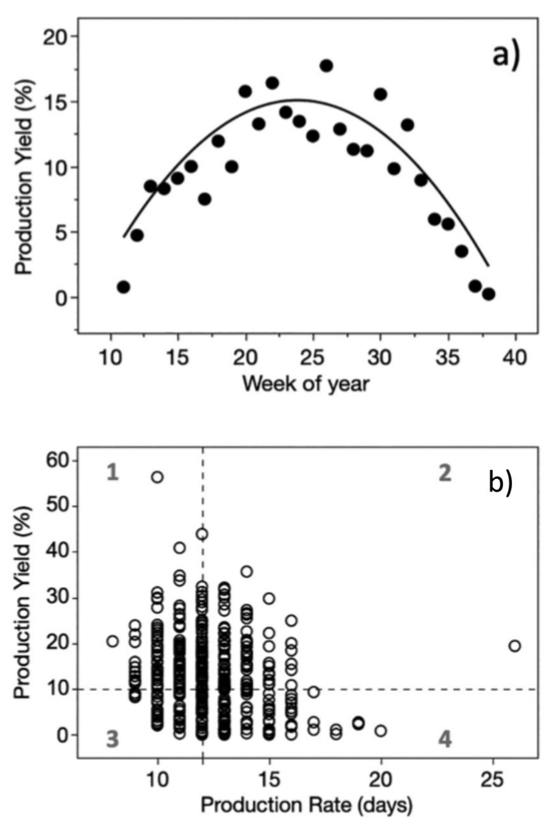

Figure 1. a) Average hatchery production yield over the week of the year; b) production yield over production rate divided among four quadrants separated by median values (dashed lines).

PARTIAL AND TOTAL REPLACEMENT OF SOYBEAN MEAL WITH A HIGH-PROTEIN YEAST FERMENTATION PRODUCT IN RAINBOW TROUT *Oncorhynchus mykiss* FEEDS

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Soybean meal is a widely used protein source in finfish feeds, however, high inclusion rates of soybean meal (above 20-25%) can lead to gut inflammation and reduced growth efficiency in many carnivorous species. High-protein feed products extracted from yeast fermentation of corn (HPF-YFC) are potential alternatives to soybean meal that could meet the dietary protein demands of carnivorous finfish without the drawbacks commonly associated with soybean meal. This investigation examined the culture performance, intestinal histology, and expression of gut inflammation markers in rainbow trout fed experimental diets with graded levels of soybean protein and a HPF-YFC.

\Rainbow trout (48.9±1.3g) were randomly distributed to twelve 100-L tanks within a recirculating aquaculture system (9 L/min inflow in tanks) with an initial stocking density of 30 fish/tank. Fish were fed four experimental diets for 70 days on a restricted feeding regime (1-3% biomass/day), and tanks were weighed and every 14 days. The four experimental diets were formulated to contain 40% protein and 20% lipids. The diets had 0% (Control), 25%, 50%, or 100% replacement of soybean meal with HPF-YFC

The mean weight, specific growth rate, and feed conversion ratio of fish were not significantly different (α=0.05) between diet groups after 69 days of feeding (139.6±7.5g final weight). Histological analysis of distal intestines showed a significant increase in villi length (p=0.012) and villi length:width ratio (p=0.005) associated with the 100% HPF-YFC diet group (Figure). The expression of gut inflammation markers TNF-α, IL10, and MMP9 tended to be lower in the 50% and 100% HPF-YFC diet groups, relative to the soybean control group, although these differences were not statistically significant. These results suggest that the replacement of soybean protein with HPF-YFC protein in rainbow trout diets does not impair growth performance and improves gut health.

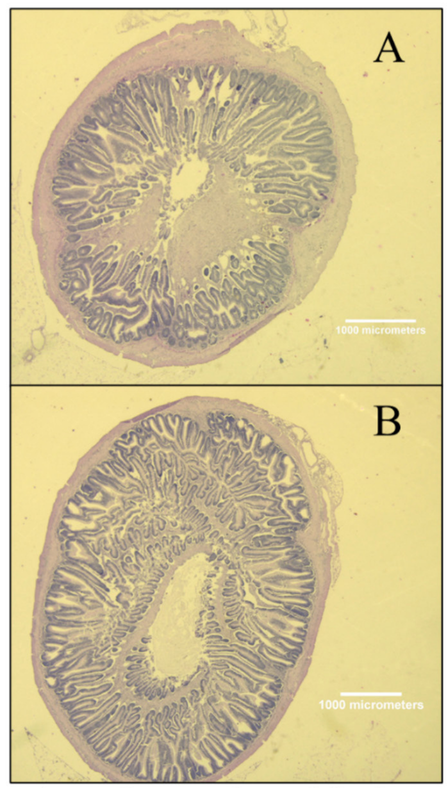

Figure. Cross sections of distal intestine from rainbow trout in the soybean meal control group (A) and 100% HPF-YFC group (B).

FEAST OR FAST: THE EFFECTS OF FEEDING RESTRICTIONS ON RAINBOW TROUT *Oncorhynchus mykiss* GROWTH AND FEED UTILIZATION

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The efficiency with which fish can convert dietary macronutrients and energy into bodily growth (feed conversion ratio; FCR) is one of the most important economic considerations for finfish production. Two important factors that influence this process are the amount of food provided and the timing of feeding events. Studies over the last 40 years have suggested that restricting feeding rates below satiation levels can improve FCR values, and that recurrent short-duration fasting events (intermittent fasting) can improve FCR and greatly increase appetite. This investigation examined the culture performance and expression of appetite control markers in rainbow trout fed to apparent satiation, on a restrictive feeding regime, or on a restrictive feeding regime with partial weekend fasting.

Rainbow trout (48.9±1.3g) were randomly distributed to nine 100-L tanks within a recirculating aquaculture system (9 L/min inflow in tanks) with an initial stocking density of 30 fish/tank. Tanks were fed three times per day either to apparent satiation or on a restricted feeding regime (75-85% of satiation feeding rate; NRC 2011). A third experimental group was included that was fed on the restricted feeding regime with partial fasting two days per week (1 feeding/day, 33% of recommended feeding rate). Fish were weighted every 14 days for the 70-day experiment, and feeding rates were adjusted based on fish size.

The mean weight (p=0.008), specific growth rate (p=0.007), and FCR (p=0.040) of fish were significantly different between diet groups (122-163g/fish final weight; Figure). Initial results suggest that growth is maximized when feeding fish to satiation, but that restrictive feeding and partial fasting on weekends can greatly improve FCR. The expression of appetite controlling hormones CCK, GRP, ghrelin, and leptin in intestinal tissues, as well as histological analysis of distil intestines, will be evaluated for this presentation.

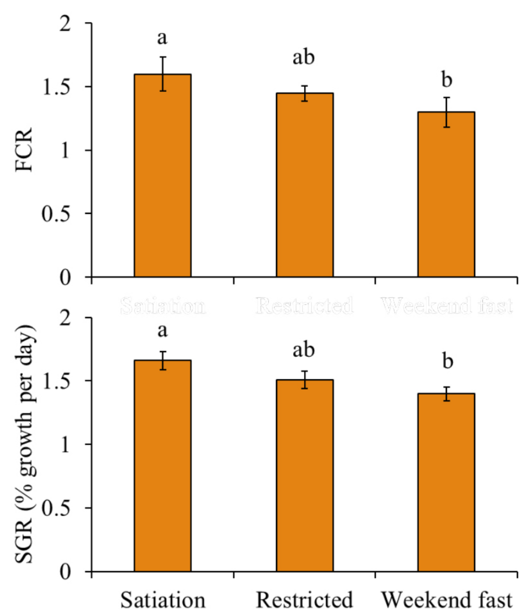

Figure. Specific growth rate (SGR) and food conversion ratio (FCR) of rainbow trout after 70 days of feeding three times a day to satiation, under a restricted feeding regime (75-85% of satiation amount), or under a restricted feeding regime with one meal/day on weekends (weekend fast). Different letters represent significant differences (ANOVA) at α=0.05.

DEVELOPMENT OF CULTIVATION SYSTEMS AND BEST MANAGEMENT PRACTICES FOR SEAWEED AQUACULTURE IN THE TROPICAL U.S. AND CARIBBEAN

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The Caribbean's small countries and island nations are experiencing a loss of resources due to climate change, nutrient pollution, ocean acidification, seagrass bed habitat loss, fishing pressure, and lost tourism revenues due to COVID-19. We believe that well-managed development and growth of tropical seaweed aquaculture in the region may help to assuage these issues while also providing a new source of seaweed biomass for the existing carrageenan, new food and textile and possibly future biofuel markets. We are exploring the opportunities for expanded seaweed aquaculture in the Caribbean and the Gulf of Mexico in collaboration with partners across 15 institutions and research sites in Puerto Rico, Florida, and Belize.

In this presentation, we will explain how insight from our field research is being combined with consultation from local stakeholders to guide the development of Ecological Best Management Practices (BMP) for seaweed aquaculture in the Gulf of Mexico and the Caribbean Sea. Our intention is that these ecological BMPs will support prospective seaweed farmers, resource managers, and buyers through ecologically and socially responsible decision-making around seaweed aquaculture. Fostering sustainable expansion of Caribbean and Gulf of Mexico macroalgal cultivation will encourage production in these regions to expand beyond the existing small-scale farms producing seaweed for artisanal beverage and cosmeceutical markets. Establishing larger-scale farms in a responsible manner will enable production of algal biomass for additional local and global markets and allow resource managers to consider seaweed farming as an extractive component of an integrated water quality management strategy.

SPATIAL AND TEMPORAL PATTERN OF NOROVIRUS DISPERSAL IN AN OYSTER GROWING REGION IN THE NORTHEAST PACIFIC

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Contamination of Pacific oysters, *Crassostrea gigas*, by human norovirus (HuNoV) is a major constraint to sustainable shellfish farming in coastal waters of the Northeast Pacific. HuNoV is not a marine virus and must originate from a human source. A barrier to effective management is a paucity of data regarding HuNoV dispersal in the marine environment. The main objective of this study was to identify the spatial distribution and persistence of HuNoV in an active shellfish farming region in the Northeast Pacific. Market-size *C. gigas* were sequentially deployed for two-week intervals at 12 sites during the 2020 winter risk period from January to April. HuNoV quantification was performed by reverse transcription real-time PCR (RTqPCR) according to method ISO 15216-1:2017, with minor modifications. The estimated prevalence of genotypes GI and GII HuNoV associated with human illness in oyster digestive tissue was 0 ± 0 and 0.8 ± 0.3 %, respectively. Spatiotemporal analysis performed using the Bernouilli model available in SaTScan v9.6.1 revealed contamination of oysters with GII HuNoV changed through time and space during the surveillance period. The largest GII HuNoV cluster had a radius of 8.4 km and the longest duration of a cluster was ≤ 6 weeks. These results will help mitigate HuNoV illnesses by informing the shellfish industry and regulators on the size and duration of harvest closures following HuNoV outbreaks.

OCEAN ACIDIFICATION ALTERS DEVELOPMENTAL TIMING AND GENE EXPRESSION OF ION TRANSPORT PROTEINS DURING LARVAL DEVELOPMENT IN RESILIENT AND SUSCEPTIBLE LINEAGES OF THE PACIFIC OYSTER (*Crassostrea gigas*)

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Ocean acidification (OA) adversely impacts initial shell formation of bivalve larvae. Despite many studies observing large differences in developmental success between distinct genetic populations of bivalves exposed to OA, few studies have investigated the molecular mechanisms that enable resilient larvae to build their initial shell. This knowledge is key to their ecological and economical conservation. Herein, we used a genetic-selection program for *Crassostrea gigas* to produce a resilient and susceptible larval lineage to OA. The resilient and susceptible larvae were sampled every 3-hours over a 24-hour period in OA and control conditions. The susceptible lineage failed to develop a larval shell in OA conditions, whereas 52 % of the resilient lineage developed to D-larvae by 24 hours post fertilisation. We measured the expression of 23 genes involved in initial shell formation by RTqPCR, which revealed significant genotype-by-time and environment-by-time interactions for the transcription of these genes. OA upregulated a single gene encoding a protein involved in ion transport, Na^+ K^+ ATPase, in both the resilient and susceptible lineage. These results were corroborated by a second experiment involving 25 pair-mated *C. gigas* families exposed to OA and control conditions. Our findings indicate *C. gigas* have a fixed capacity to modulate expression of genes involved in initial shell formation in response to OA. Thus, phenotypic differences to OA between the resilient and susceptible lineage are likely explained by other cellular processes, such as bioenergetics or protein translation.

CATCHING FISH (NEWS): AQUACULTURE AND AQUATIC HEALTH EDUCATION ON AQUADOCS PODCAST

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Podcasts are the next frontier for educational and promotional resources. As new technology continues to dominate in all aspects of modern life, there is a growing need for aquaculture professionals to engage in non-traditional communication to highlight the sophistication and importance of the industry. Multiple studies demonstrate the utility of podcasts in public education, particularly with respect to specialized content such as aquaculture. Podcasts establish a personal interaction between the listener and the topic. They create an engaging and memorable experience through storytelling and community formation. Podcasts also offer information and news in smaller bite-sized chunks, making information readily and easily accessible, perfect for daily commutes, exercise routines, and busy schedules. The positive experience created through podcast listenership and the emergence of consistent followers cultivates a meaningful and personal network connecting individuals on a global scale.

Amongst the public, there is a lack of knowledge about basic aquaculture protocols, standards, and significance in the food animal industry. Aquaculturists can receive negative or neutral press often because people do not have proper knowledge to understand or recognize the intricacies and necessity of the industry. Podcasts can highlight the distinctive qualities of aquaculture and describe the essential components required for success. By doing so, they elevate the overall knowledge of aquaculture practices and in turn, increase the public's positive perception of the industry. While there are a handful of successful aquaculture podcasts currently available, one key component that is often overlooked is the role of the aquatic herd health professional. There are currently few resources available for both aquaculturists and the public to learn about aquatic veterinarians. *Aquadocs Podcast* has become that resource.

Since launching in September 2020, *Aquadocs* has become the #1 podcast on aquatic veterinary medicine and a top 50 life sciences podcast streaming in over 90 countries. To date, 40 episodes have been published, each averaging 28 minutes in length, and resulting in over 20,000 downloads. Listeners are distributed geographically across 90 countries and located on all continents excluding Antarctica. *Aquadocs* demonstrates the process, feasibility, and utility of creating podcasts that provide useful information to inform aquaculturists, consumers, industry leaders, and the public about aquaculture medicine. The positive audience response generated by *Aquadocs* encourages further exploration for the aquaculture community to highlight niche aspects of the industry as well as adapt non-traditional communication methods.

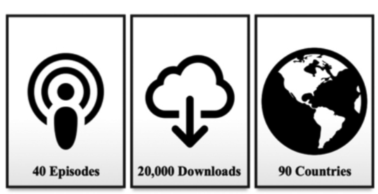

Figure 1. *Aquadocs* Metrics
(September 2020 – 2021).

COLLECTING ACCURATE DATA FROM THE AQUACULTURE INDUSTRY: A MINNESOTA EXAMPLE

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A major obstacle for U.S. aquaculture is the lack of reliable industry data collected from producers. Accurate reporting and data collection mechanisms form the basis of industry understanding and are necessary to guide resource allocation, research topics, technological needs, and policy changes in the aquaculture industry. The United States Department of Agriculture (USDA) attempts to track the industry through the Census of Aquaculture, released every five years. Although producers are required to submit data for the census, many businesses do not report, resulting in an unreliable source of data, minimal production estimates, and an inability to track credible industry change. The absence of accurate reporting makes understanding the effects of both local (employee shortages, weather events) and global (COVID-19 pandemic, supply chain disruptions) events on the industry difficult or impossible to quantify.

Our case study aims to 1) determine how well the USDA Census of Aquaculture represents the status of the aquaculture industry in Minnesota and 2) establish an accurate baseline that reflects the status and needs of the Minnesota aquaculture industry. We compared three data sources: 1) USDA Census of Aquaculture, 2) state aquaculture license information from the Minnesota Department of Natural Resources (MNDNR), and 3) phone and in person interviews with Minnesota aquaculture producers. The interviews prompted producers to answer basic questions about their farm status, what they see as current industry needs, and how COVID-19 has affected their businesses and the industry overall.

Our results highlight disparities between the USDA census information and data collected from MNDNR aquaculture licenses and phone/in person interviews with producers. Comparing output from the three methods showed different results and highlighted the importance of accurate reporting to understand how industry needs can be met. For example, if there are no accurate baseline data, it is difficult to determine how COVID-19 affected the industry overall in terms of businesses lost or added and what could have protected against losses. In addition to highlighting how data gaps might be filled, we provide a baseline for the current status of the Minnesota industry and quantify current producer needs in light of the COVID-19 pandemic. Although we acknowledge that our methods are more costly and time consuming, they may provide a better framework for future data gathering and help move sustainable aquaculture forward in other regions of the U.S.

DIRECT COMPARISON OF FECAL AND GUT MICROBIOTA IN THE BLUE MUSSEL *Mytilus edulis* DISCOURAGES FECAL SAMPLING AS A PROXY FOR RESIDENT GUT COMMUNITY

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Bivalves have ecological and economic importance but information regarding their associated microbiomes is lacking. As suspension-feeders, bivalves capture and ingest a myriad of particles, and their digestive organs have a high throughput of particle-associated microbiota. To better understand the complement of transient and resident microbial communities, standard methods need to be developed. For example, fecal sampling could represent a convenient proxy for the gut microbiome and is simple, nondestructive, and allows for sampling of individuals through time. The goal of this study was to evaluate fecal sampling as a reliable proxy for gut microbiome assessment in the blue mussel (*Mytilus edulis*). Mussels were collected from the natural environment and placed into individual sterilized microcosms for six hours to allow for fecal egestion. Feces and gut homogenates from the same individuals were sampled and subjected to 16S rRNA gene amplicon sequencing. Fecal communities of different mussels resembled each other but did not resemble gut communities. Fecal communities were significantly more diverse, in terms of amplicon sequence variant (ASV) richness and evenness, than gut communities. Results suggested a mostly transient nature for fecal microbiota. Nonetheless, mussels retained a distinct resident microbial community in their gut after fecal egestion that was dominated by ASVs belonging to *Mycoplasma*. The use of fecal sampling as a nondestructive substitute for direct sampling of the gut is strongly discouraged. Experiments that aim to study solely resident bivalve gut microbiota should employ an egestion period prior to gut sampling to allow time for voidance of transient microbes.

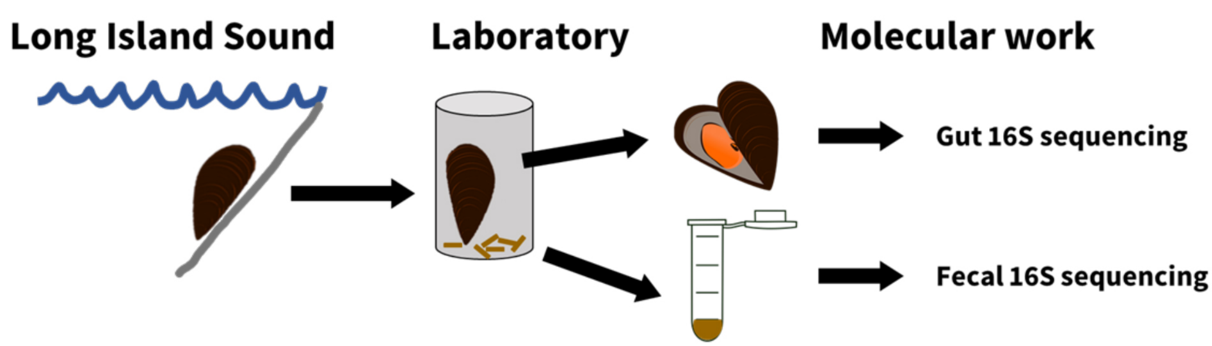

FIGURE 1. Experimental design flow diagram outlining the sampling of feces and gut tissues from mussels

APPLICATION OF ELECTRIC FIELD POTENTIAL TO ENHANCE SHELLFISH AQUACULTURE

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Major innovations in culture practices will be required if bivalve aquaculture is going to adapt to the challenges of changing ocean chemistry and rising temperatures due to climate change present. The shellfish industry must adapt to the reality that environmental conditions are going to be increasingly unpredictable, thus practices that increase growth rate and shorten the time to harvest will be beneficial. One such innovation would be the application of electric field potential to produce improve bivalve growth rate. The application of an electric field potential (aka 'Biorock' technology) is based on the discovery that the passage of a low voltage current through a conductive material drives the accretion of dissolved minerals to form limestone deposits. While the original goal of the technology was to construct mineral material from seawater, observations revealed that organisms that relied on carbonate ions to build their skeletons or shells recruited to these accreted structures and exhibited elevated growth rates. The most high-profile application of this technology has been in coral reef restoration in which steel rebar is used as a conducting substrate for mineral accretion and coral reef restoration. Similar potential benefits of electric field potential technology have been reported for shellfish, with published research showing that Pacific, Eastern, and Pearl oysters that were grown on charged structures showed elevated growth rates and improved survival as compared to oysters grown on control structures. One advantage of applied electric field potential technology is that it is inexpensive to deploy, since it requires low voltages (<12 volts) that are easily provisioned by low-cost renewable energy sources such as wind and solar, and low-cost metal cages or ropes. This allows the technology to be deployed in remote grow-out locations.

The shellfish industry is under already under strain because of ocean acidification (OA) which is associated with poor seed production, reduced growth rates, and thinner shells. Electric field-driven accretion of carbonates results in localized increases in aragonite. We hypothesize that this localized carbonate increase may be advantageous to bivalves that require carbonate ions for shell development. Therefore, we hypothesize that electric field potential technology may serve to offset the effect of OA which may otherwise depresses calcification rates in shellfish. There is also considerable interest in the restoration of the west coast's reefs of indigenous Olympia oysters, which are seen as playing a key role in defending coastal ecosystems from climate change and protecting the coast from sea level rise and wave-driven erosion. Electric field potential may prove to have utility in the restoration of this important natural resource.

To the best of our knowledge, electric field potential technology has never been deployed in a commercial aquaculture setting, and so it's benefits to the industry remains unknown. Evidence that electric field potential technology improves shellfish resilience and growth will have considerable ramifications throughout the industry because this is a technology that can be easily deployed. We believe that innovative farming practices such as those proposed here could benefit the industry and spur further innovations. The type of work described in this abstract is also technical and therefore benefits from an academic-commercial partnership.

RECENT ADVANCEMENTS IN SLAUGHTER TECHNOLOGY TO INCREASE FISH QUALITY FOR SMALL-SCALE SALMONID PRODUCERS AND PROCESSORS

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With the closing of seafood processors and restaurants due to the Covid 19 pandemic, many aquaculture producers pivoted to direct sales and began exploring small-scale processing. As the majority of food fish in California are sold to live markets or delivered to processors, few aquaculture producers in California have experience with fish processing and associated technology available to large seafood processors. Given the many challenges of increasing domestic finfish supply in the west, reducing loss by extending the shelf life and improving the quality of fresh and frozen fish products is critical to maintaining the growth and profitability of the fish producers in the Western Region of the US. Since fish products are highly perishable, it is necessary to reduce the post-harvest waste due to low meat quality of fish products. Stress generated by animals during the slaughtering process may cause abnormal ultimate pH of muscle, resulting in reduced water holding capacity, increased growth rate of microbes that have adverse effects on meat quality, and shortened shelf life of the products. Slaughter methods for salmonids in the Western US-primarily consist of compression, electrical stunning, percussive blunt force trauma for first stage methods followed by exsanguination in an ice slurry as a second stage method. This presentation addresses the immediate needs of small-scale finfish producers and processors by evaluating novel percussive and electrical slaughtering technology advancements to increase fish quality while reducing stress during slaughter.

UGLIES FOR RESTORATION: KEY RESULTS AND LESSONS LEARNED USING ADULT OYSTERS ON REEF RESTORATION IN THE GREAT BAY ESTUARY, NH EXPANDED UNDER THE SOAR PROGRAM (SUPPORTING OYSTER AQUACULTURE AND RESTORATION)

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Through a spatially explicit planning process and stakeholder engagement, The Nature Conservancy in New Hampshire identified stock enhancement of adult oysters from aquaculturists onto restored oyster reefs as a potential method of oyster restoration. In 2019, TNC performed a pilot study in NH where adult oysters “uglies” that could not go to market were purchased from oyster growers and deployed on a reef site in the Great Bay estuary to provide ecological benefit such as water filtration, reproduction, and the production of habitat. This study yielded a 71% survival rate with growth on a subset of oysters and recruitment of conspecifics. When the global pandemic hit in 2020, restaurants shut down and oyster growers lost their half-shell-market. This pilot study served as the backbone for the Supporting Oyster Aquaculture and Restoration (SOAR) program, a partnership between TNC and PEW and funded through a private foundation, where TNC across 7 states purchased 3.5 million oysters from local farms to deploy on restoration sites. SOAR provided an economic relief to the oyster aquaculture industry, supported jobs, and provided an ecological benefit to these coastal systems. In NH, 657,562 adult oysters were purchased from farms in NH and ME and deployed in 2020-2021 on 2 acres in the Great Bay Estuary. In fall of 2021, monitoring was performed through dive surveys and tonging to evaluate the survival of those oysters deployed and the natural recruitment on the 2020 site. This presentation will dive into the restoration process adopted by the SOAR program, monitoring results, and key lessons learned.

DEVELOPMENT AND EVALUATION OF HIGH-DENSITY SNP ARRAYS FOR THE EASTERN OYSTER *Crassostrea virginica*

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The Eastern oyster *Crassostrea virginica* is a major aquaculture species for the United States. The sustainable development of Eastern oyster aquaculture depends on continued improvement of cultured stocks through advanced breeding technologies. The Eastern Oyster Breeding Consortium (EOBC) was formed to advance genetics and breeding of the Eastern oyster. Members of EOBC have developed oyster strains with improved disease resistance through selective breeding and sequenced the Eastern oyster genome. To facilitate efficient genotyping needed for genome-based analyses and breeding, the consortium developed two single-nucleotide polymorphism (SNP) arrays for the Eastern oyster: one with 566K SNPs and the other with 66K SNPs. The 566K array was developed based on resequencing data from 293 oysters from Atlantic and Gulf populations. It contains 566,262 SNPs including 47K from protein coding genes and 33 SNPs from the mitochondrial genome. The 566K array was evaluated with 960 oysters yielding a sample passing rate of 87.89%, cluster calling rate of 98.89% and sample reproducibility of 99.24%. Of all SNPs on the array, 299,899 or 52.99% were called at high resolution and recommended for downstream applications. The 66K array was developed using the best-performing 65,893 SNPs from the 566K array. Evaluation of the 66K array with 384 oysters yielded a sample passing rate of 94.00%, cluster calling rate of 99.34% and concordance of 99.81%. Of all SNPs on the array, 60,673 or 92.08% were called at high resolution and recommended for downstream applications, a big improvement in efficiency over the 566K array. Both arrays contained 756 probes from 13 oyster and human pathogens for their detection in genotyped oysters. The high-density 566K array is designed for marker screening and high-resolution genome-wide association studies (GWAS), and the 66K array can provide efficient and cost-effective genotyping needed for routine GWAS and genomic selection. The development and implementation of these tools are expected to advance genomic research and accelerate genetic improvement of the Eastern oyster, by delineating genetic architecture of production traits, enabling genomic selection, increasing selection precision and lowering long-term breeding costs.

Table 1. Number and chromosomal distribution of SNP markers of the 566K and 66K SNP arrays of *Crassostrea virginica*.

Chromosome: ID	Size (bp)	566K		66K	
		No of SNPs	Interval (bp)	No of SNPs	Interval (bp)
1: NC_035780.1	65,668,440	54,804	1,197	6,116	10,733
2: NC_035781.1	61,752,955	50,784	1,216	6,824	9,048
3: NC_035782.1	77,061,148	63,744	1,207	7,747	9,944
4: NC_035783.1	59,691,872	49,618	1,185	6,123	9,593
5: NC_035784.1	98,698,416	81,629	1,209	10,130	9,737
6: NC_035785.1	51,258,098	42,246	1,213	5,128	9,995
7: NC_035786.1	57,830,854	47,878	1,207	5,576	10,371
8: NC_035787.1	75,944,018	62,886	1,207	6,713	11,308
9: NC_035788.1	104,168,038	85,854	1,214	8,807	11,823
10: NC_035789.1	32,650,045	26,786	1,213	2,698	12,097
Total/mean	**684,723,884**	**566,229**	**1,207**	**65,862**	**10,465**

STANDARDIZATION OF THE BIOASSAY PROTOCOL FOR THE IDENTIFICATION OF FEMALE CONTACT SEX PHEROMONES IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Currently, the control of reproduction in the commercial culture of *Litopenaeus vannamei* is carried out by ablation of the ocular peduncle, however, this endocrine manipulation presents a series of problems associated with the production of postlarvae and the survival of the broodstock. For this reason, the search for alternatives that can replace or complement the use of this technique has gained interest, with the use of pheromones being one of the alternatives with the greatest potential.

However, the investigation of these molecules in crustaceans has received little attention despite of their the potential uses in the field of aquaculture, and although in *L. vannamei* the existence of two types of female pheromones (distance and contact) is hypothesized, to date these have not been identified and there is no bioassay protocol for their identification. Therefore, to establish the bases for future investigations that allow pheromones to become a feasible alternative or complement to ablation, the objective of this work was to standardize a bioassay protocol for the identification of female contact pheromones in *L. vannamei*.

To this end, the effect of (1) light during acclimation, (2) acclimation time and (3) "artificial female" material on male shrimp behaviour was evaluated. Subsequently, different methods of introducing the "artificial female" coated with liposoluble molecules extracted from the cuticle of mature females on the reproductive behaviour of male shrimp was evaluated. All results (Figures 1, 2, 3 and 4) were analyzed using the Bartlett's exact test, concluding that the following conditions are needed for a clear evidence of the potential effect that female contact sex pheromones can have on the reproductive behaviour of male shrimp: 1) a conditioning period of at least 1 h with the tank covered; 2) an introduction of the "artificial female" made of rigid PVC tubes coated with liposoluble molecules while the tank is still covered; and 3) an observation period of at least 20 min.

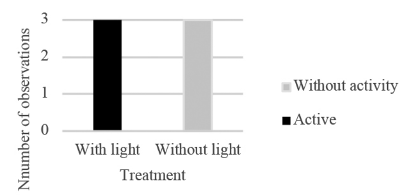

Figure 1. Activity of *Litopenaeus vannamei* males when exposed to different light conditions during the acclimation period (n=3 replicates / treatment).

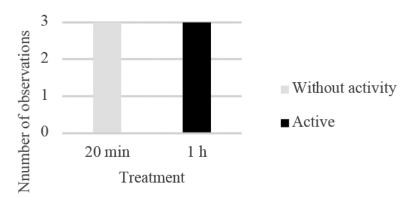

Figure 2. Activity of *Litopenaeus vannamei* males when exposed to different acclimation times (n = 3 replicates / treatment).

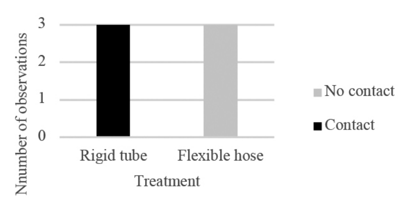

Figure 3. Contact response of *Litopenaeus vannamei* males versus "artificial females" made of different materials (n = 3 replicates / treatment).

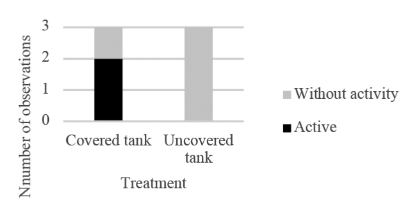

Figure 4. Activity of *Litopenaeus vannamei* males against different methods of introducing "artificial female" to the tank (n = 3 replicates / treatment).

LEVERAGING OPEN FABRICATION FOR COMMUNITY DEVELOPMENT AND AQUATIC GERMPLASM CONSERVATION

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Germplasm conservation is driven by the need to preserve genetic diversity in natural populations, and to back up lines of genetically modified and improved organisms for current and future applications. Advances in plants and terrestrial animals used for food have been significant for several decades. In contrast, germplasm preservation of aquatic species important for aquaculture, fisheries, conservation, and biomedical research has advanced at a slower pace. The large variation of reproductive mechanisms and the number of species involved requires considerable efforts to prevent loss of genetics in these organisms. Although diverse, aquatic germplasm preservation requires tools and hardware that can be similar in function but adapted to particular characteristics of each user and species. Open fabrication makes possible custom production of hardware (e.g., tools and devices) in ways that traditional proprietary development cannot.

The shared information for open fabrication (e.g. design files) should be suitable for transfer in electronic format. This can be initiated by single users, or groups of users with common or similar problems. These can include researchers, or innovators that design particular solutions. These solutions can then be distributed to early adopters, who will provide insights on the usability of the proposed solution, in real-life situations, and provide feedback to the innovator group. This will result in a diversified set of options, that can be integrated into the design cycle. After the design and usability steps proceed, the technology can be put in the hands of users. For example, repository developers, germplasm centers, or hatchery managers that want to maintain the genetics of broodstock. At this point the possibility of transferring electronic files such as CAD/CAM (computer aided design and manufacturing), provides a fast and cost-effective system and allows users to become makers, that is fabricate their own tools or devices, for example through 3-D printing. Furthermore, these makers can eventually become developers and design their own technology. These changes, from acquiring technology to fabricate and developing their own hardware can be greatly enhanced by the formation of communities that allow the exchange of ideas, tips, and troubleshooting in the way that old sewing and carpenter groups use to do, but a much faster digital-era speed (Figure 1).

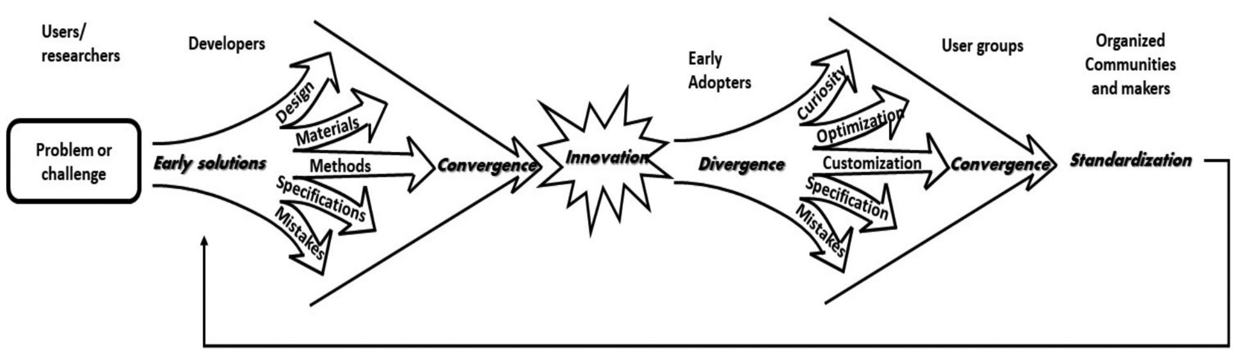

Figure 1. Open fabrication (e.g., 3-D printing) systems offer a mechanism to move from problem to solution through involvement of multiple users and associated community development.

QUALITY ASSESSMENT OF CRYOPRESERVED SMALL ABALONE (*Haliotis diversicolor*) SEMEN USING FLOW CYTOMETRY

Jin-Chywan Gwo*, Zhi-Wei Zhang, and Ting-Yung Kuo

We study the effect of cryopreservation on the quality of small abalone (Haliotis diversicolor) sperm and describe the sperm pre-and-post freezing using sperm motility and fertilizing capacity. Mean (±SE) percentage of motility, motility score, and fertility of post freezing sperm were 61 ± 2, 2 ± 0, and 67 ± 1%, respectively; for pre-freezing sperm, they were 90 ± 4, 3 ± 0, and 92 ± 1%. Sperm were stained with several fluorescent dyes to assess viability, plasma membrane integrity, mitochondrial activity, acrosomal membrane integrity, oxidation level, and DNA compation, respectively, and examined with flow cytometry. Flow cytometry assays were adapted and slightly modified from the methods used for evaluating domestic livestock semen quality. Compared to pre-freezing sperm, significantly ($P < 0.05$) higher mitopotential activity (damaged mitochondria; 25.01±1.18) and very significantly ($P < 0.01$) higher oxidation level (free radicals; 63.79±3.93) were observed after frozen-thawed. The oxidation level was found to be the most sensitive indicator of the cryopreservation-induced small abalone sperm damage. We aimed to evaluate the use of flow cytometry to measure the qualitative and quantitative characteristics of small abalone sperm pre and-post freezing. This is the first study in quality assessment of small abalone sperm using flow cytometry. In conclusion, we demonstrated flow cytometry was valuable and beneficial for objective, accurate, and rapid assessment of pre-and-post freezing small abalone sperm quality.

THE EFFECTS OF CLAM MEAL SUBSTITUTION ON THE GROWTH, DIGESTION CAPACITY AND HEALTH OF FLORIDA POMPANO, *Trachinotus carolinus*

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Florida pompano (*Trachinotus carolinus*) is one of the promising warm water marine aquaculture species in the United States. There are reported nutritional studies on this species. To the best of our knowledge, there is no reported information on the substitution of fish meal (FM) by clam meal (CM) in the diet of this species. Therefore, this study was conducted to assess the potential use of clam meal (replacing FM) in the diets of Florida pompano juveniles using growth performance, physio-biochemical and gut microbiome approaches.

A 12-week growth trial was carried out in an indoor recirculating system (RAS) at Florida Atlantic University - Harbor Branch Oceanographic Institute (FAU-HBOI). For this purpose, 20 feed-trained juvenile fish (initial weight, 6.04 ± 0.22 g) were stocked into each of 16 experimental tanks. A control diet (FM-based diet), and three test diets (clam meal at 10%, 20% and 30% inclusion of the diet) were evaluated as a completely randomized design with four replicates. Diets were fed to juvenile Florida pompano to apparent satiation three times daily.

The results indicated that: (1) survival was equal to or greater than 97%; and CM substitution did not affect feed efficiency (FE), Fulton condition factor (K factor) and intestinosomatic index; (2) juveniles fed 10% and 20% CM had a significantly higher final weight than the group fed the control (0% CM); and with the lowest weight gain and the highest hepatosomatic index (HSI) were obtained in the group fed the control diet; (3) hepatic trypsin and lipase activities were not significantly affected by the substitution of CM, but it did affect amylase activity with lower level was found in fish fed 10% CM compared to 30% CM; (4) hepatic peroxide (malondialdehyde, MDA level) and antioxidant enzymes activity showed no significant differences among the treatments; (5) there was no significant effect of CM substitution on the plasma total protein (TP), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and immunoglobulin M (IgM) in Florida pompano; and (6) there were no statistical differences between the treatments in the gut microbiome (gut prokaryotic community). Overall, this study concluded that CM has comparable benefit in the diet of Florida pompano as FM does.

GENOMICS RESEARCH CAN UNLOCK THE MICROBIAL "BLACK BOX" OF AQUAPONIC SYSTEMS

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Low-cost sequencing and -omics technologies can provide key insights into the community structure and function of microbial populations in aquaponics systems. This technology has been widely utilized to characterize the microbiome of agricultural and ecological systems. Despite the essential role of bacteria in aquaponic systems, investigation into the taxonomic structure and function of microorganisms has been limited. This presentation will highlight the different mechanisms by which genomic research can improve our understanding of how aquaponics system function. Specific emphasis on genomic techniques and pipelines will be covered, as well as broader applications of this technology to improve nutrient cycling, plant-driven rhizosphere development, nitrification, and food safety. Current and future aquaponics microbiome research at Kentucky State University will be discussed. Using a combination of both real-world and lab-scale objectives, we will compare the taxonomic profile of aquaponics systems across the United States and evaluate how environmental parameters influence the composition of microorganisms responsible for phosphorous cycling.

NEW ENGINEERING APPLICATIONS OF MULTIPLE UNCREWED VEHICLE SYSTEMS IN OYSTER AQUACULTURE

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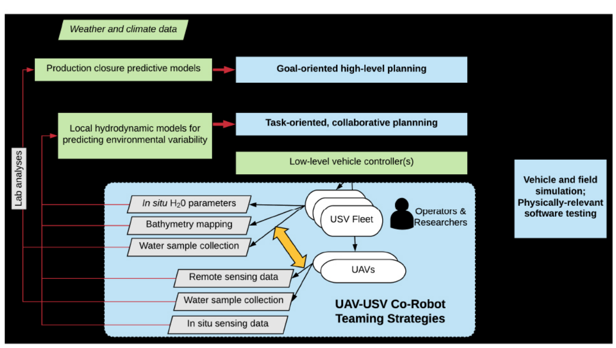
Autonomous and automated vehicle systems (e.g. surface, underwater, aerial) are now more commonly available and applications in aquaculture are being explored. This project focuses on using multiple vehicles (primarily aerial and surface) to assess and manage oyster aquaculture, with coupling to enhanced data collection and management. In particular, assessing water quality locally to better determine optimal timing for harvest is being addressed with this project. This project is also focused on developing a hybrid system that includes smaller and larger surface vehicles, as well as rotor and fixed wing vehicles for application to aquacultural and related aquatic enterprises.

Specifically, this project will test these in simulated and nearshore applications (specifically bivalve culture operations), with an eye to future offshore and more highly automated systems. Focus areas include software for both "digital twin" modeling in different situations; open source code including enhanced learning and use of automated systems; and testing of specific monitoring, mapping and aquaculture management techniques. In particular, sensors to assess location, time, dissolved oxygen, pH, temperature and other relevant parameters have been or are in development. This work should also provide humans with improved information to help make better decisions. These have been deployed in nearshore water bodies and will be tested with collaborators in near shore oyster leases to assess effectiveness, speed, accuracy and improve understanding of limits to autonomy and human-robot interfaces.

Previous work focused on ponds and other onshore applications, and/or single vehicle deployments nearshore. This expands on both of these, testing multiple different vehicles in the nearshore/offshore environment; and studying interactions between vehicles; and with humans. 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.

This paper documents applications of individual and hybrid systems to date in aquatic and aquaculture applications; briefly presents current work and outlines needs for future research.



IMMUNOMODULATORY EFFECTS OF GHRELIN ANALOGS ON PRIMARY HEAD-KIDNEY CELLS FROM RAINBOW TROUT (*Oncorhynchus mykiss*)

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Ghrelin is peptide hormone/cytokine that is mainly known to regulate metabolic processes including food intake, appetite, growth and energy balance, but it also plays an essential role in immune system. To evaluate the immunomodulatory actions of ghrelin analogs in rainbow trout, primary isolated head-kidney cells were treated with rainbow trout ghrelin and its truncated analog desVRQ-ghrelin (rt-desVRQ-Ghrl) under *in vitro* culture conditions and over a time-course (0, 2, 4 24 h). The mRNA levels of genes of interest relevant to pro-inflammation, chemotaxis, phagocytosis, leukocyte migration, antigen presenting process, myeloid differentiation and cytokine-JAK/STAT signaling were measured by real-time quantitative PCR. Treatment of primary cells, with these peptides, resulted in perturbations that displayed overlapping and divergent actions. The differing actions between the two ghrelin analogs, on various immune processes, and at differing time points, suggests the two analogs may have divergent roles in fish immunity. These results confirm the immunomodulatory effects of ghrelin peptides, providing a better understanding of these peptides in immune system of rainbow trout.

STATUS OF THE U.S. FARM-RAISED CATFISH INDUSTRY – 2021

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U.S. farm-raised catfish acreage decreased by 3% from 24,785 ha in 2020 to 24,000 ha in 2021. The 2021 acreage (and percent change from 2020) for Mississippi was 14,045 ha (-2%), Alabama was 6,313 ha (-2%), and Arkansas was 1,457 ha (-10%). These three states production area are 91% of the industry total. The primary factors associated with this decrease were Covid-19 uncertainty, higher feed prices, improved production efficiencies, and other higher land use enterprises (soybean, corn).

Through September 2021, catfish round weight processing (102,511 MT) was 5 percent less than the January through September 2020 period (107,500 MT). Foodsize catfish feed (32% crude protein) delivered for the first 9 months of 2021 was 12% higher than for the same period in 2020. Catfish feed prices increased sharply in August 2020 through May 2021 by 38% due to soybean and corn production issues and resulting shortage of these two primary catfish feed commodities. By September 2021, feed prices had dropped from the May 2021 high by 8%, but still much higher than over the past 5 years. The trend in feed price is downward. Fingerling and broodstock feed delivered was 14% lower in 2021 than in 2020.

High feed costs were concerning but higher prices paid to producers for their live fish helped mitigate this rise. Through September 2021, the price paid for premium (0.45 – 1.81 kg) sized catfish was $0.24 to $0.35 per kg higher than for this same period in 2020. The trend is price paid to producers for live fish is upward. Interestingly, the price paid for very large (2.7 – 3.6 kg) sized catfish has become equal to the prices paid for small (< 0.45 kg), premium, and large (1.8 – 2.7 kg) size catfish. In 2020, this was not the case with lower prices for large sized fish. This is an indication of the scarcity of available catfish from producers and the lack of labor in processing plants, each causing less fish going out to the marketplace.

During the first 8 months of 2021, the U.S. imported 11,793 MT of Siluriformes products, a 21% increase over the same period in 2020. Fresh product exports are lower, while frozen product exports are higher over this first 8 months of 2020 and 2021.

WITHIN-GENERATION RESPONSES TO SELECTION ACROSS HABITAT HETEROGENEITIES – WHEN, WHERE, AND WHY DOES IT MATTER?

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Evolutionary adaptation is a primary mechanism through which species can avoid extinction from climate change effects. When and where the multigenerational process of local adaptation will be rapid enough to prevent extinction is an important question. Within a life span, adaptive plasticity can generate phenotypic differentiation at microgeographic scales where gene flow prevents local adaptation. Natural selection (differential survival) also causes differentiation of genotype frequencies across habitat heterogeneities. At large spatial scales relative to the average dispersal distance this can lead to local adaptation, but at smaller spatial scales gene flow erases the differentiation each generation so there is no cumulative adaptation. Population genetic theory largely ignores the impact of selection within a cohort under these conditions, but that does not mean there is no impact. For high-fecundity species experiencing high early mortality (many marine species), a considerable portion of microspatial differentiation in each cohort can be due to within-generation selection. We refer to this as "cohort adaptation" because it can increase population fitness. Traits experiencing spatially heterogeneous selection are likely to be polygenic, with small contributions to trait variation from many loci, making detection challenging. In this presentation we will review empirical demonstrations of within-generation selection, outline the life history and environmental contexts where cohort adaptation is more likely to be important, and discuss why this relatively unstudied mechanism may be important in the context of selective breeding for aquaculture as well as for predicting population responses to climate change.

CONSERVATION AQUACULTURE IN THE ELKHORN SLOUGH, CA: SYNTHESIZING PHYSIOLOGY WITH RESTORATION FOR OLYMPIA OYSTERS

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Wild Olympia oysters have not recruited in the Elkhorn Slough since 2012 and the risk of local extinction is looming. Mortality in the surviving adults from this last recruitment continues to decrease their population density, reducing opportunities for successful reproduction. Habitat degradation from a century of land-use changes additionally decreases available substrate and their potential population size. In response, The Elkhorn Slough National Estuarine Reserve partnered with Moss Landing Marine Laboratories (MLML) and California Sea Grant to breed oysters from the remaining population sites in the hatchery and repopulate the estuary.

This report describes our conservation aquaculture effort to support native oyster recovery by outplanting hatchery-raised juveniles into the wild population. In addition to monitoring growth and survival, we conducted focused experiments in the lab to examine different responses to varying food and temperature conditions between each stage. This information complements our aim to reestablish a self-sustaining Olympia oyster population by both improving hatchery methods and identifying optimal outplant locations. We will highlight our experiments that characterize the physiological effects on food availability on assimilation efficiency in adult and juvenile Olympia oysters.

Adult oysters were collected from the Elkhorn Slough, CA in June 2021 and spawned at the MLML hatchery facility. Oysters in both life-stages were fed a mixed live microalgae diet cultured on site. Consumption and subsequent excretion were evaluated from three food availability treatment levels between 1 and 6 x 10^5 cells mL^{-1}. Filtration feeding was measured as clearance rate. The excreted feces and pseudofeces were then collected and the total organic and energy content excreted was determined using ash-free dry weights. Assimilation efficiency was calculated from the percentage of food energy absorbed in digestion. Results will be integrated with metabolic data measured as respiration from oxygen consumption in closed chambers. These values together form the baseline for separate energy budgets between life stages.

By studying changes to the energy pathway with age or with environmental conditions, this study aims to identify differences in the amount of energy being allocated for growth and reproduction. Changes in the energy balance indicate potential population bottlenecks during early life stages. In addition, understanding differences in the oysters' energy budget will optimize hatchery processes and grow-out procedures to support aquaculture efficiency.

SOCIAL LICENSING FOR OFFSHORE KELP FARMING IN CALIFORNIA

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Within the funding framework of the ARPA-E Mariner Program, Ocean Rainforest launched a demonstration project in the Santa Barbara Channel to test the durability of an offshore seaweed cultivation system, as well as better understand how to minimize impacts on local communities, marine industries and surrounding ecosystems. During the initial stages of the project, Ocean Rainforest saw first-hand the skepticism, hesitation and apprehension that surrounds seaweed aquaculture. In early discussions with various community stakeholders, Ocean Rainforest's proposed demonstration project was met with quick and forceful resistance. Conversations with regulatory agency representatives during the preparation and submission of an individual permit application further evidenced a collective confusion and uncertainty surrounding the economic, social and environmental feasibility of seaweed aquaculture in the U.S.

In an effort to improve public awareness and understanding of seaweed aquaculture, Ocean Rainforest has dedicated substantial time and effort to engaging relevant community members in early project siting analysis, as well as shared relevant scientific information on environmental, economic, and social costs and benefits of seaweed aquaculture. Irrespective of attempts at community engagement, the Ocean Rainforest team continues to face questions related to the nature, scope and ultimate benefit of aquaculture from within the local community. Given our experience and preliminary outreach efforts, it has become apparent that having a well-researched framework that describes the best strategies and approaches to building social license is essential for the growth and development of the regenerative industry.

Recognizing the need for improved messaging and education related to seaweed cultivation, a team of Masters of Environmental Science and Management students at UCSB's Bren School of the Environment have worked with Ocean Rainforest to conduct an extensive public opinion survey of their sustainable kelp cultivation strategy. In the coming months, this survey will inform an associated guide detailing opportunities in seaweed aquaculture to use for strengthening social licensing. The framework for this project is specific to California; however, the resulting body of work will lay the foundation for similar social licensing campaigns for aquaculture in the United States and internationally.

Thus, the contents of this presentation would be two-fold: first, to discuss Ocean Rainforest's efforts to build social license for a proposed demonstration project in the Santa Barbara Channel; and second, share preliminary results of a survey intended to inform a framework that would improve understanding and support for offshore seaweed cultivation in the state.

FURTHER NORTH THAN EXPECTED: POPULATION CONNECTIVITY AND GENETIC DIVERSITY ACROSS "SOUTHERN" ATLANTIC SURFCLAM *Spisula solidissima similis* POPULATIONS

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Until recently, the reported range of *Spisula solidissima similis*, known as the "southern" surfclam or Raveneli's surfclam, extended from the Gulf of Mexico to Cape Hatteras. Recent documentation shows *S. s. similis* far north of its prescribed range: in Long Island Sound New York and Massachusetts (New England hereafter), including populations of *S. s. similis* which co-occur with its morphologically cryptic and commercially harvested sister taxon *S. s. solidissima*. With no reports of geographically intermediate populations between the Cape Hatteras and New England populations, it is unclear if the New England populations were initiated by contemporary, possibly anthropogenic, founding events or if the "southern" surfclam has been present but undetected in this expanded range historically. This talk will address population structure analyses using genomic data from 130+ *S. s. similis* samples from Georgia and New England, using *S. s. solidissima* as an outgroup. This analysis allows for the comparison of population connectivity and genetic diversity among the New England sites and between them and Georgia. Patterns of DNA sequence variation will be used to infer the demographic history of New England populations and whether there is ongoing gene flow across the Atlantic coastal population gap. With interest growing in surfclam aquaculture, understanding population relationships and the distribution of genetic diversity in this species can help plan surfclam breeding strategies.

OBSERVATIONS OF AN *Akashiwo sanguinea* BLOOM IN COASTAL GEORGIA USA AND ITS IMPACT ON OYSTER LARVAE

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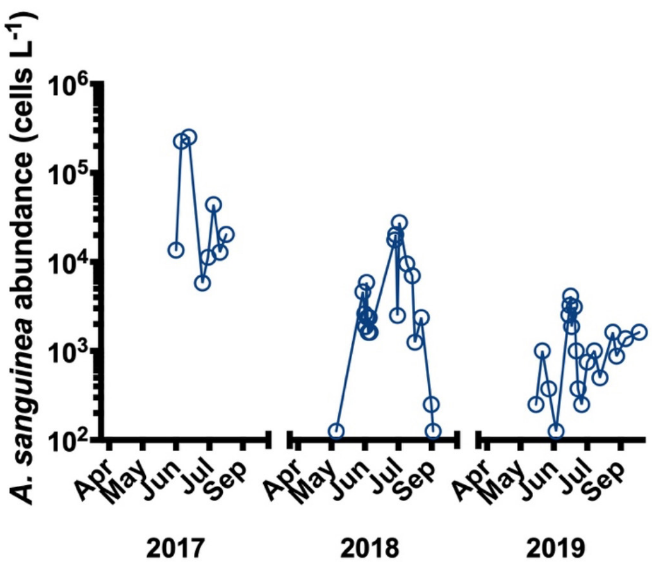
Harmful algal blooms (HABs) are a growing concern worldwide. In the state of Georgia, USA harmful algae have been observed in coastal estuaries, however bloom concentrations have not been observed. In the summer of 2017, in response to significant oyster larvae mortality at the University of Georgia's Shellfish Research Laboratory (UGA SRL), a large, novel bloom of the dinoflagellate *Akashiwo sanguinea,* was recorded. In 2017, bloom concentrations peaked at ~2.5 x 10^5 cells L^{-1} in early July, followed by enhanced abundance in August. In 2018, peak abundance was significantly lower (~2.7 X 10^4 cells L^{-1}), and no bloom occurred in 2019. Abiotic and biotic factors were also investigated to explore drivers of *A. sanguinea* accumulation in the SRE, including transparent exopolymer particles (TEP), temperature, and concurrent community abundance. Investigation of past documentation found that periods of enhanced *A. sanguinea* abundance were coincident with previous oyster larvae mortality events experienced by the UGA SRL. The work presented here highlights the importance of understanding the ecology of harmful algal bloom species in the Skidaway River Estuary, in light of the growing oyster aquaculture industry in coastal Georgia.



RE-FRAMING WASTE AS A VALUABLE RESOURCE: A METABOLIC APPROACH TO VALUE ADDED PRODUCT VIA CYANOBACTERIAL CULTIVATION

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Aquaculture has increased faster than any other food sectors over the last few decades due to its potential as a provider of sustainable and healthy protein source for future populations. This growth has also led to an increase in the waste generation from fish farming, which is one of the major challenges that this sector is facing currently. Therefore, an environmentally friendly, sustainable waste management practice is essential for the growth of aquaculture. Photosynthetic microorganisms such as cyanobacteria that can uptake the harmful nitrogen and phosphorus nutrients from wastewater are an excellent remediation choice to deal with this issue. The major advantage of using cyanobacteria is that the biomass produced during the bioremediation of aquaculture wastewater can be used to produce economic commodities such as sugar, biofuels, and other bio derived products. Several cyanobacterial species accumulate carbohydrate in their biomass during nutrient limiting conditions which can further in several bioprocessing applications. The presented research studies the potential of a fast-growing cyanobacterial species *Synechococcus elongatus* UTEX 2973 as a bioremediation agent for aquaculture wastewater and a raw material for sugar production. The growth and carbohydrate content of this particular strain during bioremediation of aquaculture wastewater was investigated to further use that biomass for sugar production. To complement the experiments, stoichiometric metabolic modeling of this strain was performed to predict the trend in the growth of the species and carbohydrate accumulation in biomass under different environmental conditions (by varying nutrients). Furthermore, the metabolic model of this strain was used to predict optimal nutrient levels in aquaculture wastewater for carbohydrate accumulation. Hence, by combining two important bioprocesses, this study addresses one of the most discussed issues of recent times, turning wastes into resources.

MICROALGAE CONCENTRATE AS A PARTIAL AND COMPLETE REPLACEMENT OF LIVE MICROALGAE DIET FOR HARD CLAM, *Mercenaria mercenaria*, LARVAE

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The requirement of live microalgae as a food source for larval stages represents a significant challenge for bivalve hatchery operations. The cost of live microalgae production represents 30- 50% of operation cost for a bivalve hatchery. The requirement for multiple species and contamination/culture crash of live microalgae poses an additional challenge to hatchery operations. To assess the efficacy of an alternative diet, commercially available microalgae concentrates were evaluated as a partial or complete replacement to live microalgae for culture of the hard clam, *Mercenaria mercenaria*, larvae. Larvae were fed one of six diets from 2-14 days post fertilization and growth, development and survival were assessed as response variables. Larvae fed a live microalgae diet performed better than those fed partial or complete replaced diets. Larvae fed partial and complete replaced diets were significantly smaller than those fed live microalgae. Foot development was significantly delayed in those fed complete replacement diets compared to partial replaced or live microalgae diets. Although survival at 5 days post fertilization (DPF) was similar among the dietary treatments, survival at 14 DPF varied significantly (Table 1). These results suggest that replacing live microalgae with a microalgae concentrate is likely to affect growth, development, and survival. However, live microalgae may be replaced partially for a short duration without affecting production performance.

Table 1: Survival rate (mean ± SD) of hard clam, *Mercenaria mercenaria,* larvae fed live microalgae, partial and complete replacement diets (n=3, 50 larvae/replicate). DPF: Days Post Fertilization. Different letters in same the column indicate significant differences among the treatments (one-way ANOVA, α=0.05, a > b > c).

Table 1: Survival rate (mean ± SD) of hard clam, *Mercenaria mercenaria,* larvae fed live microalgae, partial and complete replacement diets (n=3, 50 larvae/replicate). DPF: Days Post Fertilization. Different letters in same the column indicate significant differences among the treatments (one-way ANOVA, α=0.05, a > b > c).

Diets	Age of larvae			
	5 DPF	8 DPF	11 DPF	14 DPF
1. *Isochrysis galbana,* T. Iso (100%)	89.3 ± 4.7	81.8 ± 3.0^{a}	77.2 ± 4.3^{a}	69.8 ± 5.2^{ab}
2. T. Iso (50%) + *Chaetoceros gracilis* (50%)	91.5 ± 2.2	86.9 ± 2.8^{a}	81.7 ± 3.2^{a}	76.6 ± 4.2^{a}
3. T. Iso (50%) + Isochrysis 1800® (50%)	88.7± 3.5	81.5 ± 1.8^{a}	69.8 ± 2.1^{a}	60.1 ± 4.5^{b}
4. T. Iso (25%) and *C. gracilis* (25%) + Shellfish diet 1800® (50%)	87.1 ± 2.1	79.0 ± 4.0^{a}	70.7 ± 7.2^{a}	61.2 ± 6.0^{b}
5. Isochrysis 1800® (100%)	87.1 ± 5.1	58.4 ± 2.8^{b}	35.0 ± 6.6^{b}	15.7 ± 4.8^{c}
6. Shellfish diet 1800® (100%)	81.2 ± 5.8	63.0 ± 5.3^{b}	40.7 ± 8.9^{b}	13.6 ± 2.3^{c}

HATCHERY CULTURE OF THE LONG SPINED SEA URCHIN *Diadema antillarum* FOR REEF RESTORATION

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The long spined sea urchin *Diadema antillarum* used to be a keystone herbivore on Caribbean reefs, with an average density 5-10 individuals per square meter. In 1983-84, an unknown disease wiped out 93-98% population resulting an ecological shift from hard coral to macroalgae dominated reef. The recovery of natural populations has been very slow over subsequent decades and *Diadema* restocking has emerged as a strategy of interest to improve local recovery of coral reefs.

Our lab has been working to develop techniques for hatchery propagation of *Diadema* larvae over the past four years. *Diadema* larvae have a lengthy larval cycle (35-90 days) to reach competency for settlement. Negative buoyancy and fragile body features pose challenges to keep larvae suspended in the culture system using water circulation. At the late stages, larvae succumb to a progression of dark pigmentation in the body tissue, which is believed to be an immune response to bacterial infection.

A novel recirculating system has been developed in our lab, which to date has produced settled urchins in multiple cohorts. A recent trial has indicated higher growth and survival of larvae with mixed diet *Rhodomonas lens* and *Chaetoceros calcitrans* than single species diet *R. lens*. In addition, inclusion of 10 μM ethylenediaminetetraacetic acid in the larvae culture system improved growth, survival, and health status. In a recent batch, over 500 settled *Diadema* juvenile were produced from ~9,000 competent larvae.

We are now trying to identify settlement cues using histamine, potassium chloride, macroalgae and coralline algae. In addition, the efficacy of axenic microalgae food on larval performance will be tested using the next batch of larvae. Systematic improvement of larvae culture parameters has the potential to reach a production goal of thousands of settled juveniles from fertilized eggs.

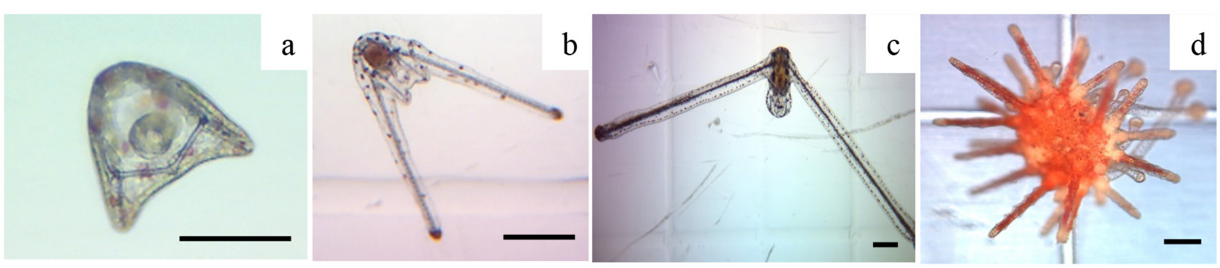

Fig 1: Representative photographs of *Diadema antillarum* larval development. (a) prism stage, 2 days post-fertilization (DPF), (b) early pluteus stage, 5 DPF (c) echinopluteus stage, 25 DPF and (d) settled juvenile, 36 DPF. bar = 100 µm.

LEVERAGING INTERNET OF THINGS (IOT) AND MACHINE LEARNING TECHNOLOGIES FOR AUTOMATED MONITORING OF MARINE MAMMALS

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Expansion into offshore waters has become an important issue in the aquaculture industry, as the global blue economy seeks to expand seafood production. While offshore aquaculture has significant potential to increase total capacity the concept of shifting aquaculture further from the coastline has brought with it several environmental concerns, which in turn has created a challenging permitting landscape.

To fully bring the promise of offshore aquaculture to bear, tools to help facility operators effectively prevent and mitigate entanglement events and reassure federal stakeholders are critical. Effective monitoring systems can help 1) increase understanding of population dynamics and animal behavior to better characterize entanglement risk, and 2) alert operators of imminent entanglement events.

Current solutions for monitoring protected species near offshore facilities can be time intensive and critically, do not provide real-time information that can be used to trigger rescue or other mitigation measures. There is then a need in the current monitoring landscape for tools which are both *automated,* to reduce manpower needs of wildlife observation, and *real-time,* and therefore able to prompt timely intervention and rescue efforts. The evolution of two major technological focus areas, machine learning (ML) and the internet of things (IoT) provide potential solutions. Machine learning uses large amounts of annotated (i.e., preprocessed) data to train software algorithms to perform tasks previously reserved for human operators, and can automate the detection of species of interest from video and audio sensors. The Internet of Things refers to a range of technologies facilitating deployment of embedded sensors and processors, allowing real-time communication between offshore unmanned systems and onshore users, via cellular networks, satellites, or other wireless communication protocols.

Case studies are underway to harness these technologies to address the challenge of marine entanglement. A prototype system developed by Synthetik Applied Technologies as part of a NOAA funded research program, provides an integrated hardware and software platform for the real-time automated detection of marine mammals at offshore facilities and is currently being demonstrated at the University of New Hampshire. The integrated hardware platform consists of embedded processors, communication hardware, and visual and audio sensors. Collected data is processed onboard the device to detect signatures of species of interest from cameras and hydrophones using machine learning models. Detection events are uploaded to an online dashboard where users can view paired video and audio recordings.

AQUACULTURE ROBOTICS AND DIGITALIZATION – INCREASING AUTONOMY IN UNDERWATER OPERATIONS IN FISH FARMS

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Aquaculture is a very important contributor to the production of seafood for human consumption on a global scale. The industry is facing major challenges related to HSE, securing fish welfare during operations, preventing fish escape, parasite and disease control, mortality, feed spill and nutrients release, structure and environment monitoring and maintenance, biofouling mitigation, and more. To address these challenges, reach the demand for food production and increase fish farming production efficiency, novel technological solutions need to be developed and/or adapted for use in aquaculture fish farms. These solutions rely on new tools, sensors, machines, algorithms, and methodologies. Furthermore, there is a trend of moving fish farms from traditional sheltered farming sites to more exposed areas, increasing the requirements of the technological solutions as well as introducing new challenges.

One potential solution to address some of these challenges is adapting the use of unmanned underwater vehicles (UUVs). UUVs such as remotely operated vehicles (ROVs) have become an important part of the operations conducted in the aquaculture industry. Typical operations are inspection of nets and mooring systems, net cleaning, and biomass inspection. However, the potential of ROVs is not fully exploited and several operations are still conducted manually, either by divers or using ROVs. The ROV is equipped with a camera and is controlled by a human operator, but controlling the ROV in a fish cage while inspecting the live video stream is challenging. The environment is dynamically changing due to ocean waves, currents, flexible structures and living fish, and it is critical to avoid damaging and/or influencing these. The challenges can be mitigated by increasing the autonomy level of the UUVs and properly instrumenting such vehicles with sensors required for autonomous operations. Together with research and industry partners, SINTEF has developed novel methods for autonomous UUVs in net pens through projects such as, e.g., CageReporter, Artifex, Netclean 24/7, CHANGE, ResiFarm, SFI Exposed. We will present some of the works targeting autonomous navigation concepts, advanced motion planning and resilient perception for UUVs operating in fish farms. Through field demonstrations in our SINTEF ACE facilities, the group of SINTEF ACE Robotic Lab aims to realize new methods and robotic solutions, thus contributing to increased efficiency and objectivity during current and future fish farm operations with the overall goal to increase sustainability and healthy production of fish.

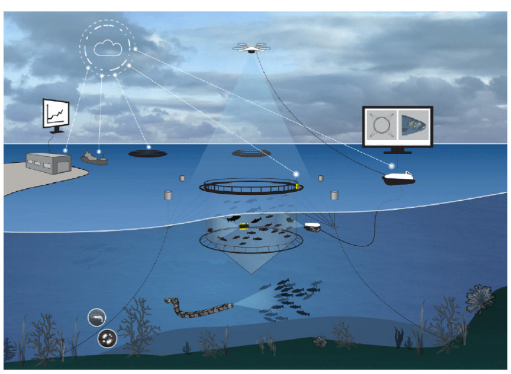

Figure 1 SINTEF ACE – Robotic Lab: Knowledge and technology for optimized operations in fish farms.

Figure 2 Technology-Biology Interaction – Aquaculture challenges and need for increased use of UUVs in fish farms.

RECENT PROGRESS ON THE USE OF LIPOSOMES FOR THE DELIVERY OF WATER-SOLUBLE NUTRIENTS TO MARINE FISH LARVAE

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Marine fish larvae are among the smallest vertebrates on Earth and are particularly challenging to culture in captivity. One of the key challenges in rearing these animals through the early life stages is providing adequate nutrition to support larval growth, health and survival. Marine fish larvae often require cultured live feeds, such as rotifers and *Artemia*, for their primary food source. However, cultured live feeds are an inferior food source when compared to the natural prey of marine fish larvae, copepods, and require enrichment to improve their nutritional value. Water-soluble nutrients are particularly challenging to manipulate in culture live feeds since the very small size of the enrichment particles are inherently prone to nutrient leaching when suspended in water. Liposomes are a type of microparticle that encapsulate water-soluble nutrients by means of a phospholipid bilayer (lamella) and are commonly used in human medicine for the delivery of pharmaceuticals and other bioactive compounds. We have found that liposomes can be adapted for use in aquaculture and are highly efficient for the delivery of water-soluble substances to aquatic organisms. We have used liposomes to control the concentrations of vitamin C and taurine in rotifers and *Artemia*. We then performed larval feeding trials with increasing concentrations of taurine and/or vitamin C and measured the resultant changes in larval growth and survival. The response of marine fish larvae to these manipulations has varied by species and will be reported in this presentation. In addition, we have conducted trials to evaluate the effects of the carrier particle on the growth and survival of two species of marine fish larvae. Our results suggest that liposomes produced with saturated soy phospholipids can be used for the enrichment of live feeds without negatively impacting the growth or survival of California halibut (*Paralichthys californicus*) or white seabass (*Atractoscion nobilis*) larvae.

WRAC AS A SPRINGBOARD FOR ADVANCEMENTS IN MARINE LARVAL FINFISH NUTRITION

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Marine finfish larvae often experience poor larval growth and high rates of mortality when cultured in captivity leading to economic inefficiencies in commercial hatcheries. Providing marine fish larvae with adequate nutrition is challenging due to: 1) rapid losses of water-soluble nutrients from microparticulate diets and live feed enrichments when these particles are suspended in water, 2) poor acceptability of microparticulate diets by larval fish, 3) challenges associated with determining the nutritional requirements of the larvae and 4) poor digestion of artificial diets by larvae due to the ontogeny of the digestive system. In previous research funded by the Western Regional Aquaculture Center we investigated nutritional strategies to address several of these challenge areas. During a previous WRAC-funded research project, we developed and evaluated liposome-based technologies for the enrichment of live feeds as well as for direct feeding to marine fish larvae. Specifically, we've used liposomes for the enrichment of essential water-soluble nutrients in live feeds and evaluated their impact on the growth and development of several species of marine finfish. Furthermore, we've found that liposomes can be included in larger carrier particles, termed 'complex particles' which can be used to: 1) improve feed acceptability and uptake of artificial diets by marine fish larvae though improved understanding of compounds that influence feeding, 2) provide a platform for the oral vaccination of marine and freshwater fish and 3) deliver a broad range of nutrients and bioactive compounds to marine fish larvae. The purpose of this presentation is to provide an overview of the progress made in these challenge areas as well as address opportunities for future research.

TECHNOLOGICAL PROGRESS IN THE U.S. CATFISH INDUSTRY

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The U.S. catfish industry has undergone significant technological advancements in an attempt to achieve cost efficiencies. Producers have increasingly adopted improved production practices such as split ponds and intensively aerated ponds in recent years. Subsequently, there has been increased adoption of complementary technologies such as fixed-paddlewheel aeration, automated oxygen monitors (AOM), and hybrid catfish. Similarly, fingerling producers are relying on an oral feed-delivered vaccine against Enteric Septicemia of Catfish (ESC). This study monitored the progress of the adoption of alternative and complementary technologies in the U.S. catfish industry. A 2019-2020 multi-state in-person survey in Alabama, Arkansas, and Mississippi (n=68), revealed increased adoption of intensively aerated ponds (6,315 ha) and split ponds (1,176 ha). Adoption of such intensive practices has also led to greater use of complementary production technologies. As a result, the average aeration rate in the tristate region has increased to 7.8 kW/ha with 97% of catfish farms adopting automated oxygen monitors. About 53% of the water surface area in the tristate region was used for hybrid catfish production. Eighty-three percent of the fingerling farms and 73% of the fingerling production area were vaccinating against ESC at the time of the survey. The increasing adoption of productivity-enhancing technologies in the U.S. catfish industry explains the increases in foodfish productivity (59%) from 2010 to 2019. Monitoring the progress of adoption of productivity-enhancing technologies will guide researchers and Extension personnel involved in the refinement and dissemination of these technologies.

Table 1. Adoption of productivity-enhancing technologies in the U.S. catfish industry, 2013 and 2020.

Regions	…………Total area under…………..			…………..Average……………	
	Intensive aeration (ha)	Split ponds (ha)	Hybrid catfish (ha)	Aeration rate* (kW/ha)	Foodfish yield Kg/ha
Alabama	1,588	13	1,635	7.40	6,847
Arkansas	254	115	551	6.92	6,608
Mississippi	4,102	1,048	9,830	8.27	8,176
Tristate in 2020	6,315	1,176	12,016	7.75	7,621*
Tristate in 2013	475	670	~5,000	5.30	5,850*

*weighted averages

PRODUCTION ECONOMIC RELATIONSHIPS IN INTENSIVE U.S. CATFISH PRODUCTION SYSTEMS

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The U.S. catfish industry is evolving by adopting intensive farming practices such as intensively aerated ponds and split-pond systems. The functional relationship between fish yield and key production inputs in these intensive systems was analyzed based on commercial catfish production data from 143 pond observations (2010-2018). A Cobb-Douglas production function was employed for the intensively aerated ponds and a modified translog production function was used to define the production relationships in split ponds. Both models were tested for goodness of fit and econometric issues such as multicollinearity and heteroskedasticity. The results indicated that the size of fingerlings at stocking, stocking density, aeration rate, feeding rate, survival, and harvest size of the fish were statistically significant variables influencing fish production in intensively aerated ponds. Initial fingerling stocking biomass, feed conversion ratio, feeding rate, and pond size were the most important variables influencing production in split-pond systems. All statistically significant variables had a positive relationship to fish yield in both production systems except for the feed conversion ratio. Being an efficiency indicator, a negative relationship of feed conversion ratio to fish yield in split ponds indicated that a lower feed conversion ratio represents higher fish biomass in the ponds. Feed fed, as well as stocking biomass (interaction of stocking size and stocking density in intensively aerated ponds), were the significant variables found in both models. Both production functions indicated further room for improvement in the use of inputs to increase production, especially in feed management. This study provided insights into input elasticities and direction of effect of key production inputs on fish production in intensive catfish production systems.

Table 4. Cobb-Douglas production function explaining the production relationships in intensively aerated ponds (N= 52 commercial pond observations).

Variable	Coefficient	Standard Error	P - value
Stocking size (g)	0.121	0.058	0.042**
Stocking density (dummy)[a]	0.096	0.053	0.076*
Total aeration (kW)	0.343	0.069	0.000**
Feeding rate (kg/ha)	0.596	0.082	0.000**
Survival (%)	0.782	0.094	0.000**
Harvest size (g)	0.239	0.120	0.052*
Intercept	0.635	1.115	0.572

[a] Ponds stocked <20,000 fish/ha assumed value of 0 while those with higher densities took a value of 1.

ECONOMIC CONTROBUTION OF THE US CATFISH INDUSTRY

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The catfish industry is the largest US aquaculture sector and a major economic contributor to the rural economies of the Southern states of Alabama, Arkansas, and Mississippi. It is vital to accurately portray the economic contribution of this industry that includes supply chain actors such as hatcheries, feed mills, farms, and processing facilities. Economic contribution of the catfish industry in the tristate region was estimated employing the input-output (I-O) modeling approach, and the IMPLAN database and software (Impact Analysis for Planning MIG, Inc.). An analysis-by-parts approach was employed because the IMPLAN database does not disaggregate a catfish sector from other animal livestock industries with expenditure patterns that differ substantially from those of catfish. All major supply chain actors of the catfish industry viz., feed mills, hatcheries, foodfish farms, and processing plants were surveyed to obtain their expenditure patterns and output in terms of sales. The survey data consisted of 68 farms (hatcheries and foodfish combined), four feed mills, and eight processing plants in the tristate region. Standard enterprise budgeting techniques were used to convert the sales and expenditure values to coefficients to be imported to the IMPLAN model to estimate industry contributions for 2019. The direct output from the catfish industry ($1.1 billion) generated a total economic impact of $1.9 billion. The industry directly employed 4,298 people and created an additional 4,868 jobs in the tristate economy for a total employment effect of 9,166 jobs. Catfish industry spending created an indirect economic effect of $552 million in other secondary sectors, that supplied production inputs and services. The induced economic effect generated from spending by employees within the catfish industry and secondary sectors amounted to $254 million. Some of the key sectors influenced by the catfish industry are grain farming, banking and financial institutions, truck transportation services, electricity generation, equipment, and machinery manufacturing, etc. The industry also generated $78 million in local, state, and federal taxes. Results of this study provide critical insights for policymakers and others into the contribution of the US farm-raised catfish industry to local and regional economies as well as its diverse industry interconnections.

Table 1. The economic impact of the catfish industry in the tristate region, 2019.

Impact	Employment	Labor Income	Value Added	Output
Direct	4,298	$192,678,334	$216,124,324	$1,102,136,449
Indirect	3,066	$151,354,974	$231,455,974	$552,099,888
Induced	1,802	$72,974,473	$138,884,686	$254,498,153
Total	9,166	$417,007,781	$586,464,035	$1,908,734,490

EFFECTS OF HYDROPONIC SYSTEM TYPE ON GROWTH AND NUTRIENT UPTAKE IN LETTUCE *Lactuca sativa* 'Rex' IRRIGATED WITH AQUACULTURE EFFLUENT

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Aquaponics, which is the combination of hydroponics and aquaculture, is a rapidly developing technology that has potential to increase food production in urban and urbanizing areas. An experiment was conducted at Auburn University to determine the effects of hydroponic system type on mature size and nutrient uptake in lettuce (*Lactuca sativa* L. 'Rex') irrigated with aquaculture effluent. The experiment consisted of three treatments and three replicates. Each replicate contained 48 individual plants. Treatments were: 1) substrate trough culture with a perlite substrate; 2) nutrient film technique; and 3) deep water culture. Aquaculture effluent was supplied from a biofloc-type recirculating aquaculture system producing Nile tilapia (*Oreochromis niloticus* L.). Nitrate concentrations in aquaculture effluent ranged from 330 to 550 mg L^{-1} during the experiment while pH and electrical conductivity remained relatively constant from 6.4 – 7.0 and from 1.1 to 2.35 mS cm^{-1}, respectively. There were no significant differences in head fresh weight after 30 days. Chlorophyll content (SPAD index) was highest for the substrate troughs and lowest in nutrient film technique. There were slight differences in foliar nitrogen concentrations between hydroponic system type. Micronutrient concentration was significantly increased in substrate culture culture compared to deep water culture and nutrient film technique. The pH between repetitions varied slightly due to fluctuations in the tilapia aquaculture environment. Aquaculture effluent from the biofloc filter was not screened when filling the reservoir tanks. Micronutrient supplementation may not be necessary in aquaponics as long as particulate matter is allowed to interact with plant root systems. Further research should focus on identifying and quantifying plant growth promoting bacteria in aquaponic systems to further analyze nutrient acquisition relationships.

THE CREATION, LAUNCH AND SUSTAINED DELIVERY OF THE NORTH CAROLINA SHELLFISH FARMING ACADEMY: AN AQUACULTURE TRAINING AND WORKFORCE DEVELOPMENT INITIATIVE –IN THE MIDST OF A GLOBAL PANDEMIC

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Abundant natural resources present significant potential for the expansion of the shellfish aquaculture industry in the Carolinas and Georgia. However, one barrier to continued industry development that has been identified is the lack of training opportunities for prospective shellfish growers. Workforce development is a fundamental component for the growth and expansion of any industry, including shellfish aquaculture.

The North Carolina Shellfish Farming Academy (NCSFA) is the product of a regional project "Developing a Framework to Expand Comprehensive Training Opportunities for Prospective Shellfish Growers in North Carolina, South Carolina, and Georgia" funded by NOAA Sea Grant's New Aquaculture Opportunities - 2019, NOAA-OAR-SG-2019-2005960, and directly addresses this barrier. NCSFA is an 8-week course comprised of 24 hours of classroom instruction and 24 hours of hands-on field day experience. The course is offered through the Carteret Community College Department of Continuing Education and was designed to prepare students for entry into the NC shellfish aquaculture industry.

This paper will cover the approach taken to acquire resources, form partnerships and provide solutions for the challenges presented in the creation, launch and sustained delivery of the North Carolina Shellfish Farming Academy in the midst of a global pandemic.

Table 1. North Carolina Shellfish Farming Academy coursework modules
Introduction to shellfish aquaculture in North Carolina
Biology of bivalves
Hatchery techniques
Nursery design and strategies
Hard clam grow-out
Oyster grow-out
Risks to shellfish crops
Farm management and BMPs
Harvesting regulations and shellfish dealership
Properly siting and applying for a shellfish lease
Business planning and marketing

Table 2. North Carolina Shellfish Farming Academy metrics	
Inaugural Course Offering	Summer 2020
Total Course Offerings to Date (Including Fall 2021 –In Progress–)	5
Total Enrollment to Date	65
Current Enrollment (Fall 2021)	11
NC Shellfish Farming Academy Graduates (not including Fall 2021)	55
No. of Students Who Already Had Their Own Shellfish Farms	3
No. of Graduates Who Have Started Their Own Shellfish Farms Since Graduating	3
No.of Graduates Pending Lease Approval	7

THE DEVELOPMENTAL BIOLOGY OF PENAEID SHRIMP

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Fertilization in penaeid shrimp occurs externally immediately after spawning, when the female releases both eggs and stored sperm. Like all crustaceans, penaeid sperm are non-motile and immediately bind to eggs and undergo an acrosomal reaction for entry. Penaeid eggs are activated by Mg^{2+} in seawater, not sperm, resulting in cortical “rounding up”, the completion of meiosis, and formation of a hatching envelope. Embryos undergo rapid, complete, and stereotyped cleavage divisions, in contrast to other decapod crustaceans. Equal and synchronous divisions produce a 32-cell stage blastula, when two mesendoderm cells (mesendoblasts) temporarily arrest their cell division while the remaining cells continue to divide. Cleavage produces a unique pattern of interlocking bands of cells dividing in similar directions.

Gastrulation begins when the two arrested mesendoblasts ingress at the vegetal pole and are internalized inside the blastula wall. This is followed by the oriented division and invagination of prospective naupliar mesoderm cells at the vegetal pole. Cell lineage analysis from the 4-cell stage shows that the animal pole A and B blastomeres give rise to ectoderm, the vegetal C blastomere produces ectoderm and naupliar mesoderm, and the vegetal D blastomere gives rise to ectoderm, naupliar mesoderm, endoderm, endodermal yolk cells, the teloblastic mesoderm, and the germ line. An RNA-containing granule has been identified as a possible germ line marker and can be traced from the egg to one of the mesendoblasts. Blastomere isolation and recombination experiments suggest that the mesendoblasts display both autonomous specification properties and the ability to induce morphogenesis in the rest of the embryo.

Post-embryonic growth occurs by addition and differentiation of segments to the posterior of the nauplius and subsequent larval stages. Growth occurs through the anterior-posterior division of teloblastic stem cells (ectoblasts, mesoblasts, and endoblasts). The maxillae and first two maxillipeds form in the final naupliar stages. The third maxillipeds and five pereopods form during the protozoeal larval stages, while the abdominal limbs (pleopods) form during the mysis stages. Muscle development has been followed by fluorescent phallotoxin staining, and naupliar limb muscles begin forming soon after the limbs bud. The major abdominal muscles develop in a complex interlocking pattern during the mysis stages. The development of the nervous system has been recently described in detail by fluorescent antibody and dye staining. Germ line and gonad development has been studied using the vasa probe. Studies on the development of other organ systems using molecular probes are limited.

The publication of penaeid shrimp genomes and developmental transcriptomes has allowed the rapid identification of developmental genes and their temporal transcription patterns. Further progress in understanding development will require a robust, high resolution fluorescence in situ hybridization method for detecting spatial patterns of mRNAs of interest in embryos and larvae.

IDENTIFICATION AND DIFFERENTIAL EXPRESSION OF SMALL NON-CODING RNAS IN TES-TES AND SPERM OF BLUE CATFISH *Ictalurus furcatus*

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Catfish farming accounts for ~70% of total U.S. freshwater aquaculture production, where the channel catfish, *Ictalurus punctatus* ♀ × blue catfish *I. furcatus* ♂ hybrid constitutes >50% of the harvest. Current technologies to produce hybrid embryos are labor intensive and require the sacrifice of males that do not reach maturity for 4-7 years. Catfish sperm are then often of inadequate quality/quantity and do not necessarily yield high fertility. Thus, our objectives were to i) identify miRNA in testes and sperm; ii) compare differential expression of testes sRNA between males; iii) decipher differences in sRNA from testes and sperm; iv) describe relevant biological pathways for mapped miRNA; and v) describe sequence length differences in sRNA between tissue types.

Five mature blue catfish males (2.67 ± 0.72 kg) were harvested from aquaculture ponds. Testes and sperm were then snap frozen for molecular analyses. Samples were shipped to Novogene Corporation for sequencing and bioinformat-ic analysis using *I. punctatus* (v. IpCoco_1.2) as the reference genome.

There were 174 known mature miRNAs detected. All known miRNAs from sperm samples were also detected in testes samples, and 5 novel miRNAs were unique to sperm samples. sRNAs with differential expression were clus-tered using DESeq2, revealing that testes and sperm show different expression patterns (Fig. 1). KEGG enrichment showed that detected miRNAs were primarily devoted to metabolism and endocytosis. Sperm samples peaked at 33 nucleotides (nt) displaying more piRNA, whereas testes peaked at 22 nt, displaying more miRNA. Overall, this study describes the small RNA profiles in *I. furcatus* male gonadal tissue and gametes, including core miRNAs that should be further characterized as biomarkers.

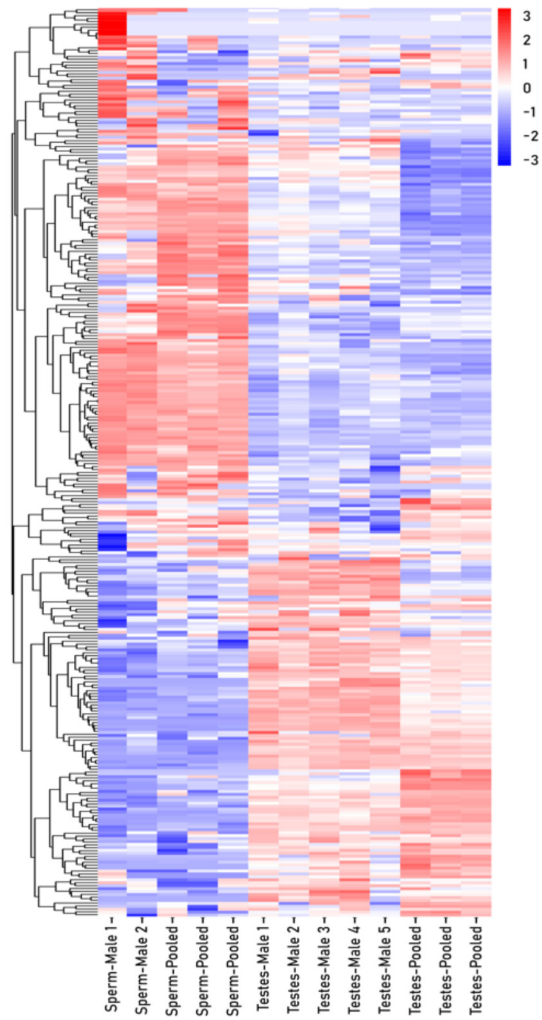

Fig. 1. Heat map used to assess expression patterns of sRNAs found in blue catfish, I. furcatus testes and sperm. Red represents upregulation and blue downregulation.

PROBIOTIC-INDUCED DISEASE RESISTANCE OF PACIFIC OYSTER *Crassostrea gigas* LARVAE TO THE BACTERIAL PATHOGEN *Vibrio coralliilyticus*

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On the US West coast, Pacific oyster larvae often experience severe mass mortality events caused by the bacterial shellfish pathogen *Vibrio coralliilyticus* (*Vcor*). This study evaluated the efficacy of four probiotic bacteria strains to increase the survival of Pacific oyster larvae when exposed to *Vcor*. Each probiotic strain was chosen due to its ability to inhibit the growth of *Vcor* on an agar plate or improve the survival of infected larvae. Individually, the probiotics displayed variable improvements in larval survival, but when used in combination, survival of infected larvae significantly improved (Figure 1). The probiotic cocktail has also been found to improve the overall survival of larvae and increase settlement rates.

An experiment assessing timing of probiotic application showed that larval survival rates improved when receiving the probiotics earlier in their development. Larvae that received the probiotic mixture one-hour post-fertilization had 20% lower mortality compared to the larvae that received the probiotics at 24 hours post-fertilization (Figure 2). This finding suggests that probiotics stimulate the innate immune response, leading to significantly higher larval survival and benefiting hatchery production.

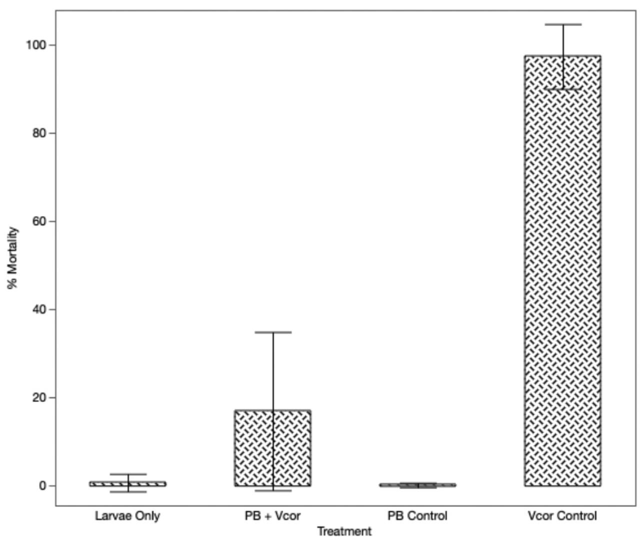

Figure 1. Mortality of *C. gigas* larvae (4 days post-fertilization) exposed to *Vcor* strain RE22 in the presence and absence of the probiotic cocktail. Treatments included a larvae-only control (Larvae Only), larvae who received both probiotics and *Vcor* (PB+Vcor), larvae who received probiotics only (PB Control), and larvae who received *Vcor* only (Vcor Control). Error bars represent one standard deviation from the mean.

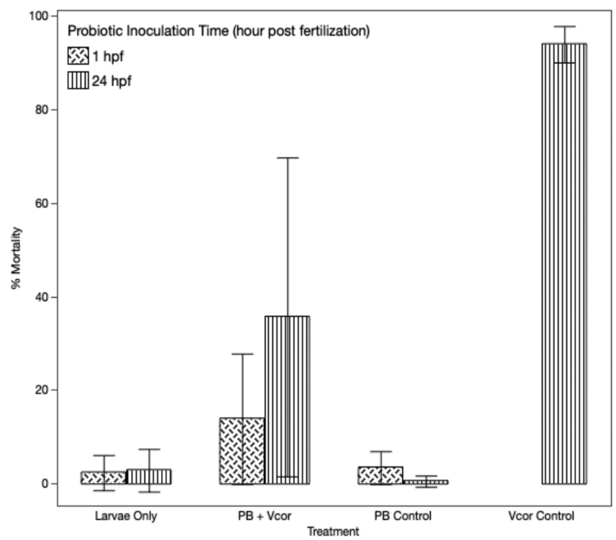

Figure 2. Mortality of *C. gigas* larvae (4 days post-fertilization) exposed to *Vcor* strain RE22. Probiotics added either to the hatching water within 1 hour (1 hpf) or at 24 hours post-fertilization (24 hpf). Treatments included a larvae-only control (Larvae Only), larvae who received both probiotics and *Vcor* (PB+Vcor), larvae who received probiotics only (PB Control), and larvae who received *Vcor* only (Vcor Control). Error bars represent one standard deviation from the mean.

ADAPTING TO THE INVISIBLE: THE pCO_2 TO GO AS A LOW-COST TOOL TO ADDRESS OCEAN ACIDIFICATION IMPACTS IN HATCHERIES

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Ocean acidification--the ongoing increase in dissolved carbon dioxide (pCO_2) and decreased pH in seawater--presents a burgeoning challenge for aquaculture of potentially-sensitive animals, such as molluscs, crustaceans, and even fish. Hatcheries offer a relatively-controlled environment in which to adjust conditions to be more optimal for survival, growth, and calcification, especially for the most vulnerable early life stages. Yet, it is challenging to measure incoming seawater conditions well enough to understand when and to what degree stressful conditions are present. The pCO_2 to Go is a hand-held, low-cost analyzer that can be used to measure the amount of dissolved CO_2 in seawater. Using regional knowledge of alkalinity characteristics, which can be developed in partnership with local oceanographers, an included manipulation application allows hatchery staff to calculate how much buffering solution to add to tanks to create optimal conditions for organismal calcification. The buffering solution recipe is included with the analyzer, and it is relatively easy and inexpensive to create. Additionally, aquaculturists can bring the pCO_2 to Go with them to the field to measure the conditions in understudied habitats, such as clam gardens, and better understand what their species contend with in the natural environment. The analyzer, application, and methodology are currently being tested at the Alutiiq Pride Marine Institute, ensuring this approach will provide an easy-to-use system when made available to the industry. Access to and training with this analyzer system will empower hatcheries around the globe that have many factors to consider during production--temperature, food availability, oxygen--to more easily monitor another important parameter.

ARE U.S. WILD SALMON PRODUCTS AFFECTED BY FARMED SALMON? A COINTEGRATION ANALYSIS

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We investigate the degree of market integration several product forms of U.S. wild salmon and Norwegian farmed salmon. While several studies have investigated the link between farmed salmon and fresh and/or frozen wild salmon markets, we expand the literature with the inclusion of canned salmon and salmon roe. Understanding how canned salmon and salmon roe are related to the broader salmon market is of importance to U.S. fisheries, as these products are a high price export for the U.S. Our results find evidence of cointegration between the Norwegian farmed salmon market and all U.S. salmon markets. Domestic and international economic conditions, such as production technological advances within farmed salmon production, environmental challenges, and changes in trade regulations, which affect the market for farmed salmon, will hence also influence U.S. prices of frozen and canned salmon, as well as salmon roe.

COPPER ALLOY MESH (CAM) DEVELOPMENT BY LUVATA APPLETON

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Copper naturally has antimicrobial properties. Using a copper alloy mesh (CAM) can reduce biofouling, especially in an offshore environment.

Luvata Appleton developed a unique alloy called Seawire and deployed a few small trial cages. Several years later, Mitsubishi Materials Corporation (MMC) acquired Luvata and suggested we produce their alloy, UR30 in the United States.

The UR30 alloy has a strong commercial history with hundreds of large-scale installations. Follow Luvata Appleton's journey on development of copper alloys for use in Aquaculture.

Luvata Appleton is a brass mill located in Wisconsin.

DEVELOPING TECHNIQUES FOR THE CULTIVATION OF THE HERBIVOROUS MONKEYFACE PRICKLEBACK *Cebidichthys violaceous* AS A SUSTAINABLE ALTERNATIVE TO UNAGI

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Monkeyface pricklebacks *Cebidichthys violaceous* are eel-like fishes in the family Stichaeidae that live in rocky intertidal and shallow subtidal habitats from Oregon to northern Baja California. The herbivorous nature of this species along with other life history characteristics, make them an attractive new species for aquaculture development in California. Their richly flavored flesh gives this fish potential for developing a new market as a sustainable product, and its flavor profile can be adopted in sushi restaurants an alternative to freshwater eels traditionally used for unagi. Our study is currently assessing reproductive performance in captivity, their cranial morphometrics for sex determination, as well as a dietary study comparing how different feed types maximize growth.

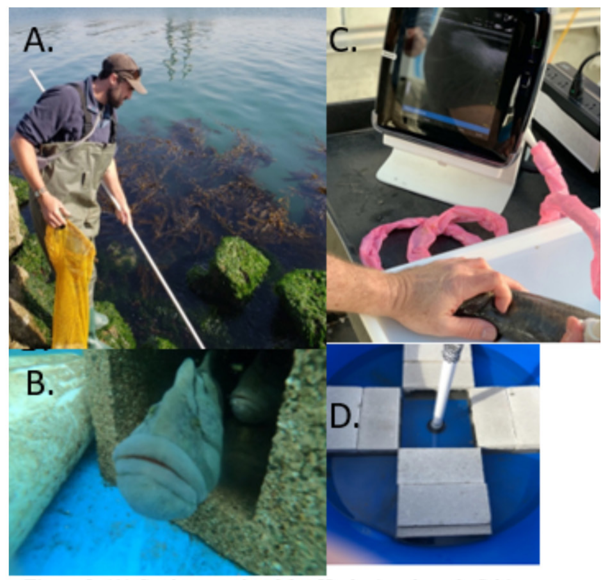

Figure 1. (A) Graduate student Matt Hoehn is poke pole fishing in the intertidal off the Moss Landing jetty to collect Monkeyface pricklebacks (*Cebidichthys violaceus*) for the project. (B) Images of Monkeyface pricklebacks in tanks at the aquaculture facility at MLML. (C) Erick Sturm from NOAA Southwest Fisheries uses a portable ultrasound to identify this Monkeyface prickleback as a female based on the presence of ovarian tissue (seen as a granular solid mass in the body cavity). (D) Photograph of the inside of a tank showing the replicate spawning "caves".

Our reproductive study assesses monkeyface pricklebacks' potential for reproduction in captivity. Reproductive success from this experiment will indicate what stocking density/ sex ratio/ and nesting habitat is preferred for the species. Sex determination in monkeyface pricklebacks is unknown. Ultrasound and Passive Integrated Transponders (PIT) tagging of individuals helped with sex determination and tracking for our reproduction experiment. After the conclusion of the reproduction experiment, we will then examine cranial morphometrics to evaluate whether the size of the fish's supraorbital crest is a sexually dimorphic trait. Results will inform us and other resource managers for distinguishing males and females when an ultrasound is not available. We are also conducting a 12-week feeding study on juveniles comparing algal-based and terrestrial-based feed types to see if Specific Growth Rate (SGR), Feed conversion ratio (FCR), and other animal health/ growth metrics are influence by diet type.

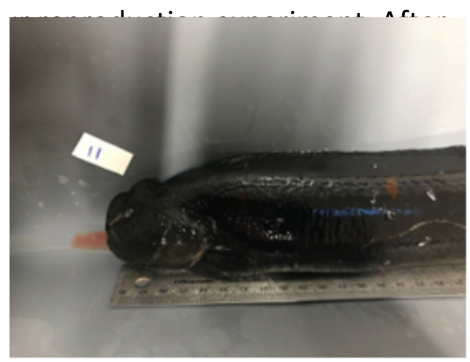

Fig. 2. Example image of a Monkeyface Prickleback that will be used for morphometric analysis of cranial features.

CONTINUOUS LIGHT TREATMENTS REDUCE PRECOCIOUS MATURATION IN AGE 1+ MALE SPRING CHINOOK SALMON (*Oncorhynchus tshawytscha*)

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Conservation hatchery programs designed to increase the abundance of Chinook salmon (O*ncorhynchus tshawytscha*) have reported high rates of precociously maturing males at age 2, referred to as minijacks. The negative consequences of releasing large numbers of minijacks into a population have driven the development of methods for reducing the frequency of this life history strategy. In this two-trial study, the effect of rearing juvenile Chinook salmon under varying continuous light regimes to reduce the number of minijacks was tested. At the end of the trials, the effect of these light regimes was measured by comparing the ratio of males that were maturing at 1+ age to non-maturing males (%MT) between treatments. Twenty-four-hour light treatment (LL) start/end dates were selected based on solstice/equinox dates which are hypothesized to queue physiological factors related to reproduction. Controls were maintained on a simulated natural photoperiod for both trials. For trial 1, the start date for one LL treatment began in June 2019 on the summer solstice and was maintained through late March 2020 (LL-Jun-Mar). The second LL group began on the fall equinox in September 2019 and ended in late March 2020 (LL-Sep-Mar). A significant difference in %MT was observed between the control (28.4%) and both LL groups (LL-Jun-Mar = 5.4% and LL-Sep-Apr = 9.4%) at the end of the trial. Trial 2 was undertaken to evaluate the length of LL treatment necessary to significantly reduce %MT relative to controls. It consisted of a control group, an LL group maintained from June 2020 on the summer solstice to April 2021 (LL-June-Apr) and an LL group beginning in June 2020 and ending in December 2020 (winter solstice), and then was returned to a simulated natural photoperiod until April 2021 (LL-Jun-Dec). Large differences in %MT was observed between control and LL groups. %MT in the control group (66%) was significantly higher than LL-Jun-Apr (10.3%) and LL-Jun-Dec (11.6%) by the end of the trial. For both trials, treatment and maturation category showed an effect on size parameters. Minijacks had higher body weights, were longer, and had increased condition factor when compared to female/immature fish (no treatment effect for female or immature fish was observed weight/length measurements). Weight and length amongst minijacks in the LL groups were larger than minijacks in the control groups and female/immature fish in LL groups had increased condition factor compared to the controls. The conclusion is that exposing male juvenile Chinook salmon to long term LL significantly reduces the number of fish precociously maturing at age 1+ relative to fish exposed to a natural photoperiod.

EFFECT OF DIFFERENT WASTE SUBSTRATES ON THE GROWTH, DEVELOPMENT AND PROXIMATE COMPOSITION OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE

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Black soldier fly larvae (BSFL) is considered as an alternative sustainable protein source for pigs, poultry and fish feed as well as a means of converting and utilization of organic waste. To achieve these roles, it is necessary to develop appropriate culture methods and understand their growth on different feeding substrates. This study therefore investigated the effect of three different substrates on the growth, development and proximate composition of BSFL. Feeding substrates mainly from Hotel Wastes (HW), Market Wastes (MW) and mixture of Market and Hotel Wastes (MHW) were used separately for the BSF culture. Efficiency of BSFL to consume different substrates and reduce the waste load to manure was also investigated. HW was found to be more attractive to the BSF eggs (5.90kg) but not significantly different ($P>0.05$) to MW substrate which had 5.34kg. MHW had the lowest production of (4.53kg) which was significantly different ($P<0.05$) to HW and MW. In evaluating the efficiency to digest the waste load, it was observed that younger larvae of 3 days old were able to consume and reduce the waste load faster within 4 days than larvae of 6 days' old that took 9 days to reduce the same amount of waste load. Larvae fed on HW was seen to take a shorter time (7 days) to develop into pupae with significance difference for all treatments ($P<0.05$). BSF larvae of 0.019 kg subjected to HW grew faster to a final weight of 0.68kg while MHW and MW production was lower with 0.567kg and 0.41 kg respectively. All treatments were seen to be significantly different ($P<0.05$) on the production of the final weight of BSFL. The study concludes that the larvae reared in MHW substrate is a better option to grow BSF larvae compared to MW and MHW due to its high crude protein content of 51.57%, ($P<0.05$).

Table 1: showing mineral content and proximate composition of BSF larvae fed on Market Waste (MW), Hotel waste (HW) and Market and Hotel Waste (MHW) substrates.

Parameter (% dry matter)	Market waste (MW)	Hotel waste (HW)	Market and Hotel waste (MHW)
Crude protein	46.52±0.55	45.29±0.52	51.52±0.01
Calcium as Ca	3.30±0.06	3.15±0.03	3.50±0.01
Crude fiber	4.92±0.01	4.12±0.01	4.02±0.01
Crude Ash	10.08±0.01	9.03±0.02	9.55±0.02
Moisture	50.05 ±0.01	54.78±0.02	51.06±0.02
Energy (DM) Kcal/kg	3,924.18 ±0.01	3,801.89±0.00	3,847.06±0.01

LATITUDE OIL™ AS A SUSTAINABLE ALTERNATIVE TO DIETARY FISH OIL IN RAINBOW TROUT *Oncorhynchus mykiss*: EFFECTS ON FILLET FATTY ACID PROFILES, INTESTINAL HISTOLOGY, AND PLASMA BIOCHEMISTRY

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Alternative oil sources are needed to meet the growing demand for highly digestible sources of energy and fatty acids in fish feeds. Historically, marine oils have met this need; however, diminishing supplies cannot continue to meet the demand of a rapidly growing aquaculture industry. Furthermore, the primary dietary source of long-chain polyunsaturated fatty acids for humans is seafood, but for farmed fish to meet the dietary LC-PUFA requirements of human consumers, aquafeeds must contain oil sources high in these fatty acids, such as fish oil (FO). The aim of this study was to evaluate the effects of Latitude™ oil (Transgenic canola) inclusion in fish feeds on growth performance, non-specific immune responses, histology, and fillet omega-3 fatty acid contents in rainbow trout, *Oncorhynchus mykiss*, fed for 52 weeks. Latitude oil (LO) is highly digestible (93%), containing omega-3 fatty acids eicosapentaenoic acid (EPA, C20:5n-3) docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Three isonitrogenous (49.8%), isolipidic (20.4%) and isocaloric (24.2 MJ/kg) diets differing by lipid source (0, 8, or 16% LO, replacing FO and poultry fat) were fed over an entire production cycle beginning with 19g juvenile fish. At the end of 52-week feeding trial, final body weight, weight gain and specific growth rate of fish fed 8% LO (LO-8) and 16% LO (LO-16) diets were significantly higher than those fed the 0% LO (LO-0) diet ($P < 0.05$). Phagocytic respiratory burst in fish fed the LO-16 diet was significantly higher than those fish fed the other 2 diets ($P < 0.05$). There were no significant differences in superoxide dismutase ($P=0.295$), catalase ($P=0.078$) and lysozyme ($P=0.075$) activities among different dietary groups. Histological examination of the distal intestine indicated reduced inflammation in fish fed the LO-8 diet but not the LO-0 and LO-16 diets. Inclusion of LO enhanced the omega-3 fatty acid concentrations of EPA and docosahexaenoic acid (DHA, 22:6n-3) in the fillet. Fillet DHA content of fish fed the LO-8 and LO-16 diets were similar to those of fish fed the LO-0 diet. As these diets had lower DHA content, this suggests dietary EPA and DPA from LO was converted to DHA and deposited in the fillet. This is supported by increased expression of genes involved in fatty acid elongation, desaturation and beta oxidation in both liver and muscle of fish fed LO ($P < 0.05$). Total EPA+DHA content of the edible fillet ranged between 1079 to 1241mg/100g across treatments, each providing the recommended daily intake for human consumption. Overall, this study demonstrated that LO is a highly digestible lipid source suitable for meeting the fatty acid requirements of rainbow trout, as well as consumer expectations for fillet omega-3 fatty acid content.

THE INNOVATION SYSTEM OF COMMERCIAL AQUAPONICS: A QUALITATIVE INVESTIGATION OF PATHWAYS FOR INDUSTRY SUCCESS

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Aquaponics has gained notoriety as a resource efficient food production typology in light of the growing promotion and awareness of regenerative approaches to resource management in food systems. Though its touted benefits have been widely discussed, commercial systems and growers remain a minority as the success of the field and the number of commercial aquaponics operations established is lagging in relation to the comparable parent industries of hydroponics and aquaculture. To identify current drivers and barriers to the success of aquaponics as an emergent commercial food production typology and strategy for resource circularity, we apply the Technical Innovation System (TIS) framework, incorporating aspects of broader sustainability transitions theory. The TIS framework focuses on the innovation, development, use, and diffusion of a technology and has become a core component of sustainability transitions research. Utilizing this framework as an interdisciplinary and international team of collaborating researchers in sustainability, aquaponics, aquaculture, engineering, and built environments, in this study we therefore investigate what dynamics are currently driving or preventing the aquaponics industry from realizing its commercial and sustainable potential.

Although such attempts of analyzing aquaponics as an emerging TIS have already been made for Europe and the Netherlands, an analysis in the context of North America and other parts of the world is still lacking. Likewise, more recent analyses are needed given rapid developments in recent years. Within our TIS analysis of aquaponics, a set of seven functions assessing aspects which impact innovation such as policy, market development, knowledge generation and dissemination, business models, and more, are evaluated to help characterize the performance and dynamics of commercial aquaponics. The assessment of the TIS is supported by data from literature review, policy review, semi-structured interviews with commercial aquaponics practitioners, and a collaborative analysis and review working structure integrating the interdisciplinary expertise of the team. Semi-structured interviews with commercial practitioners in North America (n=25) were conducted in 2021 and assessed through qualitative coding which corresponds to the TIS framework. Through the TIS analysis, pathways for the emergence of aquaponics as a significant contributor to the food system and as a successful strategy to close resource loops are explored to support a future in which the promise and performance of commercial aquaponics align to help achieve crucial circular sustainability transitions.

EVALUATION OF A HIGH PROTEIN CORN DISTILLERS' DRIED GRAINS WITH SOLUBLES (DDGS) AS A COMPLETE SOYBEAN MEAL REPLACER IN PRACTICAL DIETS FOR ATLANTIC SALMON (*Salmo salar*)

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Distillers dried grains with soluble (DDGS) is a co-product from ethanol industry. Corn DDGS is low in lysine and contains moderate crude protein concentrations (28 to 32%) and relatively high fiber, thus limiting the inclusion level of DDGS in practical fish feed. However, through applying advanced processing techniques, the nutritional quality of corn DDGS can be modified, and recently Marquis Grain Inc. developed a high protein (48%) DDGS product. Until now, this new DDGS product had not been tested as a protein substitute in practical diets for Atlantic salmon (*Salmo salar*). Therefore, a 12-week feeding trial was conducted to evaluate this high protein DDGS as a soybean meal (SBM) replacer for Atlantic Salmon diets and determine growth, nutrient digestibility, intestinal morphology, and health performance. A low fish meal (15%) based control diet (Diet-1, Control) was produced, then 25%, 50%, 75% and 100% of SBM protein from the control diet were replaced with DDGS protein for diet groups Diet-2, Diet-3, Diet-4 and Diet-5, respectively. All experimental diets were fed to satiation, twice daily to triplicate groups of Atlantic salmon juveniles (initial weight 21g). Following the 12-week growth trial, *in vivo* digestibilities of the five diets were also determined. After the feeding trial, final weight, specific growth rate, weight gain and feed utilization were not significantly different among the treatment groups. Significantly higher and lower whole body moisture content was observed in fish feed diet group Diet-3 and Diet-4, respectively; other groups showed intermediate values. In comparison to the control diet, increased final whole body protein content was observed when DDGS was included and was significantly higher in Diet- 3. Except for cystine, lysine, tryptophan, tyrosine, proline and taurine, all other measured amino acids were significantly influenced by dietary treatments. Higher and lower values were observed in fish fed Diet-3 and Diet-5, respectively; other dietary groups showed intermediate values. DDGS inclusion significantly increased dietary lipid digestibility, with fish fed Diet-4 and Diet-5 having higher values, followed by Diet-3. Diet-2 resulted in intermediate values, with significantly lower values observed in fish fed the control diet. In terms of immune response and oxidative stress, DDGS inclusion increased indices for nonspecific immune response and oxidative stress tolerance. Based on the overall findings, we can conclude that the high protein DDGS tested can be effectively used as a SBM replacer in Atlantic Salmon diets.

NEKTON USE OF OYSTER *Crassostrea gigas* FLIP-BAG CULTURE IN NATIVE EELGRASS *Zostera marina* HABITAT

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In recent years, shellfish farms in Washington and California have been exploring the use and expansion of novel, off-bottom grow-out methods, including “flip-bags” – buoyed mesh bags that rise and fall with the tide -- to produce high-quality oysters for the singles, half-shell market. The interactions of these complex systems with the surrounding environment have yet to be comprehensively explored. Intertidal shellfish farms in parts of the Pacific Northwest overlap with native eelgrass (*Zostera marina)* habitat. *Z. marina* beds are designated as Essential Fish Habitat (EFH) for multiple Pacific salmon species (Pacific Fishery Management Council 2008) and provide nursery habitat for many other species within the Pacific Coast Groundfish Fisheries Management Plan. Regulators require robust scientific information to support decision making for ongoing and proposed shellfish aquaculture activities in our region. Our study is the first to systematically explore nekton use of oyster flip-bag culture and directly supports the permitting and regulatory process of these systems within and adjacent to *Z. marina* habitat.

In 2020 and 2021, we collected nekton within oyster flip-bag arrays, inside and outside *Z. marina* beds at six farm locations, representing key shellfish growing areas in Washington state. Four habitat types were surveyed: flip-bags with eelgrass, flip-bags without eelgrass, eelgrass with no culture and bare mudflat. Sampling occurred in both spring and summer seasons using a modified seine net with cod end to collect nekton >5mm in size. All nekton were counted and identified to species from three replicate tows within each habitat. Sampling within the highly structured arrays presented some logistical challenges compared to the unstructured habitats with the seine net. A multi-methods approach within these complex, dynamic systems is recommended to capture and observe all potential nekton utilizing these habitats.

In general, spring had the greatest total nekton abundance compared to summer. Total nekton abundance was greatest in dense eelgrass beds with no oyster culture in both spring and summer. Both habitats with eelgrass, no culture and flip-bag culture, had greater nekton abundances compared to habitats without eelgrass during the spring season. Oyster flip-bags without eelgrass had the least total nekton compared to all other habitat types in both seasons.

Our study results further indicate the critical value of *Z. marina* to support abundant nekton communities in Pacific Northwest estuaries. At our study sites, *Z. marina* was able to grow within the flip-bag arrays and support nekton communities in greater abundance than flip-bags without eelgrass. Further analysis of community structure within these habitats will improve our understanding of ecological function. Our study results provide scientific information to key stakeholders producing and regulating shellfish aquaculture in our region.

SAXITOXIN ACCUMULATION AND DEPURATION IN ROCK SCALLOPS *Crassadoma gigantea*: A PREREQUISITE FOR CULTURE ON THE WEST COAST OF NORTH AMERICA

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The rock scallop *Crassadoma gigantea* can achieve market size in 2-4 years and shows promise for commercial culture along its native range, from Baja California to southeastern Alaska. One serious unresolved issue, however, is the lack of information on accumulation and retention of algal toxins that can cause paralytic shellfish poisoning (PSP) in humans. This is critical because toxins associated PSP, e.g. saxitoxin (STX) and its derivatives, are currently the most widely reported toxins in bivalves along the west coast of North America. To address this issue, hatchery-reared rock scallops were deployed at three locations prone to PSP closures in Washington State, and sampled when a closure occurred and weekly thereafter until the closure was lifted. Induced toxicity under controlled conditions of algal cell density, temperature and salinity were also conducted at the NOAA Northwest Fisheries Science Laboratory in Manchester, WA. This work represents the first attempt to induce toxicity in *C. gigantea*.

The overarching goal of this research was to address the needs of public health agencies and shellfish producers by investigating where biotoxins accumulate in scallops, and how long the toxins remain. Temporal field exposure trials and lab experiments examined bioaccumulation and subsequent detoxification of STX in the adductor muscle and viscera of individual scallops. Both field and lab studies demonstrated that rock scallops can attain very high toxin loads for long periods of time, but toxicity was generally confined to the viscera (gut). Mean toxicity levels in rock scallop adductor muscles (the part that is consumed) were below the regulatory limit for STX (80µg/100g tissue). Induced toxicity experiments revealed a pattern of toxification and detoxification similar to other studies of bivalves fed toxic dinoflagellates, with rock scallops increasing toxin loads in digestive gland tissues at significantly higher rates than for other tissues (Bricelj and Shumway 1998). It is not clear from any of our datasets, field exposure or laboratory, how long rock scallops take to completely depurate STX from all tissues. This aspect of research will need to be further explored with future monitoring efforts to identify complete depuration rates. This data gap aside, it is clear that very high, persistent levels of STX in scallop viscera will likely preclude this species from safe, whole product consumption. The potential for a shucked, adductor only market will also require careful scrutiny due to persistent toxicity and variability of toxicity among individual rock scallops. This work was supported by NOAA-OAR-SG-2016-2004807.

EFFECT OF MANAGEMENT STRATEGY USING DIFFERENT COMMERCIAL PROBIOTICS ON NURSERY CULTURE PERFORMANCE OF PACIFIC WHITE SHRIMP, *Penaeus vannamei*

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This study aimed to evaluate the effects of using a combination of commercial probiotic products as a feed supplement and as water additive on the water quality, growth, and survival on nursery culture performance of Penaeus vannamei. A four-week nursery trial was conducted using bacterial based water treatment. Two commercial probiotics were used as water additives singularly and in combination with a probiotic supplemented- feed, resulting in four treatments using static biofloc type systems. The fifth treatment was used as the reference by connecting to a recirculating aquaculture system (RAS). Results showed that the shrimp post larvae cultured in the four probiotic treatments had significantly higher final biomass (g) and survival (%) as well as significantly lower FCR than the reference ($p < 0.05$). There were no significant differences between dissolved oxygen (mg/L), temperature (ºC), pH, and total ammonia-nitrogen (mg/L) of the five treatments. However, a peak of total ammonia-nitrogen (1.11 ± 0.08 mg/L) and nitrite (0.74 ± 0.06 mg/L) was observed in the four probiotic treatments around day 20 of the experiment, that was rapidly decreased by day 22 (Figure 1). This is assumed to be due to the continuous addition of the fermented probiotic additive to the water as well as the restricted feed during these peaks. Overall, results of this study and other published studies indicated that biofloc type systems have a number of advantages over clear water systems and that the use of commercial probiotics helps to develop and maintain biofloc based systems for enhancing shrimp performance.

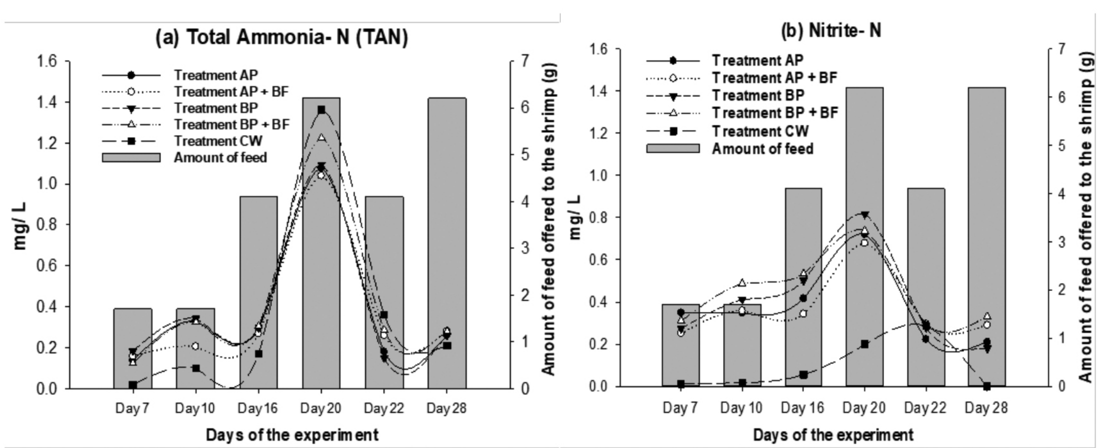

Figure 1: Fluctuation in total ammonia-nitrogen (a) and nitrite-nitrogen (b) over the rearing period of Pacific white shrimp post-larvae in each treatment (probiotic treatments n = 5, reference treatment n = 4). AP: Alltech® probiotic, BF: Biowish® probiotic-supplemented feed, BP: Biowish® probiotic, CW: clear water.

MANIPULATING ESCAPE RESPONSES OF THE CYCLOPOID COPEPOD *Oithona colcarva* AND THE CALANOID COPEPOD *Parvocalanus crassirostris*

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Copepods are an essential live prey item for the larviculture of many marine ornamental fish species. Copepods are generally small, nutritious, and exhibit movement patterns thought to elicit feeding behaviors in larval fishes. Larval fish must be able to efficiently capture live prey items to ensure ample energy reserves for growth and development. However, most copepod species have strong escape swimming behaviors. The parameters of copepod escape swimming, such as speed and response latency, can be species-specific and influenced by a myriad of environmental variables. The goal of these experiments was to suppress copepod escape responses to improve prey capture by larval fishes.

Pilot studies were conducted to determine copepods' lethal limits to acute exposure to environmental stressors. Treatments were selected based on the following factors: ease of use, cost effectiveness, and relative safety to fish larvae. Three sets of experiments examined the copepods' behavioral effects to temperature, salinity, and water viscosity (Table 1). The viscosity experiments tested two common live feed enrichment products, "Easy DHA Selco" and "AlgaMac 3050."

For each experiment, three containers with clean saltwater and a 35 micron nested screen were stocked with 100,000 newly hatched nauplii. Nauplii were transferred to a treatment cup for a set period of time and returned to the recovery cup with clean saltwater. Copepod escape behaviors were recorded hourly using an Edgertronic high-speed video camera (500fps) paired with a 4x long working distance objective lens, piezoelectric stimulus probe (predator mimic), 10 MHz pulse generator, and fiber optic light. Videos were recorded of copepod nauplii responding to the stimulus probe to capture how and when nauplii reacted. For each escape response recorded (n=30), the nauplii's trajectory over time is measured using ImageJ software. From that data, the average and maximum speed, total distance travelled, response latency, and total escape duration are measured. Survival and response percentages will also be reported.

The most successful treatment(s) will show reduced speeds and/or distances and longer response latencies, with these affects lasting for a long period of time. These factors should improve prey ingestion rates by larval fishes and will be tested in future experiments.

	Temperature (°C)			Salinity (g/L)			Enrichment (g)		
	High	*Low*	*Control*	*High*	*Low*	*Control*	*AlgaMac*	*Selco*	*Control*
P. crassirostris	40	1	26	60	0	30	1	1	0
O. colcarva	40	1	26	80	0	30	1	4	0

Table 1: Stressor treatment levels used for temperature, salinity, and enrichment experiments. Similar experiments were conducted for two copepod species, *Parvocalanus crassirostris* and *Oithona colcarva.* Treatment duration varied between species based on their lethal limits.

DETECTION OF DISEASES IN WILD ORGANISMS (CRUSTACEANS) IN NORTHWESTERN MEXICO

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In Mexico in 2020, shrimp farming represented approximately 70% (155 thousand),of its total aquaculture production with a volume close to 219 thousand tons and with an estimated value of 1,700 million dollars. Other species such as catfish, tilapia, carp, trout, oysters, marine fish and molluscs, represent a low percentage, but in view of accelerated growth. The biggest problem that has been occurring in recent years in shrimp farms is the high mortality rates presented by the various pathogenic agents, whether they are bacteria or viruses.

To prevent the introduction of pathogenic organisms to shrimp farms. The objective of this study is to determine the presence of diseases in wild aquatic organisms (crustaceans), by the Real-time PCR method.

Samples of pleopod, gill and hemolymph were taken from wild organisms (shrimp and crab), they were organized by month of reception (Table 1), the total number of samples was 54 from three states: Baja California Sur (16), Sonora (22) samples and Tamaulipas (16). The samples were processed to extract DNA using the lysis buffer method and RNA using the RNA extraction solution method. Molecular detection was determined using the IQ REALTM IHHNV, WSSV, TSV and NHP-B kit (GeneReach Biotechnology), the 7500 Fast-Real PCR System Real-Time PCR kit (Applied Biosystems), and the 7500 Fast system software V1.4.2

The RT-PCR technique was performed for the following pathogens (WSSV, IHHNV, TSV and NHP-B), the results are presented in Table 1 and Figure 1.

It can be concluded that of the 4 pathogens analyzed in wild crustaceans, only the IHHNV virus was detected with a prevalence of 20%, which indicates a serious problem in wild populations, this could suggest that IHHNV could somehow be introduced to shrimp farms with negative results in production.

	No. of samples	IHHNV positive
November ´17	7	0
January '18	15	1
February '18	5	1
March'18	18	6
July'18	9	2

Table 1. Samples tested and positive.

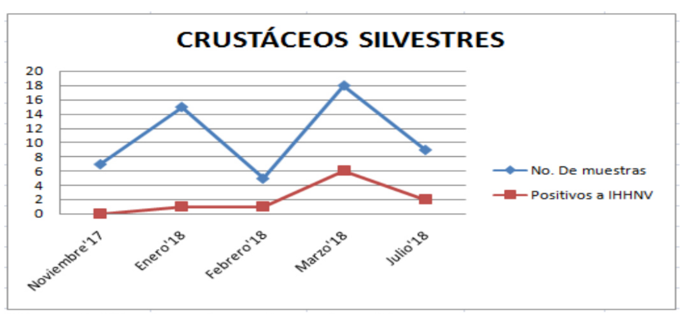

Figure 1. Number of samples and positive cases.

DIFERENT EXPERIENCES AT FIRST FEEDING IN JUVENILE FISH PRODUCTION FOR SEVERAL MARINE SPECIES

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Life-history evolution has generated many successful reproductive strategies in teleost fishes with different maternal contribution levels (e.g., energy content and quality of yolk and oil globule) to the next generation. The first feeding is a crucial bottleneck in fish production. It is often associated with 'mass mortality' events in different species raised to date, such as snapper (e.g., *Lutjanus guttatus*, *L. peru*, *L. argentiventris*, *L. campechanus*, and *Ocyurus chrysurus)*, snooks (*Centropomus viridis* and *C. undecimalis*), croakers (*Cynoscion nebulosus*, *Sciaenops ocellatus, and Totoaba macdonaldi*) and others species. After hatching, the larvae have to be ready for the transition from endogenous nutrition to exogenous food sources. This includes the development of their active sensory systems (pigmented eyes, pectoral fins, swimming behavior, jaw gape) as well as physiological-digestive parameters such as liver, pancreatic, intestinal, and stomach enzymes that appear heterochronically between different fishes. Mass mortality events in captive-bred fish are linked to this transition in food source and need to be optimized for every species. For example, snappers (egg size between 600-800 mm and oil droplet not more than 125 mm) sometimes have to have their yolk sac entitrely depleted before first feeding, while snooks and croakers (same egg size range but with oil droplet between 150 to 250 mm) can feed before yolk sac depletion and accelerate their growth rates. In those fishes, three critical parameters playing a role at first feeding are the mouth gape (restricting particle size), larval length (restricting swimming capacity and hunting success) and handling. In addition, the quantity and quality of prey at the appropriate time are important to maintain the correct feeding behavior and long-term survival. Our results with more than 20 diferent marine fish species rely on the use of healthy enriched rotifers at this stage. Consequently, our different results have shown between 10% to 40% survival in snappers and more than 60% in snooks and croakers. Based on those previous experiences, an innovative red drum semiautomatic larval rearing system was designed and tested at the Whitney Laboratory. With this system, we reached an average of 50 juv/l and more than 60% survival. Hence, we need to keep in mind that at first feeding, all larvae can ingest, digest, and assimilate food that permits them to continue to grow to the juvenile stage. In conclusion, it is clear that the reliable production of high-quality juveniles is indispensable for success in new aquaculture species, and the first feeding is a crucial step.

SUPPLEMENTAL DIETARY CHOLESTEROL LIMITS FILLET BLEACHING BUT DOES NOT IMPROVE THE GROWTH PERFORMANCE OR UPPER THERMAL TOLERANCE OF FEMALE TRIPLOID ATLANTIC SALMON

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The salmon aquaculture industry must be proactive at developing mitigation tools/strategies to offset the potential negative impacts of climate change. Therefore, this research examined if additional dietary cholesterol would: (1) enhance the upper thermal tolerance of female triploid Atlantic salmon (*Salmo salar* L.), or their growth performance when exposed to elevated temperatures; (2) prevent/limit the fillet 'bleaching' (loss of astaxanthin) that has been observed in sea-caged fish exposed to high temperatures; or (3) affect fillet lipid and fatty acid content/profiles in the context of membrane fluidity, or basal and post-stress cortisol levels (the latter measured as cholesterol is the precursor of steroid hormones).

Salmon, initially acclimated to 12°C, were exposed to an incremental thermal challenge wherein temperature was raised by 0.2°C day^{-1} to mimic conditions that these fish experience when reared in sea-cages; with temperature held at 16 and 18°C for several weeks to prolong their exposure to elevated temperatures. From 16°C onwards, the fish were fed either a control diet, or one of two nutritionally-equivalent experimental diets containing supplemental cholesterol (+1.30%, ED1 or +1.76%, ED2). The additional dietary cholesterol did not improve the salmon's incremental thermal maximum (IT_{Max}) or growth, and may have negatively impacted survival (at the highest inclusion level). In contrast, it reduced fillet 'bleaching' between 18°C and when the fish reached their upper temperature tolerance limit (as measured using SalmoFan™ scores) as compared to fish fed the control diet.

Although further analyses are being conducted (e.g., fillet lipid class and fatty acid composition; plasma cortisol levels), the current results suggest that supplementing salmon diets with cholesterol would have few/minimal benefits for the industry. That said, ≤ 5% of the female triploid Atlantic salmon used in this study died before temperature reached 22°C, and the mean temperature at which 50% mortality occurred was 23.2 + 0.1°C. These latter data suggest that it is possible to produce all female populations of reproductively sterile salmon that can withstand summer temperatures that are likely to occur in Atlantic Canada.

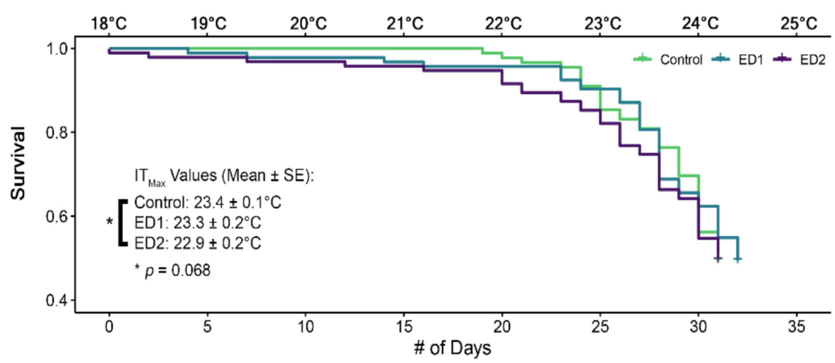

Figure 1: Salmon survival during the IT_{Max}

GILL HISTOLOGY OF FARMED FLORIDA POMPANO (*Trachinotus carolinus*) CULTURED UNDER DIFFERENT SALINITY CONDITIONS

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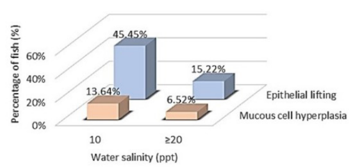
The Florida pompano, *Trachinotus carolinus*, a euryhaline Carangid species, found along the eastern Atlantic Ocean, is a prime candidate for aquaculture under low water salinity conditions. To test the effect of different salinities (10, 20 and 30 ppt) on the gill histology, a total of 68 fish from 1 to 24 days post hatch were euthanized and fixed in 10% neutral buffered formalin. Then, they were processed for histology (4 µm cuts, H&E stains) and the results were analyzed with SPSS 15.0 and WinEpi. Pathological alterations in the secondary gill lamellae of the fish were observed, with hyperemia/haemorrhage, mucous cell hyperplasia and epithelial lifting (cell detachment) (Fig. 1) as the most frequent.

The number of gills with epithelial lifting was significantly higher (by Pearson Chi Square, $p<0.05$) in fish reared at 10 ppt, with 45.45% of the sampled fish, ($n = 22$) compared to ≥20ppt (15.22%, $n = 46$) (Fig. 2), and this may constitute a risk factor for pre-metamorphosed pompano, and approximately 5 times more likely to suffer from gill morphological alterations.

Our results show that rearing Florida pompano under 10ppt salinity conditions can affect the gill's histological integrity during early development, which can be frequently associated, according to other author's, with respiratory and osmoregulatory distress, and a higher susceptibility to pathogen infections, resulting in health impairment and even mortality.

Figure 1 – Gill secondary lamellae showing epithelial lifting (x400, 20 µ scale bar)

Figure 2 – Relative percentage of sampled fish with epithelial lifting and mucous cell hyperplasia



HISTOLOGICAL AND SKELETAL DEVELOPMENT IN FARMED FLORIDA POMPANO (*Trachinotus carolinus*) LARVAE AND JUVENILES

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The Florida pompano, *Trachinotus carolinus*, is a euryhaline Carangid species found along the eastern Atlantic Ocean and considered a prime candidate for the US food aquaculture. However, few studies have evaluated the histological development of this species. In order to discriminate induced changes from environmental and culture conditions, a baseline of normal histological structure is necessary.

From the first day post hatch (DPH), through metamorphosis (24 DPH), and until the juvenile stage (~20 g, 95 DPH), a total of 152 fish were sampled from the broodstock system at the aquaculture facilities located at the FAU-Harbor Branch Oceanographic Institute (Fort Pierce, FL).

Fish were euthanized and fixed in 10% NB formalin. Then, they were dehydrated, embedded in paraffin, cut (4 µm), stained with H&E (Figure 1) and other specific stains (PAS, Masson's trichrome, silver impregnation), and the slides were scanned and digitalized. To reveal the morphology of the cartilage and the skeleton, the *clear-stain* technique was used (Alcian Blue and Alizarin Red stain; Figure 2). Finally, the visual information was interpreted and used to illustrate study the different landmarks of pompano's development.

This work will serve as a reference for the aquaculture industry. More specifically, it will facilitate disease diagnosis and management, and the early detection and reduction of malformations, thus helping in the process of selective breeding and genetic improvement of the species.

It will also help to establish a reference baseline for larval quality control in nutritional studies and development of production technology.

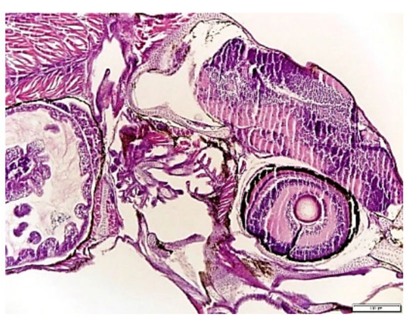

Figure 1 – Pre-metamorphosed pompano larvae at 12 DPH (H&E stain, x100, 100 µ scale bar)

Figure 2 – Pompano juvenile at 95 DPH (Alcian Blue & Alizarine Red stain)

CURRENT AVAILABILITY OF VACCINES AGAINST POTENTIAL FLORIDA POMPANO (*Trachinotus carolinus*) PATHOGENS – A REVIEW

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The genus *Trachinotus* consists of 40 species of Carangid fish, 6 of which are farmed in tropical and subtropical areas worldwide. After an extensive literature review carried out in 2021 by the FAU-Harbor Branch Oceanographic Institution, 3 species of viruses, 11 species of bacteria, and more than 70 species of parasites were identified as potential pathogens of the Florida pompano (*T. carolinus*), some of which are responsible of important direct and indirect economic losses to the aquaculture industry every year. As part of the prophylactic measures that can be implemented by the fish farmers, vaccines have been effectively used in food fish for approximately 30 years. This review provides an overview of those commercially available for this species, as well as those that are still in an experimental stage, with the aim of suggesting and facilitating a wider use by the farmers.

In 2009, the Center for Veterinary Biologics of the Animal and Plant Health Inspection Service (APHIS, USDA) licensed 17 fish vaccines, 2 modified live and 14 killed vaccines. Since then, and in the last few years, they have become commercially available for most of the pompano's potential pathogens, including viruses such as the *Red Seabream Iridovirus* (RSIV) and the *Red Spotted Grouper Nervous Necrosis Virus* (RGNNV), and bacteria such as *Listonella anguillarum* (syn. *Vibrio* a.), *Tenacibaculum marinum* (syn. *Flexibacter* m.), *Photobacterium damselae* subsp. *piscicida*, *Streptococcus agalactiae*, and *Aeromonas hydrophila*.

Vaccines for some other pathogens, however, still need more research, being only at initial experimental stage. Such is the case for acid-fast bacteria (*Mycobacterium marinum* and *Nocardia seriolae*), *Pseudomonas* spp. and some *Vibrio* spp. (*V. harveyi*, *V. ponticus* and *V. vulnificus*), the dinoflagellate *Amyloodinium ocellatum*, and some parasitic copepods (e.g., *Caligus* sp. and *Lepeophtheirus* sp.). However, with the advances of the new biotechnology and molecular biology techniques, new options in the form of subunit vaccines, recombinant vector and DNA vaccines, are becoming increasingly available. Furthermore, although some progress has also been made on the study of the immune host reaction against other parasites (ciliates such as *Cryptocaryon irritans*, and some micro- and myxosporidians protozoans), these studies are still at a very early stage.

On the other hand, new vaccination methods, such as immersion (less cost and time consuming, and more effective in the case of anorexic fish), still demand much work. And what is more, these vaccines, which seem to work in other species, still need to be tested for efficacy in Florida pompano and other finfish marine species. In addition to the forgoing, we cannot forget that the first step in order to develop a specific vaccine is the isolation, ideally from the same fish species, and correct identification of the pathogen's species and strain, and frequently this work cannot be done without involving the joint efforts of the industry, health specialist, and public/private research institutions.

RECEPTOR-MEDIATED PEPTIDE-ASSISTED DELIVERY OF dsRNA VIA dsRNA-BINDING DOMAIN INTO CRUSTACEAN OOCYTES

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Gene silencing at the transcription and translation level is a powerful tool for studying gene function and for molecular therapeutics. RNA interference (RNAi) is a key method that enhances scientific knowledge by discovering the function of a wide array of genes and molecular mechanisms. The knowledge obtained from RNAi studies led to novel gene therapies, improved agriculture crops, and new gene intervention technologies for the aquaculture industry. However, a stable silencing and successful introduction of long dsRNA stretches into cells and oocytes of oviparous animals still needs investigation.

In the current study, a chimeric protein composed of dsRNA binding domain retrieved from *M. rosenbergii* dsRNA binding protein and an oocyte-specific targeting amino acids sequence (Vg-dsRBD) was cloned, expressed, and purified. The newly developed chimeric protein was found to interact with dsRNA. The Vg-dsRBD bound with dsRNA was characterized by EMSA and TEM. Moreover, the endocytosis capability of dsRBD-Vg bound dsRNA into the oocytes of *M. rosenbergii* and its capability to silence specific genes was tested by *in vitro* and *in vivo* experiments. Indeed, our *in vitro* results demonstrate that the novel dsRBD-Vg protein can deliver the bounded dsRNA into *M. rosenbergii* oocytes. Moreover, the dsRNA introduced to the oocytes by the Vg-dsRBD could silene the eye development *PAX6* gene in an *in vivo* experiment. Such a tool can affect thousands of embryos by administering the complex of the chimeric protein attached to the gene-specific dsRNA into vitellogenic crustacean females. Therefore, the newly developed chimeric protein tool possessing efficient gene silencing can be used for silencing crustacean's aquaculture-relevant genes that may enhance the aquaculture industry.

ULTRASOUND IMAGING – AN INSTRUMENTAL REPRODUCTIVE TECHNOLOGY FOR TILAPIA BROODSTOCK MANAGEMENT

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Ultrasonography interconverts electric and acoustic energy to create a gray-scale image of internal anatomy for diagnostic procedures. Since the early 1980s ultrasonography has been conducted on a variety of important fish species such as salmon, catfish, sturgeon, and bass to identify sex and to evaluate gonadal development. Understanding the reproductive cycle is crucial for management of broodstock and increased control of fish reproduction. Female Nile Tilapia are asynchronous spawners (follicles in all stages of development present in ovary). This hinders broodstock from spawning at the same time. Non-invasive methods for assessing and monitoring tilapia reproductive biology are limited. Ultrasonography provides a real-time and safe method for monitoring, evaluating and segregating broodstock females based on ovarian development. Systematic fish handling and ultrasound imaging procedures and interpretation guide based on the ovarian cycle for Nile Tilapia was developed at Kentucky State University. This will be used to develop educational materials to provide training to hatcheries for improving broodstock management, spawning synchronization, and hatchery productivity (Figure 1). Female broodstock can be grouped and separated into a scheme of tanks based on ultrasound imaging and the ovarian cycle. This will be instrumental for ease of egg production that is predictable, reduces fish handling and labor, and uses time and space affectively for increasing reproductive efficiency and hatchery productivity.

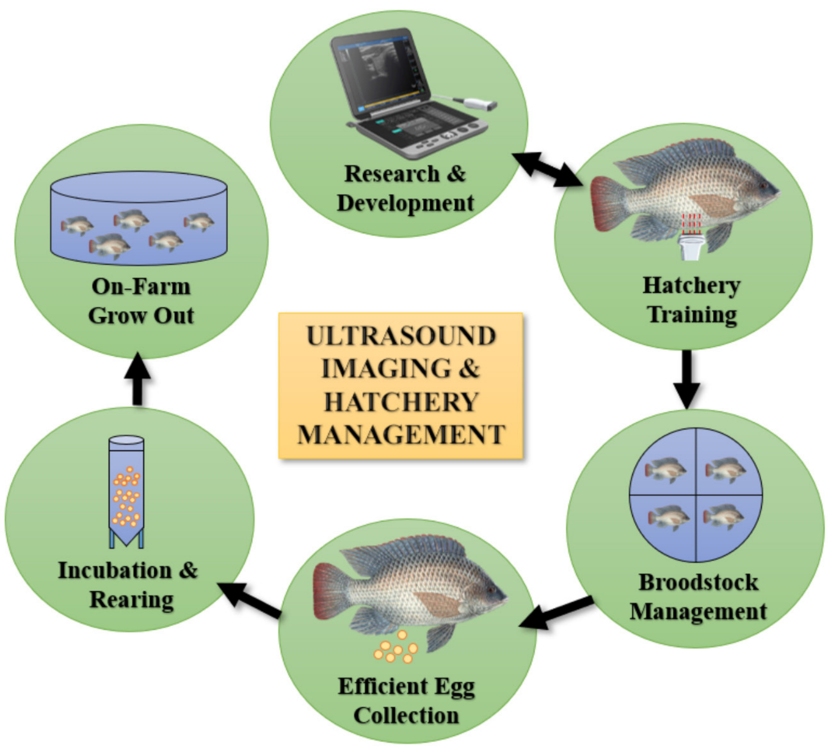

FIGURE 1. The systematic fish handling and ultrasound imaging procedures developed at Kentucky State University will be used for training of hatchery personnel to increase reproductive efficiency and productivity.

TRANSGENESIS PIPELINE FOR ENGINEERING SPECIES LIKE BARRIERS TO REPRODUCTION in *C. carpio*

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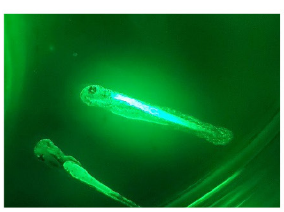
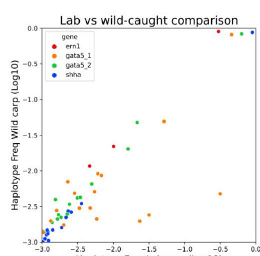
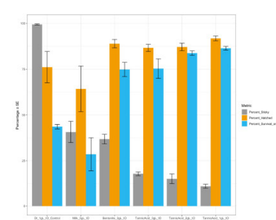
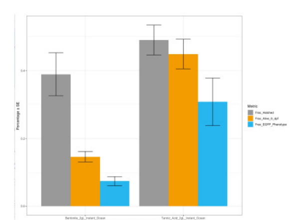
There exists a need to construct intraspecific genetic barriers to reproduction in a variety of important fish species. In food-fish aquaculture, this would mitigate the risk of undesirable breeding between farmed escapees and their wild conspecifics. In invasive fish management, these genetic barriers can be erected to create reproductively isolated strains for genetic biocontrol. Here we present engineered genetic incompatibility (EGI), a genetic strategy to produce intraspecific incompatibility. EGI relies on CRISPR activation (CRISPRa) to induce lethal gene expression in the hybrid offspring of a wild type/EGI cross. Our lab has demonstrated EGI's effectiveness in *D. melanogaster* and preliminary experiments indicate effectiveness in zebrafish. In *Cyprinus carpio*, we detail our progress at creating EGI, assessing wildtype genetic diversity of candidate EGI carp promoters, and our pipeline for attaining year round transgenesis.

EGI relies on targeting genetically conserved promoter regions in wild populations. To assess wild carp genetic diversity, we sequenced four targetable promoters (shha, gata5_1, gata5_2, and ern1) from 250 fish samples from 6 different environments (5 lakes and 1 domesticated lab strain). Results show that all surveyed populations shared a few highly conserved targetable haplotypes for each promoter. To begin the steps of EGI in *C. carpio*, a facility for year-round spawning was produced. One year old carp were introduced to an indoor flow through facility kept at 24C 16L/8D for 3 months. Then, fish were acclimated over 8 days to 16C 12L/12D for periods ranging from 3-21 months. Finally, mature fish were warmed to 24C 16L/8D over the course of a month and induced to spawn with Ovaprim injections. After in vitro fertilization, transgenesis was performed in *C. carpio* embryos by co-injecting tol2 mRNA and a plasmid containing GFP driven by the carp beta-actin promoter. Experiments comparing deadhesion solutions (milk, bentonite, and tannic acid) and their effects on survival were evaluated. Females were spawned successfully even when held at 16C for 21 months. GFP-positive transgenic fish were successfully produced. It was demonstrated that stripped oocytes can be kept at room temperature for up to 5 hours before ensuing embryo survival drops below 30%. It was found that a 3 minute 500 ppm tannic acid treatment was the most effective for reducing egg adhesion and resulted in the highest embryo survival after microinjection.









IMPACT OF TRANSPORT ON SUBSEQUENT ROE ENHANCEMENT OF THE SEA URCHINS *Strongylocentrotus droebachiensis* AND *Paracentrotus lividus*

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Introduction

There is growing interest in Europe and worldwide in the capture and roe enhancement of various sea urchin species. There has been a significant amount of research focused on feed development and protocols for sea urchin roe enhancement. Less focus has been directed on the protocols for, and the impact of, transport on subsequent survival, and roe enhancement of sea urchins. This is a crucial step in the value chain as sea urchins need to be transported from the point of harvest to enhancement facilities as well as onwards to markets (sometimes via live holding hubs). This study describes research being undertaken to understand what the stressors are, and what their impact is on sea urchins during transport, particularly when this is followed by roe enhancement.

Materials and methods

A series of experiments have been conducted to determine the optimal transport methods for *Strongylocentrotus droebachiensis,* in terms of both 'in water' (road and sea) and 'out of water' (road and air) transportation. This presentation will summarise the findings of multiple trials over a number of years. Additional trials have been conducted on *Paracentrotus lividus* to see how they compare in terms of sensitivity to transport stress. This presentation will focus on the former species but will discuss possible implications for the latter.

Results and Discussion

Figure 1 shows the typical results from a post transport roe enhancement trial where sea urchins were held in simulated transport (static seawater tanks with aeration) for 0 days, 7 days and 14 days showed no significant differences in GI after the enhancement period. There was also no significant difference in the final GI of sea urchins held at low (4kg) or high (8kg) densities during the transport or between sea urchins transported for 7 days or for 14 days prior to enhancement (Figure 1).

However, mortality data shows significantly higher mortality in sea urchins exposed to longer transport periods. Preliminary testing on *P. lividus* suggest this species is much more sensitive to transport stress than *S. droebachiensis.* The results from this, and a series of studies will be discussed in terms of the future development of a sea urchin roe enhancement industry and what role transport plays in this.

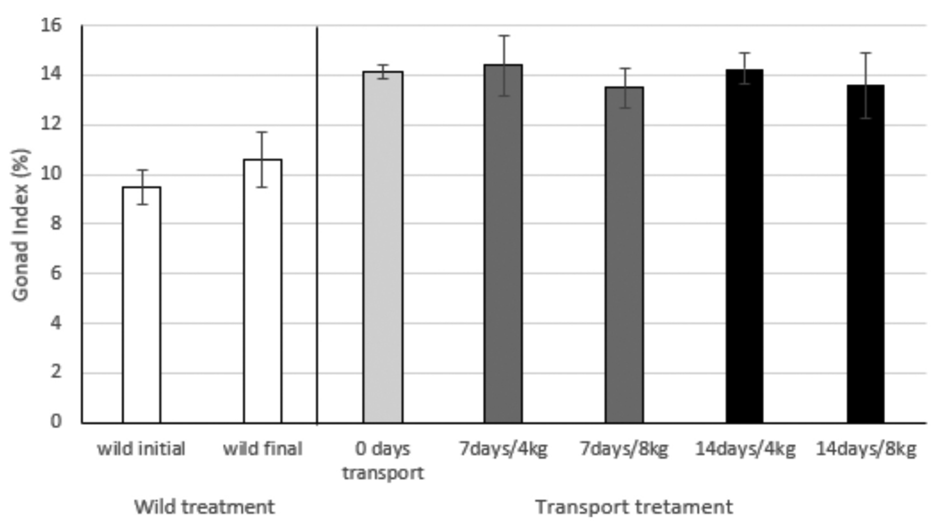

Figure1: The final GI of sea urchins collected from the wild compared to those held for 0, 7 and 10 days simulated transport followed by an 8 week enhancement period.

POLYCULTURE OF RED SWAMP CRAWFISH *Procambarus clarkii* AND PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN LOW SALINITY WATER

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Polyculture of Pacific white shrimp, *Litopenaeus vannamei* and red swamp crawfish, *Procambarus clarkii* is a new production strategy being explored by commercial shrimp farmers in west Alabama. Crawfish are being produced on a pilot-scale in earthen ponds and a split pond on one commercial shrimp farm. Traditional crawfish production relies on primary productivity of the pond to fuel growth and reproduction of the crop, while low salinity shrimp culture involves annual stocking and supplemental feeding. The excess nutrients generated during shrimp production may be beneficial to crawfish growth. The main area of concern is that crawfish are known to feed on small fish, insects, detritus, and plant material. This feeding strategy raises the question of whether crawfish would feed on juvenile shrimp, causing a negative impact on shrimp survival and production. Two controlled experiments were set up using two different systems to investigate the polyculture potential for these two species. The first experiment consisted of a 24-tank system (75 L per aquaria) with water recirculated through a sand filter, biofilter, and sump. The salinity was maintained at ~2.3 g/L using reconstituted seawater. Three treatments (with 8 replicates), each with 20 shrimp (0.53 g mean weight) were used to evaluate the impact of crawfish presence on shrimp growth/survival: shrimp only with shrimp ration; shrimp + 1 crawfish with shrimp/crawfish ration; shrimp + 1 crawfish with only shrimp ration. After 21 days of rearing, shrimp were harvested and evaluated for survival and growth. The second experiment used a green water on-levee flow-through (4.9 L/min) 12-tank system (800 L per tank) at Greene Prairie Aquafarm (salinity of 2 g/L). This system had three treatments (with 4 replicates) with similar treatments, as above (each with 35 shrimp at 0.15 g mean initial weight and 4 crawfish). The first experiment indicated crawfish presence had a significant negative effect on shrimp survival (Fig 1A). However, the second experiment showed no significant differences among treatments for survival and growth (Fig. 1BC). These results indicate that although crawfish will prey on shrimp in a clear water system, the need or ability to prey on shrimp did not occur in a green water system.

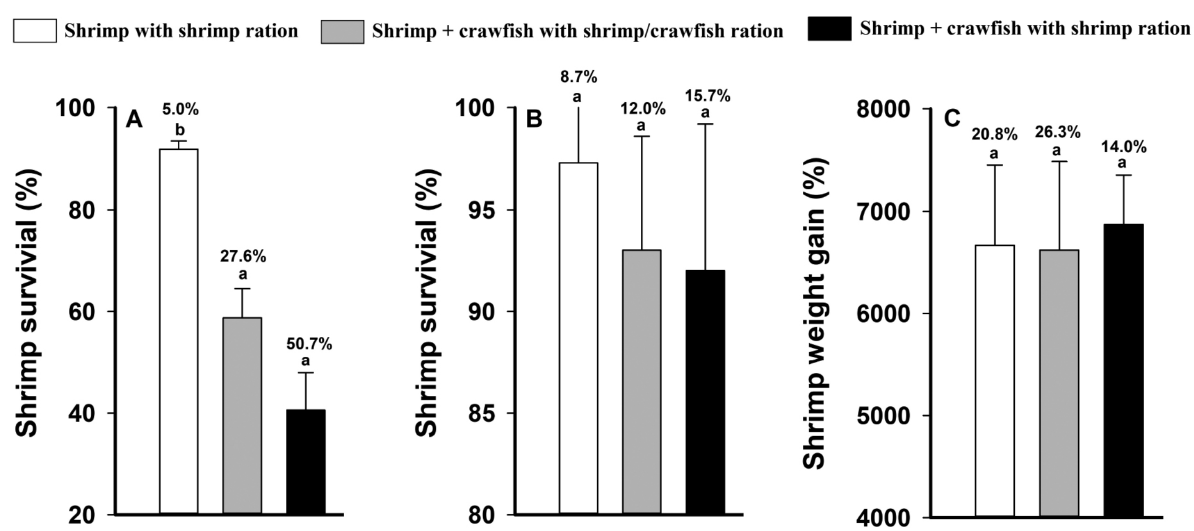

Fig. 1. Impact of crawfish on shrimp survival (A for Exp. 1 and B for Exp 2) and growth (C for Exp. 2). The coefficient of variation for each treatment is provided above each bar.

DEVELOPING POLICY CONSENSUS TO FACILITATE STATE REGULATION OF SEAWEED AS FOOD PRODUCT

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The emerging seaweed industry in the United States presents novel legal considerations. There is currently little federal guidance on the food safety risks of seaweed in its whole form, leaving states unsure how to proceed with their own laws and regulations. In 2019, the National Sea Grant Law Center (NSGLC), in partnership with Connecticut Sea Grant, received funding from the National Sea Grant College Program to enhance coordination and cooperation among states to build policy consensus as to the preferred approaches for regulating the sale of seaweed in its whole form for food.

While this project is ongoing, the NSGLC has completed the first two phases of the project. In 2020, the NSGLC hosted a webinar series to build a foundational base of knowledge and gather input from a broad range of stakeholders to inform workshop discussions and the project as a whole. The webinars on federal considerations and industry barriers and challenges were advertised widely, recorded, and posted on the NSGLC project page. A third webinar focused on state efforts was by invitation only and not recorded so state regulators could discuss the issues openly and "off the record."

In March 2021, the NSGLC hosted a virtual workshop over 8 sessions during a two-week time frame. Since only some workshop registrants had participated in the webinar series, in the weeks before the workshop, the NSGLC hosted a series of informal video "coffee chats" for participants to drop by and discuss different topics the NSGLC was researching, including the federal regulatory framework, state of the science regarding hazards and international models.

32 state regulators representing 11 states participated in at least one session of the March workshop. Participants assisted the NSGLC with the development of an FDA workflow, developed their own draft state workflows, and brainstormed food safety hazards of concern and possible control methods. The NSGLC has prepared workshop proceedings, which will be available late 2021. On December 8, 2021, the NSGLC will host a workshop to kick-off Phase 2 of the project- developing a guidance document for states.

SOFTSHELL CLAM (*Mya arenaria*) CULTURE AT THE ALUTIIQ PRIDE MARINE INSTITUTE, ALASKA

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Softshell clams (*Mya arenaria*) are a clam species endemic to the East coast of North America but have been introduced to a large portion of the Northern Hemisphere, including coastal Alaska. These Clams inhabit upper to mid tidal zones in marine and estuary environments across a range of substrates. Anecdotal evidence from local harvesters indicates softshell clam populations have been increasing in Southcentral Alaska, while other populations of native clams have experienced significant decline. APMI has developed techniques for producing softshell clams for enhancement and study of life history. APMI is exploring the optimal substrate to determine the optimal outplanting substrate. The work presented here describes substrate preference and digging efficacy for juvenile clams. In this study, we examined the habitat, culture, settlement rate, and theorized outplant success of softshell clams in Resurrection Bay, Alaska as a model for suitable substrate for shellfish enhancement. In the spring of 2021, a habitat suitability study was conducted across several transects within known softshell clam harvest areas. Habitats during these collections were evaluated for sediment composition and total softshell clams observed within each sediment type and depth. A total of 220 softshell clams were collected as broodstock during this effort. These clams were held at the Alutiiq Pride Marine Institute (APMI) in a downwell tank system at 12°C, and were successfully spawned in early July 2021 using heated water to 17°C and the addition of *Chatocerous* spp. and *Isochrysis* spp. as feed. An estimated 5 million eggs at 70 μm were counted, yielding approximately (2.7 million larva for setting by day 17 (235 microns). Estimated setting success was 50% and the water was maintained at 19°C to accelerate growth. Clams at 10 mm and 14 months of age were placed in varying substrates and settlement and survival rates were both evaluated as a method to determine preferred habitat for outplanting. APMI hopes to utilize the methodologies developed in this study to further softshell clam enhancement for shellfish harvesters. We believe that Softshell clam enhancement can be used as an adaptation strategy for mitigating tradition shellfish harvest loss and increase food security across Southcentral Alaska.

METABOLIC STATUS AT SPAWNING AND POST-SPAWNING NUTRITION INTERACT TO INFLUENCE CONSECUTIVE OR SKIP REPEAT SPAWNING IN SUMMER-RUN STEELHEAD TROUT

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The consecutive (1-year spawning interval) and skip (≥2-year spawning interval) repeat spawning life histories are found throughout seasonally breeding iteroparous teleosts, including anadromous steelhead trout. Initiation of a reproductive cycle in salmonids is thought to be determined by energetic status during a seasonally defined critical period ~1 year prior to spawning, during which energy levels must exceed a threshold or spawning will be deferred for a future annual cycle. Evidence in steelhead and rainbow trout indicates that reproductive schedule is determined during the period following spawning. However, the relevant aspects of energetic status and the importance of energetic status at spawning versus post-spawning nutrition are not well understood. The growth hormone–insulin-like growth factor-I (GH–IGF-I) endocrine axis indicates metabolic status. During fasting, circulating IGF-I levels decrease and GH levels increase, indicating a catabolic state. Elevated GH levels stimulate lipolysis, enabling access to endogenous energy reserves, while reduced IGF-I levels curtail growth.

We hypothesized that reproductive schedule in female steelhead trout would be determined during the 10 weeks after spawning based on an interaction between metabolic status at spawning and post-spawning nutrition and conducted an experiment to investigate these effects. Females collected at spawning were either fed to satiation or fasted for 10 weeks before being returned to satiation feeding. Plasma samples and morphometric data were collected every 10 weeks until terminal sampling at 40 weeks. Plasma GH, IGF-I, and estradiol (E2) levels were measured using established and validated assays. Reproductive status was assigned based on complete separation of E2 levels into two groups at 40 weeks. Some fish in both the fed and fasted groups became reproductively active on a consecutive spawning schedule. Fasted females that became reproductively active had lower GH levels at spawning than fasted fish that became non-reproductive, indicating a less catabolic state before the fasting treatment was begun. Of the fish that were fed after spawning, reproductively active females displayed both a decrease in GH from spawning levels and lower GH than non-reproductive fed females at 10 weeks, suggesting greater feed consumption. GH increased strongly in fasted fish after 10 weeks of fasting, and then decreased during refeeding and recovery from spawning. IGF-1 levels were lower in fasted fish after 10 weeks of fasting, and then increased during refeeding and recovery from spawning. These results suggest that the GH-IGF-I axis operates to access stored energy and regulate growth in the post-spawning state. Our results show that both metabolic status at spawning and feeding during the first 10 weeks after spawning influence reproductive decisions in repeat spawning female steelhead.

YAKAMA NATION COHO HATCHERY: CHILLER AND PRAS SYSTEM INTEGRATION AND PROCESS FLOW

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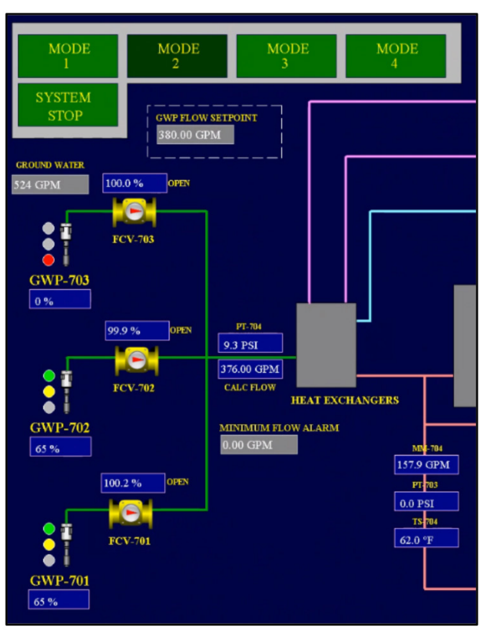
The Melvin R. Sampson (MRS) Coho Hatchery near Ellensburg, Washington is part of the Northwest Power and Conservation Council's (NPCC's) Fish & Wildlife program (Program) and is a component of the Fish Accord between Action Agencies and the Lower River Tribes. Under the Program, the YN and Yakima-Klickitat Fisheries Project (YKFP) are managing Coho reintroduction efforts in the Yakima River Basin by using artificial propagation to re-establish, supplement, and/or increase natural production and harvest opportunities of anadromous salmonids. The MRS Coho Hatchery is an extension of these efforts. The MRS Coho Hatchery includes Coho spawning, incubation, and rearing facilities, as well as operations that will integrate with the overall YKFP. Target production goals for the facility include the incubation of 1,080,000 Coho eggs and the subsequent production of up to 700,000 Coho smolts per year.

Important objectives in the facility design included 1) maximizing energy efficiency, 2) providing quality process water from both groundwater and surface water sources, and 3) accommodating anticipated changes in water temperature due to climate change. To meet the production goals of the project while satisfying these ancillary objectives, a partial recirculating aquiculture system (PRAS) was coupled with an energy recovery and water-cooled chiller system to provide chilled makeup water for grow-out, chilled water augmentation for adult holding, and cool water for incubation. The PRAS system includes four recirculation modules providing 75% re-use water to ten 26-foot diameter grow-out tanks, with each module outfitted with a 40-micron drum filter and gas transfer tower with CO_2 stripper and low head oxygenator. Chiller operations were established to meet four operational modes that differ based on ambient water temperature and the reporting needs of the facility. Due to the complexity of the operational scheme, a fully integrated SCADA package was implemented that includes a human-machine interface (HMI; see example screenshot) with full remote manual control of key system components, a programmable logic controller (PLC) designed to run the chiller system's four operational modes, and an alarm system with pre-established thresholds to notify personnel when monitoring thresholds have been exceeded.

This presentation will walk through the overall facility design and then dive deeper into some of the process flow details, with a focus on the chiller and PRAS systems.



Partial Screenshot of Facility HMI

WHAT MAKES A FARM SUCCESSFUL? VIRTUAL FARM TOURS WITH GREAT LAKES AQUACULTURE COLLABORATIVE

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The COVID-19 pandemic still continues to provide a challenging atmosphere for many including those providing in-person educational programs and outreach. We will share how the Great Lakes Aquaculture Collaborative (GLAC) utilized feedback from previous events and advisory groups to design and deliver Great Lakes Aquaculture Days (GLADs) in 2021.

GLADs 2021 featured virtual tours of six "successful" farms from different states in the Great Lakes region (Fig. 1). The GLAC events committee defined a successful farm as a farm which has been in business for longer than five years, is the sole source of income for the farmer, and continues to make a profit. To highlight the diversity of farming success in the region, the events committee invited farms that utilized a variety of systems, species, and markets (Fig. 1). The events team then coordinated the production of a 20-30 minute farm tour video that was designed to show the viewer the inner workings of the farm. During GLADs 2021, the farm video was aired, followed by a 30 minute live interview, and question and answer session (not recorded) with the farm owner. GLADs 2021 had national and international reach with over 223 registered attendees from 31 U.S. states and 15 countries.

GLADs 2021 offered opportunities for Great Lakes' farmers to open up their businesses to share their experiences and to discuss topics of interest pertaining to their successes and failures in aquaculture. Common themes that were discussed include: family owned and operated businesses, starting slowly and growing gradually, unique system designs, fish mortalities are inevitable, the importance of water quality, the critical importance of backup systems, and being adaptable moving forward. This presentation will expand on these themes by compiling and describing thoughts and discussions elicited during the virtual farm tours and live discussion events.

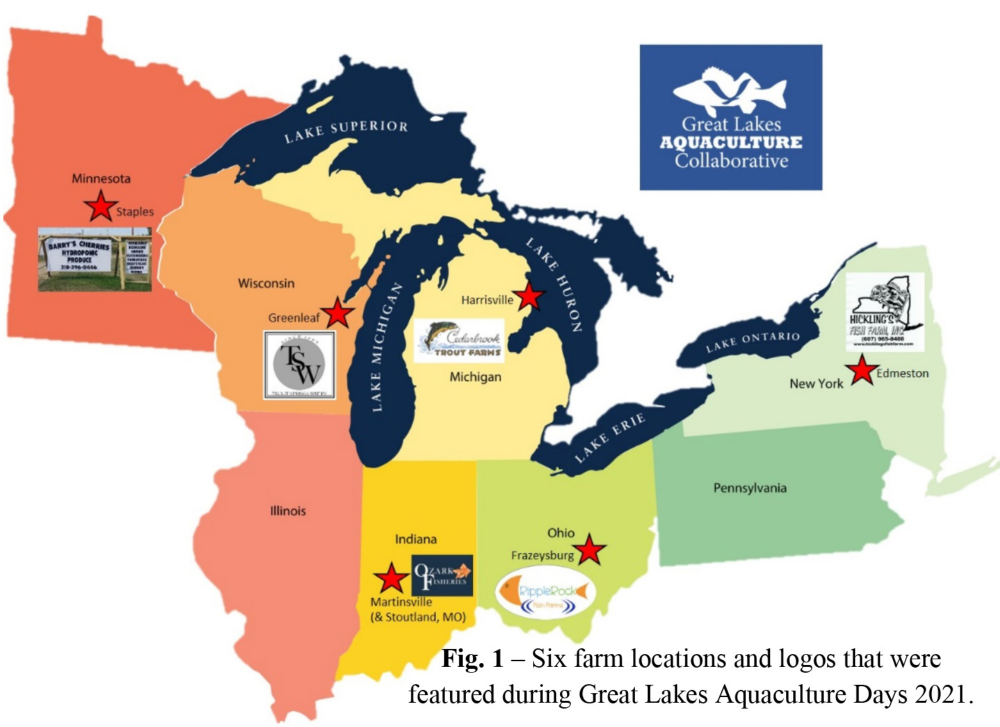

Fig. 1 – Six farm locations and logos that were featured during Great Lakes Aquaculture Days 2021.

ENVIRONMENTAL HYPOXIA IN THE INDIAN RIVER LAGOON AND ITS EFFECTS ON NATIVE FISH SPECIES DURING EARLY DEVELOPMENT

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The Indian River Lagoon (IRL) is one of the most species-diverse estuaries in North America. However, the distribution and intensity of hypoxia (low dissolved oxygen, DO) is increasing due to eutrophication, 'dead zones', algal blooms, etc. Effects of coastal hypoxia are well-studied in adult fish, yet gaps of information remain in terms of early life stages, including potential consequences to the development, hatching success, or future recruitment of economically important native species. These include Florida pompano, red drum, snook, and grey snapper. To test effects of severe hypoxia, fertilized eggs will be incubated in 10, 20, and 100% DO saturation. Samples will be collected at 10, 20, 30, and 40 hours post-fertilization. Lipids will be extracted from each sample and separated into neutral (used for energetic purposes) and polar (used for membranous development) fatty acids. Concentration of these fatty acids will inform us of the potential effects of severe hypoxia on energy demand and membrane remodeling. It is expected that we will see a decrease in hatch rate, survival, and overall development under severe hypoxia treatments (20 and 10% saturation). This project is a direct implementation of the HBOI-Florida Atlantic University (FAU) strategic plan to increase understanding of marine ecosystem function, especially in the IRL. By investigating potential impacts of hypoxia on early larval stages, conservation and management strategies will be more well-informed and executed.

APPLICATION OF GENOME EDITING TOWARDS IMPROVED DISEASE RESISTANCE IN AQUACULTURE SPECIES

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One of major problems facing sustainable aquaculture is infectious disease outbreaks, which can result in significant mortality and animal welfare issues. Disease resistance can be improved by selective breeding, but genetic gain can be relatively slow for species with long generation time, and is limited to the existing genetic variation in the population. Combining genomic analyses and genome editing tools such as CRISPR-Cas9 has potential to accelerate the process by identifying causative genes and variants, or by transferring disease resistance alleles between populations or even species[1,2].

Our typical workflow begins with high-throughput genomics research leading to potential targets underlying intra- or inter-specific genetic variation in disease resistance. The function of target genes is then evaluated in cell lines using CRISPR-Cas9 mediate targeted genome editing, which has now been optimised in various salmonid cell lines[3]. These shortlisted candidates are evaluable *in vivo* by microinjecting CRISPR-Cas9 molecules into zygotes, followed by disease challenge experiments to assess phenotypes.

One recent example of combining genomic studies and genome editing to identify a causative gene is elucidation of NEDD-8 activating enzyme 1 (*nae1*) gene as a causative gene underlying the major QTL affecting resistance to IPNV in Atlantic salmon[4]whole genome sequencing and functional annotation approaches were applied to characterise genes and variants in the QTL region. This was complemented by an analysis of differential expression between salmon fry of homozygous resistant and homozygous susceptible genotypes challenged with IPNV. These analyses pointed to the NEDD-8 activating enzyme 1 (nae1. In this study, whole genome sequencing and functional annotation approaches characterised genes and variants in the QTL region. In addition, differential expression analysis between homozygous resistant and susceptible salmon fry challenged with IPNVs pointed to *nae1* as a putative causative gene underlying the QTL effect. The function of *nae1* in IPNV resistance was evaluated via CRISPR-Cas9 knockout of the *nae1* gene and chemical inhibition of the Nae1 protein activity in Atlantic salmon cell lines, both of which resulted in highly significant reduction in productive IPNV replication, indicating that *nae1* is the causative gene underlying the major QTL affecting resistance to IPNV in salmon.

Our research is currently focussed on applying similar technologies to tackle sea lice and other infectious diseases affecting salmonids. The sea lice projects consist of cross-species genomics comparison approaches between salmonid species with differential susceptibility to sea lice to identify genomic features, followed by genome editing to precisely transfer the resistant alleles between species[2]. Other foci include development of CRISPR-Cas9 mediated genome-wide screens to identify *de novo* resistance alleles, and optimising methods of *in vivo* editing to improve editing efficiency and reduce mosaicism and routes to application, including via surrogate broodstock[5]. Depending on acceptable regulatory and public perceptions, genome editing technology holds the potential to develop disease-resistant fish strains with significant downstream positive impact on aquaculture sustainability and animal health.

1. Gratacap, R. L., Wargelius, A., Edvardsen, R. B. & Houston, R. D. Potential of Genome Editing to Improve Aquaculture Breeding and Production. *Trends Genet*. 35, 672–684 (2019).
2. Houston, R. D. *et al*. Harnessing genomics to fast-track genetic improvement in aquaculture. *Nat. Rev. Genet*. 21, 389–409 (2020).
3. Gratacap, R. L., Jin, Y. H., Mantsopoulou, M. & Houston, R. D. Efficient genome editing in multiple salmonid cell lines using ribonucleoprotein complexes. *Mar. Biotechnol*. 22, 717–724 (2020).
4. Pavelin, J. *et al*. The nedd-8 activating enzyme gene underlies genetic resistance to infectious pancreatic necrosis virus in Atlantic salmon. *Genomics* 113, 3842–3850 (2021).
5. Jin, Y. H., Robledo, D., Hickey, J. M., McGrew, M. J. & Houston, R. D. Surrogate broodstock to enhance biotechnology research and applications in aquaculture. *Biotechnol. Adv*. 49, 107756 (2021).

SALMON MOVEMENT WITHIN A CAGE IS RESTRICTED BY A COMBINATION OF WAVES AND CURRENT

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Due to increasing consumer demand for salmon, salmon farmers are constantly seeking to expand their production. However, environmental barriers such as parasite load and site carrying capacity are preventing expansion in established sites. Therefore, salmon farmers seek to move or expand their production to more exposed sites. In exposed sites, salmon are likely to experience stronger currents and larger waves than what they would normally do. Some previous work has established some baseline information on swimming capacity and behaviour of salmon in currents, but it is not well established how currents and waves interact, and how well salmon are able to cope in conditions where they are exposed to both.

In this study we investigate the effects of strong currents and waves on the behaviour of salmon and how they choose to use the space available to them. Using video cameras and echo sounders, we show that fish prefer to use the entire water column, narrowing their range only as a response to cage deformation (Figure 1), waves, or daylight.

Conversely, fish show strong horizontal preference, mostly occupying the portions of the cage exposed to currents. Additionally, waves cause salmon to disperse from the exposed side of the cage to the more sheltered side. Even when strong currents decrease the amount of available space, salmon choose to occupy the more exposed part of the cage.

This indicates that at least with good water exchange, the high density caused by limited vertical space is not so aversive that salmon choose to move to less desirable areas of the cage. However, the dispersal throughout the entire available water column indicates that making cages deep enough to compensate for deformation in strong currents would be beneficial to salmon welfare.

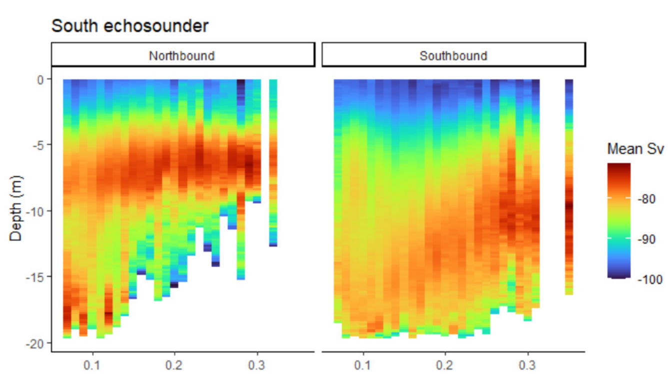

Figure 1 Fish depth over current speed. Lighter colour signifies more fish. Panels are the two main current directions "Northbound" and "Southbound".

ADDITION OF THE RED MACROALGAE PACIFIC DULSE *Palmaria mollis* TO ALTERNATIVE PLANT BASED FEEDS FOR JUVENILE SABLEFISH *Anoplopoma fimbria*

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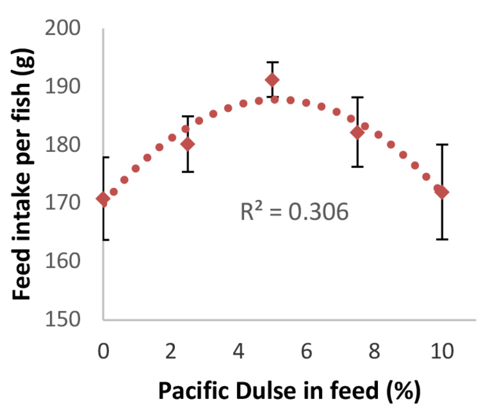
The demand for fishmeal and fish oil in aquaculture feeds, has increased dramatically in recent years. Alternative protein and oil sources are needed if further development of the aquaculture industry is to be sustained. Terrestrial plant ingredients can replace a portion of the fishmeal used in feeds for a number of species. Complete replacement, however, is seldom achieved. Macroalgae may prove a more appropriate feed ingredient than terrestrial plants products for marine fish feeds as macroalgae contains many essential nutrients from the marine environment that are limiting in terrestrial plants. Additionally, there appears to be health benefits associated with the use of some macroalgae species for some marine fish species. In previous studies at our laboratory, the addition of the red macroalgae Turkish Towel to sablefish feeds improved liver health and increased fish survival when exposed to the pathogen *Aeromonas salmonicida*.

In this study, we incrementally added the red macroalgae Pacific Dulse to alternate plant based feeds for juvenile sablefish to evaluate the effect of this ingredient on fish growth, feed intake, feed efficiency, whole body nutrient composition, and liver condition. Five isonitrogenous feeds were prepared with 0, 2.5, 5, 7.5, and 10% dulse. As dulse was added, soy, corn, and wheat ingredients were proportionately removed from the diets. Dulse was sourced from Sol-Sea LTD, Seattle, WA, USA and was produced intensively in land based tanks in 2020.

Fish grew well during the 8 week the study with 100% survival. Feed intake increased non-linearly with the addition of dulse to the feeds and peaked at 5% inclusion (P =0.045, Figure). There was no effect of dulse addition on feed efficiency (P = 0.225). While not significant, fish weight gain mirrored feed intake and was numerically highest among 5% dulse fish (P =0.234). There was no effect of dulse addition on fish length, condition factor, or hepatosomatic index. Effects on whole body nutrient retention and liver histology will be presented. Results from this research indicate Pacific Dulse may be a promising alternative feed ingredient for cold water marine fish. Further research is needed to explore reasons behind the observed decrease in feed intake with feeds containing over 5% dulse and to fully evaluate potential fish health benefits associated with this ingredient.



EVALUATING A NOVEL ORAL VACCINE DELIVERY PLATFORM IN RAINBOW TROUT *Oncorhynchus mykiss*

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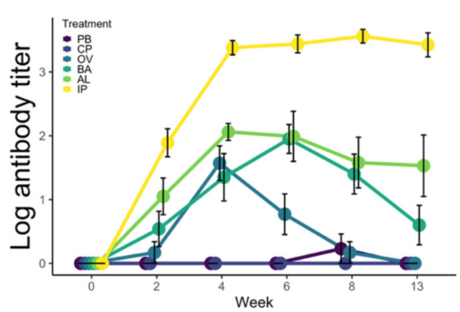
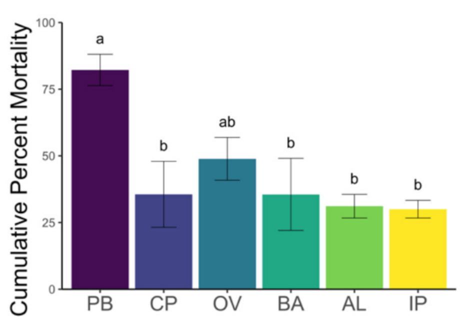
As commercial aquaculture operations grow and intensify, growers continue to adopt vaccination programs to prevent losses from disease. Oral vaccination can significantly reduce costs of these programs while allowing for low stress vaccine delivery to small fish. This study evaluates the ability of a novel alginate-based oral vaccine particle to stimulate immunity and provide protection against the causative agent of furunculosis, *Aeromonas salmonicida*.

A formalin-killed *A. salmonicida* vaccine was produced and used to vaccinate juvenile rainbow trout via four routes: intraperitoneal (i.p.)injection (IP), anal intubation (AL), immersion (BA), and the experimental oral vaccine particle (OV). Control groups included a particle containing no bacterin (CP), and an i.p. injection with sterile PBS (PB). All treatment groups received a booster 2 weeks after the initial vaccination. Specific antibodies in serum were measured at 0, 2, 4, 6, 8, and 13 weeks post vaccination. After 13 weeks, fish were challenged with *A. salmonicida* via immersion and monitored for 28 days. The OV group had an antibody titer peak at 4 weeks, but then steadily declined. The IP group maintained the highest titers, followed by the AL group. In the pathogen challenge, cumulative percent mortality (CPM) of the PB group (82%) was significantly higher than all treatments except for the OV group (48%) which was not significantly different from any treatments. Other groups, including CP, showed significant protection and their CPM ranged from 30-35%.

The oral vaccine particle successfully stimulated an antibody response and both groups fed the alginate-particle showed resistance to *A. salmonicida* in a pathogen challenge. The anal vaccination demonstrated the ability of killed vaccines to stimulate gut associated lymphoid tissue (GALT) which can provide protection against pathogens. Interestingly, the control particle without vaccine provided equal protection to other vaccine treatments. The reason for this is not clear but may be due to an adjuvant effect of the alginate particle. The oral vaccine stimulated a specific antibody response, which provides evidence for oral delivery of vaccines but adjustments to particle formulation, dose and delivery strategy requires further research.





PRODUCTION OF A MONOCLONAL ANTIBODY AGAINST SABLEFISH *Anoplopoma fimbria* IGM AND ITS USE IN AN ELISA TO MEASURE CIRCULATING ANTIBODIES

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Sablefish (*Anoplopoma fimbria*) are an emerging aquaculture species native to the northern Pacific Ocean along the coasts of North America, Russia, and Japan. Recent investment in culture techniques has optimized and reduced the costs of juvenile production, however further investigation of sablefish is required to understand and manage diseases associated with their production.

This work produced a monoclonal antibody (mAb), designated UI-25A, against the heavy chain of sablefish IgM and subsequently developed an ELISA to measure circulating antibodies within sablefish plasma. Plasma collected from adult sablefish was donated by Memorial University, and IgM was isolated using a mannan binding protein column (Thermo Fisher Scientific). Purity of IgM was confirmed via SDS-PAGE before it was used to immunize BALB/c mice. Once specific antibodies to sablefish IgM were detected, spleen cells from mice were harvested and fused to mouse myeloma cell line X63 AG8.653. This resulted in 32 unique hybridoma colonies producing antibodies specific to sablefish IgM. Of these candidates, 4 were chosen for further testing (1, 9, 25, 30) and were confirmed to bind to the heavy chain portion of sablefish IgM (Figure 1).

An ELISA was developed using UI-25A to detect anti-*Aeromonas salmonicida* antibodies within sablefish plasma. This tool successfully detected high antibody levels in vaccinated fish and low signals in unvaccinated fish. The UI-25A mAb has potential to aid in the development of sablefish vaccines as well as further research into mechanisms of their immunity during pathogen infection.

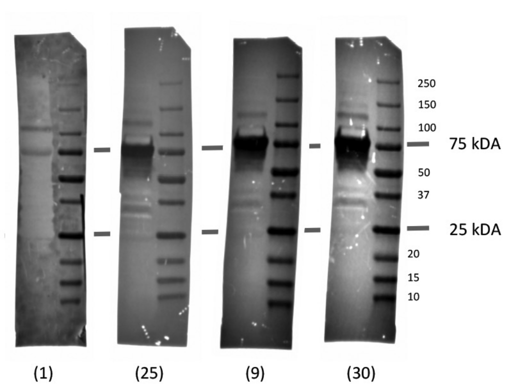

Figure 1: Western Blot of sablefish plasma probed with various mAb candidates. Bands at ~75 kDA show reactivity to the heavy chain portion of sablefish IgM

POPULATION DYNAMICS OF TOTAL AND POTENTIALLY PATHOGENIC *Vibrio parahaemolyticus* IN TEMPERATURE-ABUSED AND RESUBMERGED OYSTERS IN THE NORTHEAST U.S.

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Increasing shellfish-borne illnesses from *Vibrio parahaemolyticus* (*Vp*) in the Northeast US during the past 10 years has triggered intensive management practices based on a limited understanding of risks. Current gear-based production requires extensive pre-harvest handling that, during warm months, can increase *Vp* levels in oysters and potentially increase the risk of illness. Field-testing of *Vp* control measures for pre-harvest practices was conducted in Maine, New Hampshire and Massachusetts where aquaculture practices, regulations, environmental conditions, and *Vp* populations all vary. Oysters were temperature-abuse in Little Bay NH, Spinney Creek ME, and Plymouth Harbor and Katama Bay MA. The abundances of total (*tlh*), potentially pathogenic (*tdh* & *trh*), and *tdh* allele-variants using assays developed to identify regionally important pathogenic strains, were measured in bottom and surface cultured oysters exposed to air for 3-48 hours from sub-tidal and inter-tidal locations. Temperature abused, un-exposed and re-submerged exposed oysters were sampled after 1-10 days. Total *Vp* concentrations significantly increased in abused oysters and remained higher than in unexposed oysters for up to 8 days, depending on treatments. Both *tdh* and *trh* were elevated in abused oysters compared to control oysters but these pathogenic markers returned to environmental levels quicker than total *Vp*. The prevalence of specific *tdh* alleles as proxy for *Vp* strain types 36 and 631 (*tdh3*) varied and provided further resolution of this specific pathogenic population. The study provides scientifically sound findings for each state to use in managing pre-harvest practices to reduce *Vp*-related public health risks.

ROBOTIC PLATFORM FOR AUTOMATED MICROINJECTION OF *Zebrafish* EMBRYOS

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Microinjection is a widely used technique employed by biologists with applications in transgenesis, cryopreservation, mutagenesis, labeling/dye injection and in-vitro fertilization. However, microinjection is an extremely laborious manual procedure, which makes it a critical bottleneck in the field and thus ripe for automation. We have recently developed a computer vision-guided robotic platform that automated the targeted microinjection of zebrafish embryos, one of the most important model organisms in biological and drug discovery research. A computer vision guided microinjection robotic platform uses a series of cameras to image a Petri dish containing embryos at multiple magnifications and perspectives. This imaging is combined with a machine learning algorithm and computer vision algorithms to automatically detect 100s of embryos on a Petri dish and pinpoint a location on the embryo for targeted microinjection with microscale precision. Once located, the robot automatically guides each embryo on the Petri dish to the micropipette for microinjection. We demonstrate the utility of this microinjection robot to successfully microinject zebrafish embryos. Preliminary results indicate that the robotic microinjection has the potential to significantly increase the throughput as compared to manual microinjection. The performance of an automated microinjection robotic platform can be validated by microinjecting the cryoprotectants into the yolk of the zebrafish embryos for cryopreservation, which is a safe and non-toxic method. Survivability of zebrafish embryos in cryopreservation experiments is mainly affected by the variability of manual microinjection, physical damage due to microinjection and toxicity of the solution injected. The experiments to manually investigate these parameters to improve the survivability of zebrafish embryos will be time consuming and laborious. Therefore, we are using this robot to study the effects of microinjection on zebrafish embryos and seek to derive fundamental principles that can be generally applied in other contexts and species. We are also using this robot to develop novel cryopreservation and transgenesis strategies of zebrafish embryos. In the future, this robotic platform can be used to perform high throughput microinjection experiments and it can be extended to automatically microinject a host of organisms such as fruit fly (*Drosophila melanogaster*) embryos, roundworms (*Caenorhabditis elegans*), mosquito (*Culicidae*) embryos, sea urchins (*Echinoidea*), and frog (*Xenopus*) oocytes.

SUSCEPTIBILITY OF SHELLFISH AQUACULTURE SPECIES IN THE CHESAPEAKE BAY AND MARYLAND COASTAL BAYS TO THE OSTREID HERPESVIRUS-1 MICROVARIANTS

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The Ostreid herpesvirus 1(OsHV-1) and its microvariants are highly virulent pathogens that cause mass mortalities of oysters and pose a threat to the shellfish aquaculture industry globally. OsHV-1 causes economically devastating mass mortality events up to 100% in the pacific oyster (*Crassostrea gigas*). However, OsHV-1 and its variants lack host specificity and are known to infect a range of bivalve species, such as bay scallops (*Argopecten irradians*), and be carried by the European green crab (*Carcinus maenas*). A recent laboratory study indicates that the eastern oyster (*Crassostrea virginica*) can experience infection and mortality from OsHV-1 which has significant implications for other aquaculture species used in Maryland and globally. Therefore, determining the susceptibility of economically and ecologically important United States bivalve species to OsHV-1 is an essential step in improving biosecurity and disease management to protect the sustainability of the aquaculture industry. There is a lack of monitoring and research on OsHV-1 on the East coast of the United States, including in eastern oysters grown in the Chesapeake and Maryland Coastal Bays where aquaculture is an important industry for food production, job security, and restoration efforts. Chesapeake and Maryland Coastal Bay species are already threatened by various parasitic and viral diseases, indicating that they may be vulnerable to OsHV-1. Surveys were conducted in June-August 2021 in the Maryland portion of the Chesapeake Bay to determine the prevalence and viral load of OsHV-1 at five aquaculture farms. Using quantitative PCR, OsHV-1 was not detected at any sites. However, continuous surveillance is crucial in mitigating possible introductions to the area. Experiments will be conducted in the Fall 2021 and Spring 2022 at the University of Arizona to determine if natural infection and mortality are possible in economically and ecologically important species, including the eastern oyster, bay scallop, and hard clam (*Mercenaria mercenaria*). These same species will be used in a vector study to determine if they are able to transmit the virus to naive individuals, specifically pacific oysters.

PROSPECTION OF NATIVE MICROALGAE TO FEED *Crassostrea gasar* LARVICULTURE

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Microalgae are an important source of food for aquaculture and one of the main food groups sources to cultivate bivalve molluscs. Even though those organisms are very versatile and have been studied for years, their full potential is yet to be explored. Primar Aquicultura is an estuarine organic farm that is equipped with the only oyster seed breeding hatchery, focused on the *Crassostrea gasar*, located in the northeast of Brazil. Although still not very expressive on the national scenario of bivalve molluscs, the production of native oyster seeds is a new approach and possibly viable in order to evolve the culture of oysters in Brazil. In a way to contribute to the cultivation of native oysters, native microalgae obtained from areas with natural occurrence and production of *C. gasar* in the state of Rio Grande do Norte, Brazil, were studied in regards to their potential of inclusion in the nursing of the *C. gasar*. In this study, 18 microalgae were isolated at LARBIM/UFPB, three of those presented good performance in massive cultive and were utilized in the nursing of *C. gasar*. The native and traditional microalgae were selected according to the morphology and size. The feeding during the larviculture was tested in two different treatments, one with native algae an another with traditional algae. This two treatments were performed in triplicate. Each tank was populated with 10,3x10^6 larvae. Survival of larvae in the treatment with traditional microalgae was significantly higher than the larvae fed with native microalgae

Figure 1 - Massive production of traditional e native microalgaes at PRIMAR´s hatchery.

Figure 2 - Native microalgaes strains isolated at LARBIM/UFPB.

STUDYING THE UNDERLYING MECHANISM OF GROWTH IN RESPONSE TO COMPENSATORY FEEDING REGIME: A PROTEOMICS APPROACH

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Aquaculture Research Institute at University of Idaho in collaboration with USDA has genetically selected the several lines of rainbow trout (*Oncorhynchus mykiss*) that show higher growth rates when fed all plant protein diet (PPD) than non-selected lines of trout fed a fishmeal-based diet. We propose to test if feed intake and body weight variations during successive periods of feed deprivation (FD) and re-feeding (RF) are correlated using compensatory feeding regime and has examined the proteomics profile of these fish based on their feed efficiency phenotype during FD and RF periods.

We have used 1600 fish from 12 families of the selected trout lines, fed PPD (50% soy). Fish were tagged individually, reared in common environment, went through the first feeding challenge, alternate months of FD and RF for 4 months, and performance was recorded. Thereafter, fish were separated into four groups *(1331 fish)* based on individual performance during FD and RF challenge studies. Feed Conversion Ratio (FCR) was recorded for 3 months in all four groups followed by measuring the stability of response to the FD and RF periods (second feeding challenge) which was the same as the first one. Liver samples were collected after each month of the challenge and protein extraction and analysis were done down the line.

The major goal of this study is to understand the underlying molecular mechanism on how some animal can conserve energy more efficiently compared to others and loose less weight over a feed deprivation period (FD^-) and gain more weight over a re-feeding period (RF^+). For that, a proteomics approach was taken to investigate such mechanisms at the protein level. We were able to detect ~3000 proteins in the liver tissue for each phenotype and as shown in our preliminary data in figure 1, there are multiple proteins with different responses to the feeding regimen identified in each group. A deep analysis of the results is ongoing and will be presented at the conference.

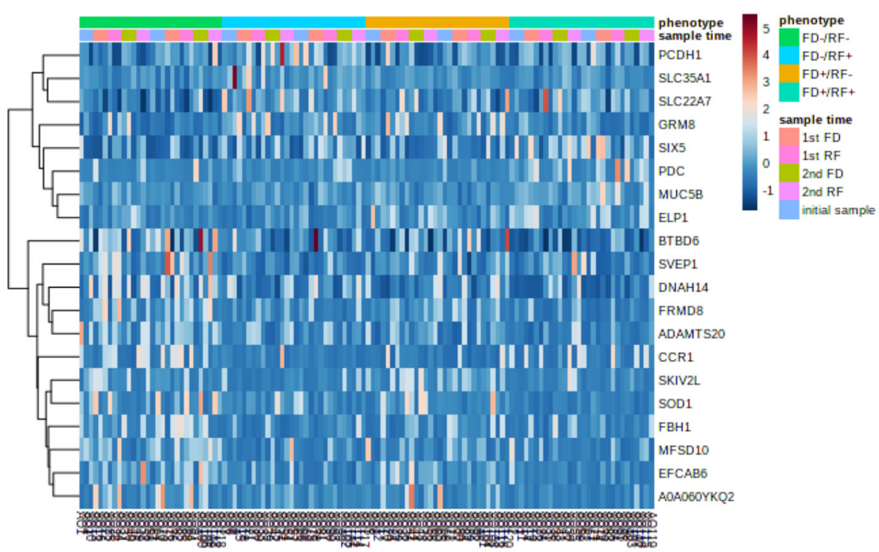

Figure 1: Heat map of the top twenty proteins from two-way ANOVA results using phenotype and sampling time.

L-ARGININE MODULATES INSULIN RESISTANCE, GLUT4 PROTEIN, AND BIOCHEMICAL INDICES IN FRUCTOSE-FED JUVENILE AFRICAN CATFISH

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Increasing industrialization or production of large amounts of fructose (FRU) consumed in syrup, foods, and vegetables culminates a food chain problem including aquatic toxicity. Thus, this study investigated the potential modulatory role of L-arginine (L-ARG) supplementation, a protein biosynthesis amino acid, during FRU feeding in juvenile African catfish (JAC). Using standard methods of probit analyses, fresh FRU solution was prepared 24 hours a day and introduced to the aquaria containing dechlorinated tap water (pH 6.7-7.1). Oral acute toxicity of FRU was done by the adding 0, 0.5, 1.3, 5, 10 and 15 g of FRU per liter of water to each of the fish groups (n = 10) respectively daily for 96 hours. Signs of toxicity, behavioral changes, food consumption, movement abnormality, and morphological changes were recorded. The lethal dosage was calculated by studying the mortality rate in each pond for 24, 48, 72, and 96 hours respectively. Also, 108 JACs (average weight, 25.4±7.1) were exposed to sublethal (14 days) FRU alone (1/12th and 1/2th lethal concentration, LD_{50} = 1156.11 mg/kg after 96 hours) or with divided doses of L-ARG (0.05 and 0.2 mg) and assessed for fasting blood glucose, biochemical parameters, insulin level, GLUT-4 level, weight and histology of gills, liver and heart respectively.

FRU (0.1 and 0.5 g) administrations in the JAC diet showed elevated (p< 0.05-0.01) serum blood glucose levels (BGLs) (110.28 mg/dL - 147.09 mg/dL versus control (94.1 mg/dL), 1.2 - 1.6 folds), aspartate aminotransferase (266.27 - 297.07 IU/L versus control 147.53 IU/L, 2 folds), and total cholesterol (1.17 - 1.69 IU/L versus 1.09 IU/L, 1.1 - 1.6 folds) levels. This was accompanied by increased insulin (41.53 - 59.48 ng/mL versus control 34.74 ng/mL) and decreased GLUT-4 protein (0.118 - 0.510 ng/mL versus control 0.175 ng/mL) levels in the JAC groups. Also, FRU-fed JAC demonstrated elevated malondialdehyde plus lowered antioxidants reduced glutathione levels as well as activities of superoxide dismutase and catalase. FRU intoxication increased gill (1.23 - 1.72 versus control 1.15 g) and liver (1.32 - 1.38 g versus control 1.03 g) weights and also decreased the body weight (2 folds). The addition of L-ARG improved antioxidants status and lowered hyperinsulinemia. However, L-ARG supplementations appear to modulate the changes observed in BGLs, liver biomarkers, lipids, GLUT-4 protein as well as necrotic damage of the gill. Bioaccumulation of FRU in the fish may contribute to metabolic disorders including type-2 diabetes symptoms and other cardiovascular risk factors which may require public health intervention. Although, a high FRU level can cause damage to aquatic life, however, L-ARG supplementations prove to be beneficial via modulation of the antioxidant defense system and biochemical parameters. Thus, both short-term and long-term tests are essential to evaluate the toxicity of sugar effluents to aquatic organisms using the One Health approach in order to ascertain the production of healthy fish for healthy people.

ADSORPTION OF AQUACULTURE WASTEWATER CONTAMINANTS ON N-DOPED BIOCHARS

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The aquaculture industry has witnessed tremendous growth globally in the last 50 years and currently contributes to a production of over 50 million tons. Consequently, this growth has environmental impacts as well. Aquaculture wastes contain nitrogenous compounds such as ammonia, nitrite, and nitrate as result of uneaten fish feed and excretion. Thus, there is a significant interest in mitigating these wastes from aquaculture streams. The goal of this research is to use low-cost adsorbents to treat aquacultural nitrogenous wastes. Pine bark biochars-doped with nitrogen were synthesized and characterized for physical and chemical properties. Current research is underway to determine the efficacy of these nitrogen-doped biochars via batch adsorption systems for removing ammonia and nitrate from model aquacultural wastewater streams.

FISH DISEASE RISK ASSESSMENTS AND REGULATIONS

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Risk assessments have a practical application in developing a production medicine plan for fish farms. There has been limited discussion advising practitioners how to use risk assessments to build their fish medicine practice services.

We are finding new fish pathogens, but what is the significance of these findings? Fish farmers want to know whether to worry about these newly discovered organisms. How do we prioritize pathogens? What should a practitioner know about risk assessments? These are some of the questions that will be addressed in this presentation. Risk analysis involves risk identification, risk assessment, risk management, and risk communication. This presentation will present risk assessments in a practical easily replicated manner for practitioners and attempt to provide options for responding to the questions above. Risk assessment discussions can help focus the conversation with fish farmers on the most important issues on their farm.

Examples of how risk assessments have been applied in regulatory realm will be presented along with non-regulatory cases demonstrating how being well versed in risk can aid your fish farm clients. Known regulatory fish pathogens include: Viral Hemorrhagic Septicemia, Tilapia Lake Virus, and Infectious Hematopoietic Necrosis among many others. Our view of risk changes with time. Several years ago, Largemouth Bass Virus was viewed as a great risk to largemouth bass. Now the concern is generally less. Supposedly it has been freely spread throughout the U.S., yet it has not been associated with declines of largemouth bass. Heterosporis was also a big concern and similarly became less of a concern. We will use these and other examples to demonstrate how to assess risk. What constitutes severe fish diseases? We will discuss potential zoonotic concerns, economic impact, and environmental harm, among other considerations.

Fish farmers vary greatly in the level of concern about introducing a fish pathogen to their farms. Some are extremely cautious and other are unconvinced of the risks. What is the likelihood that a currently unknown pathogen will appear and cause severe disease? How should we put that in perspective for our fish farm clients? These issues will be discussed, and the author will highlight that risk assessment discussions are a great opportunity to demonstrate the value of the fish veterinarian to fish farms.

GROWTH PARAMETERS IN NORTHERN LARGEMOUTH BASS *Micropterus salmoides salmoides* RAISED NEAR THEIR UPPER THERMAL TOLERANCE FOR 28 DAYS

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Commercial largemouth bass producers indicate that when water temperatures exceed 29.5 °C, a significant reduction in feed consumption occurs and that fish lose biomass. Typically, producers feed LMB four times a day until water temperatures exceed 29.5 °C, after which they feed only once or twice a day as fish do not consumption is drastically reduced. Climate models predict that temperatures will continue to rise approximately 5 °C by 2100. The increase in water temperatures over time will inevitably result in warmer water temperatures in the southeastern US. This study assessed growth, feed conversion efficiency, and fatty acid composition of three size classes of northern largemouth bass *Micropterus salmoides salmoides* (80 g, 105 g, and 137 g) subjected to a 35 °C water temperature for 28 days. A completely randomized design was conducted, with ten fish per tank and treatments triplicated. The trial was conducted in 180-L tanks in a recirculating system, and fish were fed a commercial diet formulated for largemouth bass consisting of 48% protein and 18% lipid at approximately 0800 and 1500 hrs daily. The experimental period lasted for 28 days. Water temperature was a significant contributor to the feeding and growth results observed in this study. While the 137 g group did experience some growth, it was not biologically significant (Table 1). The percent body weight consumed by all treatment groups was less than 0.21% day/d. Feed conversion was best for the 137 g group at 4.0 ± 0.44 compared to 5.5 ± 2.57 and 5.8 ± 2.77 for the 80 g and 105 g groups, respectively. Fatty acid profiles of the groups remained similar except eicosapentaenoic acid, which was lower in the 80 g fish. This study was the first study to examine the growth of northern largemouth bass at temperatures exceeding 32 °C and indicate extended periods of exposure to 35 °C or higher water temperatures resulted in thermal stress and the inability of these fish to grow at rates necessary to make them profitable for commercial producers.

Table 1. Mean ± SE of growth parameters of three different size classes of northern largemouth bass *Micropterus salmoides salmoides* raised for 28 days at 35 °C in a recirculating system. Means within a size class with different letters indicate significant differences ($P<0.05$).

Growth parameters	size classes		
	80 g	105 g	137 g
Initial weight (g)	79.5 ± 0.4 [a]	105.3 ± 0.35[a]	137.1 ± 1.24[a]
Final weight (g)	83.8 ± 2.47[a]	110.1 ± 2.16[a]	142.2 ± 0.87[b]
Initial length (mm)	18.4 ± 0.22[a]	19.6 ± 0.16[a]	21.2 ± 0.10[a]
Final length (mm)	18.6 ± 0.32 [a]	20.0 ± 0.21 [a]	21.3 ± 0.46[a]
Fulton's Condition Factor (K_i)	1.32 ±0.02[a]	1.39 ±0.03[a]	1.44 ±0.02[a]
Fulton's Condition Factor (K_f)	1.31 ± 0.03[a]	1.37± 0.02[a]	1.39 ±0.02[b]

Table 2. Mean ± SE of growth parameters of three different size classes of northern largemouth bass *Micropterus salmoides salmoides* raised for 28 days at 35 °C in a recirculating system. Means between size classes with different letters indicate significant differences ($P<0.05$).

Growth parameters	size classes		
	80 g	105 g	137 g
% BWt consumed per day	0.21 ± 0.07	0.21 ± 0.05	0.19 ± 0.01
Weight gain (%)	5.36 ± 2.98[a]	4.76 ± 1.73[a]	3.72 ± 0.41[a]
Specific Growth Rate (%/d)	0.18 ± 0.10	0.17 ± 0.06	0.13 ± 0.02
Feed Conversion Ratio	5.5 ± 2.57[a]	5.8 ± 2.77[a]	4.0 ± 0.44[a]
Survival (%)	86.7 ± 6.67[a]	79.3 ± 9.67[a]	93.3 ± 6.67[a]

OVERCOMING OPERATIONAL CHALLENGES; AN OVERVIEW OF UTILIZING RECIRCULATING AQUACULTURE SYSTEM (RAS) AND PARTIAL RECIRCULATION AQUACULTURE SYSTEM (PRAS) TECHNOLOGY FOR SALMONID SPORTFISH RESTORATION AND ENHANCEMENT PROGRAMS IN VERMONT

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Producing quality smolts and yearlings requires providing rearing strategies that create an optimum culture environment for promoting growth, health and welfare, and ultimately post release performance. Challenges associated with energy use, regulatory parameters related to discharge and limited water resources are factors that can compromise the ability to conduct fisheries restoration and enhancement program objectives at state and federal fish culture facilities.

Utilizing the State Resource Management Revolving Fund (SRMRF) available for “green” projects that promoted energy conservation, the Vermont Fish and Wildlife Department (VFWD) Ed Weed Fish Culture Station in Grand Isle Vermont constructed an early rearing RAS system to produce landlocked Atlantic salmon *Salmo salar* parr as well as two PRAS modular systems adapted to existing production raceways to produce steelhead *Oncorhynchus mykiss* smolts. Positive results led to the installation of two additional RAS systems for the early rearing of all salmonid species produced at the facility.

Over a ten-year period, the combined heating and electrical cost savings achieved has exceeded nearly two million dollars, while providing improved growth and development for all species being reared. This convincing performance established confidence in the technology within the fisheries division’s fish culture section leading to the installation and operation of RAS and PRAS for salmonids at three additional VFWD fish culture stations.

LARGE SCALE INTENSIVE CULTURE PRODUCTION OF WALLEYE *Stizostedion vitreum* FINGERLINGS IN A RECIRCULATING AQUACULTURE SYSTEM (RAS)

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There has been an identified need to develop large scale production methods for the larviculture of walleye (*Sander vitreus*), R.C. Summerfelt et al (2011). Since 2011, intensive culture of walleye fry/fingerlings has been conducted at the Vermont Fish and Wildlife Department's Ed Weed Fish Culture Station in Grand Isle, Vermont. Large scale intensive culture production has been the goal from the facility's program inception to supplement and enhance existing extensive pond culture efforts of fingerlings conducted by the Lake Champlain Walleye Association (LCWA) used for fisheries restoration on Lake Champlain. Tank volumes of 1,940 liters are currently being used in a RAS system dedicated exclusively for intensive walleye culture. Proof of concept techniques have been applied with successive production years to duplicate identified advances related to feed and feeding rates as well as various rearing environment conditions such as the use of greenwater (algae) for turbidity, applying low light intensity throughout the larval production cycle and the utilization of self-cleaning tanks. Application of these techniques is resulting in high quality fingerlings yielding larviculture survivals from day one post hatch (1 dph) through 34 dph greater than 60 % while achieving 50 mm in length. Analysis of oxytetracycline (OTC) otolith marking of stocked fingerlings is documenting recruitment that is making a significant contribution to the fishery.

CALIFORNIA NEEDS TO GROW WHAT PEOPLE WANT TO EAT!

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California's agriculture industry annually produces about $50 billion in farm gate value growing over 350 commodities and employing from 500 to 800 thousand farm workers. However, the State's aquaculture industry only produces around $100 million annually. Considering the length of its coastline, the size of its population and its status as a major farm producer, the State's marine aquaculture industry is limited by the lack of political and regulatory vision, environmental insight and economic commitment.

In May 2006, Governor Schwarzenegger signed the Sustainable Oceans Act (SB 201), the intent of which was for the State to develop a Programmatic Environmental Impact Report (PEIR) that would guide the development of marine farming, including finfish, along California's coast. SB 201 mandated the PEIR, but 15 years later, there has still not been a draft PEIR available for public review even though the Department of Fish and Wildlife has expended close to one million dollars to develop the document. Recently, California developed a policy, contrary to SB 201, that would limit marine farming to only seaweeds and shellfish and the development of finfish farming in land-based, Recirculating Aquaculture Systems (RAS).

This limitation is contrary to the reality of what America consumes. Of the almost $20 billion of seafood imports annually, seaweed is less the 0.3% of that value, while finfish (salmon and others) averages around $6 billion, or 46% of import value. If we are going to feed America, we need to grow what America wants to eat. Constraining marine finfish farming to only land-based systems significantly limits opportunity and puts more pressure on already impacted coastal areas and energy resources. There are many examples of finfish sustainably grown domestically and internationally in marine pens, with *de minimis* impacts to the environment, and these fish are readily available in U.S. markets, including California.

If we are to reap the Blue Economy benefits of marine farming to sustain coastal communities and minimize impacts to global warming, then we cannot forego the opportunity for using small tracts of the open ocean to grow a significant portion of our Nation's animal protein needs. As a center for global innovation, California should not limit finfish farming to RAS farms, but rather encourage marine aquaculture for finfish, shellfish and seaweed using effectively demonstrated technologies for both the nearshore and offshore environments.

WE SHOULD USE THE REGULATIONS WE ALREADY HAVE TO GROW THE FOOD WE NEED

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The productivity of U.S. seafood resources must be expanded to meet increasing domestic demand and to offset the growing seafood trade deficit. The greatest opportunity to realize this needed production 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 response to this necessity, Hubbs-SeaWorld Research Institute has worked over the past two decades with several investment groups, most recently Pacific6 based in Long Beach, California, to obtain permits to grow local species 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.

Specific permits are required 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.

The U.S. already has the laws and regulations it needs to govern the permitting and operation of marine farms located in the EEZ. But, with multiple agencies reviewing permits, one agency needs to serve as the lead for the NEPA review. While permitting authority is one factor for consideration in lead agency determination, as provided in the Council on Environmental Quality (CEQ) regulations, it is neither the only nor the most important factor. In a precedent setting manner, NOAA Fisheries has agreed to serve as the lead agency for the NEPA review for the the POA Project, and has codified this role through a Memorandum of Understanding with the federal permitting agencies. This is consistent with the May 7, 2020 Presidential Executive Order (13921) as well as with the reintroduced *Advancing the Quality and Understanding of American Aquaculture (AQUAA) Act*.

If the U.S. is to reap the Blue Economy benefits of marine farming to sustain coastal communities and minimize impacts to global warming, then we cannot forego the opportunity for using small tracts of the open ocean to grow a significant portion of our Nation's animal protein needs. As a center for global innovation, the U.S. should not limit finfish farming to RAS farms, but rather encourage marine aquaculture for finfish, shellfish and seaweed using effectively demonstrated technologies for both the nearshore and offshore environments.

COMPOSITION, SAFETY, AND SENSORY COMPARISON OF AQUAPONIC TOMATOES AND SOIL-GROWN TOMATOES *Solanum lycopersicum*

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Background: Aquaponics – the integration of hydroponics (water-grown produce) with a recirculating aquaculture system – is a sustainable alternative to current food production practices. A better understanding of the impact of aquaponics on produce quality is essential for the success of this novel production method. The goal of this study was to compare the composition, safety (pathogen presence), and sensory profiles of tomatoes grown in an aquaponics system with soil-grown counterparts.

Methods: Two tomato varieties (Early Girl, a slicing tomato; and Sugar Rush Hybrid, a bite-size tomato) were grown in soil and in an aquaponic system in the same greenhouse. The aquaponic system consisted of three tanks (two tanks of yellow perch (*Perca flavascens*) followed by one tank of calico crayfish (*Orconectes immunis*), which evenly fed water to the tomato raceway. Tomatoes were picked at peak ripeness and analyzed for firmness, skin strength, color, moisture content, titratable acidity, brix, total phenolics, and antioxidant capacity. Semi-trained panelists evaluated samples for aroma, taste, flavor, and texture attributes. One hundred bacterial strains from both soil- and aquaponics-derived environments were isolated and identified. In addition, the presence of *E. coli* was also determined. [choose one of these]

Results: Aquaponic tomatoes were heavier, had a stronger skin, were lighter and more yellow in color, and had a lower brix. Common water and soil bacteria were identified in the two different environments, and *E. coli* analysis revealed no differences in pathogen load between the two growing conditions. However, sensory profiling revealed possible differences in the Early Girl variety, with aquaponic tomatoes being rated as more savory and less sweet and fruity. Successful growth of a complex crop, such as tomatoes, in an aquaponic system may require diligent micronutrient monitoring and adjustment.

TABLE 1: Quality and nutrition indicators of Early Girl (EG) and Sugar Rush Hybrid (SRH) tomatoes. * $p<0.05$

Test	EG Aqua	EG Soil	SRH Aqua	SRH Soil
Weight (g)	**112.65***	**90.82**	**8.52***	**11.09**
Firmness (N)	6.97	7.95	9.24	9.98
Skin strength (mm)	**5.96***	**4.99**	**5.77***	**4.17**
Moisture (%)	88.31	87.09	**91.45***	**89.37**
Color				
L*	**44.10***	**41.58**	**30.02***	**26.72**
a*	36.34	37.29	18.48	17.99
b*	**32.01***	**27.53**	**13.98***	**11.95**
Titratable acidity (%)	**24.63***	**32.90**	**38.70***	**32.89**
Brix (%)	**4.20***	**4.61**	**7.26***	**10.57**
Total phenolics (GAE)	0.27	0.39	0.329	0.238
Antioxidants (TE)	0.08	0.06	0.026	0.095

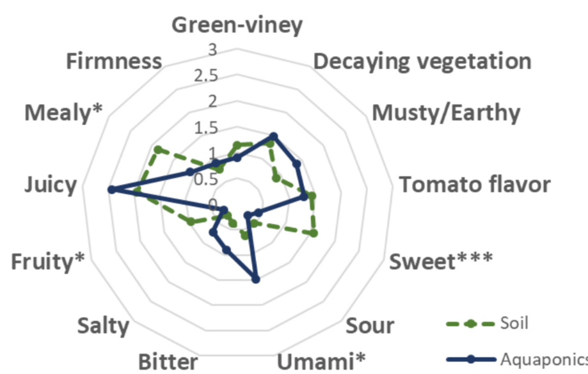

FIGURE 1: Sensory profile of Early Girl tomatoes. * $p<0.05$, *** $p<0.001$.

PEKILO PROTEIN AND SUSTAINABLE GROWTH OF AQUACULTURE

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Seafood, wild-catch and aquaculture is the largest and the fastest growing animal protein industry in the world. Wild-catch tonnage has been stable since 1990 thus, aquaculture accounts for all growth. Aquaculture´s annual growth rate is ~ 6 %.

In many cases Land-use change (LUC) is responsible for the lion's share of the fish feeds' carbon footprint. The expanding area under soybean cultivation raises environmental and LUC concerns because much of this is in tropical areas where it is a major driver of deforestation. Especially in the Amazon and Cerrado regions in Brazil.

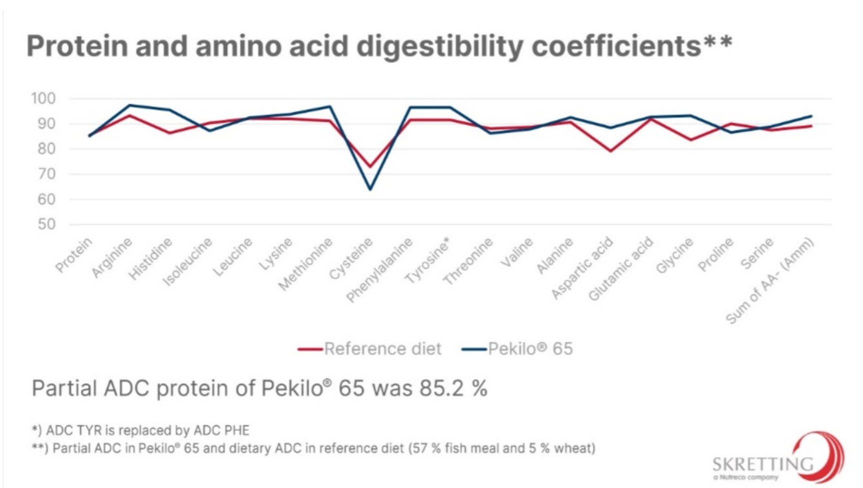
Thus, there is a growing demand for more sustainable protein ingredients that maintain feed performance, use less land, and stabilize supply and economics during industry expansion.

Pekilo protein produced from nonfood resources such as corn ethanol thin stillage can serve as a high-quality protein source for farmed fish which need high protein feed.

One 10 kT Pekilo protein plant has a footprint of 0,001 km2. To produce the same amount of soy protein, an area of land about the size of Manhattan (~60km2) is needed. Soy protein production requires 60 000 times more land than Pekilo protein production.

Recently, the digestibility of Pekilo protein in Atlantic Salmon was found to be the same as in fishmeal protein. The digestibility trial was performed by Skretting, world´s largest aquafeed producer.

Figure 1. Digestibility of Pekilo protein in Atlantic salmon compared to fishmeal protein. Picture provided by Gunvor Baardsen, Skretting ARC.



BIOTECHNOLOGY IN AQUACULTURE: A CRUSTACEAN OOCYTES DELIVERY TOOL FOR GENE SILENCING AND EDITING

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Oviparous animals are characterized by an elaborated yolk production process and packaging in the oocytes before egg-laying. The major yolk protein (vitellin) is usually produced as vitellogenin outside the egg (Vg) and internalized into oocytes by receptor-mediated endocytosis (RME). Like many other crustacean species, *M. rosenbergii* vitellogenin is expressed in the hepatopancreas. *M. rosenbergii* vitellogenin possesses 2537 amino acids and shares at least 33% identity with other decapod crustacean vitellogenin such as shrimps, crabs, and crayfishes. Vg contains several domains, among them the lipid-binding domain that is involved in yolk-lipid vesicles formulation. Upon arrival to the oocytes, the Vg-receptor (VgR) extracellular domain interacts with a distinct amino acid sequence of the Vg and internalizes it to form yolk droplets. One distinctive characteristic of the VgR family is their role in massive internalization and accumulation of lipoproteins. Vitellogenesis is recognized by an immense accumulation of the Vg in the oocyte that will serve the embryo's metabolic needs for development and growth. For that reason, we predicted that Vg endocytosis could be used as a valuable tool for oligonucleotides' high throughput delivery into the oocyte. Indeed, a specific Vg-derived peptide sequence (Vg24) was found capable of oocytes' specific entry by *in vitro* and *in vivo* means. However, a peptide with the same amino acid composition but scrambled order (scVg24) could not enter the oocytes. Vg24 synthesized with nine Lysine-Histidine repeats or recombinantly expressed downstream of a double-strand RNA binding domain (dsRBP) were conjugated successfully with dsRNA by electrostatic or affinity interactions, respectively. The KH-Vg24 and the dsRBP-Vg24 were able to *in vitro* piggyback *PAX6* dsRNA into *M. rosenbergii* oocyte. The piggybacked *PAX6* dsRNA led to eye development retardation in embryos of a treated mother. Alignment analysis of the *M. rosenbergii* Vg24 shared 85% identity with the corresponding Vg peptide sequences from other decapod species. The peptide similarity proposes cross-reactivity between the *M. rosenbergii* peptide and the VgR of other decapod species. The developed tool might serve to deliver other than dsRNA molecules into crustacean oocytes, which may prove to be a powerful high throughput tool for functional genomics investigation in crustacean embryos, immunity, and transgenic animal production.

ADVANCED TECHNOLOGIES FOR PRESERVATION OF AQUATIC EMBRYOS AND LARVAE: A NEW PARADIGM IN CONSERVATION AND AQUACULTURE BIOTECHNOLOGIES

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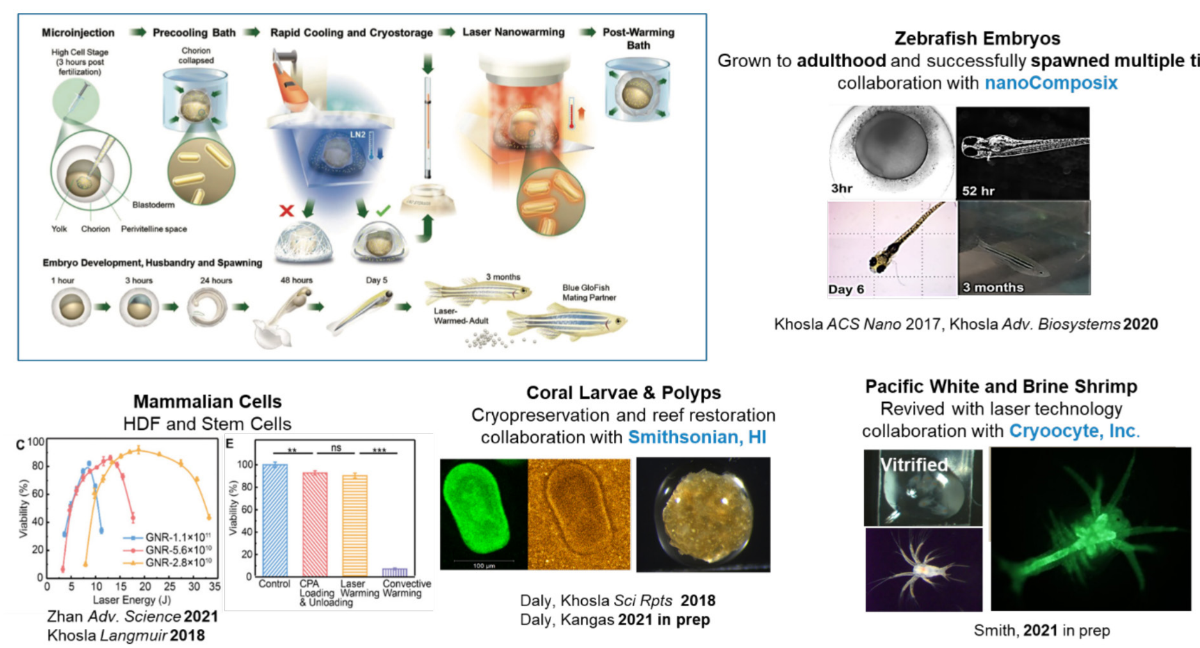
The development of reliable methods for cryopreservation of fish gametes and embryos will be highly impactful for aquaculture, fisheries research, and biodiversity preservation. However, success has been elusive for aquatic and other yolk-laden embryos due to their large size (mm scale) and low permeability to anti-freeze chemicals (cryoprotectants). Our group has developed an ultra-rapid laser warming technology that can reliably rewarm different millimeter scale biological systems (cells, embryos, and larvae) with minimal use of cryoprotectants. Recently, we demonstrated that rapidly cooled (i.e., vitrified) zebrafish embryos, when microinjected with CPA and gold nanoparticles, can be revived with a millisecond pulsed laser. Since gold nanoparticles are highly efficient plasmonic absorbers, they can induce warming rates exceeding 10 million K/min to overcome dangerous ice formation and revive viability. The rewarmed zebrafish embryos were able to hatch, swim and mature to produce viable offspring. Beyond our success in zebrafish, the laser nanowarming platform has enabled the successful cryopreservation of embryos from other fish, corals, and shrimp. With improvements in the automation of droplet vitrification and storage, large-scale adoption and dissemination of this technology can be made possible. In this talk, we will discuss recent improvements and limitations of this approach towards building a "Cryo-Seed Bank" for aquatic species.



OYSTER AQUACULTURE SITE SELECTION THROUGH A DYNAMIC ENERGY BUDGET MODEL COUPLED WITH HIGH RESOLUTION SATELLITE PRODUCTS

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Site selection is a vital step in starting a successful aquaculture business. Bivalve growth is largely controlled by temperature and food (phytoplankton or suspended organic matter). However, the tools and methods that are required to measure these parameters over a typical growing season are often expensive and time consuming. Site selection can be especially difficult in Maine, where the coastline is convoluted. Most Maine aquaculture occurs in bays and estuaries that have vastly different environmental parameters despite close proximity to each other. Our work reduces the barriers of site selection by coupling high-resolution satellite data from Landsat 8 and Sentinel 2A/B with a dynamic energy budget (DEB) oyster growth model. The high spatial resolution as well as short revisit times of these satellites offer unprecedented farm scale site characterization.

Landsat 8 temperature products are provided at a 100 m resolution with a 16-day revisit time, while chlorophyll and turbidity can be derived from Sentinel 2 at 20 m resolution at a 5-day revisit time (Figure 1). Previous work validated satellite products on a network of monitoring buoys along the coast of Maine. To validate the growth model, we tracked tissue and shell growth of oysters at four farms with varying food and temperature conditions over 6 months. Predicted growth from DEB models run on hourly in situ data collected at the study sites were compared to observed growth. The final model was applied to monthly climatologies (2016 – 2020) of chlorophyll from Sentinel 2 (Figure 1) and daily climatologies of temperature (2013 – 2020) from Landsat 8 to predict time to market. While this work focuses on reducing the barriers for expansion of oyster aquaculture in Maine, it has the potential to inform siting of other emerging culture species such as sea scallops or established species in different environments such as offshore locations.

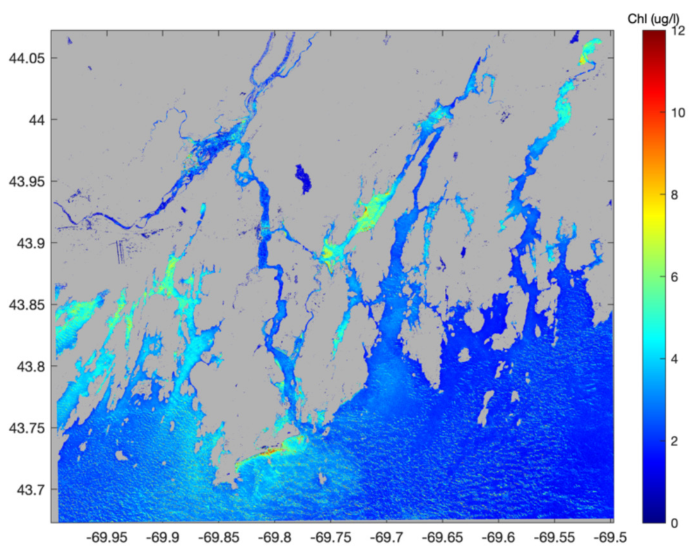

Figure 1. Average July chlorophyll a data from cloud free Sentinel 2 products (2016-2020) over a portion of Maine’s coast

Sargassum horneri EXTRACT ENHANCES THERMAL TOLERANCE AND ANTIOXIDANT ACTVITIES IN A RED ALGA *Neopyropia yezoensis*

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Neopyropia yezoensis is one of the most important aquaculture species in Korea. Due to global warming, it is important to develop new cultivars with thermal tolerance. Acadian Marine Plant Extract Powder (AMPEP) is known as a biostimulant, enhancing growth and resistance to temperature stresses. This study compared the growth and antioxidant activity of *Neopyropia yezoensis* exposed to AMPEP and *Sargassum horneri* extract (SHE). Blades were exposed to 1 g L^{-1} of AMPEP and SHE solution at 10 °C for 10 days. At the end of exposure, they were transferred to flasks with fresh medium and cultivated at 10 and 20 °C for 15 days. At 10 °C, no significant differences in terms of growth were observed. However, at 20 °C, the growth at control decreased, whereas the samples with SHE and AMPEP showed growth rates similar to those of 10 °C. The total protein contents of the SHE and AMPEP groups at 10 °C were higher than those at 20 °C, while the SHE and AMPEP groups at 20 °C showed similar results to the control group at 10 °C. These results suggest that SHE has similar effect to the commercially available seaweed derived biostimulant, AMPEP, enhancing the temperature tolerance in *Neopyropia yezoensis*.

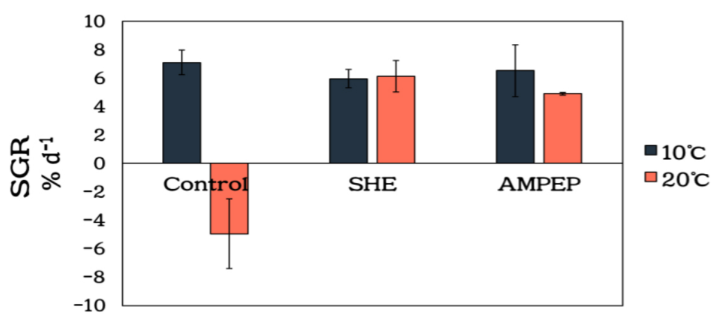

Figure 1. Growth rates of *Neopyropia yezoensis* at different biostimulnts and different temperatures (10 and 20 °C)

USING 2B-RAD SEQUENCING TO IDENTIFY GENETIC VARIATION IN THE POPULATION OF FLORIDA POMPANO (*Trachinotus carolinus*) FOUND OFF THE EAST COAST OF FLORIDA

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Lack of genetic information is a hurdle to implementing an effective and efficient selective breeding program in many aquaculture species. The examination of genome-wide variable sites within a species allows for the identification of genes which are associated with desirable traits for aquaculture, such as increased growth rate and decreased inbreeding. Once identified, these genes can be positively selected for in a genetics based selective breeding program. Restriction site-associated DNA sequencing (RADSeq) is a quick and cost-efficient sequencing method used in genome-wide genotyping studies. 2b-RAD sequencing, a type of RADSeq, uses a type IIB restriction endonuclease to cut the genome into small, equally sized DNA fragments that can be sequenced faster than whole-genome sequencing. It allows for higher power with a smaller number of samples when conducting high throughput genotyping in populations.

The Florida Pompano (*Trachinotus carolinus*) is a warm-water, marine teleost species found in the Atlantic Ocean off the coast of Massachusetts, U.S. down to Brazil. It is commonly found off the east and west coasts of Florida, U.S. where it is known as a popular food fish. There is great interest in bringing this species to large scale aquaculture due to its popularity and high market value. A genetics-based selective breeding program would allow for increased production rates in an aquacultural setting resulting in greater profits for farmers. An assessment of the genetic variability of the wild Florida Pompano populations is necessary to select the best candidates to include in a selective breeding program (broodstock).

Towards this end, this project genotyped n=30 Florida Pompano caught off the east coast of Florida. Total genomic DNA was extracted from fin clips. Genomic libraries were prepared following the 2b-RAD sequencing protocol and sequenced on Illumina HiSeq 3000 platform. Sequenced reads were filtered for low-quality and uninformative reads and mapped using a Florida Pompano reference genome developed by our team. Multi-loci genotypes are being analyzed to estimate levels of genetic variation in the wild population. This information will lay the foundation to identify genes of interest that can be used in a genetics-based selective breeding program for enhanced seedstock.

DEVELOPMENT OF A BREEDING PROGRAM FOR GULF OF MEXICO EASTERN OYSTER USING COMMUNAL REARING OF FAMILIES AND MARKER-BASED PEDIGREE RECONSTRUCTION

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In recent decades, wild stocks of eastern oyster *Crassostrea virginica* have suffered major declines across most of the species' range including in the Gulf of Mexico, the largest producing region in the US. In 2019, the SALT consortium initiated a breeding program to support the developing industry in the Gulf of Mexico with oysters bred for improved performance in different salinity environments. The program is evaluating the effectiveness of a family selection approach where families are mixed at fertilization and reared communally until phenotyping. The research team works in collaboration with a business advisory council to discuss objectives of the program and orient activities to meet the demand of the industry.

The first generation was produced by crossing 102 males and 102 females collected from 17 natural reefs across the northern Gulf of Mexico between San Antonio Bay (Texas), and Cedar Key (Florida). Founders were bred according to 51 non-overlapping 2 males x 2 females mini-factorial crosses to produce 204 families. Crosses were produced between August 31 and September 3 2020 by strip spawning and in vitro fertilization. Families produced on the same day were pooled after fertilization for larval culture. Eyed larvae from each group were harvested over a 4-day period and set separately set on microcultch to produce single-seed oysters. All groups were pooled for common garden culture on October 10 (38-41 days post fertilization). Random samples of the pool were deployed in April 2021 on 7 growout sites expected to experience different salinity conditions. Four of the sites were selected at the end of the growout period based on the salinity conditions recorded during the growing season. Oysters from these four sites were retrieved between mid-October and early November 2021. Most of the harvests were frozen until measurements (shell height, length, width, and scoring of back-bend deformity) and tissue sampling for genotyping and parentage assignment. A sub-sample (600 specimens per site) was kept alive as candidate broodstock for the second generation. The candidate oysters were individually tagged with Passive Integrated Transponders (PIT-Tags) and a non-lethal biopsy was taken for genotyping. Parents and all offspring are being genotyped at 192 Single Nucleotide Polymorphism markers. Pedigrees will be reconstructed using a likelihood ratio approach. The dataset will be used to estimate genetic parameters for growth and conformation traits for each salinity environment, genetic correlation across environments, and breeding values of candidate broodstock. Future development of the program including assessment of additional traits and incorporation of genomic information will be discussed.

RESTORING COASTAL SALT MARSHES THROUGH THE CREATION OF INTERTIDAL EASTERN OYSTER REEFS IN SOUTH CAROLINA, USA

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Dating back to 2001, the majority of the SCDNR's intertidal oyster (*Crassostrea virginica*) restoration efforts have focused on increasing environmental awareness and stewardship and the evaluation of the ecosystem service of habitat provision to a diversity of other organisms. More recently, however, the ability of these intertidal oyster reefs to protect and restore fringing salt marshes (as illustrated in *Figure 1*) has become particularly relevant in the context of shoreline erosion and habitat loss. The loss of salt marsh habitats is particularly concerning as salt marsh is one of the most biologically-productive and ecologically-valuable habitats in the coastal region.

This presentation will present project results derived from both on-the-ground and remote sensing monitoring efforts, specifically related to temporal patterns of oyster reef development and salt marsh habitat accretion. The information presented will focus on selected case studies for sites established and monitored through research funded by the NOAA's NERRS Science Collaborative (2015-2019) and the SC Department of Health and Environmental Control's Office of Ocean and Coastal Resources Management (2019-2022). This information was used to help develop new state of South Carolina regulations and is continuing to support their implementation. Through these monitoring efforts, researchers have also developed some important lessons learned regarding living shoreline performance that will be shared in this presentation. Important considerations include physical shoreline parameters, where materials are placed on the shoreline, and the relative needs for periodic maintenance for different living shoreline materials.

Figure 1. Photograph from Big Bay Creek, a tributary of the South Edisto River, taken on August 19, 2021. The photograph includes a number of living shoreline materials installed on June 1, 2016. Over the course of 5 years there has been expansion of salt marsh habitat seaward from the naturally-occurring marsh edge for all treatments except the negative control (fourth experimental plot from left to right).

OPERATING A MARINE BAITFISH AND FOOD FISH HATCHERY: TRIALS, TRIBULATIONS AND OUTLOOK FOR THE FUTURE

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LAB was founded in 2013 as the first and only commercial marine baitfish aquaculture company in the USA. Through years of innovation and optimization, LAB has since become the premier source of warm water marine baitfish, food fish fingerlings, and a leader in applied aquaculture innovation, restoration, and conservation. This journey has not been smooth and without tribulations. But a bright future is within our grasp.

This presentation utilizes past experiences and current realities to describe the warm water marine bait and food fish industry today. From there we can contemplate what an ideal industry may look like. And focus on the steps and opportunities to get us there.

THE MASSACHUSETTS SHELLFISH INITIATIVE: A MULTIYEAR STAKEHOLDER FORUM TO DEVELOP A STATEWIDE HOLISTIC STRATEGIC VISION FOR SHELLFISH MANAGEMENT

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Modeled after NOAA's National Shellfish Initiative, the Massachusetts Shellfish Initiative (MSI) governed by a state sanctioned task force and steering committee, developed a strategic vision for shellfish management in the Commonwealth of Massachusetts. The MSI was a stakeholder driven process with a mission to enhance the economic, social, and environmental benefits of the state's shellfish resources and was born out of a recognition that current and future management challenges would be best addressed by incorporating stakeholder feedback. Several interim projects were completed including conducting a statewide assessment, describing how shellfish are managed in Massachusetts, as well as what institutional infrastructure and capacities exist to support shellfish resources and shellfish fisheries, a task not previously completed. Additionally, public feedback was solicited and consolidated regarding issues of public concern pertinent to shellfish. These efforts were used as the foundation with which to develop a five-year strategic plan, which was completed in the winter of 2021. Will the newly minted strategic plan alter the trajectory of shellfish management toward MSI's mission?

TECHNO-ECONOMIC PATHWAY TO BIOFUEL-SCALE SEAWEED FARMS

Hauke Kite-Powell*, Erick Ask, Simona Augyte, David Bailey, Julie Decker, Clifford A. Goudey, Gretchen Grebe, Yaoguang Li, Scott Lindell, Domenic Manganelli, Michael Marty-Rivera, Crystal Ng, Loretta Roberson, Michael Stekoll, Schery Umanzor, and Charles Yarish

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Seaweed farming has the potential to produce feedstocks for many applications, including food, feeds, fertilizers, bio-stimulants, and biofuels. Seaweeds have advantages over land-based biomass in that they require no freshwater inputs, little or no fertilizer, and no allocation of arable land. But seaweed farming has not been practiced at scales relevant to meaningful biofuel production. We describe a techno-economic model of large-scale seaweed farms and its application to the cultivation of the temperate seaweed *Saccharina latissima* and of the tropical seaweed *Eucheumatopsis isiformis*. At farm scales of 1,000 ha or more, our model suggests that farm gate production costs in waters up to 200 km from shore are likely to range between $200 and $300/dry tonne. The model also suggests that production costs below $100/dry tonne may be achievable in some settings, which would make these seaweeds economically competitive with land-based biofuel feedstocks. We also outline a techno-economic pathway from small-scale (1-10 ha), semi-mechanized farms that are viable today to large, biofuel-scale operations.

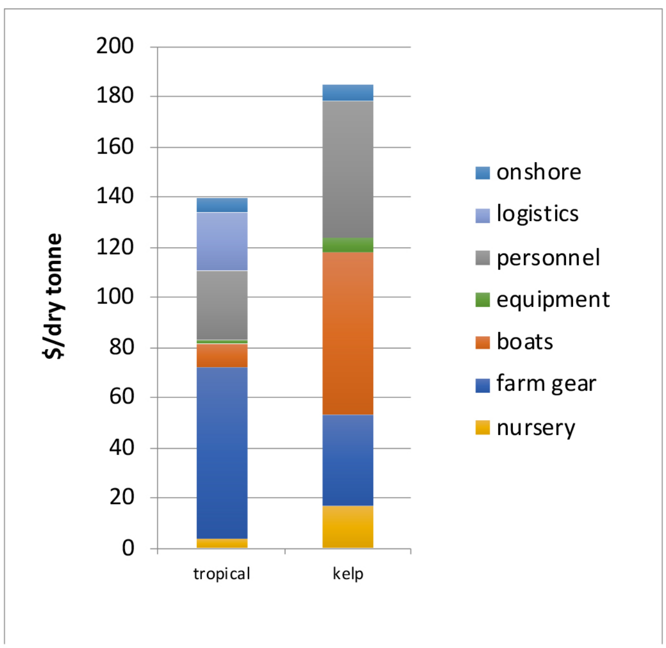

Figure 1: Estimated farmgate production cost components for 1,000 ha seaweed farms at 75 km distance from shore base.

LONG-TERM PERFORMANCE OF GENE EDITED, STERILE ATLANTIC SALMON – GROWTH, SMOLTIFICATION, WELFARE INDICATORS AND FILLET COMPOSITION

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Using sterile salmon in aquaculture could mitigate sustainability challenges including precocious male maturation and genetic introgression from farmed escapees to wild populations. By knocking out *dead end* (*dnd*), we have created germ cell-free (GCF), sterile salmon with the potential to remain immature throughout life. We are also developing a method to produce large quantities of GCF salmon. In this context it is also necessary to characterize the production traits of these fish, and to distinguish the phenotypic effects caused by lacking the *dnd* gene from effects of lacking germ cells. Being GCF, and hence sterile, is an interesting commercial trait. In this study, we produced GCF and wild type (WT) Atlantic salmon and reared them indoors in a common garden setup with natural light and temperature for 3 years. Fish performance in terms of growth, welfare indictors, gene expression in non-target tissues and fillet quality were evaluated throughout the study**.** There were little differences in growth performance between GCF and control early in life. However, condition factor (CF) was lower in GCF at harvest size. Smoltification markers displayed normal levels before and after seawater (SW) transfer for both WT and GCF. Plasma stress indicators including lactate and osmolality concentrations were higher in GCF than WT plasma 24 hours after transfer to SW, but these differences disappeared after 6 months in SW. In adults at harvest size, lactate concentrations were higher in WT than in GCF for both sexes. Transcriptome profiling of muscle and pituitary revealed minuscule differences between WT and GCF in postsmolts after 6 months in seawater. Prevalence of vertebra deformities was similar and within a normal range in both WT and GCF fish. No differences were found in hepatic or cardio somatic indexes (HSI/CSI) in postsmolts after 6 months in seawater, however in adults at harvest size, HSI was higher in WT than in GCF fish, while CSI was unaffected. No sexual maturation was detected in GCF fish of either sex throughout the study period, in contrast to their WT counterparts. Fillets from WT and GCF salmon at harvest size showed no significant difference in proximate composition (protein, dry matter or total fat). Interestingly, the relative content of the omega-3 fatty acid DHA 22:6n-3 was higher in GCF compared to WT males despite having the lowest total amount of fatty acids.

GCF fish of both sexes showed lower CF at harvest size, as well as smaller livers, which may reflect altered metabolism in line with the onset of sexual maturation, indicating that nutritional needs may differ between WT and GCF fish. Since GCF individuals were not more prone to develop deformed vertebra than their WT counterparts, GCF farmed salmon may potentially have less problems with quality downgrading losses at harvest with respect to deformities than the currently used triploid sterile salmon. Although the fillet transcriptome and proximate composition did not differ, the finding of increased relative amount of DHA 22:6n-3 in GCF males compared to WT males may represent a more favorable fillet composition for consumers.

PRESERVING A HISTORIC FACILITY WHILE UPDATING LSS INFRASTRUCTURE: THE STORY OF THE KEY WEST AQUARIUM

Travis Knorr

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Built between 1933 and ‘35 as an open-air public aquarium, the Key West Aquarium, placed at the shoreline of Key West Harbor, poses a unique set of challenges to maintain over 30 exhibits of marine life in a historical facility. Originally built in a remarkably efficient design, utilizing only two water pumps and a series of aqueducts, 21st Century technology has been retrofitted to allow more reliable water quality, and resilience in the face of ever-increasing storm impacts. Installations of a saltwater production well, degasification tower, injection well, and backup generator all while preserving the irreplaceable features of the building, provide a unique experience for guests, and importantly, the capability to provide habitats for iconic Atlantic species such as Tarpon, Goliath Grouper, Horseshoe Crabs, and Seahorses.

GARAGE-A-CULTURE: SMALL-SCALE ORNAMENTAL AQUACULTURE

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Ornamental aquaculture has many advantages compared to seafood aquaculture; perhaps the biggest advantage is the value of product per size or weight. While ornamental aquaculture products are rarely sold by the pound, these small aquarium fishes and invertebrates yield a high value when compared to seafood aquaculture products. This small product size opens the (garage) door to operations working in small spaces. With less area required for production, places such as garages, green houses, or even spare bedrooms can be appropriate for ornamental aquaculture systems. This presentation will discuss advantages and strategies to maximize production in small spaces, and species that work well on a small scale which may have a relatively high return on invested time, money and space of an aquaculture operation.

Summerland Ocean Life is an S-Corp created in 2019 as a side project by T. Knorr and M. Walsh. The operation focuses on simple aquaculture techniques that require little effort and high-value products. The use of technology to monitor and control the aquatic habitats is utilized to minimize required efforts and allow for autonomous operation. The site of the operation is in the Florida Keys and takes advantage of the South Florida aquarium trade, networking with local collectors and regional wholesalers. Of the species cultured, a particularly ecologically important species, the blue neon goby, *Elacantinus oceanops*, remains a high priority as their abundance within the coral reef ecosystem has been shown to be related to increased biodiversity. Additionally, the Banggai cardinalfish, *Pterapogon kauderni*, has been greatly impacted by over-harvesting. This species has been placed on the IUCN Red List of threatened species. Aquaculture of *E. oceanops* and *P. kauderni*, is an important alternative to wild collection, and a main objective of Summerland Ocean Life.

BUILDING A GOOD PROBIOTIC FOR AQUACULTURE

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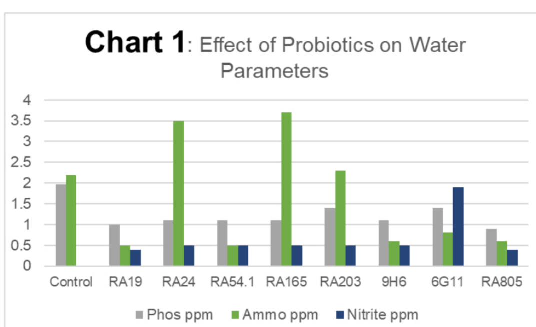
Probiotics improve water quality by decreasing concentrations of ammonia and nitrite and inhibit pathogens by producing antibacterial substances, by competitive exclusion, and by stimulating the host immune system. We have collected isolates worldwide and by using are high-throughput screening techniques we have tested them for growth at different pH, salinities and temperatures and for inhibition against pathogens that are pervasive in aquaculture and for their ability to reduce ammonia, nitrite, nitrate and phosphorus. We selected a handful to demonstrate how the testing we have done can lead to better probiotic combinations. For example, RA203 and 9H6 can grow at 15°C, RT, and 30°C and inhibit all but one pathogen tested. (Table 1 and 2). Isolate 6G11 has a stronger inhibition than the rest against our target pathogens.

RA19 or RA54 could be good candidates to reduce ammonia and phosphorus. RA805 a new isolate that needs more testing has shown to be the best at reducing phosphorus (Chart 1). Also, the isolates are tested for enzyme activity: amylase, protease, cellulose, citrase and numerous others. The only enzyme tested not produced by RA203 and 9H6 is amylase. Therefore, we would choose another isolate to add to fulfill this need. A combination of RA203, 9H6 and 6G11 and possibly RA805 would make a probiotic combination good for use in different environments.

Table 1				Oysters							shrimp pond	shrimp						
Salt water environment				Hawaii					WA		Brazil	Vietnan	Lab Isolates					
Origin	Local	ID	*Vibrio*	*owensii*	*harveyi*	*vulnif*	*ponticus*	*alginol*	*anguil*	*anguil*	*alginol*	K144	Ecu. 1	Ecu2	A3	O3K6	*coralyt*	*tubi*
			Genus Species									*parahemolyticus*						
Ecu	water	6G11	*B. altitu grp*		+	+						+	+	+		+		
Ct	ocean	9H6	*B. amylo grp*				+	+	+	+		+				+		
MAL	shrimp farm	RA203	*B. amylo grp*	+	+	+	+			+	+	+	+	+	+			+
HI	oyster	RA19	*B. methylo.*		+	+		+	+	+			+	+	+	+		+
HI	oyster	RA24	*B. subtilis*		+	+						+	+	+				+
WA	oyster	RA54	*B. subtilis*		+	+						+	+	+	+			
VA	oyster	RA165	*B. subtilis*		+	+						+	+				+	

Table 2	*Flavibacter spp*		*F. limicola*			*F.sychrophilium*	*salimonicida -1*		*salmonicida-2*	*E. ictaluri*
	15°C	30°C	15°C	RT	30°C	15°C	RT	30°C	30°C	RT
6G11	-	-	-	+	+	+	?	+	-	-
9H6	-	+	-	+	+	+	+	+	+	+
RA203	+	+	+	+	+	+	+	+	-	+
RA24	-	+	+	+	+	+	-	-	-	-
RA54	-	+	-	+	-	-	+	-	+	+
RA165	-	+	+	+	+	+	-	-	+	-



OPPORTUNITES AND CHALLENGES IN DEVELOPING A GERMPLASM REPOSITORY PATHWAY FOR THE SEA HARE, *Aplysia,* GENERALIZABLE TO OTHER MARINE INVERTEBRATES

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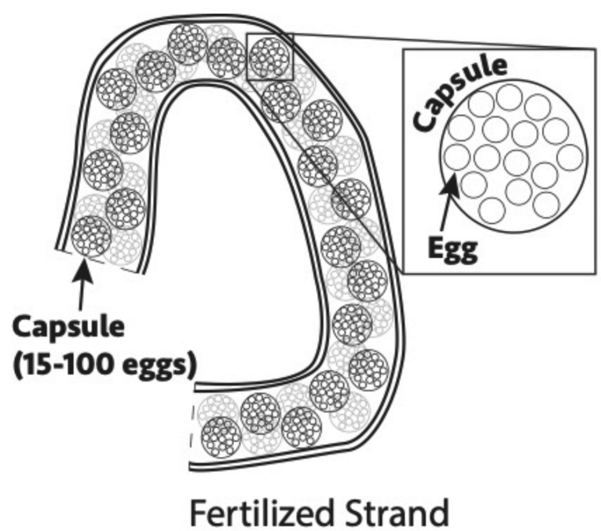
The safeguarding of economically relevant agricultural species has been driven by storing, evaluating, and distributing genetic resources as cryopreserved germplasm maintained in repositories. However, the shift to cryopreservation has been slow within the broader scientific community, especially for fish and invertebrates. To advance within aquatic species, it will be advantageous to develop generalizable pathways that can provide a foundation for repository development and a means for addressing cross-taxa challenges. Aquatic biomedical model organisms and imperiled species are examples of groups with great needs to begin broadening the scope of germplasm repositories. The California brown sea hare, *Aplysia californica*, is a biomedical model largely used to examine neural development, behavior, and aging. There are many challenges associated with maintaining *Aplysia*, for example, their production of copious mucus and toxic ink expulsion, which require flow-through aquarium systems or extensive filtration. In addition, culture of multiple algal species is required for feeding, and inbreeding is considered to have detrimental effects on development in this and many other species. Repository storage of frozen material will provide opportunities for the research community to preserve genetic diversity and to create and maintain mutant and transgenic lines. Repository development itself presents challenges, and there are specific problems related to tissue types and developmental stages. A relevant example is the encapsulation of multiple embryos within capsules and semi-rigid strands produced by many gastropods (Figure 1). In collaboration with the National Resource for *Aplysia* (NRA, University of Miami), our goal is to develop a generalizable cryopreservation pathway, including quality management and economics, that can be applied to this species, with the intention of extending the pathway to other aquatic invertebrates such as oysters and imperiled corals.

FIGURE 1. *Aplysia* enclose a variable number of eggs (15-100) within capsules that are helically organized within a semi-rigid strand. Typical cryopreservation focuses on freezing of single cells (e.g., sperm) but becomes more challenging as the number of cells and internal compartments increase. Organisms that lay eggs enclosed within a strand present new challenges as the embryonic and larval stages are multicellular and the capsule and strand inhibit penetration of cryoprotective molecules.



USING NEURAL NETWORKS TO BETTER UNDERSTAND THE BIODIVERSITY NEAR KELP FARMS

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Seaweed aquaculture has myriad benefits running the gamut from meeting increasing seafood demands to enriching ecosystems by producing more oxygen-rich waters around them. Taking into account the current surge of interest in eco-friendly fuel sources, kelp has the potential to replace gasoline, if cultivation can be efficiently scaled up. Kelp farms have been shown to boost marine biodiversity in nearby waters similar to kelp forests, so kelp cultivation could benefit local wild populations and potentially fisheries as well, through added habitat, but the tools to understand these relationships in situ remain underdeveloped.

Differentiating marine species from kelp can be efficiently achieved by applying object-detection techniques to underwater visual data. Neural networks have yielded promising results in numerous fields and applications, including natural language processing and medical imaging. Object detection in videos is one of the more popular applications, for which the current industry standard is the You Only Look Once (YOLO) algorithm. YOLO is a convolutional neural network which detects objects in real time and assigns probabilities of likelihood to the detected objects. Prior literature has used YOLO-based neural networks for object detection, although not for assessing the impact of seaweed aquaculture on marine biodiversity.

Our aim was to classify fish and kelp in underwater video data using the YOLOv5 model. Video data obtained from a test farm in the Santa Barbara channel were captured by the UCSB Coastal Oceanography and Autonomous Systems Lab. Because the fish do not stand out in the video frames as clearly as the kelp, we made adjustments to a large pre-trained YOLOv5 model and retrained the model using sampled video frames from the kelp farm. Figure 1 shows example results from testing the trained model. Through this work the network's confidence levels have been improved and the technique has been applied to multiple species of fish to identify which species are most commonly found associated with seaweed farms. Classifying fish and kelp provides a better understanding of the biodiversity near kelp farms, increasing the importance of kelp farms to the marine ecosystem, and should in turn inform management of sustainable farming practices.

Figure 1 YOLOv5 Results with Confidence Levels

PHYTONUTRIENT FEED ADDIVITE IMPACT ON FISH FILLET FLAVOR

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A research study was conducted at a contract research facility located in Stuttgart, AR and the sensory lab located in Marshall, MN with a third-party sensory research company, to evaluate the effects of an essential oil feed additive on fish fillet flavor. ONE CURRENT® is a proprietary feed additive that contains a patent pending blend of phytonutrients including Microfused® Essential Oils, Actifibe® Prebiotic and *yucca schidigara*. The product is a natural daily feed additive that increased fish growth, decreased mortality during challenge and enhanced protection against disease demonstrated in previously published research.

Hybrid striped bass are considered a mild white fish that produce a fillet with a delicate, slightly sweet flavor. These fish are raised in conditions that ensure adequate aeration and strict water quality control to ensure consistent flavor. They are considered versatile fish adaptable to most cooking methods and popular in white tablecloth restaurants. Because of their delicate mild flavor, researchers wanted to ensure that including an essential oil-based feed additive would not impact the flavor of the fish fillets.

Multiple studies have been conducted looking at the impacts of essential oils on meat quality and organoleptic properties of food including chicken breast meat and found no difference in the flavor of breast meat between birds fed various combinations of essential oils as compared to a control diet or found positive impacts on chicken breast flavor and acceptability.

This study was conducted to determine if the combination of essential oils in ONE CURRENT® impacted the organoleptic properties of hybrid striped bass fish fillets after being fed a diet containing the feed additive for 48 days.

After the 48-day feeding portion of the study, the fish were euthanized, filleted, flash frozen and shipped overnight to the sensory lab. Participants were recruited for a blinded sensory evaluation test and served fish fillets prepared with no seasoning or breading to equally compare samples. Under the conditions of this study, adding the phytonutrient feed additive to a standard commercial diet fed to fish for 48 days had no impact on fish fillet flavor.

Figure 1.0

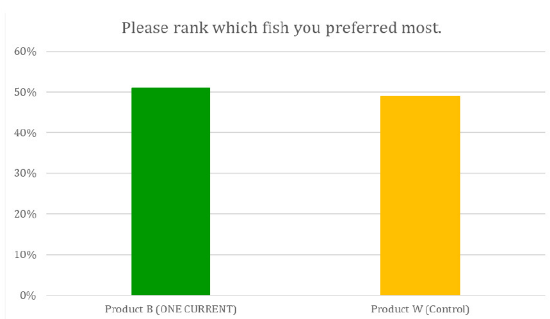
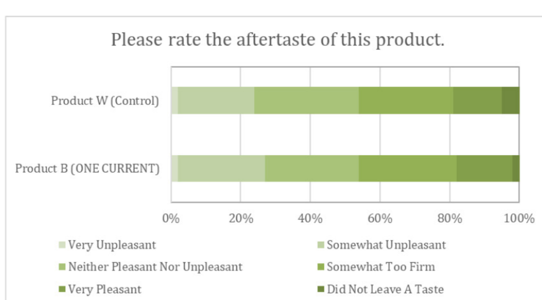


Figure 2.0



US OFFSHORE MARICULTURE: SUSTAINABLE FINFISH CULTURE THROUGH WELFARE MANAGEMENT AND ENVIRONMENTAL MONITORING

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Aquaculture remains the fastest growing food sector in the world and marine aquaculture continues to grow at about 1.5% annually. The US has been slow to adopt this growing sector of food production and continues to import over 80% of US seafood.

Blue Ocean Mariculture has spent the last decade on a mission to provide healthy, sustainable, delicious seafood produced in the USA. Working with the State of Hawaii, Blue Ocean Mariculture has created a model for sustainable offshore mariculture by developing, testing, and monitoring the production of Hawaiian Kanpachi (Seriola rivoliana) off the Island of Hawaii. Through the employment of developing technologies, strong best management practices, and robust monitoring, Blue Ocean has managed to scale its business while maintaining balance with the surrounding ecosystem.

This effort is to provide an update on the status of Blue Ocean Mariculture's offshore finfish farm, provide an overview of our current operation, and share some of the breakthroughs that have allowed our farm to grow.

THE PROGRESS AND FUTURE OF AUTOMATED PLANKTON AND WATER QUALITY MITIGATION STRATEGIES IN A CHANGING CLIMATE

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Growth in the aquaculture sector has been accelerated by new technologies that enable farmers to operate more efficiently. However, the impacts of climate change are introducing new challenges to ocean farmers including, increasing global sea surface temperatures, increasing incidence and severity of hypoxia and more intense and frequent precipitation events, storms, and heat waves often leading to more serve harmful algae blooms. Each of these impacts present operational challenges that farmers must navigate to protect their livestock and must overcome to ensure they are working toward more efficient and sustainable production cycles.

Despite these challenges, both industry and scientific researchers are working on novel technologies that are helping farmers better manage their sites. Higher water temperatures reduce the capacity of water to hold oxygen and increase fish's metabolic demands. Many farmers face low oxygen conditions in the summer season, particularly when fish are close to harvest size. In response to this, many sites have invested in substantial aeration and oxygenation injection systems that can provide life support to fish to ensure oxygen levels remain above a viable threshold. Not only this but there is an increasing trend in the number of semi-enclosed systems that require oxygen injection to provide a suitable habitat for fish. Producers are quickly realizing that these new technologies allow them to not only provide suitable habitat but by super saturating the environment with oxygen they can shorten grow-out cycles. These technologies are not only being applied to improve water quality but in the management of harmful algae that can cause major health issues and mortalities on farms.

Oxygen injection and aeration systems are a significant investment and can be very costly to operate – in particular, against harmful phytoplankton where systems can often be left running round-the-clock to protect against unknown threats. Automated and data-controlled mitigation systems are the future of this technology to ensure that systems are operating at the highest efficiency level and only when necessary. To achieve this, farms must adopt widescale environmental monitoring for individual pen control and continue to digitize operational farm metadata that can be fed into algorithms to achieve smart control systems.

ORAL DELIVERY OF A VACCINE AGAINST STREPTOCOCCOSIS IN NILE TILAPIA

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The injection route is the most common method used for the administration of commercial vaccines but it presents some drawbacks; it is time consuming, requires qualified personnel and may induce local reactions at the injection site. Mucosal vaccination is a good alternative and allows to develop protective local immunity in order to struggle against infectious diseases. Oral administration of vaccines is particularly suitable for protection of breedings with high density of animals like in fish farming. The main challenge linked with this route of administration is to preserve the integrity of the antigen from the harsh environment created by the digestive system to reach gut associated lymphoid tissue (GALT) in order to induce a potent local immune response. This issue could be gotten around by using an adapted adjuvant.

The Gastro-Resistant Adjuvant (GRA) tested here, is an oily adjuvant containing a gastro-resistant matrix to protect antigen from gastric degradation. Indeed, an *in vitro* experiment showed GRA formulated with BSA was able to retain the antigen in acidic media and release it under neutral pH conditions. Then, a study in tilapia was performed on a *Streptococcus agalactiae* vaccine formulated with GRA and mixed at 2% or 20% with feeding pellets. The tilapia have been orally vaccinated under 2 periods one week apart as follows : first, during 4 consecutive days, then during 3 other days. Each vaccine group was constituted with 3 replicates of 40 tilapia. At D49, the tilapia were challenged with *S. agalactiae* serotype III by intraperitoneal route. The protective rate of GRA groups has reached 91.1% and 88.9% for vaccines formulated at 2% and 20% respectively while it was only 73.3% in the control group (vaccination with non-formulated antigen).

Taken together, these results show that GRA is well adapted for the oral administration of vaccines in tilapia with a good palatability, safety and efficacy. However, more data is needed especially in a large-scale trial and also for cold water fishes to confirm these first observations before considering other animal species.

DISCOVERY AND CHARACTERIZATION OF EASTERN OYSTER *Crassostrea virginica* CIS-DEFENSIN ANTIMICROBIAL PEPTIDES

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Defensins are known and important antimicrobial peptide effectors essential to innate immunity. While previous proteomic and genomic studies revealed multiple small members of the β-like *cis*-defensin family in *Crassostrea gigas* and other commercially important bivalve molluscs, no cis-defensin genes have been identified from the *C. virginica* reference genome. A bioinformatic approach including a custom profile hidden Markov model was applied to search the *C. virginica* transcriptome for defensin motifs and signal peptide sequences. A multigenic cluster of six cis-defensins previously described as noncoding RNA was identified, as well as two additional cis-defensins residing on separate chromosomes. Included in this discovery is the gene that codes for American Oyster Defensin, an antimicrobial peptide isolated in 2005 using a protein chemistry approach that had eluded genome mapping until now. The predicted peptide structures indicate that seven of the defensins contain three disulfide bonds and one defensin has four disulfide bonds, and all show strong sequence and structural homology with known antimicrobial defensins. Quantitative gene expression analyses confirm differences in defensin mRNA expression among tissues and following immune stimulation. The diversity of cis-defensin sequences in the eastern oyster and high sequence and structural polymorphism may be important for the oyster's adaptation and resistance to multiple potential pathogens. Additionally, these antimicrobial peptides may constitute a potentially rich source of antibacterial or antiviral compounds for use in aquaculture and human medicine.

BEYOND RESTORATION AQUACULTURE: TESTING THE PROPAGATION, REARING AND DEPLOYMENT OF FRESHWATER MUSSELS FOR WATER QUALITY ENHANCEMENT OF IMPAIRED HABITATS

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Freshwater mussels can furnish diverse and valuable ecosystem services in rivers, streams and lakes when they are healthy and abundant. The service generating the greatest interest is their propensity for filtering large quantities of microparticulate seston, thereby improving water clarity and potentially removing or transforming particulate pollutants such as nitrogen, phosphorus and some human pathogens. The magnitude of these services depends on complex relationships among the population biomass, physiological status, temperature, seston quantity and composition, and system hydrodynamics. The species diversity, abundance and distribution of natural mussel populations has been in steep decline worldwide, however. Hatchery propagation and aquaculture practices are seen as useful tools to help stem the decline, driven foremost by conservation interests and laws (e.g., the Endangered Species Act in the United States). In the last 10 years, there has also been a growing interest in the restoration of natural mussel assemblages that form mussel beds, driven by emerging interest in ecosystem services (e.g., the Clean Water Act in the U.S.).

The significance and viability of these restoration efforts is an active area of research, but the prospects for recovery of natural populations appears to be plausible at least for some areas thanks to advances in the restoration aquaculture of freshwater mussels. Thinking beyond the restoration of natural assemblages in natural settings, can freshwater mussels be deployed in designed, nature-based systems aimed at delivering specific ecosystem services where those services are most needed? Results from ongoing projects suggest that hatchery-propagated mussels of two species native to the Delaware River Basin can persist, grow and contribute water quality benefits in various man-made aquatic systems, including a living shoreline, impoundment, reservoir, and stormwater ponds (Table 1). When their requirements are met, freshwater mussels can therefore be included in engineered aquatic systems, helping to address water quality impairment closer to pollutant sources and in urban landscapes where traditional restoration practices are constrained.

Table 1. Daily increase in shell length of juvenile *Utterbackiana implicata* held in man-made aquatic habitats during two different study periods.

Habitat	2017-2019 Growth Rate (mm/day)	2020-2021 Growth Rate (mm/day)
Reference Pond (Winterthur, DE)	0.06	0.02
Water Reservoir (Green Lane, PA)	0.10	
River Impoundment (Van Sciver, PA)	0.06	
Stormwater Pond (Talley Day, DE)		0.07
Stormwater Pond (Rockwood, DE)		0.06
Stormwater Pond (Papermill, DE)		0.04
Stormwater Pond (Airport, DE)		0.03

DETECTION OF FISH FARMING EQUIPMENT USING 3D MULTIBEAM SONAR

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Aquaculture food production grows faster than other major food production sectors, and in the Faroe Islands, salmon accounts for nearly half of the countries export value. In order to to keep up width the global trend, fish-farming in the Faroe Islands has moved from sheltered locations, to more exposed farming sites. Understanding the behaviour of fish-farming equipment and their inhabitants at exposed sites is important for ensuring fish welfare, and by extension, profits. Measurement equipment and methods make used today make it difficult to obtain an accurate description of the spatial extent and dynamics of the cage and the fish distribution and movement within, since the cages are very large and in exposed sites are subject to large deflections and deformations. Sonars have a comparatively high range, compared to optical cameras, but lack the ability to measure in different direction. Multibeam sonars allow for spatial information of its surroundings to be gathered, both in the spatial and temporal domain.

In this study we investigate imaging a fish-cage with a iXblue Seapix 3D multibeam sonar. The sonar is suspended 2 meters from the water surface, and 2 meters from the side net of a fish-farm. The preliminary results show that the extent of the cage can be determined, and within the first 10m, individual fish can be seen.

Since we have verified the information of cage extent and fish distribution can be found using this method, the next step is to develop a cage detection method that utilizes nearby angles as well as multiple pings. Combining these will result in a 3d model of the cage, from witch, the volume can be calculated.

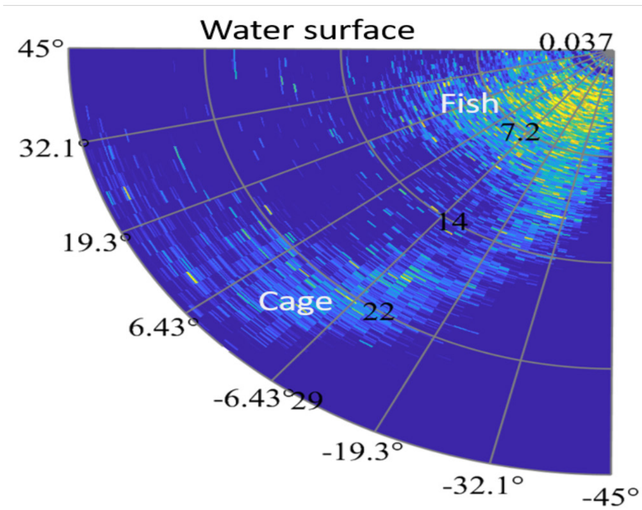

Figure 1 Sv at different angles. Higher intensity signifies stronger backscatter.

EFFECT OF DIFFERENT COLORED LED LIGHTS ON BFT CULTURE SYSTEM OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Several factors directly influence the formation and maintenance of bioflocs, such as the carbon/nitrogen ratio, oxygenation, carbon sources, light, etc. Light is considered an extremely important abiotic factor for organisms that live in the aquatic environment. Studies show significant differences in behavior, growth, food intake, maturation, reproduction, and possibly changes in swimming activity of peneid shrimp when exposed to different light conditions. For microorganisms, often especially when exposed to sunlight, it can change abruptly from a heterotrophic system (dominated by bacteria and protozoa mainly) to a predominantly photoautotrophic system (dominated by microalgae). This study aimed to evaluate the growth performance of *Litopenaeus vannamei* reared in a BFT system with supplementary colored LED light.

Five treatments were designed with 3 repetitions each, using colored LED lights, namely: yellow, blue, red, green and white. The experimental units were isolated from each other using a conical lid over the tanks, so that the influence of a color on the other would not occur. Animals weighing 0.37 g were stored at a stocking density of 500 shrimp/m^3 in experimental units of 150 L. The experiment lasted for 70 days.

Significant differences were found for water quality parameters such as nitrite, light penetration into the water column and total amount of water used during culture period. No statistical differences were found for temperature, dissolved oxygen, pH, ammonia, nitrate, phosphate, alkalinity, CO_2, total suspended solids, settleable solids, turbidity and chlorophyll *a*. The total amount of water used differ significantly, the colors of green, blue and red lights used lower amounts of water (340 liters), comparing with white and yellow lights (495 liters)(Figure 1). This difference in the total amount of water for production is related to the number of water exchange that were carried out when nitrite levels exceeded the safety levels for the specie (Figure 2). The color of green and blue light had better nitrification when compared to white light (control).

The present study demonstrated that the production of *Litopenaeus vannamei* exposed to green light showed significantly better results in water quality and zootechnical parameters when compared to other colors, we are currently analyzing the microbial community and oxidative stress in different treatments in order to elucidate these questions.

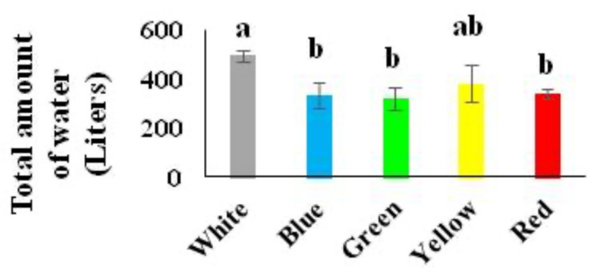

Figure 1 - Mean values (± SD) of total amount of water (liters)

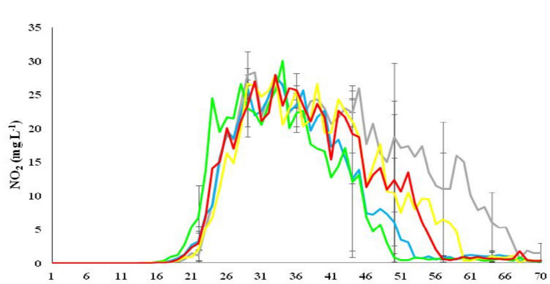

Figure- 2 Mean values (± SD) of nitrite (mg L-¹)

EFFECTS OFCOPEPODS *Apocyclops panamensis* ON THE MICROBIAL AGGREGATES ENRICHMENT IN *Litopenaeus vannamei* reared IN BFT SYSTEMS

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Copepods are important organisms in the food chain of aquatic environments, and their excretion acts as a natural substrate for bacterial production. These excretions create a nutrient- and carbon-enriched microhabitat that encourages bacterial growth. In a Biofloc Technology (BFT) system, the addition of copepods can be an advantageous strategy, as their abundance and bacterial productivity can mean improved water quality and better zootechnical performance; added to the fact that copepods can be a suitable nutrient source for cultivated shrimp. The aim of this study was to evaluate the influence of the addition of copepod *Apocyclops panamensis* on the culture of Litopenaeus vannamei reared in a BFT system.

A 60 day-trial was conducted at the VSAREC in Hampton, VA. After nursery, Pacific white shrimp (0.056±0.003) juveniles were stocked at 500/m^3 in nine, 300 L circular tanks. Three treatments (three replicates) were tested: T1 – Clear water and copepod addition; T2 - BFT and copepod addition and; T3 – BFT without copepod addition. The copepod density was keep at 5000 / L (the number of copepods in tanks were checked once a week). Shrimp were fed with a commercial diet (provide by Zeigler Bros., Gardners, PA). Weekly rations were adjusted based on shrimp consumption and growth performance. Tanks were maintained with no water exchange through the duration of the study except for CW treatment where 100% of water was change 3 times per week. Water temperature, salinity, dissolved oxygen (DO) and pH was recorded twice daily (0800 and 1600. Water was tested daily for measuring TA-N. Monitoring of NO_2-N, and NO_3-N was done every three days, while alkalinity was measured once a week. Total suspended solids (TSS) and settleable solids (SS) were measured once a week. Every week, 30 shrimps were randomly sampled from each tank and individually weighed. At the end of the trial, total shrimp biomass along with individual weights of the total of survival from each tank were recorded. Water quality parameters were compared by two-way repeated measures ANOVA (system type). Significant differences of P<0.05 was used in all zootechnical performance, Tukey's multiple-range test was applied when significant differences were detected.

The presence of copepod provided better fixation of nitrifying bacteria resulting in a better stabilization of nitrogen compounds in treatments with copepod addition (Figure 1). In the zootechnical performance there was no difference between treatments, even in clear water (Table 1).

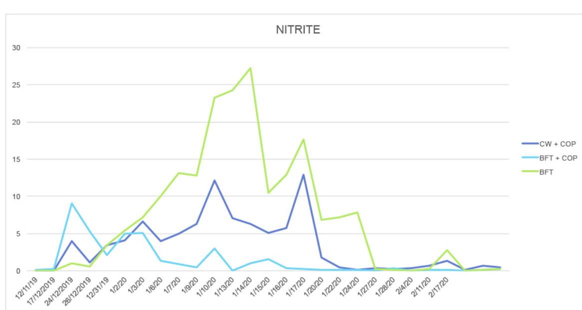

Figure 2. Mean values of nitrite (mg L-¹)

Table 1 - Mean values (± SD) zootechnical parameters

	CW + COP	BFT + COP	BFT
Initial size (g)	0.056±0.003	0.056±0.003	0.056±0.003
Final Size (g)	11.82±1.56	11.85±2.08	11.74±2.12
WGR (g)	1.37±0.41	1.37±0.17	1.30±0.30
Productivity	5.07±0.14	4.67±0.86	4.23±0.93
Survival (%)	91.25±4.57	83.54±6.53	74.14±4.57

GUT MICROBIOME AND DISTAL INTESTINAL MORPHOLOGY MODULATED BY DIFFERENT DIETARY LEVELS OF CARBOHYDRATE AND LIPID WITH OR WITHOUT BILE ACID SUPPLEMENTATION IN LARGEMOUTH BASS

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Carbohydrates are cheaper ingredients source to satiate the energy requirements of fish. Fish oil is a limited resource that needs to be reduced or replaced in fish feeds by using sustainable alternatives. Therefore, the goal of this study was to improve the carbohydrate (CHO) utilization by reducing lipid inclusion in the diets and assessing whether dietary bile acid (BA) supplementation enhanced lipid utilization. Hence, three variables such lipid and CHO inclusion level and bile acid supplementation, led to a 2 × 2 × 2 factorial experimental design. Largemouth bass juveniles (average weight: 6.0 g) were fed (8 isonitrogenous diets: 45% crude protein) different combinations of dietary high (HF: 12%) and low fat (LF: 9%) and high (HS: 30%) and low starch (LS: 20%) levels with or without bile acid (BA) supplementations at 1% for 8 weeks.

Growth performance of fish was significantly affected by dietary treatments. The weight gain of individual fish was highest in the LF/HS-BA treatment, which was significantly higher than the other diets with BA supplementations. In terms of gut microbiome results, alpha diversity was significantly unaffected by different level of fat and carbohydrate among the treatments, however a significant increase was observed by the addition of bile in the HF-LS diet. Moreover, linear mixed effects model analysis revealed significant effects coming from the starch levels on the richness of the gut communities (P=0.02), indicating decreasing richness with increasing starch levels (Figure 1A). Looking at the beta-diversity (microbial composition), the microbial communities were affected by both the bile addition (P=0.036) and the interaction between fat, starch and bile addition (P=0.01), as indicated by Permanova and Principal Coordinate analysis (Figure 1B). Among the genera, Clostridium was the most abundant taxon, followed by Lactobacillus, Paraclostridium, Plesiomonas, Candidatus Arthromitus, Cetobacterium and Lactococcus. Data for distal intestinal morphology will be presented.

Conclusively, dietary supplementation of bile acid in high dietary carbohydrate fed group can improve the growth performance and gut health via maintaining the gut microbial community.

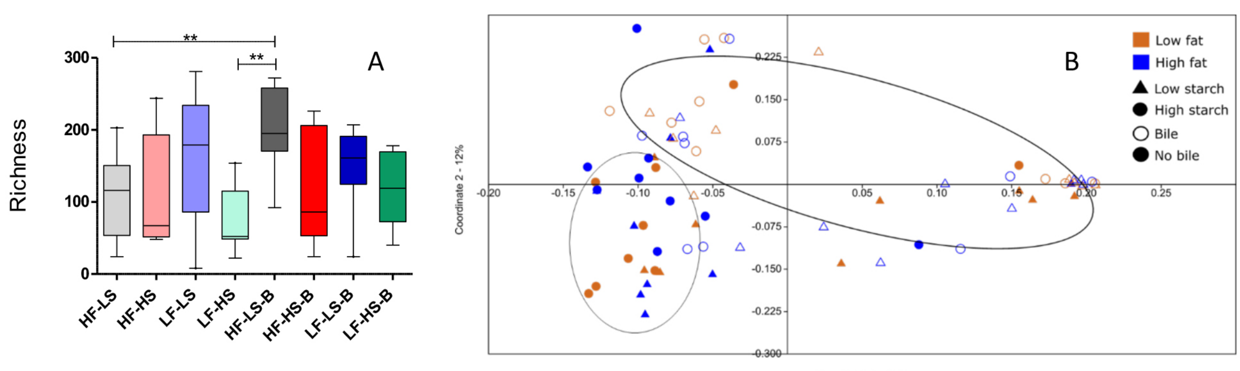

Figure 1. Alpha diversity indices between the different diets (A). Principal Coordinate Analysis using Bray-Curtis as metric. There was a significant clustering due to bile addition (P<0.01) (B).

DIETARY SUPPLEMNTATION OF WHOLE AND/ PARTIALLY DEFATTED BLACK SOLDIER FLY LARVAL MEAL ENHANCES THE PLANT PROTEIN (SOYBEAN MEAL) UTILIZATION IN RAINBOW TROUT

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Soybean meal (SBM) is one of the most commonly used alternative plant-based ingredients to replace marine derived fish meal (FM) in aquafeed. Relatively high protein content and favorable amino acid profile of SBM approaches the nutritional requirement of many cultured species. However, high soybean meal inclusions in aquafeed induced enteritis (SBMIE) and ultimately reduce the nutrient utilization and decrease the growth performance of carnivore species. Though, there is an alternative approach to mitigate the enteritis (distal intestine inflammations) by using the complementary feed ingredient such as “black soldier fly larval (BSFL) meal”. Our goal was to evaluate the effects of whole and partially defatted black soldier fly larvae (WB/PDB) as complementary feed ingredient in soybean meal based diets on growth performance, feed utilization, gut health and immune related parameters in rainbow trout (*Oncorhynchus mykiss*).

Six experimental diets (42% crude protein and 20% lipid): fish meal based diet (FM), SBM based diet (SBM), SBM+2.5% and 5% WB (WB2.5 and WB5) and SBM+2.5 and 5% PDB (PDB2.5 and PDB5) were fed twice at satiation level for 10 weeks. Each diet was fed with four replicate 30 fish (~5 g) per tank (60-L) in recirculatory aquaculture system.

At the end of the feeding trial, supplementation of WB and PDB improved ($p<0.05$) the performance rainbow trout. Feed conversion ratio was negatively correlated with growth performance. Nutrient and moisture content of whole body of fish significantly affected the dietary inclusion of WB and PDB. Gut histology, immune function related genes would be presented. In summary, the insect protein might act as an additive for plant-based diet for sustainable carnivore fish production.

HYDRAULIC IMPACT ON FISH MIGRATION IN SARIAKANDHI FISH PASS OF BANGLADESH

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The importance of open water fish in our socio-economic regime has recently drawn the attention of the policy makers of the country. FCD/FCDI projects mainly serve the agricultural interests, but it interfere fish migration. This inevitably affects the open water fisheries sector as migratory routes. Nursing grounds of many species of fish are hampered and disturbed for these projects also. In order to permit fish migration in rivers, it is necessary to maintain conditions that help migrants reach their spawning grounds. To overcome obstacles, such as hydraulic structures, placed in the path of migrating fish, structures must be designed to assist the fish to pass them. The periodic and directed travel of fish mainly for feeding, breeding and over coming adverse climatic conditions is called migration. Fish passes are constructed to allow normal breeding migration and to ensure natural route of fish movement.

The concept of a fish passes is relatively new in Bangladesh. At present, two Fish passes and two fish friendly structures are constructed. These are Fish Pass in Jamuna to Bangali River at Sariakandi in Bogra, fish Pass in Kawadighi Haor of Monu river in Moulovibazar, fish friendly structure in Lohajong river of Tangail and fish friendly structure at Morichardanra in Chapainawabganj. Fish fry, spawning and hatchling movement from Jamuna to Bangali River was the main objective of Sariakandi Fish Pass Project. The Fish Pass Project of Sariakandi is necessary for the development of the dominant fishes like catfish and small fishes. The structures will also aid in efficient development of the carp fishes. Spawning migration, mainly in carp fish, in the study area was found to begin at the 2nd week of May and continue up to the 3rd week of July. Catfish migrations began at the last week of March and continue up to the 2nd week of June.

Fish fry and hatching movement from Jamuna to Bangali river was the main objective of Sariakandi fish pass project. The study also found that there were seven major category migratory species in the project area and the fish pass is contributing positively for growth of fishery resources in then study area. During the monsoon carp fish is the dominating migratory species. Carpfish migrates in a higher velocity, whereas, catfish migrates in a lower velocity. Some problems were found in the operation and management of fish pass.

WSSV IMPACT ON GUT MICROBIOME OF THE BLACK TIGER SHRIMP *Penaeus monodon*

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The gut microbiome plays an important role in the immune system of invertebrates and vertebrates. Pre- and pro-biotic dietary could improve gut microbiome and benefit to host. It has been reported that pre- or pro-biotic could enhance shrimp immune system by increasing activities of phenoloxidase (PO), prophenoloxidase (ProPO) and superoxide dismutase. During viral infection, alteration of the host immune system could influence the gut microbiome and possibly give a chance to other pathogens. In this study, intestine immune genes of STAT-silenced shrimp were investigated. During WSSV infection, expression levels of *Pm*Vago1, *Pm*Doral and *Pm*Spätzle in STAT silenced shrimp were increased as compared with WSSV infected shrimp. The transcription levels of antimicrobial peptides including crustin*Pm*1, crustin*Pm*7 and *Pm*PEN3 were higher in WSSV challenged *Pm*STAT silenced shrimp, compared with that of normal shrimp infected with WSSV. Meanwhile, *Pm*STAT suppressed *Pm*ProPO1, *Pm*ProPO2 and *Pm*PPAE1 expressions during WSSV infection. Moreover, microbiome of *P. monodon* during WSSV infection has been studied. The result revealed that the relative abundance of phyla *Bacteroidetes, Actinobacteria* and *Planctomycetes* reduced in WSSV challenged shrimp. At the species level, the abundance of *P. damselae,* a pathogen human and marine animals, was significantly increased in WSSV challenged shrimp.

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.

QUANTIFYING IMPACTS OF FLOATING OYSTER AQUACULTURE ON NITROGEN CYCLING IN A SOUTHEASTERN MASSACHUSETTS COASTAL EMBAYMENT

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Estuaries worldwide are increasingly degraded by anthropogenic nitrogen (N) inputs, primarily from their watersheds. In southeastern Massachusetts (MA), several municipalities are implementing floating oyster aquaculture (FOA) in their estuaries as a bioremediation method to increase N removal through assimilation into oyster biomass, sediment burial, and enhanced sediment denitrification. Denitrification associated with FOA was quantified in a small Cape Cod tidal salt pond to determine the effectiveness of this nontraditional method. Dinitrogen gas, oxygen, and nutrient fluxes in sediments were measured inside (treated) and outside (control) of bottom areas affected by oyster biodeposits. Enhancement of microbial denitrification within the treated area (treated) was observed during each of the three years of the study and resulted in 5.0–12.1 kg N removal per year over control areas. The greatest mean denitrification rates were observed in sediment cores collected within the biodeposit area (treated) during July 2018, followed by August 2016, and then August 2017 (Table 1). We observed significant denitrification enhancement on 70% of the flux dates (Table 1).

The extent of denitrification enhancement varied between seasons and years; enhanced denitrification totaled 2.8, 2.4, and 3.3 g N_2-N m^{-2} for the summer and fall months of 2016, 2017, and 2018, respectively. Differences in enhanced denitrification were attributable to interannual and seasonal differences in N deposition and bottom water dissolved oxygen and nitrate + nitrite concentrations. Measurements indicated that denitrification enhancement can be increased by modifying oyster stocking density within each floating bag and density of bags over pond surface area, thereby controlling the spatial extent and intensity of N biodeposition to surficial sediments. These data are necessary to assess the efficacy of using FOA to reach N reduction goals within shallow depositional coastal systems.

Table 1 Average core incubation temperature (°C), mean ± SD denitrification (mmol N_2-N m^{-2} day^{-1}) and nitrogen (N) efflux (mmol N m^{-2} day^{-1}) measured in sediment cores collected inside the biodeposit area (treated) and outside the biodeposit area (control). The difference between the two rates represents denitrification enhancement in biodeposit affected sediments above background rates. The percentage of the total N denitrified is the portion of measured total N efflux that was N_2-N

Project Year	Date	Temperature (°C)	Denitrification (mmol N_2-N m^{-2} day^{-1})			N Efflux (mmol N m^{-2} day^{-1})		Percent N Efflux Denitrified (%)
			Treated	Control	Enhancement	Treated	Control	
Year 1	Aug ’16	27.3	3.0 ± 1.1	1.7 ± 0.3	**1.2 (69%)**	22.4 ± 12.0	8.5 ± 2.3	21 ± 12
	Oct ’16	16.2	2.8 ± 1.1	1.7 ± 0.7	1.1 (62%)	6.6 ± 2.6	3.7 ± 0.7	31 ± 14
	Apr ’17	10.5	2.7 ± 1.7	0.9 ± 0.3	**1.8 (202%)**	2.5 ± 2.5	1.0 ± 0.3	70 ± 32
Year 2	Jun ’17	22.2	1.3 ± 0.4	0.3 ± 0.4	**1.0 (343%)**	9.0 ± 5.2	6.2 ± 3.3	12 ± 8
	Aug ’17	24.1	2.1 ± 0.9	1.6 ± 0.8	0.5 (29%)	12.2 ± 7.1	7.1 ± 3.1	18 ± 9
	Sep ’17	22.9	0.7 ± 0.9	0.2 ± 0.1	0.5 (236%)	8.6 ± 5.1	6.4 ± 2.0	7 ± 11
	Oct ’17	17.7	1.5 ± 0.9	0.7 ± 0.4	**0.8 (107%)**	4.8 ± 2.5	1.6 ± 0.7	29 ± 15
Year 3	Jul ’18	23.7	3.3 ± 2.5	1.2 ± 0.4	**2.2 (186%)**	16.6 ± 10.2	9.9 ± 3.3	14 ± 7
	Oct ’18	18.8	0.5 ± 0.3	0.2 ± 0.3	**0.4 (196%)**	4.4 ± 2.4	2.2 ± 1.4	9 ± 4
	Apr ’19	12.0	1.8 ± 1.2	0.3 ± 0.5	**1.4 (354%)**	2.0 ± 1.4	3.3 ± 1.4	37 ± 19

Note: Boldface P indicates significance at $\alpha = 0.07$, which we chose given the heterogeneity of the biogeochemical fluxes.

CONNECTING SEA GRANT, NCCOS, AND COASTAL STAKEHOLDERS TO IMPROVE SUSTAINABLE AQUACULTURE SITING AND DEVELOPMENT

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Recent efforts focus on identifying Aquaculture Opportunity Areas (AOAs) in U.S. waters as Congressional interest in improving aquaculture regulation increases. Whether inshore or offshore, sustainable aquaculture wades into crowded waters where multiple, complex layers of stakeholders are present. The National Centers for Coastal Ocean Science (NCCOS) — and particularly the Coastal Aquaculture Siting and Sustainability Program (CASS) — are well equipped to meet these challenges and have created many resources to aid decision making by coastal ocean stakeholders. Key to a science-based approach, these NCCOS tools and services often use marine spatial planning as a continually evolving method to analyze and address the challenges of ecosystem and human interactions in coastal ocean areas.

This presentation describes a new project that will build the capacity of the Sea Grant Network to assist stakeholders to use and be informed by coastal science products and resources developed by NCCOS CASS. We propose a comprehensive approach that will build capacity and collaboration among Sea Grant (SG), NCCOS, and other coastal ocean stakeholders for environmentally, economically, and socially equitable aquaculture development. Central to this work is the creation of a National Aquaculture Extension Coordinator position to oversee extension of NCCOS resources, inform broad SG/NCCOS marine spatial planning efforts, and facilitate a series of collaborative, regionally-tailored workshops to advance aquaculture siting conversations. This four-year project and its workshops will take place in the Mid-Atlantic (Summer 2022); Gulf of Mexico (Winter 2023); Southern California (Fall 2023); Pacific Northwest (Summer 2024); Pacific Islands (Fall 2024); and New England (Spring 2025). Through this approach we aim to complete three project objectives: (1) Extend the reach of NCCOS aquaculture planning resources; (2) Improve Sea Grant – NSGP – NCCOS – stakeholder connections through regional workshops that co-create aquaculture siting and development roadmaps; and (3) Inform broader Sea Grant/NCCOS marine spatial planning efforts.

METAGENOMIC ANALYSIS OF THE INTESTINAL MICROBIOTA IN THE GREEN ABALONE *Haliotis fulgens* FED WITH ABKELP® FORMULATED DIETS AT DIFFERENT pH LEVELS

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Specifically, the green abalone *Haliotis fulgens* is one of the species that stands out in Mexico due to its commercial exploitation. This is an endemic species of the Baja California Peninsula of fishing importance and with great aquaculture potential. There is evidence that the formulation and preparation of diets with acidic pH favors their stability, allowing greater use and a decrease in production costs. This study investigated the intestinal microbiomes of juvenile green abalone *H. fulgens* (2.17 ± 0.04 g; 2.61 ± 0.08 cm) with a formulated diet ABKELP® isoprotein (15.52%) and isolipidic (1.51%) with three pH levels (5, 7, 8) and a control with *Macrocystis pyrifera* for 5.5 months. The intestinal samples (Bacteria 16S V3 + V4 gene, Endophytic Bacteria V5 + V7, Archaeal 16S V4 + V5 and Fungal ITS ITS1–5F, Illumina NovaSeq 6000 Systems gene) were sequenced and the intestinal microbiomes were analyzed.

COMPARATIVE TRANSCRIPTOME ANALYSIS BETWEEN *Haliotis rufescens, Haliotis fulgens* AND THEIR INTERESPECIFIC HYBRID

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Hybrids between the red abalone (*Haliotis rufescens;* RR) and green abalone (*H. fulgens;* GG) were produced at the Abalone Laboratory of CICESE with the goal to increase survival and thermal tolerance under culture conditions. Therefore, this study aims at investigating the genetic contribution of each pure species to the transcriptome of the hybrid.

Both parental (RR and GG) and the hybrid abalone (RG) were cultivated under identical conditions for 4 months. At the end of the experiment, whole-fresh tissue of three individuals per replicate were preserved in RNAlater®. Total RNA was isolated with Trizol (Thermo Fisher). After quality checking, three libraries per abalone cross were produced by pooling RNA from 3 – 4 individuals. Samples were sequenced in an Illumina NovaSeq 6000 (2 x 100 bp). The reference transcriptome was generated following the genome-guided protocol from Trinity v. 2.4.0 using the *H. rufescens* genome. Transcript abundance was calculated with Salmon v 1.4.0 and differential expression analysis was made with DeSeq2. Transcripts with an FDR corrected P values < 0.05 and log2FC $> |1|$ were considered significantly expressed.

A multivariate analysis with normalized counts displays a clear separation of RR from RG and GG (Fig. 1), suggesting that the transcriptomic profile of the hybrid RG is more similar GG than to RR. This is supported by the number of differentially expressed transcripts (DET) between each contrast, were the lowest numbers of DETs is observed between RG and GG (Fig. 2). Overall, results suggest a larger genetic contribution of *H. fulgens* to the hybrid than the red *H. rufescens*.

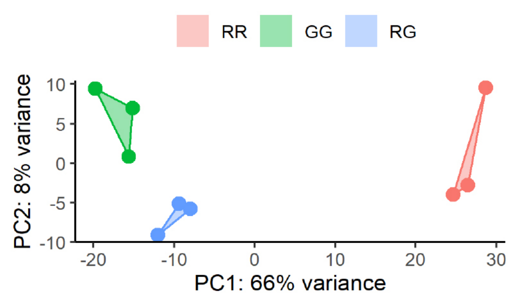

Fig.1: PCA plot

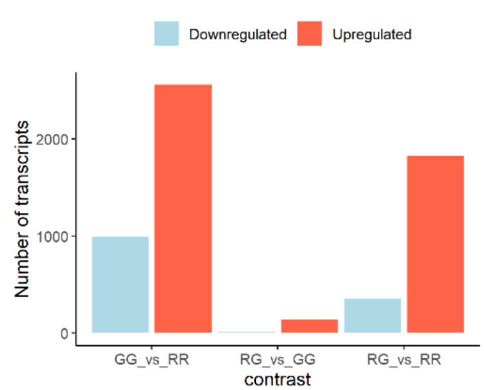

Fig.2: Number of DETs among contrasts

OFFSHORE PEN AQUACULTURE CHALLENGES & OPPORTUNITIES IN SOUTHEAST ASIA – THE U.S. SOYBEAN EXPORT COUNCIL'S SOUTHEAST ASIAN AQUACULTURE PROGRAM WORK

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The U.S. Soybean Export Council (USSEC) is a non-profit marketing agency for international promotion of U.S. soy products. As a part of this effort USSEC has provided technical servicing to the global aquaculture industry for over 35 years. Southeast Asia (SEA) is a key focus area for these efforts and USSEC has worked to improve the conditions for a larger, more efficient and productive feed-based marine fish aquaculture industry in SEA. Through these efforts USSEC hopes to show the superior value of U.S. soy in feeds for marine fish species.

SEA has unique geographical and marketing advantages for marine aquaculture. This region has long coastlines and generally clean offshore waters, and largely sits in the tropical or sub-tropical zone allowing year-round production. There are thousands of islands in the region that provide convenient bases for logistical supply and servicing for developing offshore aquaculture operations. SEA is a seafood processing hub and has close links with international seafood markets and is adjacent to one of the main aquatic product consumption markets, China.

Despite the potentials of a good environment and good markets for marine fish culture, the production volume is still quite low compared to other regions of the world. An important part of this is a need by governments to provide clear approach for zoning, licensing, and security for offshore sites, but also there is a lack of experience and knowledge by producers who are more familiar and comfortable with continuing culture in near-shore, shallow water environments. USSEC has taken a holistic approach to work with the entire marine fish production chain industry to try to educate and promote a shift to offshore pen culture for marine fish in SEA.

USSEC's approach for technical servicing for marine fish aquaculture has included:

- Guidance on hatchery operations and genetic improvement of marine fish species
- Assessment, implementation, and training for offshore marine pen culture
- Workshops with international marine fish pen aquaculture experts
- Work on marine fish health management and disease control
- Offshore pen marine fish feed feeding demonstrations using U.S. soy-optimized diets

The presentation will illustrate USSEC's SEA aquaculture program work in the marine fish aquaculture sector and will discuss the challenges and opportunities in the region. The current offshore cage aquaculture status and impact of Covid-19 pandemic will also be introduced.

VERTICAL AND HORIZONTAL PRICE LINKAGES AND DEMAND GROWTH FOR DIFFERENT SALMON PRODUCT FORMS IN EUROPE

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It has been reported in the literature that the degree of price transmission for salmon is reduced for more highly processed product forms, potentially also limiting market t integration at the retail level. This paper analyzes price linkages for Norwegian exported salmon to Great Britain, Germany, France, Italy, and Spain. We use price data on the export and retail level to analyze price transmission in the most important European salmon markets, as well as market integration between the different product forms in the market. Salmon is mostly exported from Norway as whole fresh with little processing involved. However, the salmon is processed closer to the consumer and the retail level contains a wider range of salmon products. The data shows that in most of these European countries, natural fresh salmon products such as fillets have increased their share of the retail market compared to more processed salmon products. Interestingly, prices of most of the processed products are found to be stationary, while most of the prices of natural fresh products with little processes are nonstationary. This indicates consumers are more willing to absorb price increases of natural salmon products with little processing involved. Using the Johansen cointegration framework, the results show price transmission tend to be greater for less processed products like natural fresh fillet compared to more processed salmon products. An exception is Great Britain where frozen salmon fish fingers have appeared and become a hit during the pandemic. The trends between the countries differ in other ways. If we compare the overall salmon consumption trends from 2014 to 2020, the markets in Great Britain and Italy have grown, Germany and Spain have remained relatively stable, while France has decreased. While the retail price trends are similar across countries there are differences in price levels which sources are not obvious.

HOW BLOCKCHAIN WILL TRANSFORM THE FUTURE OF SEAFOOD: TRACEABILITY, TRANSPARENCY AND BUILDING RESILIENCE IN THE SEAFOOD SUPPLY CHAIN

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It is the "Decade of Ag", the "Decade of the Ocean", and the optimum time to consider a "Decade of Ocean Ag"! While we increase production of seafood in the marine environment and on land, and as we optimize our wild capture fisheries, we must collaborate to build a more resilient seafood supply chain. This is the challenge of our lifetime – to produce healthy seafood protein to feed the world, in the face of undeniable climate change. The time is now to consider the multitude of benefits to be garnered by the seafood industry through utilization of a seafood blockchain.

Blockchain provides an immutable record which will protect our seafood supply, track provenance, and promote seafood safety, sustainability, and quality control. Allowing for instant recalls, smart contracts, waste reduction and tokenization, blockchain will facilitate a more resilient seafood supply system, and provide a platform for more efficient distribution of seafood products. Fishermen, fish farmers, seafood distributors, retail markets, brand owners and processors all stand to benefit from the increased product value and efficiency of a Seafood Blockchain!

ROLE OF JAK/STAT SIGNALING PATHWAY DURING VIRAL INFECTION IN BLACK TIGER SHRIMP, *Penaeus monodon*

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Janus Kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway plays an important role for antiviral immunity. In brief, the signal binding to DOME receptor activates JAK, leading to phosphorylated STAT and their dimerization. STATs, then, localized to the nucleus and activated gene transcription. In order to better understand the role of JAK/STAT signaling pathway in *Penaeus monodon* during viral infection, RNA interference technique was employed to silence the expression of *Pm*DOME and *Pm*STAT gene expression in *Penaeus monodon,* and the cumulative mortality of shrimp were observed after WSSV infection. From tissue distribution, *Pm*DOME, *Pm*JAK and *Pm*STAT were expressed in all tested tissues. Knockdown of *Pm*DOME and *Pm*STAT delayed shrimp mortality after WSSV challenge. In addition, *Pm*STAT silencing has altered some gene expression in the immune deficiency (Imd) pathway and antimicrobial peptides (AMPs).

EFFECTS OF MICROALGAL CONCENTRATES ON THE PRODUCTION OF POST-SET SUNRAY VENUS *Macrocallista nimbosa* AND HARD CLAMS *Mercenaria mercenaria*

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The sunray venus clam (SRV) *Macrocallista nimbosa* is an alternative species to hard clam *Mercenaria mercenaria* production in Florida due to similar culture techniques. Tropical storms, hurricanes, and contamination can affect live microalgae production in hatcheries, therefore use of alternative feeds, such as microalgal concentrates, should be considered for emergency situations or to alleviate labor and costs associated with live microalgae production.

This study explored whether 2-week post-set SRV (0.83 mm) and hard clams (0.89 mm) could be reared on partial or complete live microalgae replacement diets. Experimental treatments consisted of two live microalgae, two 50% partial replacement and two 100% algal concentrate diets (Table 1). Survival and growth were assessed at six weeks. Fatty acid profiles of clams from each treatment group, live algae and concentrates, were also analyzed.

There was a significant interaction between the effects of clam species and diet on survival, $F(5, 24)=24.1$, $P < 0.05$, as well as growth, $F(5,708)=26.1$, $P < 0.05$. For both species higher survival and growth was seen with live and partial replacement diets, and hard clams performed better with partial and complete replacement diets than SRV clams. Highest survival was seen in T2 (75%, 68%) and T4 (78%, 76%) and lowest survival in T6 (30%, 45%), in SRV and hard clams, respectively. Highest growth was seen for SRV clams fed live microalgae, and for hard clams in treatments T2 and T4. SRV clams showed lowest growth in T6 and hard clams showed lowest growth in T5.

Clam fatty acid profile was treatment dependent, and although overall trends were similar significant differences between species were seen. The fatty acid profile of clams in T1, T3 and T5 was similar, yet production was highest in the live algae treatment, and higher in the partial than the complete replacement diet. Increased clumping and settlement of the non-motile concentrates was noted and likely accounted for the decreased production. Although partial replacement diets may be a viable option when live algae production is insufficient, a complete replacement of live algae is not recommended for post-set clams.

Table 1. Dietary treatments fed to sunrvay venus (SRV) and hard clams

Treatment	Diet type	Commercial/live microalgae diet
T1	Live	*Isochrysis galbana*
T2	Live	*I. galbana* (50%) + *Chaetoceros gracilis* (50%)
T3	Partial replacement	*I. galbana* (50%) + *Isochrysis* 1800™ (50%)
T4	Partial replacement	*I. galbana* (25%) + *C. gracilis* (25%) + Shellfish Diet 1800 ™ (50%)
T5	Complete replacement	*Isochrysis* 1800™
T6	Complete replacement	Shellfish Diet 1800™

REGIONALLY ADVANCING OYSTER RESTORATION AND LIVING SHORELINES IN SAN FRANCISCO BAY: AKA WE'RE STRONGER TOGETHER

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Living Shorelines are promising nature-based climate adaptation and restoration design approaches that include strategically siting natural shoreline features, including Olympia oyster reefs, to achieve biological habitat enhancement and physical shoreline protection goals. The CA State Coastal Conservancy, Smithsonian Environmental Research Center, San Francisco State's Estuary & Ocean Science Center, UC Davis, Merkel and Associates, SF Estuary Institute, and other academic and resource agency partners are engaged in several demonstration projects in SF Bay that are linking sound science into regional management actions, testing experimental methods on the ground, and providing a model for other efforts around the state. Project manager Marilyn Latta will share the state agency perspective on current best practices with designing multi-objective projects that are the first of their kind in the bay, and lessons learned on building capacity to conduct these types of projects.

Marilyn will briefly review key results from five years of monitoring from the foundational first 2012 San Rafael project and describe how our team has integrated best practices and lessons learned into the designs of 2018 and 2019 project installations on the North Richmond Shoreline to include additional habitat types, elevations, species, and methods. The ultimate goal is to use such living shorelines as alternatives to traditional shoreline armoring where possible; however, there is a need to first test and better understand optimal methods, timing, and outcomes for Olympia oyster reef designs and other specific nature-based site approaches. With accelerating sea level rise and other climate changes, we see an urgent need to provide a model for others to take on similar demonstration projects and to grow the expertise and capabilities of contributors at all phases, from design to permitting, construction, monitoring, and interpretation of results that can inform the next projects. This rapid capacity-building will require forming diverse collaborative partnerships and will be critical to scaling up oyster restoration projects that can maintain natural shoreline habitats as we adapt to rising seas in the coming decades.

The talk will conclude with brief updates on regional and statewide permitting incentives, and plans to provide new design and constructability guidance, towards a programmatic and more efficient approach to regionally advancing high quality projects. Developing a suite of projects as a set that link with data and lessons learned and recommendations in regional plans, will allow us to advance multiple aquatic and shoreline restoration projects more efficiently and thoughtfully as a group, versus project by project, which can take years of effort. This approach will not only benefit the direct partners and projects in SF Bay but will generate new technical resources and permit pathways that will also be of benefit to additional partners baywide and statewide.

A SOLUTION FOR PROVIDING STERILE FARMED FISH

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Large scale production of sterile fish for aquaculture will increase culture performance and environmental sustainability by preventing early sexual maturation during grow-out and uncontrolled reproduction from farm escapees. While varied reproductive containment solutions have been proposed, none to date has proved fully effective, or has been widely adopted by the industry.

Here, we describe strategies to generate, breed and produce sterile fish in large scale. Our solutions rely on gene edits designed to create broodstock lines that only produce monosex, sterile populations of progeny. Thus, our design combines the benefit of sterility with sexually dimorphic performance traits in grow-out. These approaches have been validated in tilapia and are transferrable to multiple species of fish. The edited broodstock can be propagated and incorporated into breeding programs. We identified and inactivated 12 genes in two evolutionarily conserved pathways, one governing sex differentiation and the other sex competency. We isolated null alleles of genes necessary for spermiogenesis and estrogen synthesis causing male sterility and masculinization, respectively. Double edited combinations for these genes produced all-male sterile populations. Likewise, we inactivated genes which caused females to develop atrophic ovaries arrested at a previtellogenic stage or under-developed ovaries lacking oocytes. We further disrupted genes causing genetic males to sex reverse into females. Double edited combinations for these genes produced all-female, sterile populations. We successfully propagated and amplified the double edited lines via germ cell transplantation from a juvenile mutant donor into several germ cell free wild-type recipient embryos. In the resulting recipient broodstock, the edits had no effect as the genes targeted are not expressed in germ cells. With this approach, we generated fertile broodstock that successfully produced large populations of monosex, sterile progeny. Finally, we tested the performance of all-male sterile tilapia in grow-out trials. Monthly average of daily body weight gain indicated that sterile tilapia grew 12% faster than their maturing siblings starting around the time of puberty.

USE OF COMPARATIVE GENOMICS TO UNDERSTAND *Aeromonas* PATHOTYPES AND DESIGN A RECOMBINANT VACCINE STRATEGY

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Aeromonas hydrophila is a Gram-negative pathogen that causes motile aeromonas septicemia (MAS) in farm-raised catfish, which is the largest aquaculture industry in the U.S. Although it is historically considered a secondary pathogen, MAS outbreaks caused by a clade of virulent *A. hydrophila* (vAh) since 2009 have resulted in significant loss of market-size catfish in Mississippi and Alabama. vAh isolates have unique biochemical and molecular phylogenetic features. In particular, comparative genomics revealed that the vAh isolates have approximately 55 unique and distinguishable genomic features compared to historical reference *A. hydrophila*. Further analysis revealed that vAh is a distinct monophyletic clade within the *A. hydrophila* species, but it has at least two distinct subclades: one predominating in West Alabama and one occurring in the Mississippi Delta.

Analysis of the 55 vAh-unique genetic regions revealed 313 predicted vAh-specific genes; 35% of the unique genes are located within predicted genomic islands, suggesting their acquisition through lateral gene transfer. The vAh-associated regions also encode predicted prophage elements, and 34 of the genes encode potential virulence factors. Several of the unique genes encode predicted surface or secreted proteins. The coding regions of eight vAh-specific surface proteins (fimbrial and outer membrane proteins) were amplified from *A. hydrophila* strain ML09-119 genomic DNA, and recombinant proteins were expressed. Vaccination with some of these recombinant proteins provided significant protection against MAS, and these proteins also demonstrate potential for use in a recombinant vaccine strategy.

In this presentation, a summary of comparative genomics between the vAh clade and other *A. hydrophila* strains will be presented. An example of how comparative genomics can be leveraged for vaccine development will be discussed. Comparative genomics of another *Aeromonas* species, *A. veronii*, will also be presented, which revealed evidence for a virulent pathotype affecting aquaculture globally.

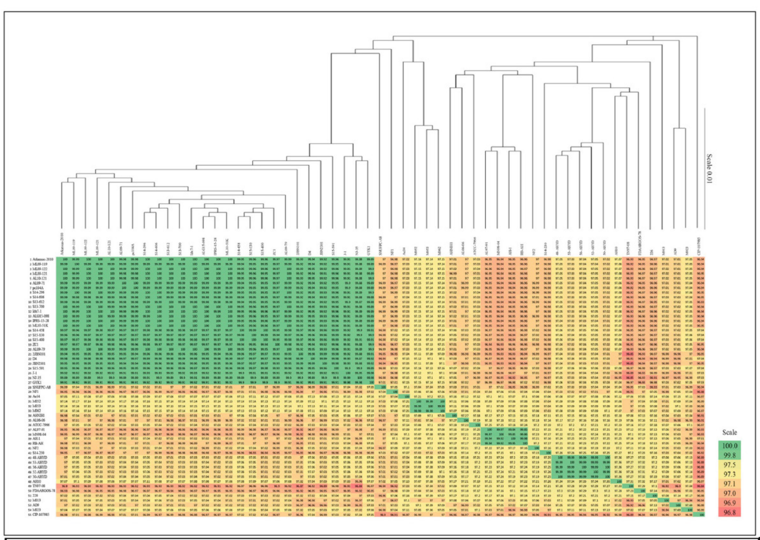

Figure 1. Average nucleotide identities (ANI) of *A. hydrophila* genomes and phylogenetic tree based on core genome comparison. vAh strains share >99.8% ANI.

EXPLORING PATHOGENICITY ATTENUATION OF THE INTRACELLULAR BACTERIUM *Piscirickettsia salmonis* THROUGH SMALL NON-CODING RNA

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Salmonid rickettsial septicemia (SRS) is one of the major diseases affecting the salmon industry in Chile. The intracellular bacterium *Piscirickettsia salmonis* is the responsible pathogen of SRS that can infect multiple tissues in its host. New approaches for its control are considered a significant challenge in the scientific community. Advances in high-throughput sequencing technologies allow a better understanding of the transcriptomic responses of organisms in several biological scenarios, such as pathogen-host interaction. In this sense, miRNAs play an essential role in the transcriptomic response of *Salmo salar* during infection with *P. salmonis*, promoting a change in the diversity of miRNA families. Also, miRNAs co-modulate the transcriptional activity of their target genes, suggesting a putative function of non-coding RNAs in the immune response of salmon infected with an intracellular pathogen. This study aimed to identify candidates for small non-coding RNA (sRNA) involved in the pathogenesis process of *P. salmonis* during infection in *S. salar* and validate at a functional level the genomic modulation of these sRNA at in *vitro* model of *P. salmonis* infection.

Transcriptome of experimental infections with *P. salmonis* EM-90 wild and attenuated in Atlantic salmon was used for sRNA candidate selection. First, putative sRNAs *P. salmonis* binding sites in up and down-regulated *S. salar* genes during infection were predicted using RNA22 version 2.0 software. Then, two sRNA were selected based on RNAseq analysis expression, synthesized as mimics (mir-222, mir 143-37), and co-transfected with the GFP reporter gene in the salmon head kidney SHK-1 cell line. After 48h, the modified SHK-1 cells were infected with $1x10^6$ CFU/mL *P. salmonis*. Cytotoxicity and cytopathic effects were monitored 24 hours after infection.

The experimental group transfected with mir-143-37 showed lower cytotoxicity against infection with *P. salmonis* compared to the other groups tested (Table 1). Moreover, quantitative PCR analysis indicated regulation of transcription of immune-related genes in SHK-1 groups transfected with mimics. In addition, an increase in the relative expression of the E3 ubiquitin-protein ligase CBL-like gene was observed, suggesting that mir-143-37 could be intervening in the regulation of ubiquitination processes in salmon cells.

Further studies will be necessary to validate these results. Nevertheless, our study suggests that the pathogenicity process of *P. salmonis* could be regulated through sRNA. This study highlights the potential use of sRNA as a bacterial attenuation technique.

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Table 1. Cytotoxicity (%) based on release of the cytoplasmic enzyme lactate dehydrogenase (LDH) from dead and / or lysed SHK-1 cells.

Experimental group	Cytotoxicity (%)
SHK-1 + *P.salmonis*	42,12
SHK-1 + mir-222 + *P.salmonis*	51,08
SHK-1 + mir-143-37 + *P.salmonis*	10,81

PACIFIC OCEAN AQUAFARMS NATIONAL ENVIRONMENTAL POLICY ACT (NEPA) ENVIRONMENTAL IMPACT STATEMENT: STATUS UPDATE

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NOAA is developing an Environmental Impact Statement (EIS) for the proposed Pacific Ocean AquaFarms development of a commercial-scale finfish aquaculture facility. The facility would be located in federal waters off the coast of southern California. The proposed project requires federal permits and authorizations including applicable permits under Section 402 of the Clean Water Act and Section 10 of the Rivers and Harbor Act. These permits and authorizations fall under the authority of the U.S. Environmental Protection Agency (EPA) and U.S. Army Corps of Engineers (USACE), respectively. The EPA and USACE are participating as cooperating agencies for the purposes of the EIS.

The Pacific Ocean AquaFarm's proposed project would consist of construction, operation, and maintenance of an offshore marine finfish aquaculture facility composed of submersible net pens off the coast of Southern California. Initial production is projected to produce 2.2 million pounds (1,000 metric tons) annually growing up to 11 million pounds (5,000 metric tons) after environmental monitoring confirms that each successive scale of expansion has not resulted in any substantial environmental or space-use impacts. California yellowtail (*Seriola dorsalis*) would be the initial farmed species. The project would utilize established and tested net pen and mooring technologies that are able to withstand storm and rough sea conditions.

In accordance with the requirements of the National Environmental Policy Act (NEPA) and the implementing regulations published by the Council on Environmental Quality (CEQ), the EIS will evaluate the environmental impacts of the proposed project and issuance of the respective permits. The EIS will consider a no-action alternative, two site alternatives, and a half scale alternative.

This talk will provide details about the proposed project, the EIS process to-date and opportunities for public engagement.

USE OF CLAY TO REPLACE ALGAE AS A TURBIDITY AGENT FOR SABLEFISH (*Anoplopoma fimbria*) LARVICULTURE

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For most marine fish species, larvae require turbid water in order to achieve proper growth and survival. Turbidity is typically achieved by mixing algae into rearing tanks, but algae is expensive and adds excess organic matter to the tanks. Clay has been proposed as a potential substitute for algae, but results have been mixed and apparently dependent on fish species and larval age.

Previously, we have shown that larval sablefish survive at higher rates when algae is used during the first week after first feeding, when compared to clay. However, transitioning to clay starting on the eighth day after first feeding leads to higher growth, and waiting until the 18th day after first feeding before transitioning to clay leads to even higher final biomass (total weight of all survivors). Turbidity costs are reduced by 80% when transitioning to clay on day-8, and by 50% when transitioning to clay on day-18.

Here, we will review these previous results and present new data that show further cost savings when clay partially replaces algae during the first week after first feeding. Consistent with previous experiments, fully replacing algae with clay during the first week after first feeding led to lower survival. Using a 25A/75C mix (25% algae with 75% clay) also led to lower survival, but a 50/50 mix (50% algae with 50% clay) led to survival that was equal to 100% algae. There were similar effects on feeding on the second day after first feeding. Feeding incidence in the 50/50 mix was higher than 100% clay and equal to 100% algae. The 25A/75C mix led to an intermediate feeding incidence. Overall, these clay studies refine sablefish larviculture methods by decreasing rearing costs while maintaining or improving larval growth and survival.

BIO-INTEGRATED AQUAPONICS: AN INTENSIVE COMMERCIAL PRODUCTION OF TILAPIA IN AN INTEGRATED AQUAPONICS SYSTEM USING ALTERNATIVE FISH FEED

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Aquaponics 5.0 is a circular production Biosystem that is based on the integration of FIVE production components to make more efficient use of FIVE natural resources with FIVE principles that promote FIVE human values for the integration of FIVE business areas. This model of Aquaponics 5.0 has been able to be implemented from a home system or a rural system in which, in addition to obtaining Healthy Food, Education and Agrotourism are obtained, to a commercial system where the same integration produces by-products of high added value that increase profitability. of the company.

Aquaponics 5.0 is an example of the cyclical model of nature, which is based on the use of resources where the reduction of elements prevails. Aquaponics 5.0 uses Biomass conversion components that generate raw materials to convert them into insect or worm protein through Permaculture techniques, which are in turn used by Aquaculture crops that produce nutrients in the water for the development of plants that They rid the water of toxic waste. All this with the help of renewable energy that gives movement to the system and the water that is stored from rain and condensation.

A commercial system of Aquaponics 5.0 not only seeks a productive profit, but also a commercial one. In production, techniques of recirculation, biofloc, microalgae, mineralization, filtration, energy efficiency, generation of raw materials, among others, are implemented so that components such as aquaculture, hydroponics and livestock are integrated into a productive circle where outputs are inputs. to another production line. And with regard to its commercial advantage, today there is a greater number of consumers and markets that seek or demand different techniques and certifications that demand Healthy and Sustainable food, so this productive-commercial integration is really the future of the Sustainable Food.

RISK ANTICIPATION IN AQUACULTURE: FOUR DATA SOURCES TO SUPPORT OYSTER FARMING RISKS

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Introduction

Since 2008, the worldwide shellfish and aquaculture industry has experienced major health crises, due to natural phenomena such as microbial infections or anoxic crises caused by oxygen depletion or Harmful Algal Bloom (HAB). As one of the main oyster farming area in France, the Thau lagoon is no stranger to those risks. Beyond just shellfish farming, lagoon environments such as Thau are real biodiversity havens that are directly impacted by these harmful events. The SENSITHAU project aims to create a Lagoon Observation Network (ROL), implementing an ecosystem database for integrated ecological, health and production management within the lagoon. Bringing together key local and national players this network will allow enhanced monitoring thanks to a multitude of sensors capable of real time and high frequency measurements across a wide range of physicochemical parameters. The SENSITHAU project focuses on (1) deploying an in situ telemetry system to monitor lagoon water conditions, and (2) developing predictive algorithms to anticipate risks associated with anoxic crisis (called "malaïgue"), microbial contamination, and algal blooms.

Material and methods

The project is conducted in the Thau lagoon, a semi-open environment in South Eastern France's Mediterranean coast. Three datasets are created to gather a maximum of information: the first dataset includes data from physical and chemical sensors (including temperature, dissolved oxygen concentration, turbidity levels, salinity…) monitoring continuously at two depths (surface and bottom). The second database analyses water samples to collect biotic data, by sampling different strategic areas in the lagoon where events have occurred over time (including *E. coli* levels, phytoplankton diversity). Several other abiotic analysis (NO3, NH4, SO4, PO4) are also collected as a complement to those biotic parameters.

A third database consists of satellite images using Sentinel satellites 2 & 3 that examine chlorophyll concentrations along with suspended particulates measurements such as phytoplankton backscattering coefficient of suspended matter and suspended particulate matter. Finally, a fourth database includes other data such as wind, air temperature, rainfall level, flow rates of rivers and releases from wastewater treatment plants around the area.

The data is collected, harmonized and stored for analysis, then preprocessed to identify global behavior patterns of different parameters (i.e., seasonality, extreme values). We also analyze correlation between various parameters to have a complete data exploration at the end. At the end we build and compare models by using statistical methods as well as machine learning methods such as deep learning to determine the optimal performance.

Results

As shown in Figure 2, the deployment of sensors and data collection on April 2021 constituted the first step for a global monitoring. The devices are continuously connected to each other and send their readings through different means (sensitives areas, susceptible for anoxic event, bacterial contamination). Data is collected at many scales: continuously with autonomous devices; locally by sampling; as well as from satellites. Those analyses allow the identification of key parameters or variations over time before they occur - or after they have taken place. Machine learning will subsequently allow to anticipate events before they happen (Lafont et al. 2019). It can also identify correlations between in situ data and satellite images so that sentinel networks can be created to monitor lagoon conditions at multiple levels simultaneously.

(Continued on next page)

Discussion and conclusion

The development of IoT (Internet of Things) technologies enables the efficient collection of large amounts and high frequency data, and their correlation with water sampling data. This information is crucial for industries dependent on water quality. Using the latest Machine Learning techniques with this data opens up the possibility of prediction, thus providing an advantage against risks that can be disastrous if not managed properly in their early stages (HAB - oxygen drop, bacterial contamination). The SENSITHAU project is designed to develop prediction tools against those risks so they can be mitigated before they happen. By improving the monitoring abilities from satellite and continuous measurements, it allows 24/7 surveying while automatically alerting end-users of potential risks. Artificial Intelligence and sensing technologies enable a wide scope of applications (including shellfish and oyster farming) around the World thanks to their usefulness in the development of sensitive areas where regular surveys or sample gathering are either impossible or prohibitively costly.

References

Garcia C, Thébault A, Dégremont L, Arzul I, Miossec L, Robert M, Chollet B, François C, Joly J, Ferrand S, Kerdudou N, Renault T (2011) Ostreid herpesvirus 1 detection and relationship with Crassostrea gigasspat mortality in France between 1998 and 2006. Vet Res 42:1–11

Hamon P-Y, Vercelli C, Pichot Y, Lagarde F, Le Gall P, Oheix J. Les malaïgues de l'étang de Thau. Tome 1. Description des malaïgues. Moyens de lutte, Recommandations.

Trotignon D, El-Farsi A, Schaeffer L, Lafont M, Dupont S, Dupont C (2020). Machine learning for aquaculture, improving water quality management and use predictive maintenance to ensure data reliability. Aquaculture Europe 2020 conference.

Lafont M, Dupont S, Cousin P, Vallauri A, Dupont C (2019) Back to the future: IoT to improve aquaculture: Real-time monitoring and algorithmic prediction of water parameters for aquaculture needs. Global IoT Summit (GIoTS), 1-6

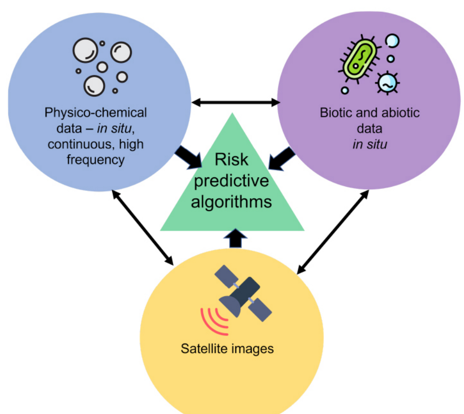

Figure 1: Global scheme of the construction of a large environmental data set

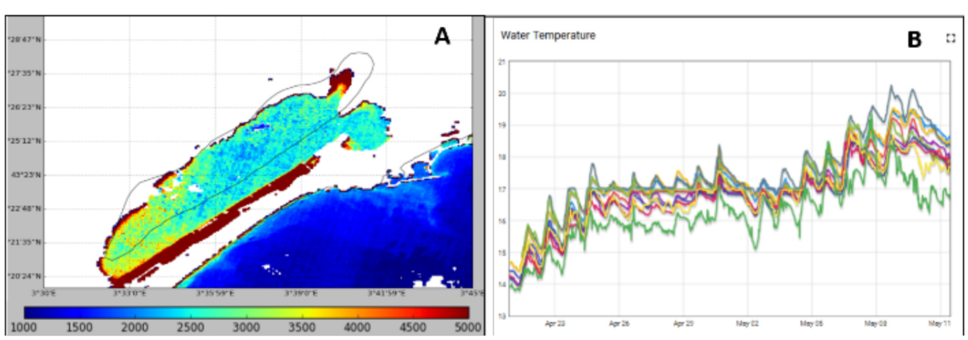

Figure 2: Example of data collected in the SENSITHAU project. A: Satellite data analysis for a-Chlorophyll (10^3 mg/m3) ; B: Water temperature monitoring thanks to autonomous devices (°C; 10 sites across Thau lagoon)

BASIC OBSERVATION BUOYS FOR HAB PREDICTION

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BOB (Basic Observation Buoy), an inexpensive, rugged, compact buoy, and FLO (Dock mounted), accommodate standard water quality sondes that can be "daisy chained". 10 BOBs & FLOs that record water temp, salinity, conductivity, DO, Turbidity, and Chlorophyll a are deployed over a 20 mile stretch of the Chester River in Maryland. The data is viewed on www.hydrovu.com using 20 minute data intervals. Available sensors include Phycocyanin/Phycoerythrin. Depth of measurement is adjustable from 22" to 45" by extending the telescoping center shaft, or cabling beneath the shaft to the bottom. Stainless hardware for anchor or handling lines is integrated in BOB. The buoy is used for aquaculture, water quality monitoring and engineering applications. It is shipped globally in stackable 24"x18"x18" cartons. Ready to sample, BOB weighs less than 20lbs and can be deployed by one person from a small boat.

BOB is assembled with a screwdriver and rubber mallet and anchored with a 5lb fluke anchor. Where docks are available, sonde and data telemetry unit can be installed inside a 4" PVC pipe affixed to a piling. The sonde is inserted through the hinged well cap and hung in the central shaft by a carabiner. The buoy was designed to use In-Situ's VuLink for data telemetry. Communication occurs through cell phone or satellite transmissions. Once powered on, the sonde transmits data to a central, Internet accessible, data portal (Hydro-Vu). The Sonde and Vu-Link contain the buoy's 3 D Cell batteries which will transmit data for over 3 years.

The objective is to saturate the nearshore, inshore and reservoirs (water security) with these low cost and BOBs and FLOs to allow high resolution spatial and temporal monitoring of water quality parameters. With increased "data fountains" and meshing with satellite imagery, the prediction of HABs onset will be markedly improved.

OYSTER MARICULTURE AND NITROGEN BIOEXTRACTION IN COPANO BAY, TX

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Nutrient discharge has detrimental effects on coastal ecosystem and leads to impaired water quality. Nitrogen from fertilizers, livestock, runoff, wastewater treatment plants, and atmospheric deposition can lead to eutrophication - resulting in harmful algal blooms, dead zones, fish kills, and restricted sunlight to plants and algae. Oysters are known to act as a buffer for eutrophication through filter feeding; ultimately bio-extracting nitrogen from the surrounding water. Quantification of this process, and its utility compared to engineered wastewater treatment processes and other nutrient management strategies is complex. To better understand this relationship, models of oyster farms are used in Copano and Matagorda Bay along the Texas coast.

This study uses a three-step approach to evaluate the potential role of oyster mariculture as a water management tool. First, the Assessment of Estuarine and Trophic Status (ASSETS) model assesses vulnerability and eutrophication status of a bay's condition based on hydrodynamic and biogeochemical measures. Second, the Farm Aquaculture Resource Management (FARM) model is used to estimate oyster harvest and associated nitrogen removal. Lastly, an economic value is assigned to oyster related nitrogen removal based on an avoided costs approach which uses costs of alternative nutrient removal strategies such as municipal wastewater treatment technologies. Nutrient credit trading is a relatively new endeavor, but it may provide a supplemental income for bivalve farmers in the future.Understanding the ecosystem services provided by oyster mariculture and natural reefs can create a sustainable management approach, and increase resiliency of our coastal bays and estuaries.

SELECTIVELY BREEDING IMPROVED STRAINS OF SUGAR KELP *Saccharina latissima;* A THREE YEAR SUMMARY

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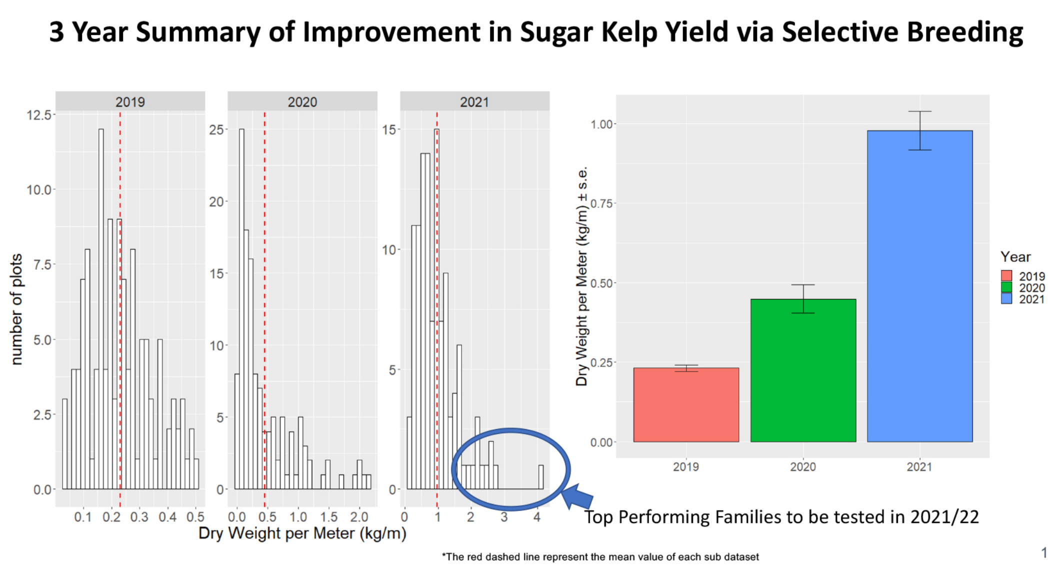
Seaweed farming in the Gulf of Maine has expanded rapidly over the past decade. As part of ARPA-E's MARINER program, we conducted a selective breeding program to improve the productivity and composition of sugar kelp which could serve new markets for food, animal feeds, bio-products and eventually biofuels. Our population genetics studies of sugar kelp prompted the development of two breeding programs: one for Southern New England and the other for the Gulf of Maine. We maintain about a thousand unique gametophytes that can be used as parents for generating crosses. We have sequenced the whole genome for 278 parents and tested their crosses. Kelp crosses were planted in "common garden" farm arrays over three seasons (2018 through 2021) in New Hampshire and Connecticut. Trait measurements and analyses of yield, composition and morphology for 734 family plots and 9,666 individual kelp blades will be presented. One highlight is that several plots exceeded 20 kg/m harvest wet weight with the top plot weighing 28 kg/m or 4 kg/m dry weight – about 4 times the commercial average. We used pedigree, genotypic markers, and harvest assessment data to predict offspring performance, and we improved the efficiency of on-farm testing and phenotyping. Ultimately, we are meeting our goal of selecting sugar kelp with 20% increased dry matter yield per unit area per generation. We also completed an annotated reference genome for sugar kelp that enables the identification of functional genes and variants, as well as natural mutations on targeted genes to potentially create non-reproductive sporophytes.



NOVEL BOTTOM CULTURE OF SUGAR KELP *Saccharina latissima* FOR DIVERSIFYING MARINE FARMS

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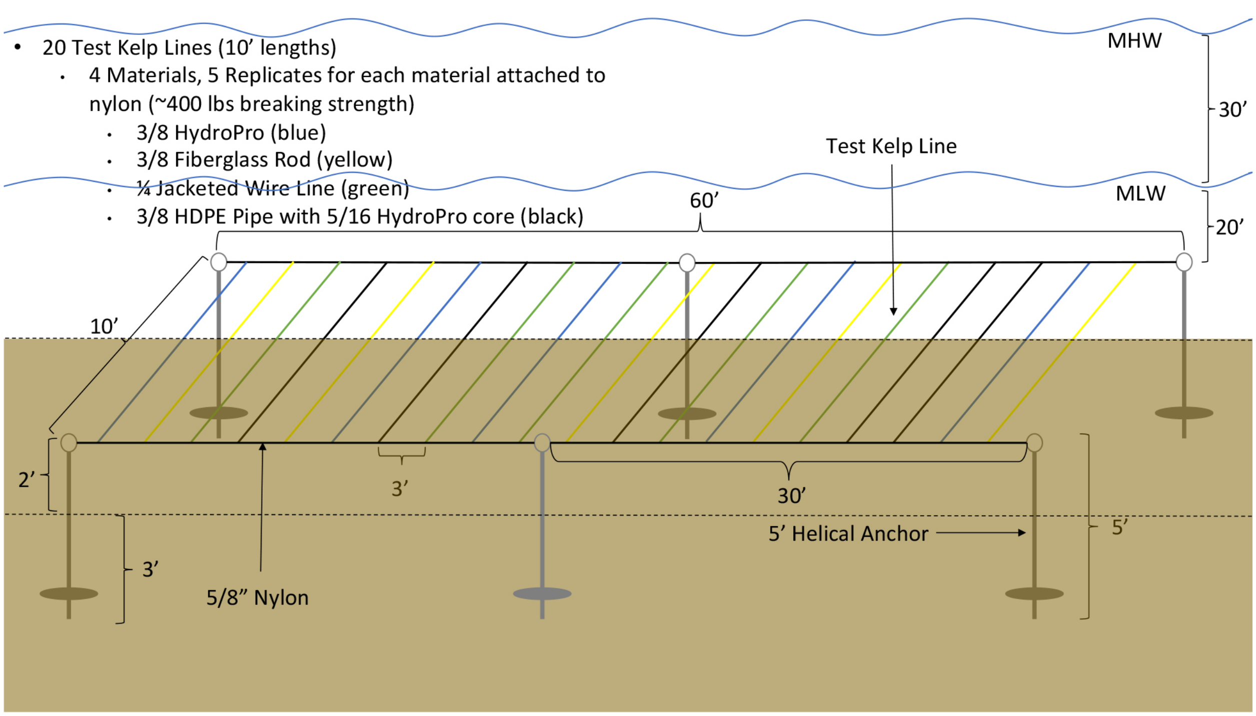
Kelp farming is a rapidly expanding industry in the Northeastern US and Alaska. Cool, nutrient rich water in the Gulf of Maine make the region ideal for growing kelp; 2021 harvests in Maine alone totaled more than 800,000 wet pounds, and the state issued 125+ permits covering 173 acres of coastal waters (Piconi et al. 2020). However, kelp farms in the southern portion of the Gulf of Maine are virtually non-existent, despite hosting healthy natural populations, due to gear restrictions in Cape Cod Bay that mitigate Right Whale entanglement during the winter months. Kelp farming provides a unique market diversification opportunity for shellfish farmers and commercial fishers in the Northeastern US. The kelp farming season ranges from November to June making it complementary to shellfish farming and many commercial fisheries which demand more attention during the warmer months. This project will test a novel on-bottom growing structure for *Saccharina latissima* with no vertical lines and stiff non-rope grow-lines. These gear modifications may make kelp farming possible in areas where traditional systems are prohibited. Deployment of the novel test structure will occur in late fall 2021 at 3 participating farm locations in the southern Gulf of Maine.

Figure: Year 1 test unit



OPTIMAL SELECTION OF OYSTER RESTORATION SITES BASED ON METAPOPULATION SOURCE-SINK DYNAMICS

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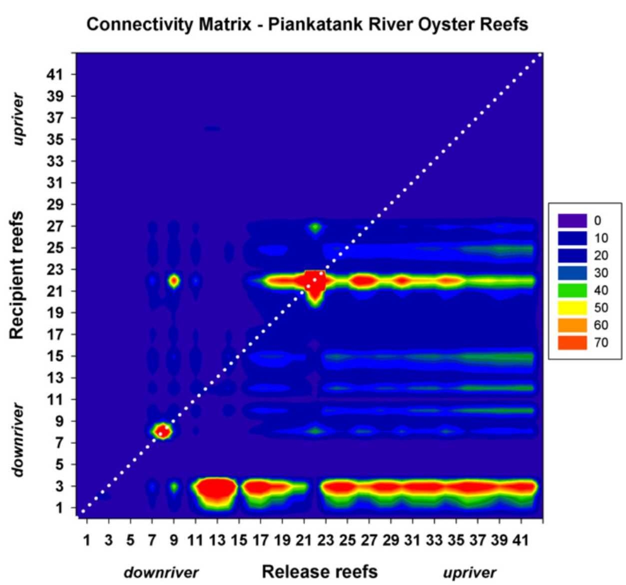
Restoration strategies for oyster populations rely critically on patterns of population connectivity (i.e. metapopulation source-sink dynamics). We present case studies from three tributaries in Chesapeake Bay where evaluation of metapopulation dynamics contributed prominently to successful eastern oyster (*Crassostrea virginica*) restoration strategies, the Piankatank, Lynnhaven and Great Wicomico Rivers. We used historical data and habitat suitability index models to parameterize biophysical model simulations that integrated larval duration, mortality, and settlement behavior to generate donor-recipient matrices, which were analyzed with cluster analyses to identify optimal restoration sites in the metapopulation. Potential restoration sites clustered into five groupings--sources, sinks, sporadic sources, closed populations, and artificial sources. Sources and sporadic sources are optimal sites for reef restoration because larvae from these sites are advected throughout the metapopulation such that both broodstock and substrate addition can enhance the metapopulation. Conversely, sinks are unsuitable for broodstock enhancement because their larvae are flushed from the system, but they are suitable for substrate addition because these sites receive larval subsidies from sources such that habitat restoration in these sites can improve water quality, refuge for small fish and invertebrates, and feeding grounds for predators. Closed populations are self-sustaining and suitable for broodstock and substrate addition, but they have negligible benefits at the metapopulation level. Artificial sources are sites where larvae from the natal or outside populations do not settle, but their larvae are advected to other populations, such as sinks. These sites require continuous larval subsidies from artificial sources (e.g. hatcheries) to be maintained. The three tributaries have met oyster restoration targets over several years to decades and can serve as models for oyster restoration strategies throughout the range of the eastern oyster and other native oyster species worldwide.



ECONOMIC CHARACTERISTICS OF FISH PROCESSORS IN ZHEJIANG PROVINCE, CHINA

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Processors are key stakeholders in the fish and fish product supply chain. With China being a major hub for global fish production and trade, there is surprisingly limited research on its fish processing industry. In 2020, we surveyed 100 fish processors in Zhejiang Province, China on their major products, markets and inputs, and their perceptions for important management and market changes in the past few years. This study applies the input-output model to understand the basic economics characteristic, and identifies important features for development. This study will contribute to the existing knowledge on the fish supply chain and for improving the cross-sector coordination in policy design.

OPEN HARDWARE TO FACILITATE DEVELOPMENT OF GERMPLASM REPOSITORIES FOR BIOMEDICAL MODELS

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Development of efficient and reliable germplasm repositories is critical for preservation of genetic resources of aquatic model organisms that are vital to advancing biomedical research. However, the most significant problem in repository development is the lack of reproducibility, posing great risks of losing valuable lines developed from billions of dollars research investment. Open hardware allows users to gain access to technologies through open-sharing mechanisms and enable individual contributions for improvement to facilitate community-scale standardization that would rarely be achieved through proprietary technologies. Our work recognizes 14 categories of open hardware for a cryopreservation processing pathway, and 6 categories for a corresponding quality management pathway to address the two impediments to establishing repositories among resource centers and research communities (Figure 1). Although cryopreservation protocols have been established through basic biological research, low quality samples are often produced that cannot be revived because of a lack of affordable, standardized, and reliable hardware to process samples along a production pathway. In addition, there are currently no cost-effective hardware options to enable quality management, including quality assessment for accurate evaluation, quality assurance mechanisms for prevention of defects, and quality control for elimination of inferior materials. Although some of these issues can be alleviated by commercial solutions, most laboratories are not willing to purchase expensive equipment (that can cost tens of thousands of dollars) when germplasm banking is not a focus or obligation of their work. Through open hardware, individuals can fabricate standardized devices in-house with low cost, offering opportunities to begin or improve germplasm preservation, and facilitate repository development with community efforts through aggregated high throughput.

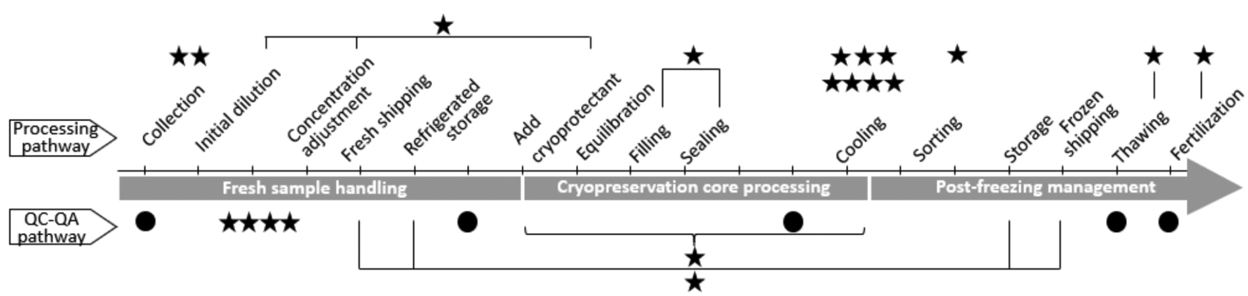

Figure 1. A generalized framework for technology development to support development of germplasm repositories for aquatic biomedical models. The processing pathway includes all activities of sample processing from gamete collection (left) through fertilization (right) with three major phases: fresh sample handing, cryopreservation core processing, and post-freezing management. The quality management (QC-QA) pathway includes repeated quality evaluation (QE) checkpoints that enable establishment of product quality control (QC), process quality assurance (QA), quality monitoring, and data transfer and management systems. Stars represent different hardware categories and black circles represent QE points.

NEUTRAL PROCESSES AND SALINITY SHAPE MICROBIAL COMMUNITY ASSEMBLY IN MANGROVE ECOSYSTEMS ALONG ESTUARY

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Mangrove ecosystems are important because they protect coasts, provide sheltering for coastal populations, and sequester carbon. Mangrove forests at different sites along an estuary from downstream region to upstream region are usually featured with different plant communities, due to the gradient of salinity and tidal inundation. We want to know whether the microbial communities also vary along an estuary and what mechanisms shape the assembly of microbial communities in mangrove ecosystems.

We compared samples collected from three mangrove species at three different locations along an estuary on Hainan Island, China. We found significant differentiation in the diversity and assembly of microbes among the three locations, as well as between rhizosphere and bulk sediments. Neutral processes have played dominant and niche-based processes have played minor roles in shaping the assembly of rhizosphere microbial communities in mangrove ecosystems. Physicochemical conditions, particularly salinity, underlie the niche-based process leading to a decline in microbial diversity and microbial network stability from the upstream region to the downstream region. However, the species identity of the host plant has only a weak influence on the assembly. Particularly, rhizosphere sediments in downstream environments are prone to the enrichment of more microbes associated with organic matter degradation (e.g., Bacteroidetes) and sulfur cycling (e.g., Deltaproteobacteria), which are likely related to carbon sequestration.

We conclude that the assembly of microbial communities in mangrove ecosystems differed along an estuary, which is dominated by neutral processes and contributed minorly by the gradient in physicochemical conditions.

BETA TESTING OF CRYO-JEOPARDY: A USER COMMUNITY DEVELOPMENT TOOL FOR OPEN HARDWARE

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Open hardware is an emerging approach to address challenging community-level problems for biological research and other applications. Open hardware allows users to gain access to powerful technologies through sharing mechanisms that enable individual contributions to facilitate community-scale improvements that would rarely be achieved through traditional proprietary mechanisms. These advantages have fueled the development of open-hardware approaches to support germplasm cryopreservation and repository establishment. For distributed systems such as these to function, community development is extremely important to recruit and advance participation through individual roles including users, makers, and developers of technology. As such, participation is the core driving force for success of open-hardware systems.

Interaction with individuals through training and workshops is traditionally used to support community development. These interactions are often performed as lectures, questions by audiences, and answers by hosts. However, these methods can lose interest and attention because of a lack of excitement and active interaction, especially in the 'Zoom Era', when hands-on activities are limited. We have developed and tested various alternative activities for community interaction, such as hierarchical thinking, show-and-tell, roleplaying, and game structures. Our goal is to promote active application of open hardware for cryopreservation and germplasm repository development in aquatic species. A 'Cryo-Jeopardy' (Figure 1) game was developed and tested within our group (alpha testing) as a learning tool to engage active thinking and maximize attention, and is available for beta-testing by outside audiences. This game includes several categories of questions. Scores are registered as temperatures associated with different difficulties of questions. The questions were designed to be fun while facilitating interaction and higher-level thinking beyond basic knowledge. Additional information is also delivered through the answers. Negative temperatures are awarded when questions are answered correctly, and positive temperatures for answering incorrectly. The team that reaches -80 °C (a common endpoint prior to storage in liquid nitrogen) wins (Figure 1). This tool was developed for open hardware, but could be adopted by general outreach and educational programs.

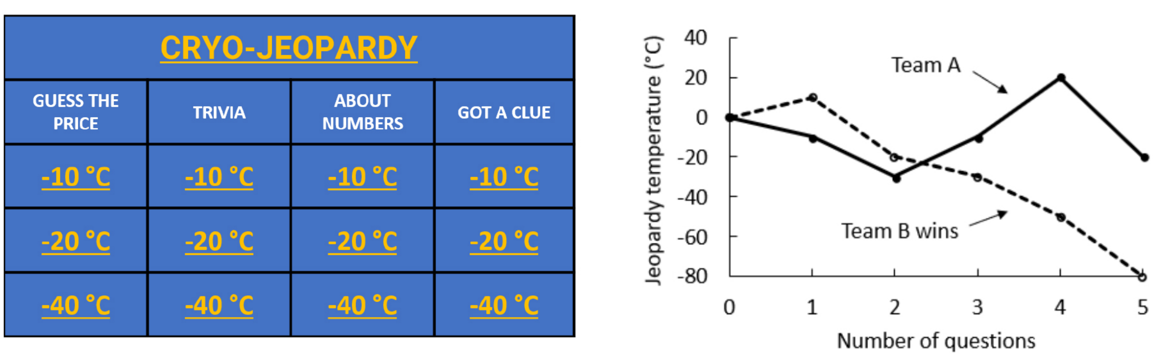

Figure 1. A 'Cryo-jeopardy' game developed as an interactive learning tool to promote audience engagement.

INCREASING FEED INTAKE AND NUTRIENT UTILIZATION OF SOYBEAN MEALS FOR LARGEMOUTH BASS THROUGH INCLUSION OF PALATANTS

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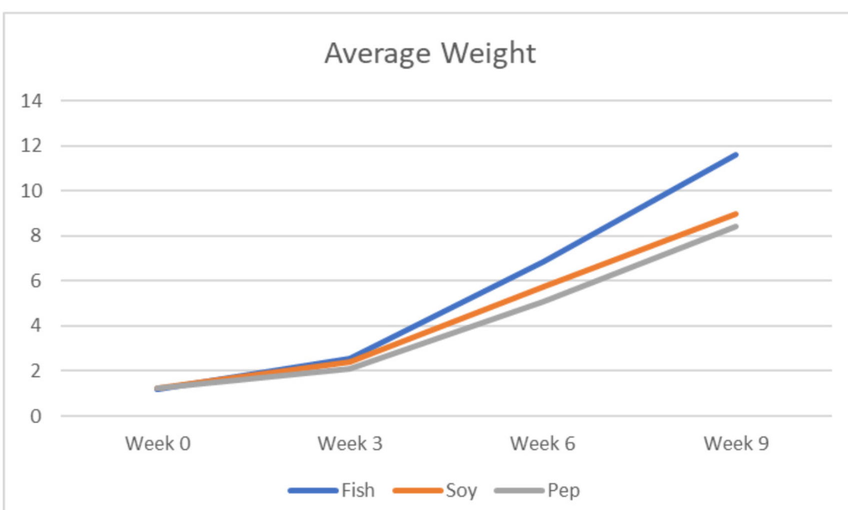
In previous feeding trials in our lab with largemouth bass (LMB) fed soy-based diets, we observed significant rejection of the diets compared to a fish-meal control. Presumably, the soy diets were less palatable to LMB, reducing feed intake. Therefore, we designed a trial to combine different palatants with the same diet formulas to see if we could improve feed intake of the soy diets. Three basal diets were formulated to contain 40-42% protein and 10-12% lipid. The main protein sources in the basal diets were fish meal/poultry meal (control), traditional soybean meal (dehulled, solvent-extracted 48% protein) or PepSoyGen™, a fermented soy protein source. Diets were extruded as 2.5 mm floating pellets by Zeigler Bros., Inc., and shipped to UAPB where palatants were added at 2% of the diet using a cement mixer to top-coat pellets. The palatants included fish silage, chicken liver, and black rice vinegar. A total of 12 diets (3 basal diets alone or combined with 1 of 3 palatants) were produced for this 12-week feeding trial.

Twenty feed-trained juvenile LMB averaging 1.23 g initially were randomly assigned to 3 replicate 110-L tanks per diet in a recirculating system. Fish are being fed twice daily to apparent satiation and bulk-weighed by tank every three weeks to monitor growth. Mortalities are recorded daily. Data is being analyzed using a 3x3 ANOVA. Nine-week average weight gain (g) data (below) showed that the fish meal diet performed better than either soy diet ($P<0.05$).

There are few significant effects of the palatants so far, but weight gain of fish fed the regular soy diet with chicken liver was not different from that of the fish meal control with no palatant. Feed intake was lower in fish fed the PepSoyGen™ diet with vinegar than the fish meal control diet (with or without vinegar). Survival of fish fed the regular Soy diet was higher than that of fish fed PepSoyGen™, and survival of fish fed regular soy was similar to that of fish fed the fish meal diet. However, cannibalism is a complicating factor in assessing diet performance. Additional results will be presented at the meeting.



gROWTH PERFORMANCE OF LARGEMOUTH BASS AND KOI FED DIETS WITH MEALWORM VERSUS OTHER PROTEIN SOURCES

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A pilot study was conducted using basal commercial diets for largemouth bass (*Micropterus salmoides*) and koi (*Cyprinus carpio*), respectively, with 10% of the basal diet substituted with either soy protein concentrate or a commercial mealworm product (YnMeal™). The three diets for each species were similar to each other in analyzed proximate composition, and met the known nutrient requirements of the target species. A modest level of mealworm substitution was chosen for the pilot study due to the unknown nutrient availability or palatability of the product.

Concurrent feeding trials were conducted with largemouth bass (LMB) and koi in a recirculating aquaculture system. Juvenile fish were stocked in each of 4 replicate 19-L tanks per diet per species. The LMB (initial weight, 2 g), were stocked at 10 fish per tank, while koi (initial weight, 8 g) were stocked at 7 fish per tank. All fish were fed to satiation twice daily with their respective diets, and both trials lasted 10 weeks. At harvest, there were no differences in weight gain, survival (≥95%), feed intake or feed conversion ratio of LMB or koi among treatments. Hematological parameters and hepatosomatic indices of LMB were also similar among diets. We observed that the bass grew significantly more (nearly 800% weight increase) compared to the koi (about 20%) during the same time period and under uniform water quality conditions. Cyprinid growth can be limited at high densities, and tank size might have restricted koi growth. Therefore, after collecting final weights from the 10-week trial, the koi were restocked into larger tanks (240 L). They are being fed the same diets and will be monitored for additional growth prior to collection of hematological and body composition data. To date, results of this pilot study indicate that diets for LMB or koi with 10% mealworm protein are consumed as readily as the commercial or SPC diets, and support growth, feed conversion and survival equally well. Additional trials are planned with higher inclusion levels of YnMeal that will include cost/benefit analysis..

gROWTH PERFORMANCE OF LARGEMOUTH BASS AND KOI FED DIETS WITH MEALWORM VERSUS OTHER PROTEIN SOURCES

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Concurrent feeding trials were conducted with largemouth bass (LMB) and koi in a recirculating aquaculture system. Juvenile fish were stocked in each of 4 replicate 19-L tanks per diet per species. The LMB (initial weight, 2 g), were stocked at 10 fish per tank, while koi (initial weight, 8 g) were stocked at 7 fish per tank. All fish were fed to satiation twice daily with their respective diets, and both trials lasted 10 weeks. At harvest, there were no differences in weight gain, survival (≥95%), feed intake or feed conversion ratio of LMB or koi among treatments. Hematological parameters and hepatosomatic indices of LMB were also similar among diets. We observed that the bass grew significantly more (nearly 800% weight increase) compared to the koi (about 20%) during the same time period and under uniform water quality conditions. Cyprinid growth can be limited at high densities, and tank size might have restricted koi growth. Therefore, after collecting final weights from the 10-week trial, the koi were restocked into larger tanks (240 L). They are being fed the same diets and will be monitored for additional growth prior to collection of hematological and body composition data. To date, results of this pilot study indicate that diets for LMB or koi with 10% mealworm protein are consumed as readily as the commercial or SPC diets, and support growth, feed conversion and survival equally well. Additional trials are planned with higher inclusion levels of YnMeal that will include cost/benefit analysis.

DIETARY THREONINE REQUIREMENT OF LARGEMOUTH BASS *Micropterus salmoides*

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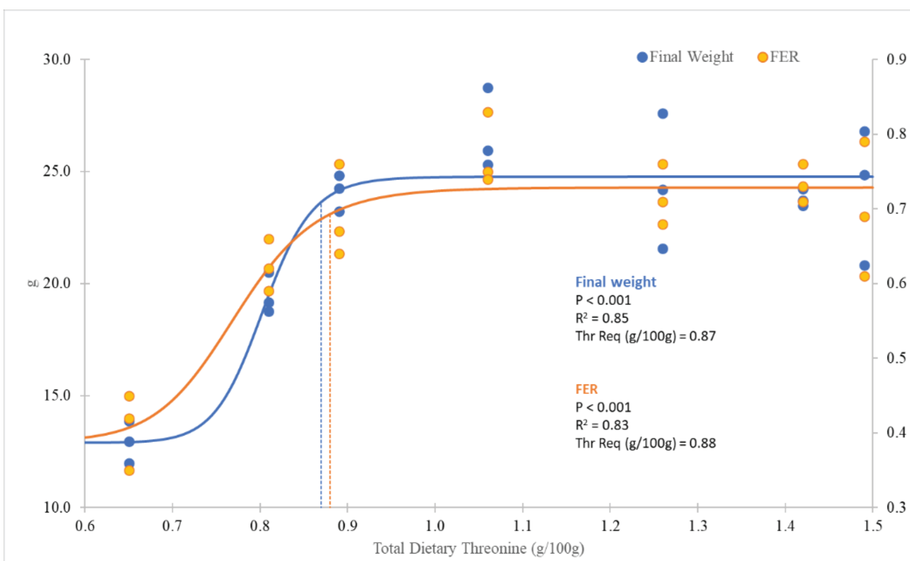
Threonine is typically the third limiting amino acid in plant-protein based diets and its deficiency leads to poor production performance and health of farmed fish. Production of largemouth bass (LMB) for food-fish markets has increased but information on its amino acid requirements remains limited. The objective of this 8-week feeding trials was to assess the dietary threonine requirement of LMB.

A semi-purified basal (Basal) diet was formulated to contain 40% crude protein (CP), 12% lipid, and 0.65 g/100 L-threonine (Thr). Crystalline L-Thr was supplemented to the Basal diet in additive amounts of 0.15 g/100g originating six additional diets with total Thr ranging from 0.65 to 1.5 g/100g. Each diet was fed twice daily to apparent satiation to triplicate groups of 20 LMB juveniles (6.6 g/fish initial weight) stocked in 110-L glass aquaria operating as a recirculating system. Water quality was kept within acceptable ranges for LMB and a 12h photoperiod was maintained by timer-controlled fluorescent lights.

Mean survival of LMB ranged from 72% (Basal diet) to 94% (remaining treatments). A four-parameter saturation kinetics model was used to fit the resulting data. The total dietary Thr requirement supporting 95% of maximum final weight and feed efficiency ratio (FER) of LMB was estimated to be approximately 0.88 g/100g (Figure), corresponding to 2.14 g/100 of dietary CP, respectively. Additional results including Thr and protein retention efficiencies of LMB will be presented. The dietary Thr requirement values determined in this study will aid in the formulation of nutritionally balanced and cost-effective diets for LMB.



FROM RESEARCH TO THE FARM: A PARTIAL BUDGET ANALYSIS OF LOW-COST LED LIGHTS FOR PRODUCTION OF BIBB LETTUCE, *Lactuca sativa* var. *Capitate*, IN A COMMERCIAL INDOOR AQUAPONIC SYSTEM

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A partial budget analysis is a decision-making tool that allows businesses to assess whether a change in production practices will result in an increase or decrease in profits. For aquaponics (AP), this tool can be useful to compare different investments as equipment costs tends to be high, particularly in colder climates that require artificial grow lights for plant growth. In this study, results from previous low-cost LED light research conducted at Kentucky State University were implemented on a semi-commercial indoor AP farm. The farm operates a 15.1 m^3 system based on the University of Virgin Islands design with 47.6 m^2 of plant growing space, 7.4 m^2 of which was utilized for this research. Four low-cost LEDs (Spider Farmer SF-2000 (SPI), Fluence RAZRx (FLU), Worldwide 4000K High Bay light (WIDE) and Designers Fountain 3500k (DES) were compared to a high-cost LED control light (Neosol DS (NEO)) for total biomass (g) of Buttercrunch lettuce, *Lactuca sativa* var. *Capitate* produced (Table 1). Each light covered a 1.5 x 1.5 m space, which contained 32 lettuce plants. Treatments DES and FLU required two lights to cover the grow area, whereas NEO, SPI, and WIDE required one. Total kWh for each light was recorded daily to determine the operational cost for the treatments and control. A partial budget analysis was performed to determine the potential net change in profits and benefit/cost ratio for each light as compared to the control. All treatment lights showed had a positive change in net profits and a positive benefit/cost ratio (Table 1). Results presented here indicate the viability of low-cost LED lights for an indoor AP farm. Utilizing financial tools for economic analysis and planning, as presented here, can aide farmers make responsible operational decisions to improve profitability.

Table 1. *Total biomass (g),energy used (kilowatt hours), selling price per kg lettuce, energy cost per kilowatt hour, lettuce cycles per year, total value of lettuce produce, total cost of electricity, purchase price for light fixtures, Net Change in Profit, and Benefit/Cost Ratio for production of Buttercrunch Lettuce,* Lactuca sativa *var.* Capitate*, grown under four different LED grow lights.*[1]

Variables	NEO	DES	SPI	FLU	WIDE
Total Plant Biomass g	1225.6	1187.6	1287.9	1001.4	809.7
Total Plant Biomass lb	2.70	2.62	2.84	2.21	1.78
kWh day	3.20	2.80*	3.17	2.36*	5.10
Price per pound (USD)	$2.50	$2.50	$2.50	$2.50	$2.50
Cost per kWh (USD)	$0.11	$0.11	$0.11	$0.11	$0.11
Grow Cycles per Year	20.8	20.8	20.8	20.8	20.8
Crop Value year	$140.38	$136.03	$147.51	$114.70	$92.74
Electric cost year (USD)	$128.57	$112.42*	$127.08	$94.75*	$204.57
Cost of Fixtures (USD)	$1,400.00	$200.00*	$300.00	$728.00*	$150.00
Net Change in Profits	NA	$1,211.80	$1,108.62	$680.14	$1,126.37
Benefit/Cost Ratio	NA	3.68	2.95	1.71	3.28

NEO=NeoSol DS; FLU=Fluence; DES = Designers; SPI=Spider Farmer; WIDE=Worldwide
*=values representative of two lights

AQUACULTURE AND THE MARINE MAMMAL PROTECTION ACT

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The primary threat to the recovery and conservation of marine mammals is bycatch in fishing gear, and along with commercial whaling, bycatch served as a major impetus for enacting the Marine Mammal Protection Act (MMPA) of 1972. The MMPA prohibits "take" of marine mammals, with limited exceptions relevant to U.S. commercial fishing operations, including aquaculture operations. These exceptions are paired with requirements to ensure the safety of marine mammals and to reduce bycatch.

In this presentation, we will discuss two exceptions to the take prohibition that are relevant to aquaculture operations. First, we will describe a provision in MMPA section 101 that allows aquaculture operators to deter marine mammals from damaging fishing gear, catch, and property, so long as the deterrence does not kill or seriously injure marine mammals. We will present NOAA Fisheries' recently proposed guidelines for safely deterring marine mammals; these guidelines are intended to provide tools to specific users, including aquaculture operators, while also protecting these users from liability.

Second, we will describe the statutory and regulatory requirements for the lawful take of marine mammals incidental to commercial aquaculture operations to help inform the design and management of farms to minimize impact on these protected species. MMPA section 118 includes prescriptive goals to reduce bycatch and a framework for evaluating and mitigating mortalities and serious injuries of marine mammals incidental to commercial fisheries. These requirements include:

- classifying fisheries according to the level of mortality and serious injury of marine mammal stocks,
- registering with NOAA's National Marine Fisheries Service,
- accommodating observers upon request,
- complying with applicable take reduction plans, and
- reporting incidental marine mammal mortalities and injuries.

These provisions not only provide options for aquaculture operators to lawfully address negative interactions with marine mammals, but they also serve to focus efforts on reducing deaths and injuries incidental to aquaculture operations where and when needed.

DIETARY SUBSTITUTION OF MICROALGAE WITH THE BAKER'S YEAST MUTANT Δ*mnn9* IN PACIFIC OYSTER *Crassostrea gigas* SPAT

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Microalgae production is one of the major limiting factors for bivalve aquaculture due to high production costs, risks of contamination and nutritional variability of algae species. Alternative diets to live microalgae have been tested in bivalves, but no satisfactory products have been developed that fulfill completely their nutritional requirements. The presence of polyunsaturated fatty acids (PUFAs), amongst others, is proven to be essential for the optimal development of marine bivalves.

This study evaluated the use of the baker's yeast *Saccharomyces cerevisiae* mutant, Δ*mnn9*, to substitute microalgae diets in Pacific oyster (*Crassostrea gigas*) spat. The Δ*mnn9* yeast is defective in the synthesis of the α-1,6-mannose arm of *N*-glycans, resulting in a cell wall that is characterized by low levels of mannan and elevated levels of chitin and β-glucan. Mutant yeasts exhibiting these characteristics have been shown to be digested more efficiently by marine species than the wild type. We replaced a microalgal diet (*Chaetoceros muelleri*:*Tisochrysis lutea*; 50/50 based on dry weight (DW)) with Δ*mnn9* at five substitution levels (0, 25, 50, 75 and 100%, based on DW; trial 1). Secondly, we compared the nutritional value of Δ*mnn9* with the wild-type *S. cerevisiae* for oyster spat by replacing 50% of the mixed algal diet with either Δ*mnn9* or the wild type (trial 2). It was observed that Δ*mnn9* could substitute 50% of the microalgae diet without significantly affecting the general performance of the spat (figure 1). Also, growth rate was significantly higher in oysters fed partly on Δ*mnn9* than those fed partially on the wild type baker's yeast, emphasizing again the role of the cell wall for yeast digestion. Gene expressions of fatty acid elongase (enzyme that participates in the elongation of long-chain fatty acids) and β-glucan-binding protein (involves in innate immune response) were also measured in the trials, and are discussed.

These results highlight that Δ*mnn9* cells are efficiently ingested and digested by *C. gigas* spat, representing a suitable alternative for microalgae in bivalve aquaculture. A higher gene expression of fatty acid elongase, which participates in the biosynthetic pathways of PUFAs, was observed in the diet with a substitution level of 75%, possibly as a response to the deficiency of PUFA.

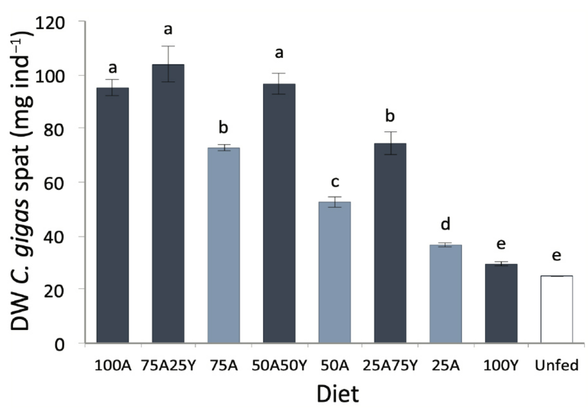

FIGURE 1. Dry weight (mg ind^{-1}) of *C. gigas* spat after 21 days feeding on a bi-algal diet (A) that is substituted at different levels (based on dry weight (DW)) with Δ*mnn9* (Y). Dark-grey columns correspond to oysters fed a 100% diet (100A, 75A25Y, 50A50Y, 25A75Y, 100Y) while the light-grey columns correspond with oysters fed the bi-algae diet of each replacement level excluding the Δ*mnn9* yeast (n=3).

INVASIVE POPULATIONS OF THE PACIFIC OYSTER, *Crassostrea gigas*, IN THE NORTHERN PACIFIC COAST OF BAJA CALIFORNIA

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The Pacific oyster, *Crassostrea gigas*, is the most cultured oyster around the world, thus it is also widely distributed as invasive. Recently it has increased its distribution in the Northeastern Pacific coast, especially in Southern California and Baja California. In order to understand the effect of these invasive species on coastal habitats, we first need to know their distribution and abundance. In order to understand the extent of the invasion in Northwest Mexico, we sampled 6 open estuaries in the northern Pacific coast of the Baja California Peninsula from La Bocana (26°N), Baja California Sur, to Estero de Punta Banda (31°N), Baja California. In 2019 and 2020, we sampled 2-5 sites per estuary using a 1m^2 quadrat every 5 m on a 50 m transect to count live oysters and empty shells. We found that the Pacific oyster, *Crassostrea gigas*, is widely distributed and is as abundant than native oysters in many estuaries (Fig. 1).

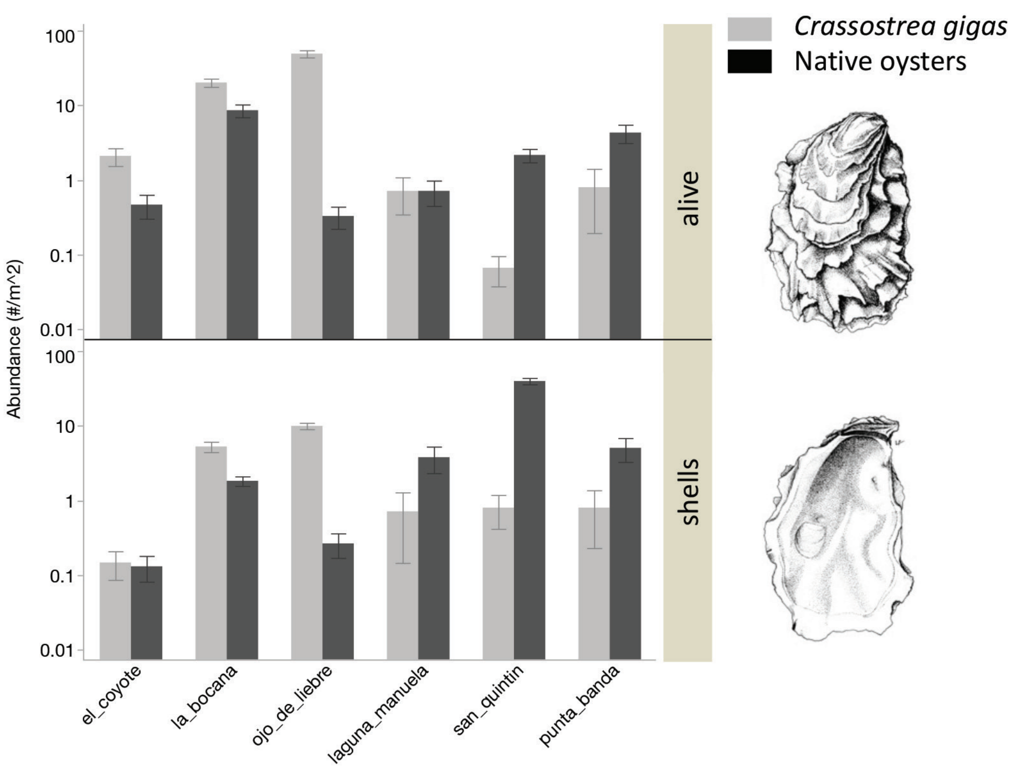

Figure 1. Mean densities of live and empty shells of Pacific oyster, *Crassostrea gigas,* and native oysters in 6 estuaries along the northern Pacific coast of the Baja California Peninsula.

AMERICAN LOBSTER *Homarus americanus* ABUNDANCE SURVEY, AND TAGGING STUDY OF THE FIRST OFFSHORE WINDFARM IN THE UNITED STATES

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The University of Massachusetts Dartmouth School for Marine Science and Technology (SMAST) conducted a standardized ventless lobster trap survey and tagging study in Vineyard Wind's Lease Area OCS-A 0501, (Figure 1). In northern portion of the Lease Area, termed the 501 North (501N) Study Area, populations of adult lobster were sampled and compared to those in the easterly adjacent Control Area. The primary goal of this project was to identify baseline conditions in the 501N Study Area and adjacent Control Area, to then compare potential impacts on several marine species of proposed wind development activities in the 501N Study Area and the Control Area between years. To establish a baseline, a Before-After-Control-Impact (BACI) design was employed to detect eventual patterns of sustained difference. Our primary objectives for this project were to: 1) Estimate the size and distribution of lobster populations in the 501N Study Area and adjacent Control Area; 2) Classify population dynamics of lobster such as length, sex, reproductivity success, age, diet, and disease; 3) Obtain movement patterns of adult lobsters through a tagging study. Catch and environmental data has been obtained in 2019 and 2020 during pre-construction and comparison between years and areas will be presented in this paper. For the 501N study area and Control Area there was no significant difference in lobster catch-per-unit-effort (CPUE) between 2019 and 2020 (K-S test, D=0.17, p-value=0.06; K-S test, D=0.16, p-value=0.13).

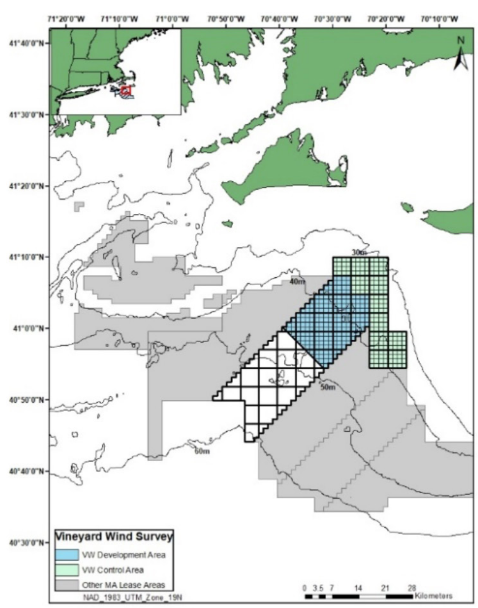

Figure 1: Vineyard Wind development area 501 N (blue), control area (green), and other Massachusetts lease areas (grey).

OPTIMISATION OF FEED MARGINS: EXTRACTING VALUE OUT OF FEED PROTEINS

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Aquaculture, which is an ancestral activity in some countries, has been modernizing and upgrading very rapidly for the last fifty years. After a first period which has allowed, to farmers , to ensure "very comfortable" margins, the global production increasing, market prices fell. Today it is essential to optimise production costs to maintain an acceptable margin for producers.

The recent dramatic situation induced by the COVID-19 pandemic, the fall of the market and the increase of production costs, has highlighted several factors that further weaken the situation of aquaculture producers, as the dependence on only a few international markets, the dependence on only few raw materials for the formulation of aquaculture feeds such as soybean meal and fish meal.

Therefore, it becomes essential to optimise the farming methods, for that purpose several tracks are possible. The feed is the one of the most important because it is the main production cost for the fish farmer, especially because of the high price of the proteins.

The incorporation of a protease in the formulation of a feed allows to seriously improve the digestibility of the long chain protein. Thus, it would allow to increase the proportion of news vegetable proteins, while maintaining a good digestibility and a correct amino acid profile. Moreover, the incorporation of an efficient protease in the formulation of the aquafeed would allow to increase significantly the percentage of digestible proteins and therefore it is possible to lower the gross protein content without affecting the zootechnical results.

Finally, when making purchases, consumers are increasingly sensitive to the environmental impact of aquaculture operations when making their purchases, especially since they are often located in fragile areas such as mangroves. Therefore, I will also discuss how the use of protease in the feed reduces the impact on the environment in three ways:

- By reducing the total protein concentration, which results in a better Protein Retention Efficiency and/or Protein Efficiency Ratio.
- By reducing the amount of marine meal and therefore reducing the need for industrial fish products.
- Minimising the percentage of non-digestible proteins that are dispersed in the environment.

Reducing the impact on the environment is important first and foremost from an ecological point of view to preserve the planet. But it is also important to ensure the sustainability of the farms and for the sake of image and sustainability of shrimp farming.

TRIPLOIDY DISRUPTS EARLY REPRODUCTIVE DEVELOPMENT OF FEMALES AND ALTERS SEX RATIOS OF SABLEFISH *Anoplopoma fimbria*

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Escapement is an ecological concern for marine fish reared in net-pen systems. To mitigate this risk, we have initiated research to develop approaches for reproductive sterilization. Sablefish (or black cod, *Anoplopoma fimbria*) is being used as a model for this line of research because of our sound understanding of its sex determination system and reproductive development, and availability of genetic/genomic information.

This study sought to induce triploidy for the first time in sablefish and assess its effects on early reproductive development. Protocols for triploidy induction using hydrostatic pressure or thermal shock were developed, as well as methods for determination of ploidy in whole larvae or blood samples. Three methods for ploidy determination were developed and validated: 1) measurement of cell size by flow cytometry, 2) counting nucleolus organizing regions, and 3) measurement of erythrocyte cell size in blood smears. Diploid (control) and putative triploid sablefish were reared until gonadal sex differentiation could be assessed by histology and ploidy and genotypic sex determined from blood samples, and then fish periodically sampled over a year.

Diploid control (XX-genotype) females 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 a portion of the triploid males (XXY) had testes that appeared similar to each other, composed of type-A spermatogonia. Interestingly, however, we found that >50% of the triploid male (XXY) sablefish developed ovaries. At one-year post-weaning, the gonadosomatic index (GSI) of phenotypic females (regardless of genotypic sex) was reduced by ~10 fold in triploids relative to diploids, while there was no difference in GSI between triploid and diploid phenotypic males.

Results from this study indicate that triploidy induction is disruptive to early ovarian development in sablefish and likely renders females effectively sterile. The observed ovarian development in over half the triploid males (XXY-genotype fish) could be due to a gene dosage effect associated with the X-chromosomes overriding the male sex-determining gene in some individuals. Future research will focus on scaling up triploid sablefish production and comprehensively assessing their survival and performance in each phase of aquaculture.

MOBILE AQUAPONICS: PROMOTING SMALL-SCALE AQUAPONICS SYSTEMS OF FARMING TO SMALLHOLDER FARMERS IN ZAMBIA

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Currently, 35 percent of fish stocks in our natural resources are over harvested, oceans contain more plastics than fish and cannot naturally provide for the 62 percent human consumption seafood demand anticipated by 2030 (Global Seafood Alliance, 2019). Aquaculture with innovations such as Aquaponics offer promising results for the future of fish farming and global consumption as they offer the best solution to food security, land conservation, and the restoration of our ecosystems.

Aquaponics is the cultivating of plants and fish together in a natural ecosystem using fish waste as plant nutrients. Aquaponic systems work in a closed loop, using natural bacterial cycles to convert the fish waste in form of ammonia to plant nutrients in form of nitrates. Small scale Aquaponics systems are the most common and offer a viable solution to rural communities in Zambia with little or no water, limited land, and unpredictable climate.

The main objective of the mobile aquaponics project is to provide a resilient response to climate change through this innovative farming technique: Aquaponics. These mobile aquaponic systems can be built at any scale anywhere in the word, using natural resources such as bamboo, using renewable energy such as solar energy and recycled materials that are already available in the communities. This project further seeks to improve food security, reduce poverty, and the unsustainable farming methods that are currently existent in Zambia. Through a demonstrative urban farm at the CAMFED climate smart center, the project will offer trainings in technical, management, leadership, business, and people skills to over 2,000 smallholder farmers especially women in the next 5 years, to create 20 new employment opportunities for women and youths to generate an extra income from the sale of both fish and vegetables for families with mobile aquaponic systems. The goal is to build healthy and resilient ecosystems and to encourage a collaborative and community driven approach to tackle issues of food insecurities caused by climate change and enable more fair and sustainable food supply chains.

Reference

Global Seafood Alliance. 2019. What Is Aquaculture and Why Do We Need It? [en línea]. [Consultado el 11 de octubre 2021]. Disponible en el World Wide Web:<https://www.globalseafood.org/blog/what-is-aquaculture-why-do-we-need-it/>

PILOT-SCALE DEPURATION DEMONSTRATES THE SUITABILITY OF NON-PATHOGENIC *Vibrio parahaemolyticus* AS A SURROGATE FOR COMMERCIAL-SCALE VALIDATION STUDIES

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Oysters are often consumed raw or undercooked; however, they are known to harbor *Vibrio parahaemolyticus*, a foodborne bacterial pathogen which causes gastrointestinal illness. Shellfish producers have proposed using depuration systems to support the oyster's natural biological function to reduce pathogen loads. For this approach to be suitable for industrial use, the efficacy of depuration must be validated in relevant species and at commercial scale using appropriate conditions (time, temperature). Validation of a new process requires several stages to verify assumptions and demonstrate method performance. This study describes the initial experimental phase to i) compare the efficacy of depuration in various oyster species, ii) to demonstrate the suitability of non-pathogenic *V. parahaemolyticus* as a surrogate, and iii) optimize depuration conditions at pilot-scale to identify process variables likely to achieve >3.0-log reduction of *V. parahaemolyticus*.

Three oyster species (*Crassostrea gigas, C. sikamea, C. virginica*) were placed in individual containers of artificial seawater containing a cocktail of either non-pathogenic (NP) or pathogenic (P) strains (n = 5 per cocktail) of *V. parahaemolyticus*. Inoculated oysters were then placed in a pilot-scale recirculating depuration system. Oysters were sampled every 24 hours from days 0 through 7 and *V. parahaemolyticus* in oyster tissue was enumerated using standard serial dilution and spread plating techniques on TCBS agar. Three replicate depuration trials were conducted, with 5 oysters of each species and cocktail sampled at each time point.

The inoculation procedure used in this study achieved at least a 5 log CFU/g *V. parahaemolyticus* in *C. gigas* and *C. sikamea*; however, *V. parahaemolyticus* accumulation in *C. virginica* was more variable and failed to achieve target density within 24 hrs of exposure to contaminated seawater. Accumulation of NP and P cocktails was similar for all oyster species. Depuration at 11°C achieved a >3 log CFU/mL reduction of the P cocktail in *C. gigas* and *C. sikamea* tissues within five days, whereas *C. virginica* averaged a 2.8 log CFU/mL reduction. *V. parahaemolyticus* clearance rate was rapid during the first 24-48 hours of depuration. NP *V. parahaemolyticus* was reduced at a comparable or lower rate than P *V. parahaemolyticus* indicating its suitability as a surrogate for commercial validations. Results from this study confirm that depuration can effectively reduce *V. parahaemolyticus* in live oysters; however, identical processes may not be suitable to achieve targeted log-reduction goals in all species.

DESIGNING AND MAINTAINING CUTTING EDGE AQUACULTURE SYSTEMS

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Intelligent and Adaptable Aquatic Facility Design is the foundational cornerstone of establishing and maintaining an aquaculture facility. Understanding and incorporating the long-term functionality needs during design planning, and periodic assessment of operations and technologies over time are key to ensuring a system that is built to withstand the tests of time. This talk will focus on key components to initial design as well as case studies in disaster operations and aging infrastructures.

ASSESSMENT OF IMMUNOTOXICITY BY QUANTITATIVE ALTERATION CORRELATED WITH ROS GENERATION IN HAEMOPOIETIC CELL POPULATION IN *Labeo Rohita*(HAMILTON, 1822) EXPOSED TO AZADIRACHTIN

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The increasing emphasis on the assessment and ecological monitoring of freshwater ecosystems has highlighted the necessity to deploy and subsequently evaluate appropriate biological indices.

In the present study, haemopoietic tissue imprints and flowcytometric analysis were employed to assess the impact of plant pesticide, azadirachtin (at No Observed Effect Limit concentration) on the cellular composition of head kidney cells from freshwater carp, *Labeo rohita*. The small lymphoidhemoblast decreased significantly throughout the experimental tenure whereas transient stages (i.e. basophilic erythroblasts, polychromatophilic erythroblasts and acidophilic erythroblasts) increased significantly suggesting the immediate requirement of younger erythroid cells into circulation post agrocontaminant exposure. Among leucocytes, the percentage of neutrophils rose and percentage of lymphocyte decreased significantly suggesting the impaired leukopoietic efficiency. Flowcytometricanalysis clearly subdivided the entire head kidney cell population into two separate groups, viz. granulocytes and lymphocytes. In a time dependent and dose independent experiment, these two populations showed significant variation (*P* value .000 and .003) and Pearson correlation suggested that both are negatively correlated (-.718).

Flowcytometric measurement of ROS production showed linear regression throughout the experimental tenure. A significant rise in ROS production indicated that these are good “oxidative stress biomarkers”for the ecotoxicological assessment of any pollutants, even in a limited exposure. Thus, this study establishes a novel and easy approach towards characterization of fish head kidney cell population which can be exploited to compare patternof quantitative cell subpopulation alteration due to external stress factor and an alternative system for immunotoxicity testing of xenobiotics in native freashwater fishes.

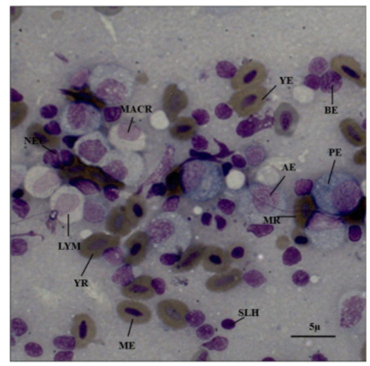

Fig 1: Major erythrocytic and lymphocytic cell types from the pronephric kidney imprint of *Labeo rohita*

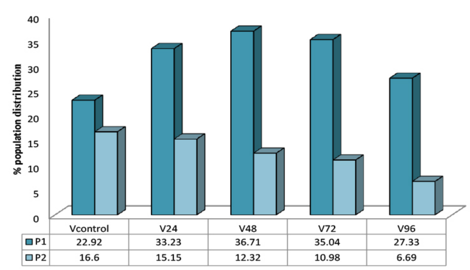

Fig 2: Comparative account of *Labeo rohita* head kidney cell subpopulation alteration owing to the treatment of with azadirachtin solution at NOEL concentration (1/7th of the LC_{50} value) at different exposure periods. The bar diagram shows two distinct population viz. granulocytes (P1) and lymphocytes (P2) respectively.

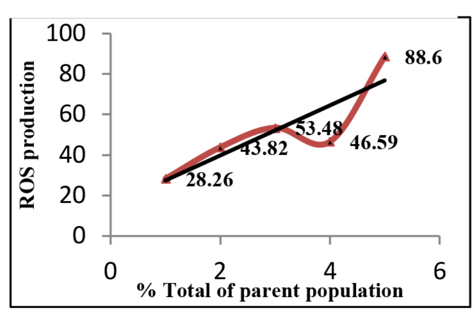

Fig 3: Graph showing the total % parent population of ROS production in *Labeo rohita* head kidney following azadirachtin exposure in a time dependent and dose independent experiment

PROGRESS ON THE PRODUCTION AND RELEASE OF CALIFORNIA HALIBUT *Paralichthys californicus* TO SUPPORT FISHERIES MANAGEMENT

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Hubbs-SeaWorld Research Institute has been stocking white seabass in coastal waters of southern California since the 1980's. Considerable expertise has been developed during this time on the culture and release of marine finfish for replenishment, and in recent years interest in expanding this approach to other species has increased. California halibut *Paralichthys californicus* have emerged as a primary candidate due to their importance, popularity, and depleted status in the region. Here we outline the progress to date in developing a replenishment program for California halibut, including the biological challenges associated with culturing this species, ongoing research to address these challenges, and next-steps for program expansion.

Efforts initially focused on establishing region-specific broodstock populations (in co-operation with local volunteer anglers) and reliable egg production in the hatchery. Once achieved, ensuring cultured juveniles intended for release are as morphologically, physiologically and behaviorally as wild-like as possible has become the primary research focus. Similar to other flatfish species, irregular pigmentation patterns (both albinism on the eye-side and excessive pigmentation on the blind-side) have become apparent in cultured California halibut. A series of experiments focusing on larval nutrition and husbandry practices have succeeded in increasing the rate of properly pigmented fish from <5% to 40%, and additional experimental work is being planned to eliminate this issue entirely. Sex determination is another important aspect in the culture process; juvenile cohorts have been male-biased to varying extents (66–96% male) and these preliminary results, supported by evidence from related paralichthids, suggest that masculinization may be occurring under certain stressful conditions during early development (e.g. suboptimal rearing temperatures). In-depth sex determination experiments are currently being designed, in addition to the use of quantitative modeling to examine the potential effects on fishery and population metrics of releasing juveniles with skewed sex ratios.

To date, small-scale releases of cultured California halibut have been undertaken in San Diego, serving as important opportunities to field test tagging methodologies, culture and release strategies, and post-release assessment. It is hoped that continued success in the prioritized areas of research (e.g. pigmentation, sex determination) will allow for larger-scale releases to occur, and incorporation of this fledgling program into the established framework for marine stock enhancement in southern California, the Ocean Resources Enhancement and Hatchery Program.

TISSUE DAMAGING AND OXIDATIVE STRESS EFFECTS ON GUPPY *Poecilia reticulata* (W. PETERS, 1859) DUE TO THE PROLONGED EXPOSURE OF ATRAZINE HERBICIDE

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Atrazine is an herbicide used to prevent pre-emergence broadleaf weeds in crops such as maize, sugarcane etc.,. But these herbicides expert a different impact on exposed organisms, when it reached the adjoining land and water. Such paradoxical events could be avoided to preserve the bio-diversity of natural population. The atrazine exposed different concentrations ranges from 70 ppm, 80ppm, 90ppm and 100ppm (Parts per million i/e μg/L) at 40 days duration. In this study the biochemical assays of molecular biomarker development of *Poecilia reticulata* Gill, Liver, Gonad of fish were made against Glyphosate exposure. The specific activity of tissue damaging enzymes such as Glutamate Pyruvate Transaminase(GPT) and Glutamate Oxaloacetae Transaminase(GOT) and Alkaline Phosphattase (ALP) were also measured. Simultaneously, the antioxidant enzymes such as Catalase(CAT), Superoxide dismutase(SOD)and Glutathion reductase also measured.. This study comes to a conclusion that, the antioxidant and tissue damaging enzymatic activity was increasing at early stage, subsequently there is a gradual decreased at the end of the exposures.Both antioxidant and tissues damaging enzymes was noted in *P.reticulata* at the lower concentration up to 70 ppm to 80ppm at 20 days duration whereas the 90-100ppm the elevation level of enzymes activity at 30days duration then declined their activities . However, GOT showed maximum activity was noted than GPT, and also reduction of GSI (Gonadosomatic index) as well as small changes in its HSI (Hepato somatic index) in all the concentrations. Biochemical and antioxidant enzymes response in fish can tolerated certain level of herbicide exposure at the low level concentrations.

DIFFERENTIALLY EXPRESSED GENES IN HEPATOPANCREAS OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE TOLERANT AND SUSCEPTIBLE SHRIMP (*Penaeus vannamei*)

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Acute hepatopancreatic necrosis disease (AHPND) is a lethal disease in marine shrimp that has caused large-scale mortalities in shrimp aquaculture in Asia and the Americas. The etiologic agent is a pathogenic Vibrio sp. carrying binary toxin genes, pirA and pirB in plasmid DNA. Developing AHPND tolerant shrimp lines is one of the prophylactic approaches to combat this disease. A selected genetic line of Penaeus vannamei was found to be tolerant to AHPND during screening for disease resistance. The mRNA expression of twelve immune and metabolic genes known to be involved in bacterial pathogenesis were measured by quantitative RT-PCR in two populations of shrimp, namely P1 that showed susceptibility to AHPND, and P2 that showed tolerance to AHPND. Among these genes, the mRNA expression of chymotrypsin A (ChyA) and serine protease (SP), genes that are involved in metabolism, and crustin-P (CRSTP) and prophenol oxidase activation system 2 (PPAE2), genes involved in bacterial pathogenesis in shrimp, showed differential expression between the two populations. The differential expression of these genes shed light on the mechanism of tolerance against AHPND and these genes can potentially serve as candidate markers for tolerance/susceptibility to AHPND in P. vannamei. This is the first report of a comparison of the mRNA expression profiles of AHPND tolerant and susceptible lines of *P. vannamei*.

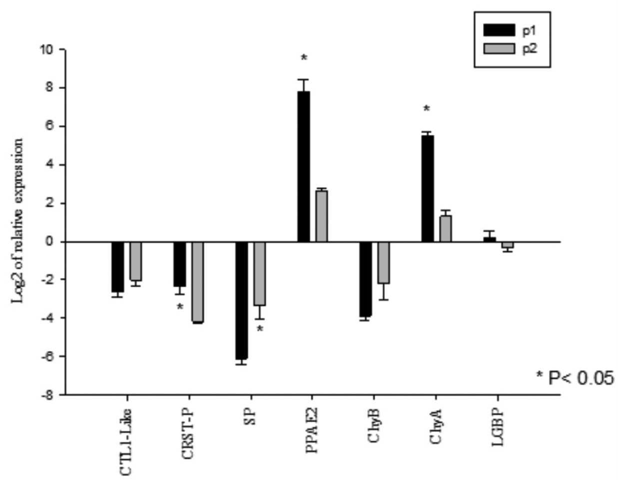

Figure. Comparison of gene expression profile from AHPND susceptible (P1) vs AHPND resistant/tolerant (P2) population. Expression levels of each gene are shown relative to the expression in P1 negative control treatment. The data are presented as log2 of relative expression ± SD. Statistical significance between control and challenged animals for each of the candidate gene was determined using Student's t-test. *=P<0.05.

COMPARING STABLE CARBON AND NITROGEN ISOTOPES TO EXAMINE FEEDING TRENDS IN BLUE CRABS (*Callinectes sapidus*) ACROSS VEGETATION ZONES IN BLACKBIRD CREEK, DELAWARE

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Blue crabs are crucial to Delaware as they account for $15 million of the annual state's economy. Evaluating the feeding habits of commercially important aquatic species such as blue crabs is necessary to manage and conserve them. C and N isotopes are used as tracers of nutrient flow in food webs. The carbon isotope $\delta^{13}C$ is used primarily to trace the food source from primary producers, while the nitrogen isotope $\delta^{15}N$ indicates the trophic level of species and dietary shifts. In this study, stable Carbon (C) and Nitrogen (N) isotope ratios were compared in blue crabs collected from various sites along Blackbird Creek, Delaware, to provide insight on their feeding habits.

Blue crab sampling was conducted in 2017, 2020 and 2021. Sampling sites were distinguished by the existent dominant vegetation along the creek: *Sporobolus alterniflorus* dominant, *Phragmites australis* dominant or mixed vegetation (Fig 1). Blue crab tissue samples were obtained from the walking legs and used in C and N isotope analysis. Based on our preliminary results from 2017, we found no significant difference for the $\delta^{13}C$ among blue crabs collected from different sites with a reported average range of -19.47 ‰ to -21.28 ‰. In contrast, there was a significant difference in the $\delta^{15}N$ values, which ranged from 9.32 ‰ to 13.44 ‰ (Fig 2). The preliminary results from $\delta^{15}N$ suggest a difference in feeding habits of blue crabs at different sites. Complementary gut analysis will be conducted to draw conclusive results on availability and type of prey.

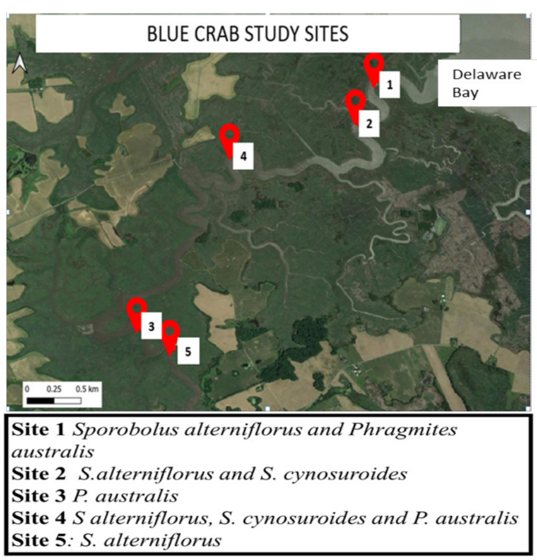

Figure 1. Map showing blue crab study sites

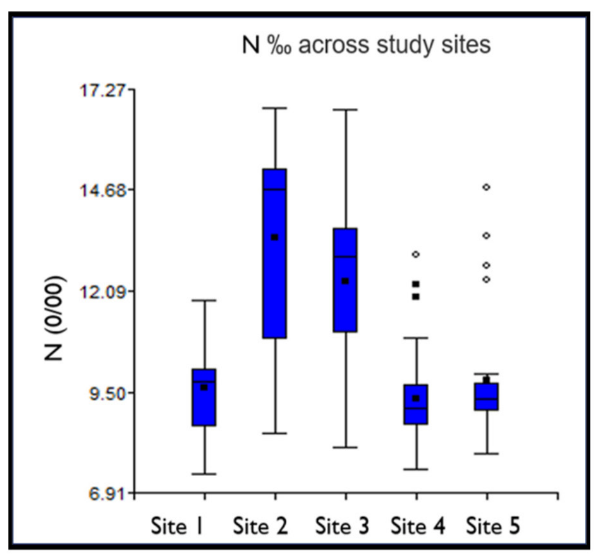

Figure 2. $\delta^{15}N$ values across sites

LINKING LOCALIZED SLUDGE DIGESTORS TO POLYGEYSERS® WITH PNEUMATIC EXCHANGE TO FACILITATE DECOUPLED AQUAPONICS OPERATIONS

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The use of airlifts to provide circulation, aeration, and carbon dioxide stripping not only saves energy, but also provides a degree of disease isolation and a relatively stable head environment facilitating implementation of pneumatic sludge discharge. Pneumatic sludge discharge utilizes head differentials generated in the cyclic backwashing of a PolyGeyser® bioclarifier to drive sludge removal eliminating the need for manual or pumped intervention for this crucial task.

Use of a RAS specific (localized) sludge basin provides for nearly 100% return of the immediate water loss associated with the sludge removal process. These discharged waters are encouraged to drop (settle) solids before being returned to the bioclarifier as pressure conditions change. Captured sludge is then dramatically reduced in mass by digestion before being finally removed after many backwash cycles.

The sludge digestor can be operated aerobically, anaerobically, or sequentially. For aquaponic applications sludge residence times of 5-10 days appear sufficient to achieve a high degree of nutrient release as the sludge volume is reduced by nearly 50% under aerobic conditions. Use of a hypolimnetic aerator (HLA) configuration maximizes clarification potential. Splitting return waters post airlift facilitates "solids free" dosing to hydroponic systems with nutrient enriched waters. Isolation of the hydroponic component from the fish component allows for optimization of water quality for plant growth while avoiding long term salt accumulation.

After considering digestion modeling analysis, a sizing recommendation of 83 liters/kg feed-day (10 gallons/lb feed-day) is being made for aquaponic applications. When pairing the digestor with a floating bead bioclarifier, a guideline of 1.3 liters-digestor/liter-beads (10 gallons/ft^3-beads) can be used when current aquaponic filter loading criteria 62 liters/kg feed-day (1 ft^3/lb feed-day) are considered.

PHENOTYPIC CHARACTERIZATION OF LEPTIN RECEPTOR DEFICIENT RAINBOW TROUT *Oncorhynchus mykiss*

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Leptin is a pleiotropic hormone that is known for regulating appetite and metabolism, and it has been implicated in many other facets of vertebrate physiology. While mammalian leptin elicits anorexigenic effects and acts as an adipostat, research on teleost leptins show varying responses to feed consumption and the hormone is primarily produced in the liver. Most fishes have only one leptin receptor (LepR/LepRA1), however, paralogs have recently been documented in a few species. We revealed a second leptin receptor (LepRA2) in rainbow trout that is 77% similar to trout *lepra1*, and the levels of these receptors are differentially expressed across tissues and during fasting, suggesting there are paralog- and tissue-specific functions.

To further understand how leptin and its receptors might function to regulate growth and energy utilization in rainbow trout, we used CRISPR/Cas9 genome editing to disrupt the leptin receptor genes. Triplicate tanks 150-L tanks (3 WT and 3 Mutant tanks) were stocked with 8-10 rainbow trout (145.9 ± 3.1 g mean body weight, BW). Fish were provided 2/3 ration of feed at 1.25% BW with automatic feeders, after which the fish were hand fed to satiation daily and were sampled at three and six weeks. LepR mutants exhibited a hyperphagic phenotype, which led to heavier body weight, faster specific growth rate, increased viscero- and hepatosomatic indices, and greater condition factor (Figure 1). Muscle glycogen and plasma leptin were elevated in LepR mutant fish at 3 weeks. Plasma cortisol was higher in mutants at 3 weeks and blood glucose at 6 weeks, however, neither was significant. A range of hypothalamic genes involved in feed regulation were measured (*agrp*, *npy*, *orexin*, *cart-1*, *cart-2*, *pomc-a1*, *pomc-b*). No differences were detected between WT and mutants except for *pomc-b*, where levels were over 7-fold higher in LepR mutant fish at 3 weeks. This suggests that leptin signaling in the brain is likely mediated in part through *pomc-b*. These studies show that rainbow trout deficient in LepR signaling display a hyperphagic phenotype, similar to mammals. Leptin clearly plays a key role in food intake and growth in rainbow trout, however, additional studies are needed on adipose- and glucoregulatory pathways to help better understand the phenotype of the LepR deficiency.

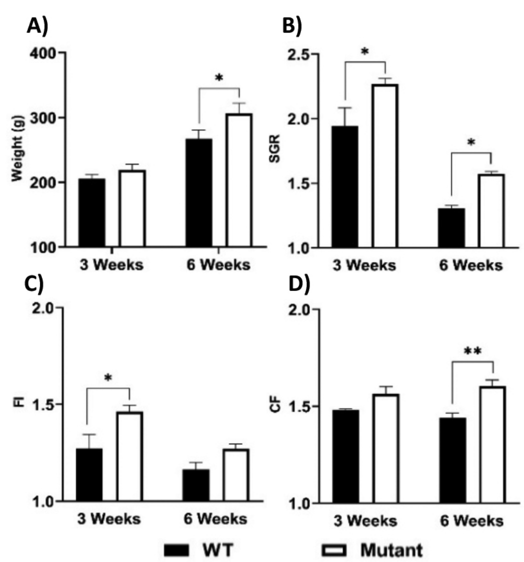

Figure 1. LepR CRISPR/Cas9 edited rainbow trout fed to satiation. WT and LepR mutant fish were sampled at 3 and 6 weeks for: **A**) body weight (g), **B**) specific growth rate (SGR), **C**) feed intake (FI), and **D**) condition factor

STUDIES OF GROWTH AND SURVIVAL DURING THE LARVAL AND EARLY-JUVENILE STAGES OF YELLOWFIN TUNA AT THE IATTC'S ACHOTINES LABORATORY IN PANAMA

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The Inter-American Tropical Tuna Commission (IATTC) has been conducting research on the early life history and biology of tropical tunas at the Achotines Laboratory in the Republic of Panama since 1986. Spawning from a population of yellowfin tuna has taken place almost daily in the Laboratory's land-based tank since 1996. The near-daily spawning of yellowfin at the Achotines Laboratory represents the only sustained spawning of yellowfin in captivity in the world. In October 2021, the Laboratory reached a milestone of 25 years of sustained spawning of yellowfin.

Eggs and larvae collected from spawning events are used to conduct ecological experiments on early life stages and estimating effects of environmental factors on pre-recruit growth and survival. Pre-recruit research on yellowfin at the Achotines Laboratory has focused on growth and survival dynamics of larvae (first 3 weeks after hatching), but in recent years the research focus has expanded to the early-juvenile stages (1-6 months). Growth rates have been estimated for all transformation and early-juvenile individuals reared at the Achotines Laboratory in land-based tanks or a sea cage over a 20 year period; the early-juveniles have ranged from 1.6 - 28.0 cm in length and up to 158 days old. Larval growth is exponential in length and weight, and early-juvenile growth is exponential in weight and non-linear in length which when linearized is estimated at 1.0 – 3.8 mm/day. In 2015, in collaboration with Kindai University, the first transfer worldwide of yellowfin juveniles from land-based tanks to a sea cage was successfully completed at the Achotines Laboratory.

The studies of yellowfin growth during the first 6 months have strong application to tuna ecology and aquaculture. Improved rearing success of early-juvenile yellowfin now provides opportunities to study density-dependence in juvenile growth, release tagged early-juveniles in coastal waters of the Panama Bight to provide rare information on pre-recruit movements and distribution, and support the potential completion of full-life-cycle rearing of yellowfin. In this presentation, the overall growth dynamics of larval and early-juvenile yellowfin will be described, and juvenile rearing studies planned for 2022 will be summarized.

WHERE, WHEN, AND HOW EXTREME CLIMATIC EVENTS AFFECT OLYMPIA OYSTER POPULATIONS IN SAN FRANCISCO BAY: IMPLICATIONS FOR CONSERVATION

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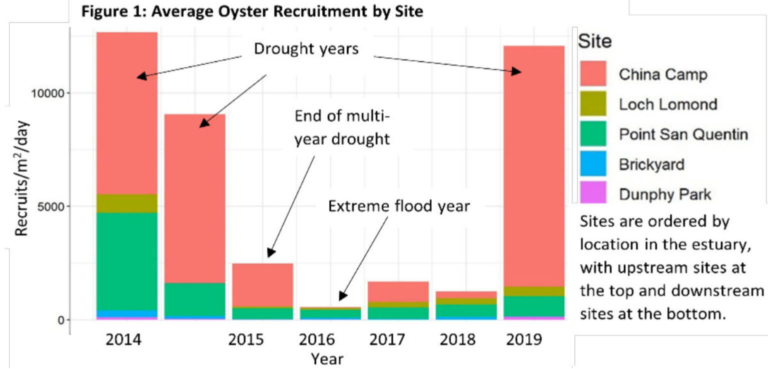
Ostrea lurida is a foundation species found along much of San Francisco Bay's rocky intertidal shoreline but has lost a significant proportion of its historic population. As a primary target for restoration, it is important to understand which sites will be most successful in the long-term when considering extreme climatic events. This field study measured *O. lurida* population density and recruitment, along with coinciding weather and water quality data from approximately 2014 to 2021. The aim of this research is to determine how extreme precipitation and temperature have affected *O. lurida* and identify potential refuges for *O. lurida* in San Francisco Bay as extreme events increase with climate change.

For sessile invertebrates such as oysters, the location where they settle is critical to prolonged survival. Benthic organisms typically experience less environmental variability toward the mouth of an estuary due to increased marine influence which modulates temperature and salinity fluctuations. In Central California, some downstream sites are more frequently protected by marine fog, reducing heat stress by scattering solar radiation. Thus, variation in conditions throughout the estuary can affect consistency of recruitment and long-term population persistence for *O. lurida* and other estuarine organisms.

While upstream sites tend to support high densities of oysters during drought years, upstream populations are also the most volatile in terms of adult density and juvenile recruitment (Figure 1). Our data indicate more stable but lower density and recruitment at downstream sites closer to the ocean. We found the greatest number of extreme heat events (in-situ temperatures >30C) at China Camp State Park, the most upstream site, which at times contains the greatest oyster density in the species' range. *O. lurida* has also experienced 97% to 100% mass mortality at this same location in response to low salinity during flood years. As climate change increases the frequency and severity of flooding and heat waves, it may become increasingly difficult for *O. lurida* populations to recover before being wiped out by another extreme event. Understanding the spatial dynamics of extreme events can help anticipate population-level impacts of climate change and inform future restoration to promote long-term resilience.

Figure 1: Average Oyster Recruitment by Site



DISTRIBUTION AND DEMOGRAPHICS OF FOSSIL OYSTERS ON THE ATLANTIC CONTINENTAL SHELF

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Oysters in the genus *Crassostrea* and their predecessors are present in fossil record to at least 50 million years ago. These species have evolved to be long lived, highly fecund, and settle gregariously to create essential three-dimensional habitat in estuaries worldwide. The underlying shell base must be maintained by accretion rates higher than the combined impacts of sedimentation, harvest, and sea level rise for reefs to persist. Fossil oysters provide a unique opportunity to understand reef dynamics during periods of rapid sea level rise. This project describes the distribution of fossil oyster shell and demographics on the mid-Atlantic continental shelf at depths ranging from 27-64 m (90-210 ft). Fossil oyster shells were found at 47 of 66 dredge survey locations. Fossil shell lengths ranged from 53 to 223 mm (Fig. 1). Collected fossil can be used to estimate reef accretion rates and carbonate production in the distant past and facilitate comparison with extant populations.

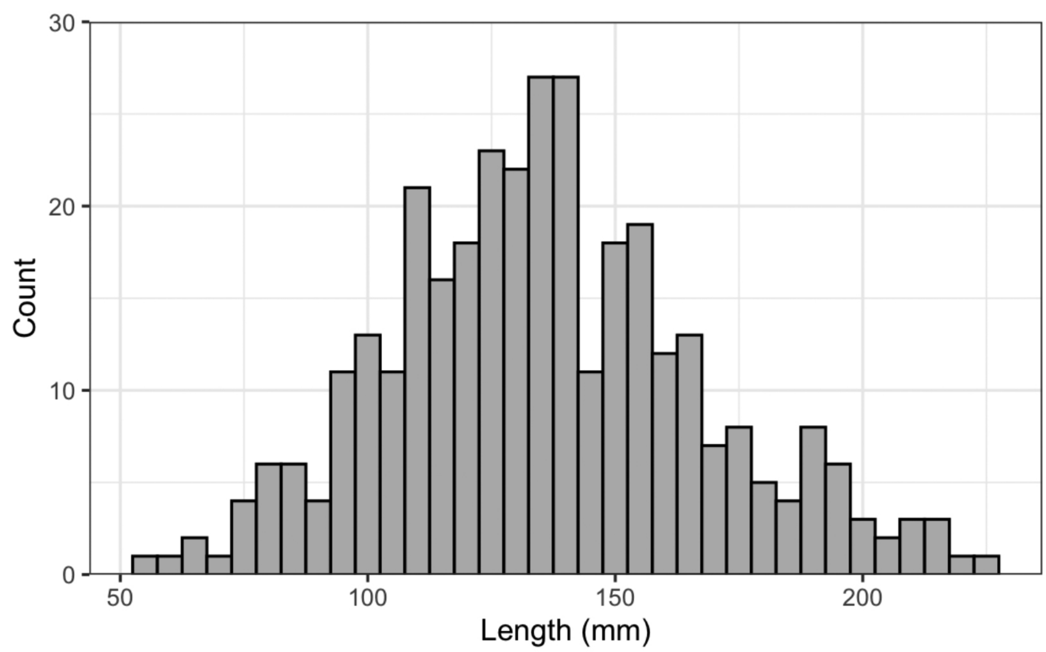

Figure 1. Length frequency for fossils oyster shells found across all survey locations on the Atlantic continental shelf.

ESTIMATING POST-SETTLEMENT GROWTH AND SURVIVAL IN EASTERN OYSTERS *Crassostrea virginica* IN THE CHESAPEAKE BAY

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Oysters are a benthic dominant, important ecosystem engineer, and provide critical ecosystem services in temperate estuaries worldwide. Despite their importance, oyster populations have declined 85% globally and are at <1% their historic harvest levels in Virginia. Many invertebrates exhibit high post-settlement mortality in early benthic life stages, but there is limited quantitative information available due to the small size of post recruit stages. The goal of this project is to estimate growth and mortality rates in newly recruited oysters in the Chesapeake Bay. In summer 2021, shellstrings were deployed at two long term monitoring sites in the James River. In summer 2022, this was expanded to include one site in both the Great Wicomico and Piankatank Rivers. At each site, six serial deployments of shellstrings were set at two-week intervals. A susbset of two shellstrings were retrieved at intervals. The number of recent oyster recruits were counted on each shellstring, a subset were photographed, and individual recruits were measured using ImageJ software. The length frequency data documents growth of cohorts over time and enables estimation of growth and mortality rates over space and time. Given that oysters have multiple spawning events throughout the summer, this approach identifies optimal time windows or conditions that improve settlement success and survival. An improved understanding of early life history recruitment, growth and mortality rates can help guide shell replenishment and oyster population management in the Chesapeake Bay.

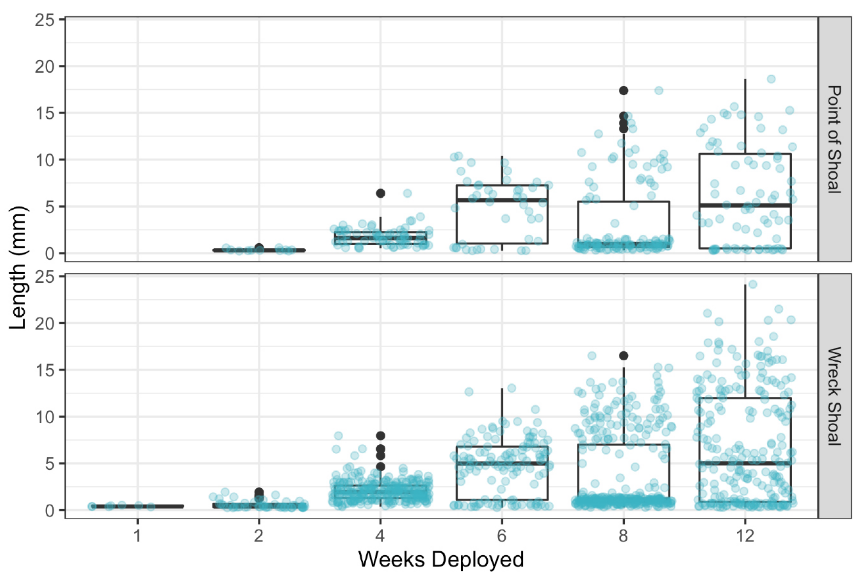

Figure 1. Size distribution of recruited oysters at Point of Shoal and Wreck Shoal in the James River during summer 2020. Plots show size distribution for each retrieval of a single deployment. Blue points indicate individual size measurements and are jittered.

CIRCUMVENTING EXTINCTION: ADAPTIVE DISEASE MANAGEMENT IN ENDANGERED WHITE ABALONE *Haliotis Sorenseni* CAPTIVE BREEDING AND OUTPLANTING EFFORTS

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White Abalone (*Haliotis sorenseni*) is an ecologically and culturally important marine species on the brink of extinction. Once found throughout the waters off the coast of Southern and Baja California, recent surveys have shown a 99% decrease in the total wild population since the 1970s. While the commercial fishery for the species was halted in 1996, their population has continued to decline throughout the first two decades of the new millennium and the density of the remaining population--estimated to be fewer than 3,000 individuals--appears to be too low for this broadcast-spawning species to reproduce. In an attempt to halt their demise, white abalone were federally listed as endangered in 2001, becoming the first marine invertebrate to receive that designation.

The White Abalone Recovery Program was founded shortly thereafter with the goals of collecting broodstock, rearing juvenile white abalone, establishing self-sustaining populations through outplanting efforts, and expanding scientific knowledge of white abalone. The program, which includes 15 institutions throughout California and Mexico, has been successful in producing new generations of white abalone who have found new homes in the wild through outplanting efforts that began in 2019. However, Withering Syndrome, a chronic degenerative disease caused by the Rickettsiales prokaryote *Candidatus* Xenohaliotis californiensis (CaXc), might present a challenge to laboratory-raised animals encountering the pathogen in the wild. While older animals may be more robust, limited laboratory rearing capacity requires optimizing age-at-outplant with husbandry resources. To develop a greater understanding of the variable susceptibility of white abalone age classes to CaXc infection, we have performed an experiment in which we exposed one-, two-, and three-year-old abalone to the pathogen to determine whether age at outplanting may influence susceptibility to CaXc infection and subsequent mortality. The result of this experiment will hopefully inform which animals stand the greatest chance of survival once they are outplanted.

Because the scale of production necessary to thwart extinction of the species will likely require the infrastructure of commercial abalone farms, we are conducting an experiment to explore the effects of culturing white abalone under typical abalone farm conditions, including exposure to pathogens in untreated seawater. It is our hope that this experiment will yield greater insight into the possibility of using farm production to restore this species throughout its native range.

INTERNATIONAL SHIPPING OF LIVE TROUT AND EGGS

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The international movement of trout and their eggs from the United States to other countries, for non-human consumption, is overseen by the U.S. Department of Agriculture (USDA), Animal & Plant Health Inspection Service (APHIS), Veterinary Services (VS). Each destination country may have different import requirements for the same type of animal so how does one keep everything straight?! This presentation will address where to find the information and requirements for export, the process of health certificate endorsement (including electronic submission), and points of contact for assistance. The pre-export requirements for an aquaculture establishment to be inspected and approved by APHIS as a Registered Aquaculture Export Facility will also be discussed, in addition to the role of an APHIS Accredited Veterinarian.

PREVALENCE, DISTRIBUTION, AND CONTROL OF SHELL-BORING POLYCHAETES ON OYSTER FARMS FROM CALIFORNIA TO ALASKA

Julieta C. Martinelli*, Megan E. Considine, Helen R. Casendino, Teri L. King, Laura H. Spencer, Carolyn Tarpey, Lorenz Hauser, Lindsay Alma, Jaqueline L. Padilla-Gamiño, Steven S. Rumrill, and Chelsea L. Wood

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Marine polychaetes in the genus *Polydora* (and other related genera) can burrow into the shells of commercially important bivalves, creating unsightly blisters and burrows. Because they are unappealing to consumers and can burst, these blisters are an economic burden on producers whose stock goes to the half-shell market. The west coast of the US is the leading producer of aquaculture oysters in the country, making it important to understand the prevalence of these pests in Pacific oysters. We obtained over 3,000 oysters from 35 farms in four states (CA, OR, WA and AK) and determined infestation prevalence across four sampling seasons. Prevalence per state ranged from 13 to 37%. Polychaetes were extracted from infested shells for molecular analyses. We used mitochondrial (CO1) and nuclear (18S) genes for species-level identification. Genetic markers confirmed the presence of *P. websteri* and *P. hoplura* at more than one site in the west coast, as well as multiple other polychaete species.

Given the difficulties faced by growers in controlling this pest, we consulted shellfish farmers from Washington State to create treatments that are both effective and easy to apply on farms. We tested three treatments in experiments carried out with diploid Pacific oysters infested with shell-boring polychaetes and collected from Washington State. Our experimental design consisted of three stages: (1) the main experiment to test treatments, (2) a growth assessment, (3) and respirometry trials. We subjected 675 oysters to three treatments: 'Dry', 'Fresh-Dry', 'Refrigeration' and a Control. The 'Dry' treatment was 100% effective at killing worms and the other two treatments were nearly as effective (Fig. 1). The growth assessment indicated that none of the treatments negatively affected oyster growth, and the respirometry trials showed no significant differences in oxygen between treatments.

Findings from this study will allow us to advise the US west coast shellfish industry on the progress of the spread of shell-boring polychaetes and strategies for reducing the economic impacts of these globally-distributed pests.

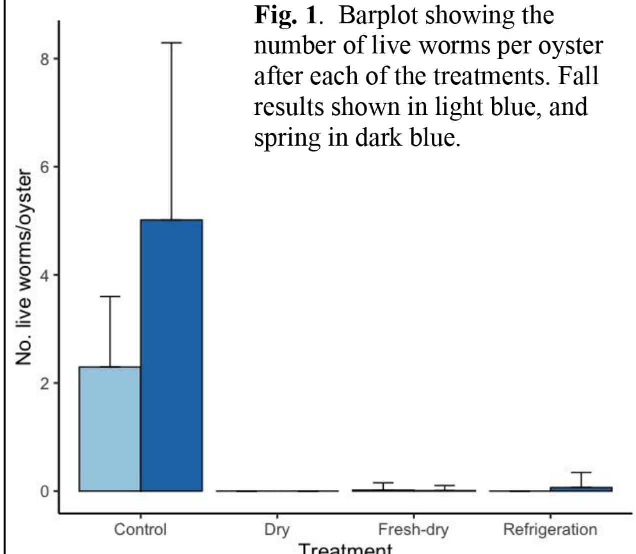

Fig. 1. Barplot showing the number of live worms per oyster after each of the treatments. Fall results shown in light blue, and spring in dark blue.

RECONSTRUCTING THE ECOLOGICAL HISTORY AND PAST ENVIRONMENTS OF NATIVE OLYMPIA OYSTERS TO INFORM THEIR CONSERVATION AND RESTORATION IN THE SALISH SEA

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Historical baselines inform restoration efforts, allowing us to interpret long-term changes in marine ecosystems prior to anthropogenic activities. In Washington State, restoration of native oysters (*Ostrea lurida*) is limited by shifting baselines and the lack of information on Olympia oyster populations before they collapsed in the late 1800s. Preserved historical and fossil oyster beds from the recent past can reveal community-level processes, such as biotic interactions, disease, and habitat provision to other organisms. Important metrics for oyster restoration such as oyster density and size frequency distributions can also be quantified from preserved oyster beds. At the individual level, past oyster shells act as archives of past water temperature, salinity and pH at the time organisms were alive and depositing their shells.

Our project seeks to answer three main questions: (i) What was the water temperature and salinity in environments where historical native oysters thrived in the past? To answer this question we will use oxygen isotopes, Ba/Ca ratios and radiocarbon dating. (ii) How does the abundance of trace metals found in past Olympia oysters compare to the abundance of trace metals found in this species today? To answer this question, we will carry out mass spectrometry (ICP-MS) analyses to measure trace metal content in shells. (iii) Can we reconstruct past biotic interactions, such as parasitism and predation, from historical oyster shells and compare the prevalence of these interactions with what we see today? To answer this question, we will observe and record traces of past biotic interaction such as those left by shell-boring polychaete worms (Fig. 1 A-C), and traces of predation by drilling gastropods. Assessing the past prevalence of these interactions together with radiocarbon dating of shells will allow us to generate a timeline for the abundance of pests and predators that may be threatening the success of contemporary restoration projects.

Historical and fossil shells are proven to be informative, quantitative tools to reconstruct past environments and biotic interactions. The insights gained from the integration of these past baselines with modern environments will help advise the planning and management of conservation and restoration efforts for Olympia oysters in the Salish Sea.

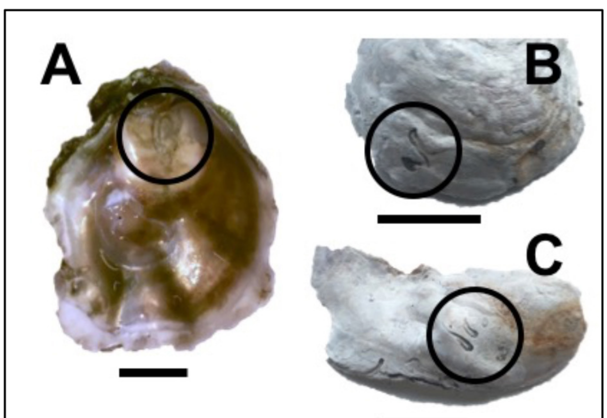

Fig. 1. (A) Modern Olympia oyster shell with traces of parasitic worms, (B-C) Historical shells with similar traces. Size bars indicate 1 cm.

VIRUS INTERFERENCE AND OCCURRENCE IN CRUSTACEANS

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Virus interference is a phenomenon first observed in the XVIII century and studied even before virology was recognized as a science. The first studies on virus interference were done in the 1930s and 1940s in viruses infecting different vertebrate or bacterial hosts. Virus interference has been reported to occur between viruses that are pathogenic to humans or to animals of economic importance.[1] Recently it has been suggested that interference may occur between influenza virus and the coronavirus causing the pandemic disease COVID-19.[2] Thus, the phenomenon may have medical or animal-production relevance due to the possibility to use it as alternative to chemical therapies against virus infections, to reduce the severity of disease/mortality caused by a superinfecting virus. Virus interference induces host resistance to a superinfection caused by a pathogenic virus causing obvious signs of disease and/or mortality, due to the action of an interfering virus abrogating the replication of the former virus. Different degrees of inhibition of the superinfecting virus can occur. Due to the emergence of novel pathogenic viruses, virus interference may become an important antiviral strategy against different pathogens in various hosts, including commercially important farmed aquatic species.

Evidence of interference has been reported between some highly pathogenic viruses mainly affecting farmed shrimp [interference between Yellow-head virus (YHV) and Taura syndrome virus (TSV)], and interference between a highly pathogenic virus [white spot syndrome virus (WSSV) and the less damaging virus [infectious hypodermal and hematopoietic necrosis virus (IHHNV)]. The latter virus interaction has by far been better studied.

This paper presents data on virus interference between WSSV and IHHNV, as well as between TSV and YHV, resulting in reduced disease and mortality of affected shrimp hosts (*Penaeus vannamei, P. stylirostris, P. monodon, P. duorarum).* This phenomenon may be applied as a potential natural strategy to control highly pathogenic virus infections in these animals.

References

[1]Escobedo Bonilla C.M. 2021. Mini Review: Virus Interference: History, Types and Occurrence in Crustaceans/ Front Immunol. 12: 674216.Published online 2021 Jun 11. doi: 10.3389/fimmu.2021.674216.

[2]Yang L, He D and Wang L. 2021. Editorial: Interference of COVID-19 and Influenza Infections. Front. Public Health 9:818199. doi: 10.3389/fpubh.2021.818199.

ALTERNATE APPROACH TO *Saccharina latissima* FARMING: DIRECTLY SEEDING JUVENILE SPOROPHYTES AND GAMETOPHYTES

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The kelp farming industry in the U.S. is highly dependent on wild population collections for a source of meiospores. Harvesting reproductive plants from the wild can have a negative impact on these populations if not enough time and care is given for them to recuperate. As the kelp industry grows this impact will likely increase as well. Therefore, we are exploring alternate methodologies where gametophytes are vegetatively cultured and held in cold storage to seasonally produce seedstring. Juvenile *Saccharina latissima* sporophytes and gametophytes can be applied by spraying directly onto the seedstring or rope instead of the traditional meiospore settling techniques. This approach has the potential to reduce pressure on wild populations, reduce nursery time, space and cost requirements for hatcheries supporting kelp farming industries. Preliminary results from experimental plots show that the direct seeding technique can produce large quantities of adult sporophytes after a growing season in the Gulf of Maine. Further investigation on application methods will be required to increase effectiveness of this technique and provide opportunities to work with strains that result from selective breeding programs of kelp.

PHYSIOLOGICAL EFFECTS OF AN ASTAXANTHIN SUPPLEMENTED DIET ON STRESSED NILE TILAPIA

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Even though aquaculture may solve the world's need for a substantial protein source, it also has its problems, with stress being the most significant issue. Stress causes fish to become more susceptible to bacterial infections and viruses, causing them to become ill. To treat the effects of stress on fish, antibiotics and other chemicals are used. The use of antibiotics and other chemicals in aquaculture are damaging ecosystems, increasing antibiotic resistance, and impacting human health. Nutraceuticals, food or food additivities with medicinal properties, are an alternative to using antibiotics and other chemicals. Astaxanthin (AST), a keto-carotenoid, has been shown to have strong antioxidant, anti-diabetic, anti-cancer, and immunomodulation properties. The effects of AST on the modulation of stress in fish has not been investigated extensively. The objective of this experiment was to investigate the effect of AST on the stress response of Nile tilapia (*Oreochromis niloticus)* by measuring the blood glucose, packed cell volume, and condition factor. The were three experimental groups: control, stressed, and stressed treated with astaxanthin. After 4 weeks we found there were only significant differences between the control and stressed groups for blood glucose. There were no significant differences between the stress and AST group and between the AST and control groups. The data suggests that a diet supplemented with the current amount of AST (200 mg/kg of feed) is not able to modulate the stress response of Nile tilapia. Further research will need to be conducted to determine if AST will have a therapeutic effect on the stress response.

INFLUENCE OF RISING OCEAN TEMPERATURE ON THE RED ABALONE *Haliotis rufescens* THROUGH CHANGES IN ITS FOOD SOURCE, MARINE SEAWEED *Palmaria mollis*

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Primary producers, such as marine seaweeds, transform inorganic nutrients into organic matter through photosynthetic processes. Their cells chemically respond to their environment by producing biomass with different chemical compositions. Temperature is one environmental factor that can affect the chemical composition of primary producers (e.g., lipid, protein, and carbohydrate content) and alter its nutritional quality. Changes in nutritional quality of marine seaweeds due to ocean warming can have cascading consequences on herbivores at higher trophic levels.

In the present study, we examined the effect of ocean warming on the marine seaweed *Palmaria mollis* (dulse) commonly used in aquaculture, as our model primary producer and cultured juvenile red abalone, *Haliotis rufescens,* as our model primary consumer. Red abalone are important in food production and conservation aquaculture in California. The effect of temperature on the growth and chemical composition (lipid, protein, and carbohydrate content) of dulse and subsequent effects on the growth and chemical composition of red abalone were studied.

Juvenile red abalone were grown for 104 days, they were fed dulse grown under three different temperatures for at least three weeks. Abalone growth was measured at days 0, 40, 75 and 104 and their chemical composition (lipid, protein, and carbohydrate content) was analyzed at the end of the experiment. Dulse was grown by tumble culture at 13°C, 15°C and 17°C under artificial light for three weeks. Dulse mass was recorded every seven days and its chemical composition was analyzed every three weeks.

This study is a first step to understanding how possible changes in ecological interactions will affect the food chain. It will help to illustrate the effects of rising ocean temperature on primary producers and the subsequent effects on primary consumers. Furthermore, this study will also assist assessment of seaweeds nutritional value to the food web for biological and ecological investigations.

IMMUNE COMPETENCY AND GROWTH RESPONSE VARIABILITY OF *Oreochromis niloticus* UNDER DIETARY SUPPLEMENTATION OF BLACK SEED (*Nigella sativa*)

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Black seed (*Nigella sativa*) used as a natural immuno-stimulant in the feed of various farmed animals. In present experiment, effect of three dietary inclusion levels of *Nigella sativa* was assessed on growth and immune response of *Oreochromis niloticus* for 8 weeks. Experimental groups received three different levels of *Nigella sativa* i.e. 2, 3, and 4 % in feed while control group received feed without *Nigella sativa*. Growth parameters (weight (g), length (cm), feed conversion ratio and specific growth rate) and immunity response (white blood cells, lysozyme activity, and globulins protein) were investigated. All the recorded growth and immunity parameters of *Oreochromis niloticus* showed a significantly different outcome at the end of trial. Among the immunity parameters, group of fish received 4% black seed in diet showed statistically higher increase in white blood cell count (198 $10^3/\mu L \pm 3.78$), globulin protein (23.66 mg/dl ±1.21) and lysozyme activity (60.66 μl/ml ±1.20) followed by 3% and then 2% supplemented groups. Group fed by 4% black seed showed statistically highest increase in weight gain (2.05 gm±0.37), gain in length (0.32 cm ±0.16) specific growth rate (1.34±0.45) and best value of feed conversion ratio (2.27±0.56) followed by 3% and then 2% supplemented groups. Results of present study reveals that *Nigella sativa* improves the growth and immunity of *Oreochromis niloticus* significantly at 4 % inclusion level in feed.

SOFTWARE DEVELOPMENT FOR MORE REALISTIC SIMULATION OF SELECTIVE BREEDING PROGRAMS FOR OYSTERS

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Software for simulating breeding programs has become a valuable tool for optimizing the design of selective breeding programs for plants and animals. Breeding programs for aquaculture of marine bivalves, such as oysters, could benefit from such technology; however, functionality that incorporates the unique genetic characteristics of oysters is lacking. Currently available technology has been designed to simulate the genetic characteristics found in terrestrial livestock and crops. However, the relatively high genetic diversity of oysters, along with a likely high mutation rate and genetic load, distinguishes them from traditionally bred species and warrants the creation of additional functions or software for more realistic simulation of oyster populations. In addition, oysters in breeding programs can be diploid, triploid, or tetraploid, making the creation and mating of polyploid individuals an especially valuable component of new oyster-based simulation technology. Here we outline goals, strategies, and progress towards the creation of new software to guide the design of oyster breeding programs and aquaculture species with similar genetic architecture.

UTILIZATION OF COPEPODS AS ALTERNATIVES TO ROTIFERS AND/OR ARTEMIA FOR WEANING OF LARVAL BURBOT (*Lota lota maculosa*)

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Burbot (*Lota lota maculosa*) are one of the most promising new species for commercialization as a foodfish. However, burbot larval culture relies solely on the use of live prey items, such as rotifers and *Artemia* for food. Production of live feed is expensive, labor intensive, and often requires substantial space allocation for an aquaculture facility. For these reasons, a study was conducted to determine the potential use of frozen copepods and an otohime commercial diet as a substitute for live feeds in burbot larval culture.

An eight-week feeding trial (8-64days post hatch [dph]) was conducted to evaluate the effect of weaning diet on burbot larval growth and survival. A total of 7200 burbot larvae were distributed into six feeding regimes (FR): FR-A (control), FR-B, FR-C, FR-D, FR-E and FR-F (Table 1), each with three replicates. At the end of the trial, results showed that survival and growth in treatment E and F (fed copepods) were significantly reduced compared to other treatments (Figures 1 and 2).

Early use of Otohime diet and copepods in treatments C, E and F demonstrated that larvae could be fully weaned from live food by 35 dph, 32 dph, or 30 dph, respectively. This reduced the length of time live prey items was required by 14 days, 17 days, and 19 days, respectively when compared to our standard control regime where larvae are weaned by 50 dph. Thus, the results of this study demonstrate the potential to utilize frozen copepods and an Otohime dry diet as substitutes for live food in burbot larval culture; however, further optimization is required to improve survival for such feeding regimes and evaluate potential long-term nutritional benefit from feeding copepods in comparison to control regimes.

Table 1. Description of larval burbot feeding trial, in which larvae were fed green algae, rotifers, brine shrimp *Artemia spp.*, frozen copepods and two different commercial diets from 8-64dph.

Feeding regimes (FR)	Green water (dph)	Green water + rotifers (dph)	San Francisco *Artemia* (dph)	Salt Lake *Artemia* (dph)	Frozen copepods (dph)	Gemma diet (dph)	Otohime diet (dph)
FR-A (control)	8-10	11-20	21-32	32-49		40-64	
FR-B	8-10	11-20	21-32	32-49			40-64
FR-C	8-10	11-20	21-35				30-64
FR-D	8-10	11-20	21-32	32-49		40-64	
FR-E	8-10	11-20	21-32		21-49		33-64
FR-F	8-10	11-30			21-49		40-64

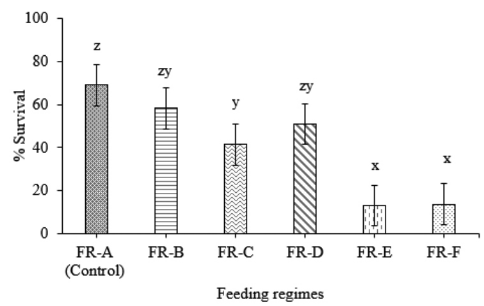

Figure 1. Mean survival of larval burbot fed green algae, rotifers, brine shrimp *Artemia spp.*, frozen copepods and two different commercial diets over time.

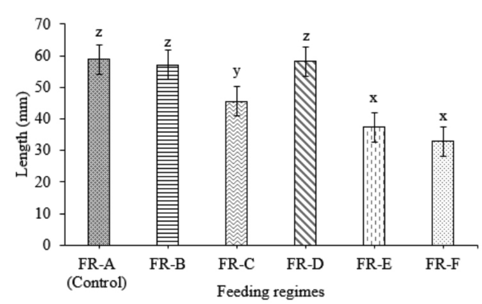

Figure 2. Mean length (mm) of larval burbot fed green algae, rotifers, brine shrimp *Artemia spp.*, frozen copepods and two different commercial diets over time.

DETERMINATION OF OPTIMUM DIETARY PROTEIN REQUIREMENT FOR BURBOT *Lota lota maculosa*

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Burbot (*Lota lota maculosa*) are the only true freshwater cod that have recently grown to become one of the most promising candidates for commercial aquaculture. This is because burbot have a high fecundity, suitable feed conversion, and fast growth rate. Burbot fillets are of high quality and their roe and liver are appreciated as delicacies. Additionally, burbot are resistant to most salmonid pathogens and require similar rearing conditions to trout production. Despite the numerous advantages and commercial benefits, there is limited understanding of nutritional (protein) requirements for burbot.To address this, a feeding trial was conducted to evaluate dietary protein requirements for sub-adult burbot.

Six experimental diets were designed to contain incremental amounts of crude protein (CP) ranging from 35 to 60% of the diet. A total of 264 sub-adult burbot(~150 g and 315 mm) were randomly distributed into 6 groups with four replicates and stocked into twenty-four 60-L tanks (11 fish tank^{-1}) in a closed recirculating system. Fish were fed the experimental diets to apparent satiation twice daily for 10 weeks. At the end of the experiment, fish in each tank were bulk weighed and sampled for fish fillet proximate and amino acids compositions, organosomatic indexes, physiological parameters in liver and tissue samples for gene expression for growth related parameters. Feed intake of each tank was measured on daily basis.

Growth performance was significantly affected by dietary treatment, and the highest weight gain percentage was observed for fish fed diet with 50% protein and lowest was observed in diet with 35% protein (Figure 1), whereas feed conversion ratio (FCR) exhibited opposite trend. Feed intake data was positively correlated with growth performance. Condition factor, viscerosomatic index, hepatosomatic index and fillet yield were not significantly different among groups.

Using second-order polynomial regression analysis, the protein requirement for optimizing growth and feed efficiency was determined to 50% crude protein for burbot *Lota lota maculosa*. Data for the chemical composition of the fish fillets, physiological parameters and gene expression will be presented in the conference.
Conclusively, optimum dietary protein requirement of burbot is 50%.

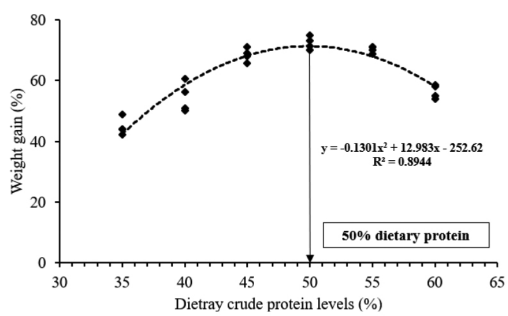

Figure 1: Optimum dietary protein level for burbot *Lota lota maculosa.*

THE EVOLUTION OF PRODUCTION PRACTICES AT A HIGH PROFILE CONSERVATION HATCHERY FOR WHITE SEABASS IN SOUTHERN CALIFORNIA

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Hubbs-SeaWorld Research Institute (HSWRI) has operated a dedicated marine finfish hatchery in Carlsbad, California since 1995 and it remains the only such facility on the west coast of the contiguous United States to this day. The primary mission of this facility and its team of dedicated staff has been to support regional conservation efforts for depleted marine fishes, with a focus on white seabass *Atractoscion nobilis*.

During this multi-decadal period, production and general operating practices have expectedly evolved and undoubtedly will continue to do so. The evolution has occurred primarily with advances in science and technology, but also with changing environmental conditions. Among the biggest changes during this period has been the shift from mostly flow through systems to recirculating aquaculture systems (RAS) for early life stages in order to maximize environmental control and biosecurity. The use of RAS has also evolved as various components and combinations of components have been tested to their limits in a saltwater environment. An improved understanding of group spawning dynamics has moved production plans towards more batches of smaller numbers of fish than the hatchery was originally designed for – a source of inefficiency that is currently being addressed. A sophisticated quality assessment and control program has been developed over time as emphasis has shifted from quantity to quality after achieving survival rates consistently greater than 20% (as high as 60%) from egg to fingerling. A clear demonstration that acclimation cages improve post-release survival by two-fold has eliminated releases directly from the hatchery and shifted practices to a minimum two-week cage acclimation period. Warming water conditions have shifted the prime 6-7 month growout season to fall and spring months. Previously, winter would be avoided for small seabass that were not otherwise physiologically adapted for cold water conditions. Now the summer is avoided to help avoid gas bubble disease, which is exacerbated in warm, shallow water. Growing fish to a larger size has also been demonstrated successful and this is driving the need for larger acclimation cages that require more resources to effectively operate; especially in relation to managing biofouling.

There are exciting opportunities for continued evolution of the Program, including applying our knowledge to new species like California halibut. The biggest challenge to the extent and pace of this evolution is the funding required to implement. Perhaps the greatest opportunity in this evolution is to adapt what has been learned and apply it to traditional farming as another means to take pressure off wild fisheries. The biggest challenge thwarting this opportunity is regulatory and not technical.

UTILIZING AERIAL IMAGERY AND REMOTE SENSING TO MONITOR EELGRASS *Zostera marina* WITHIN SHELLFISH AQUACULTURE OPERATIONS: CHALLENGES, SUCCESSES, AND LESSONS LEARNED

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Within Pacific Northwest estuaries, native eelgrass (*Zostera marina*) is regulated as critical habitat for many salmonids and other fish species under the Endangered Species Act and is recognized as an important species for ecosystem health and sustainability. Shellfish aquaculture farms operating within these same estuaries are often prohibited from impacting eelgrass beds and must demonstrate avoidance for permitting of new activities. Thus, monitoring eelgrass coverage and temporal change is of both regulatory and ecological significance. Imagery collected by unmanned aerial vehicles (UAV) or low-flying airplanes is a powerful tool for evaluating eelgrass beds at a high resolution. Compared to traditional ground-based surveys, aerial methods allow for data collection over a large area (i.e., hundreds of acres) in a short time. This can facilitate cost-effective seasonal and annual monitoring and can also present a supplementary approach for regulatory surveys.

Eelgrass data collected annually using multiple methods highlights the strengths and utilities of each. Annual monitoring of eelgrass in relation to shellfish aquaculture has occurred in Humboldt Bay, California since 2018 using a combination of high-resolution RGB imagery and ground surveys. In Willapa Bay, Washington, high-resolution RGB imagery was collected with a UAV at discrete locations in the summer of 2020, targeting eelgrass beds with and without shellfish aquaculture activity. Coinciding, RGBir aerial imagery was collected with a low-flying airplane for the entirety of Willapa Bay. These data are compared and discussed with regards to questions of scale, resolution, processing, and results.

Additionally, UAV imagery was collected seasonally at one location in Samish Bay, Washington in the spring, summer, and fall of 2021. Eelgrass surveys are typically completed during the summer growing season, but seasonal change is an important indicator of longevity and potential bed expansion. The spring and fall imagery help to illustrate a natural seasonal fluctuation in the density and extent of eelgrass beds. In the context of regulatory limitations on shellfish aquaculture activity within eelgrass, documentation of this natural variability adds to the body of science supporting proper management of these estuarine resources.

Although there are challenges to the collection and analysis of aerial imagery, monitoring of eelgrass beds using these methods has exciting potential for the ecological and regulatory understanding of interactions with shellfish aquaculture operations.

OLYMPIA OYSTER, *Ostrea lurida* (Carpenter 1864) RESTORATION: A COLLABORATION STORY. *QUID NUNC?* AN INTRODUCTION TO THE SESSION

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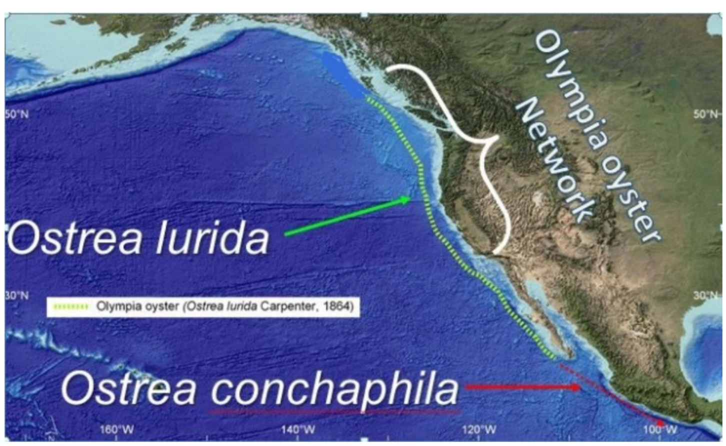
The near demise of Olympia oysters ("Olys") on the west coast of United States has led to increased efforts in the last decade to restore this species throughout much of its range (Baja, California, Mexico, to Gale Passage, Campbell Island, in British Columbia). Populations of this small oyster, once a keystone species in many estuaries, declined after the 1880s as a result of overharvest, pollution, deforestation, urbanization, and the introduction of non-native oyster species (*Crassostrea gigas*), was also accompanied by several non-native predators.

Restoration efforts began in Puget Sound in 1999 and have successfully progressed in the last two decades, including the opening of the Kenneth K. Chew Center for Shellfish Research and Restoration in Washington State, which houses a hatchery for Olympia oysters and other bivalve species, as well as a facility to culture kelp. In the last two decades there have been other achievements, including a special issue of Journal of Shellfish Research dedicated to research on the Olympia oyster; several west coast workshops for those interested in restoring Olys; confirmation of the species name and range; identification of Oly subpopulations in Puget Sound and other areas; studies on invertebrate assemblages in Olympia oyster fringing reefs; and prioritization of locations along the west coast that would best support restoration projects in the species range.

One of the latest accomplishments has been the establishment of the Native Olympia Oyster Collaborative (NOOC) that has brought together Olympia oyster restoration practitioners, shellfish growers, scientists, tribes, and agency managers in an effort to share information and restoration project results. To date there are approximately 40 Oly restoration projects along the US west coast and British Columbia, funded by various federal and state agencies, non-profit organizations, and individual contributions. Detailed information about the various restoration projects can be found on the NOOC website, and there will be more about this in some of the session presentations.

The session will also include a brief overview of the history of the Olympia oyster and presentations about other aspects of restoration and research on this species.



DO SHELLFISH AQUACULTURISTS HAVE A ‘RIGHT TO FARM’ IN DELAWARE?

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The definition of aquaculture that we prefer is a simple one, where ‘aquaculture’ is defined as the farming of aquatic organisms. This is an intentionally broad umbrella that covers everything from algae to oysters to zebra fish. Rooted in this definition is the understanding that aquaculture is agriculture. This connection was affirmed with the passage of H.R.1544 - National Aquaculture Improvement Act of 1985, that designated the USDA as the lead agency for aquaculture in the United States. Similar policies have been adopted by many states. In 1990, Delaware passed the Delaware Aquaculture Act, that declared that aquaculture is an agricultural activity, and that it would be overseen by the Delaware Department of Agriculture (DDA). Through the early 2000s, despite ongoing research, outreach and demonstration efforts, commercial aquaculture production in Delaware remained limited, though there were a handful of individuals involved with the industry to varying degrees. The majority of whom produced finfish (ex. tilapia, striped bass, hybrid striped bass).

In 2013, then Governor, Jack Markell signed legislation that would allow leasing of subaqueous lands in the Inland Bays for shellfish aquaculture. Included were changes to the Delaware Aquaculture Act that specifically removed shellfish aquaculture from the purview of the DDA and placing it under the authority of the Department of Natural Resources and Environmental Control. While these changes have facilitated a path forward for establishment of a shellfish aquaculture industry, our state legislators may have inadvertently stripped shellfish growers of the protections and benefits afforded other sectors of agriculture through right-to-farm laws, direct marketing assistance, and veterinary diagnostic services.

We examined whether shellfish aquaculture operations in Delaware’s Inland Bays fit within current Delaware Right-to-farm laws; how other states treat aquaculture for purposes of their right-to-farm laws; and potential statutory changes in Delaware that would help make clear that aquaculture operations fall within right-to-farm protections. From our analysis, we conclude that open water shellfish aquaculture like that in the Inland Bays is likely not currently protected by Delaware’s right-to-farm laws. Further, we have identified the more than 30 states whose laws currently expressly or implicitly include aquaculture under their right-to-farm statutes and offer some potential strategies for changing Delaware law to cover such operations going forward.

EVALUATING PREFERENCES FOR FISH DISEASE TREATMENT OPTIONS AND SUCCESS RATES AT KENTUCKY STATE UNIVERSITY FISH DISEASE DIAGNOSTIC LABORATORY

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Local aquaculture allows farmers to provide a fresh fish product for their community, but as with any cultured animal, disease issues arise; therefore, it is essential that farmers understand fish health management. To assist fish farmers in raising healthy fish, Kentucky State University (KSU) offers a fish disease diagnostic laboratory (FDDL) service to farmers in Kentucky and other states. Fish health professionals at the KSU FDDL obtain fish and/or water samples from farmers via an Extension visit to the farm, or farmers send samples to the KSU Aquaculture Research Center. Samples are analyzed in the FDDL and a final diagnosis is given to the farmer along with a treatment recommendation. Once a treatment option has been recommended to the farmer, a short survey will be sent inquiring what type of treatment was preferred and if the treatment chosen was successful. The objectives of this study are to 1) better understand the needs of small-scale fish farmers in Kentucky, including which disease treatments are most effective, 2) help educate farmers on pathogen clinical signs and treatment options, and 3) evaluate which treatments are preferred among fish farmers. This information, obtained by survey, will allow researchers to better understand fish disease treatment efficacies and treatment preferences of aquaculturists.

DEVELOPING A MOBILE RESPONSIVE CLINICAL FISH HEALTH DATABASE

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This project outlines the development of a clinical database for fish diseases that will allow fish professionals and veterinarians to track, record, and visualize fish disease outbreaks on their desktop or mobile device. The database and associated mobile responsive site are designed by APAX Software located in Lexington, Kentucky. Some primary functions of the database include: the ability to track treatment methods, water quality, population estimates, stockings events, mortality events, and record if the treatment was successful. The data entry fields are tailored to fish disease with the planned capability to filter and search by any field on user's diagnostics reports. Preliminary data has been collected from Kentucky State University's Fish Disease Diagnostic Laboratory, which include photographs and video footage compiled over the past 31 years of the laboratory's operation and will be linked to Oregon State University's educational website for fish diseases, www.fishpathogens.net. This supplemental material will be accessible to the public and used as an educational tool designed to inform fish farmers of possible fish diseases with example images and pathogen descriptions. The long-term goal is to create a database that will exceed the capabilities of a non-tailored entry system with data entry and searchability fields specific to fish disease that can be exported into several different formats for statistical analysis.

INFLUENCE OF OFFSHORE MUSSEL AQUACULTURE ON THE DISTRIBUTION OF EPIBENTHIC MACROFAUNA

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Mussel farming may influence the benthic environment through organic loading, mussel fall-off, and the addition of physical structure within aquaculture leases. This study evaluated near-field (distance to mussel aquaculture structures, line-scale) and bay-scale (inside vs outside a blue mussel, *Mytilius edulis*, farm) effects of an offshore mussel lease in Îles de la Madeleine on epibenthic macrofaunal communities. Benthic communities were evaluated by underwater visual counts (n = 8 sites per Period per Position – "Farm" or "Outside") using SCUBA in June and July 2014 at 4 distances from longlines – 0 m = directly below lines, and at 5, 10, and 25 m (directly between lines).

Benthic communities varied at line- and bay-scales. Overall, crabs (*Cancer irroratus*, *Pagarus pubecsens, Pagarus acadianus*), sea stars (*Asterias rubens*), Northern moon snails (*Polinices heros*), and American lobsters (*Homarus americanus*) were more abundant in farm sites than outside the farm site and ocean quahogs (*Arctica islandica)* were more abundant outside. No clear spatial trend was observed for winter flounder (*Pseudopleuronectes americanus)* and the sand dollar (*Echinarachnius parma*) but both species differed (flounder) or showed a trend to differ (sand dollar) between sampling dates. Most species were more abundant directly below and close to mussel lines and anchor blocks. Community structure analyses highlighted three distinct species assemblages: outside the farm area, within the farm area but not below mussel lines, and directly below mussel lines.

Further investigation is needed to evaluate if mussel farms serve as ecological traps for the species that congregate within them. The long-term effects of the observed spatial effects of offshore mussel culture on macrofauna fitness remains unknown.

FIGURE 1. Study site

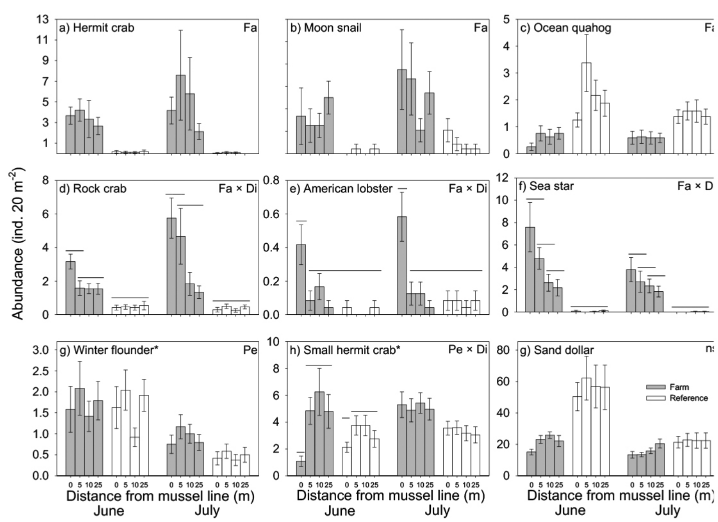

FIGURE 2. Distribution of epibenthic epifauna inside and outside of mussel farm offshore of Îles de la Madeleine. Significant effects are indicated (Pe = Period; Fa = Farm; Di = Distance).

PRACTICAL EVALUATION OF A FISHMEAL- AND FISH OIL-FREE FEED FOR SHRIMP UNDER COMMERCIAL CONDITIONS IN VIETNAM

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Shrimp consumption has been forecast to grow ~11% by 2030. Understandably, such market expansion will have to be matched by growth in shrimp feed production. At present, shrimp feeds account for around 31% of the global supply of fishmeal (FM) while shrimp only represent 5.6% of total aquaculture harvest. This clearly unsustainable position is buoyed by heavy market demand. To service the forecast 11% increase in farmed shrimp production, an additional 3% of global FM supply would be required if current feed types are retained. Abandoning reliance on FM will demand use of alternative feedstuffs that do not negatively impact shrimp production. In fact, many laboratory trials have proven that exchange of FM with alternative proteins has no, or only limited effect, on shrimp performance. These studies thereby provide strong argument for a radical redesign of shrimp feeds to enhance sustainability. However, before such reform can be more seriously contemplated, commercial style trials of alternative diets must be undertaken. At present, these remain pauce. Accordingly, we assessed differences in growth, survival and profitability of shrimp grown in ponds using a commercial (Grobest) or open-source, fishmeal- and fish oil-free (F3) diet.

Shrimp (*Litopenaeus vannamei*) were stocked into four circular plastic-lined ponds of 800 m^3 capacity at 150 PL m^3 (120,000 pond^{-1}). Ponds were then randomly assigned a diet (n=2) which was fed by hand or automatic feeder for 8 weeks. At trial end animals were assessed for growth, feed consumption, survival, and color post-cooking. After 8 weeks there were no differences in pond survival and shrimp fed the Grobest feed were smaller (18.03±1.73 g) than those fed the F3 diet (23.25±1.92 g; $P < 0.05$) with SGRs of 6.63±0.02 and 7.09±0.07% day^{-1} and final harvest weights of 1293.00±318.20 kg and 1502.00±282.84 kg respectively. FCRs of 1.15±0.00 and 1.03±0.01 ($P < 0.05$) were attained for the Grobest and F3 ponds in that order. Irrespective of diet, shrimp presented as a typical brown-grey color at harvest. After cooking, however, differences in flesh color, as assessed by SalmoFan, were apparent, with shrimp fed the F3 diet having stronger pigmentation, with a typical dorsal to ventral paling.

This study demonstrates that total replacement of dietary FM/FO in Pacific whiteleg shrimp feeds is a realistic proposition. Moreover, at trial termination an economic analysis was undertaken and revealed that return on investment for the Grobest fed ponds was 50.16%, while that for the F3 feed was 72.77%. This begs the question – why are shrimp feed manufacturers still employing FM in their diets?

AQUACULTURE AND ECOSYSTEMS AN INTEGRATED APPROACH FOR OFFSHORE AQUACULTURE AS APPLIED IN PALAU, WESTERN CAROLINE ISLANDS IN 2016

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In 2005 the National Sea Grant College Program and other NOAA agencies and the Oceanic Institute of Hawaii sponsored a workshop to identify key factors and parameters needed for ecosystem-based management relative to aquaculture and how aquaculture can contribute to ecological function and better water quality in coastal areas as well as reducing fishing pressure on local fish stocks. The workshop organizers invited 65 experts from Canada, China, Japan, South Korea, Vietnam, Chile, Sweden , Malaysia and the US to participate in developing country- based development plans and strategies. Those plans and approaches were developed for each country and may still be available in The World Aquaculture Society library as publication.

Using many of the approaches identified in that meeting in 2005 ,a new company, Indigo Seafood, was started in Palau in 2016 to grow giant clams, a herbivore rabbitfish, a valuable grouper species and a edible marine Algae(Caulerpa). We made some good progress on many fronts and found that the rabbitfish were very good for keeping the cage clean and giant clams were not compromised on the nearby reef and they had the ability to clarify the water. Caulerpa algae settled immediately on the cages at the depth that we kept them. The Aquapod cages performed very well and were the right size for small vessels available on the island. Feed supply, shipping costs and juvenile availability were the greatest challenge and cost areas.

ABUNDANCE ESTIMATES OF CRUSTACEANS ALONG THE NORTHEASTERN CONTINENTAL SHELF

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American lobsters (*Homarus americanus*), Jonah crabs (*Cancer borealis*), Atlantic rock crabs (*Cancer irroratus),* Portly spider crabs (*Libinia emarginata*), Hermit crabs (*Pagurus pollicaris* and *Pagurus longicarpus*), Snow crabs (*Chionoecetes opilio*), and Galatheid crabs (*Munidopsis curvirostra*) are both economic and ecologically important species found along the northeastern continental shelf of North America. Ecosystems are changing due to climate change, windfarm development, fishing pressure, and other drivers. It is important to establish a baseline of species abundance and density to track the changes of the areas overtime. We analyzed drop camera survey data from the 2019 and 2020 University of Massachusetts Dartmouth - School of Marine Science and Technology's optical survey. These results were comprised of nearly 8,300 stations (Figure 1), to calculate the density and biomass, and trach species distribution. The goals of this study are to test if (1) these crustaceans have an aggregated distribution over the continental shelf, (2) the aggregations of crustaceans are associated with complex substrates (gravel, cobble, rock) along the continental shelf, and (3) temperature and substrate are the primary abiotic factors influencing crustacean distribution. R studio and ArcGIS were used to map each species' distribution of abundance to explain species assemblage. When the analysis was completed, I compared changes in density and abundance of crustaceans between years and correlated to temperature, salinity, and substrates.

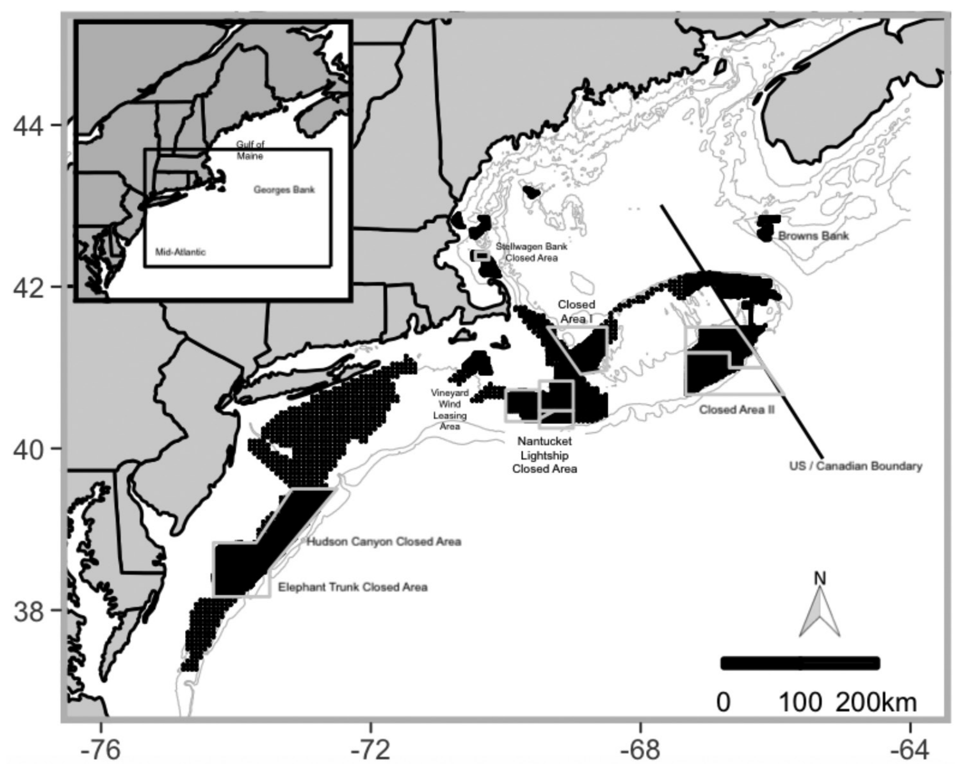

Figure 1. 2019-2020 SMAST drop camera video survey stations. This survey area ranges from Bouguereau Bank, Nova Scotia, Canada to the southern Mid-Atlantic. Survey stations are in black dots, and the management areas in light blue boxes.

PARTICLE SIZE DISTRIBUTION OF TWO DIFFERENT VOLUMES OF BIOFLOC USED IN RIVER PRAWN *Cryphiops caementarius* CULTURE

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The removal of suspended solids is typically accomplished through sedimentation and mechanical filtration processes. However, as the particle size decreases, removal via mechanisms such as mechanical filtration becomes more expensive due to pressure loss or constant backwashing.

In systems using biofloc technology, it is also critical to manage the solids produced, as high levels of solids can destabilize the system, interfering with water quality and biofloc balance.

The goal of this study was to determine the particle size of solids produced in a *C. caementarius* culture using biofloc technology with varying floc volumes (FIV). A 100 ml sample was collected from each rearing unit and filtered in series through various filter mesh (500, 350, 250, 150, 50, 20 m). This was done in triplicate, and the sample retained on each filter was removed using distilled water before being poured onto a Whatman® GF/C 1.2 μm filter paper to determine the dry weight of each particle size.

When the data was graphed, similarity in the graphs by treatment and replicas was observed (Figure 1). Similarly, when comparing both treatments (Figure 2), no significant difference was observed, as confirmed by a Kolmogorov test-Smirnov with p-value = 0.6476.

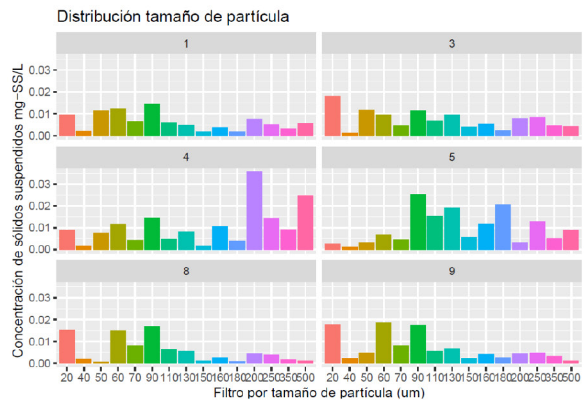

Figure 1: Treatment, replica, and mesh opening size charts.

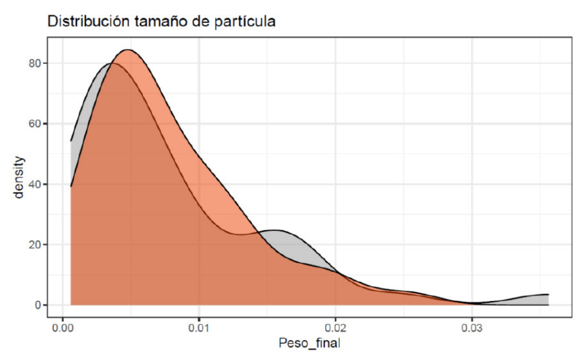

Figure 2: A graph depicting the distributions of the two treatments.

HATCHERY LARVAE AND JUVENILE PRODUCTION OF SCALLOP *Argopecten purpuratus* IN A RECIRCULATING AQUACULTURAL SYSTEM

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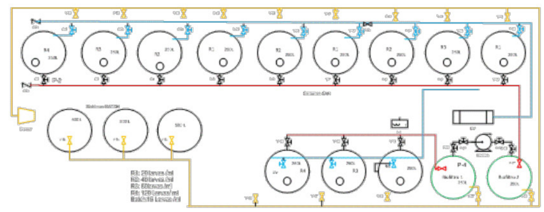
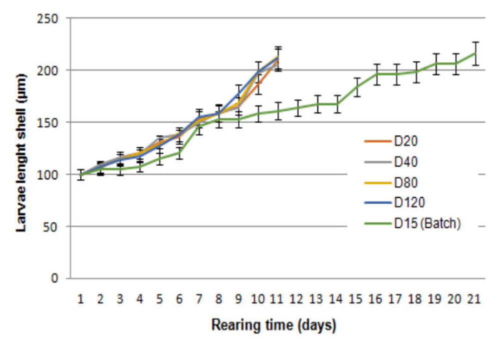
Argopecten purpuratus hatchery technology is primarily based on batch systems with 100% of water exchange per day and rearing volumes ranging from 500 L to 70 m3. Recently, a RAS technology was investigated and yielded satisfactory results for the production of postlarvae and early juvenile scallops. In this study, we assessed RAS productive performance at various rearing densities (20, 40, 80, and 120 larvae/mL), with a batch system at a common rearing density of 15 larvae/mL serving as a control. Later, the young juveniles were reared in suspended systems for 60 days at Tongoy Bay to evaluate their juveniles' yield.

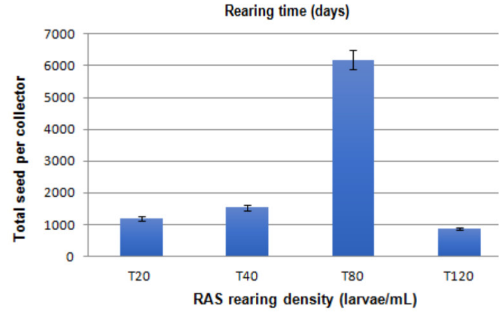
Two spawnings were performed sequentially every 20 days. Sixty scallop broodstocks with an average of 7.5 cm of shell length were taken from a population of 200 specimens from a suspended system located in Tongoy Bay for each spawning. The RAS had twelve 250 L flat-bottom cylindrical tanks, a water pump (Vulcano 0.3 hp), a UV sterilizer (Ql-40 Bioligth), 2 biofilters, and air injection from a turbo blower (Sweetwater).

RAS provided stable water quality conditions for rearing scallop larvae at densities of 40, 80, and 120 larvae/mL. Competent larvae (220 μm shell length) were obtained after 11 days of culture in all RAS, but it took at least 21 days in the batch control system. Larval morphology at high rearing densities appears uniform without shell damages as compared with those reared in a batch system, which can potentially increase the mortality of the larvae and juveniles. The production of 5-13 mm juveniles after 60 rearing days in longlines was better for RAS in comparison to the typical batch technology. Results reported in this research would significantly improve rearing protocols and the design of recirculating aquacultural systems for hatchery production of juvenile scallops.



Density Larvae/mL	Temp. (°C)	O_2 (mg/L)	Salinity (psu)	pH	TAN (mg/L)	Alkalinity (mg/L)	CO_2 (mg/L)
20 SD	17,70^{a} ±0,91	7,91^{a} ±0,48	34,63 ±0,72	8,15^{ab} ±0,17	0,00^{a} ±0,01	102,25^{a} ±4,50	28,00^{a} ±1,41
40 sd	17,70^{a}± 0,42	7,44^{a} ±0,44	34,05 ±0,91	7,73^{abc} ±0,57	0,23^{a} ±0,45	109,50^{a} ±7,37	28,00^{a} ±0,82
80 SD	18,28^{a} ±0.38	8,12^{a} ±0,53	34,78 ±0,55	8,15^{ab} ±0,17	0,02^{a} ±0,03	100,25^{ab} ±13,91	28,00^{a} ±3,16
120 SD	17,95^{a} ±0.55	8,32^{a} ±0,43	34,55 ±0,55	8,25^{a} ±0,06	0,01^{a} ±0,01	111,33^{a} ±8,33	30,67^{a} ±1,53
Batch SD	15,60^{b} ±0.66	7,20^{a} ±0,31	34,73 ±0,00	7,63^{bc} ±0,23	0,02^{a} ±0,03	96,67^{b} ±5,77	30,00^{a} ±1,00





BIOINTEGRATING RAINBOW TROUT, RIVER PRAWN, AND LETTUCE IN A COMMERCIAL AND EDUCATIONAL PROTOTYPE AQUAPONIC SYSTEM IN ARIDS ZONES

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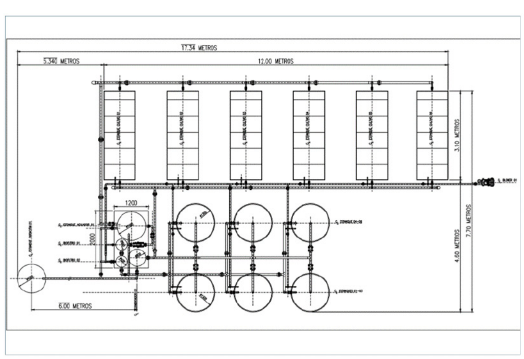
According to the agricultural history of the Coquimbo region, there was an 82 percent increase in horticultural area between 1976 and 1997. This increase in horticultural production has suffered a significant setback due to its hyper-aridity, which has had a negative impact on the sustainability of agricultural production under conventional cultivation methods in recent years. Because freshwater resources are scarce, there are no continental commercial developments on the scale that have been developed in southern Chile in the aquaculture freshwater scenario.

Aquaculture activity does not consume water, but rather temporarily uses it and returns it to the environment, creating a scenario that allows aquaculture to be integrated with agriculture. Aquaculture activity should be integrated with agriculture in such a way that water can first be used in aquaculture and then channeled for agricultural use. In terms of biomass produced per unit of water used, the integration of aquaculture and agriculture increases the productive efficiency of water resource use. Water recirculation technologies can increase the productive efficiency of water even further in aquaculture and agriculture.

Rainbow trout, lettuce, and basil are grown in an aquaponic system in Valle del Elqui. The fish rearing system consists of eight 25m3 tanks. The hydroponic component has eight 16 m^3 raceways (27 m length x 2 m width x 0.33 m depth). A local river prawn was also tested for biointegration within the vegetable raceways. Prototype systems have been installed at five Technological Agricultural Schools. These systems could include up to six 1 m^3 fish tanks and up to six 1 m^3 hydroponics raceways.

Aquaponics systems can produce rainbow trout and a variety of vegetables all year. More research is being conducted on river prawn biointegration.



WHAT DO YOU RECOMMEND? PURCHASING AND INFORMATION TRANSFER IN THE SEAFOOD SERVICE SECTOR

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To enable US aquaculture expansion, it is imperative for aquaculture producers to understand potential markets and how to tailor their practices to reach those markets. Research demonstrates that consumers are potentially willing to pay higher prices for local, farmed, and branded marine products (Atlantic Corporation 2019; Brayden et al., 2018; Chen et al., 2017; Petrolia et al., 2014), however little has been done to understand the role of distributors, chefs, and restaurant staff in guiding consumer preferences, purchasing, and markets more broadly.

This study employed a nationwide effort to understand the factors that influence purchasing as well as knowledge transfer within the US seafood service sector. Using a pandemic-adjusted approach pairing semi-structured interviews with an online survey, seafood chefs, restaurateurs, restaurant purchasers, front of house staff, wholesalers/distributors, and retailers were targeted. Questions focused on the factors that guide purchasing (i.e., why do you buy the seafood that you buy?) and how seafood-related information is obtained and shared.

Results span a range of purchasing-related topics including: seafood information needs, access, and transfer; use of seafood certification programs; distinctions between wild and farmed products; geographic preferences; ranked factors influencing purchase; and US seafood safety and sustainability. In addition, effects of the COVID-19 pandemic on present and future purchasing are considered.

By focusing on an understudied but potentially influential sector of the aquaculture industry, results from this project benefit: 1) aquaculture producers, who can realize how to better cultivate and/or market their product, 2) industry researchers and proponents who will have greater awareness of potential misconceptions and information needs, and 3) the seafood service sector, who in turn will have enhanced availability of desired aquaculture products and be better positioned to use accurate, science-based information to influence consumers' buying decisions.

TURNING UP THE HEAT: FURTHER EVALUATION OF THE EFFECT OF TEMPERATURE ON GONAD DIFFERENTIATION IN YELLOW PERCH *Perca flavescens*

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The effect of temperature on sex determination in Yellow perch was examined across two generations. The first set of experiments, previously presented in 2019, evaluated the effects of low (14°C) and high (27°C) temperature on the survival, growth, and resulting sex ratio of Yellow perch progenies (n=11) when exposed during the labile period of sex differentiation (when fish are 12-30mm total length). Sex ratio results obtained from 2 year-old fish suggested a masculinization effect of high temperature in 6 of 11 progenies exposed. Yellow perch males from this first set of experiments (F1) were used to sire an F2 generation. This F2 generation, consisting of 15 progenies from 13 different F1 sires, was then used in a second set of experiments that evaluated the effects of low (14°C), medium (23°C), and high (27°C) temperature exposure applied for 84 days from the start of first exogenous feeding.

Effects of temperature on survival and growth of the F2 progenies were evaluated throughout the temperature exposure, as well as through the first year of grow-out. Effects of sire's temperature exposure and the interaction of the progeny's and sire's temperature exposures on the growth and survival of the F2 generation were also evaluated. Survival did not differ significantly between progeny of different temperature treatments at majority of the sampling points in the F2 generation.

There was a significant effect of progeny temperature treatment and sire temperature treatment, independently, on the growth of F2 progeny. During the temperature exposure phase, both medium and high temperature progenies weighed significantly more than low temperature exposed progenies. After the temperature exposure phase, high temperature groups weighed significantly more than medium temperature groups, which in turn weighed significantly more than low temperature groups. In addition, the F2 progenies of low temperature exposed F1 sires had significantly greater length and weight than F2 progenies sired by high temperature exposed F1 sires, regardless of the progeny's own temperature exposure treatment. These results suggest a possible negative epigenetic effect of high temperature exposure during the larval stages of F1 males that results in lower growth performance of subsequent progeny, regardless of the progeny's rearing temperature. There was also some significant interaction effects of progeny and sire temperature treatment on weight and length, but they were not consistently significant along the experimental period.

The significant difference in growth resulting from early high temperature exposure during the larval and early juvenile stages continued throughout the juvenile stage and into adulthood of both F1 and F2 fish, despite transition to rearing all groups at the same ambient temperatures. These results suggest that the temperature experienced at early life stages affects the growth of future life stages of an individual as well. Sex ratio results are to be collected from the F2 progenies at the end of 2021 and will be presented along with growth and survival of F2 progenies during the second year of grow-out. This study provides the first evidence of a masculinization effect of high temperature in Yellow perch exposed during the larval stages.

EVALUATION OF SUPPLEMENTAL ENZYMES IN PLANT PROTEIN-BASED DIETS FOR HYBRID STRIPED BASS

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Inclusion of plant-protein feedstuffs in aquaculture feeds increased in recent decades, but the presence of indigestible carbohydrates and protease inhibitors in such feedstuffs can negatively affect their nutritional value to fish. While supplementation of exogenous enzymes in land-animal feeds can improve digestibility, growth rates, and feed efficiency, the efficacy of these additives in aquaculture feeds has been inconsistent. The objective of this study was to evaluate the effects of protease and carbohydrase additives in plant-based diets on the digestibility, production performance, and protein and energy utilization of hybrid striped bass (HSB).

Digestibility Trial: a 14-week digestibility evaluation was conducted using advanced juvenile HSB (~ 100g/fish) fed diets containing different levels of the enzymes. Fish in each of four tanks were fed one of five randomly assigned diets to apparent satiation once daily. Fecal matter was collected by stripping all fish once a week at ~5 h postprandial. While no significant differences in the ADCs of dry matter, protein, and GE were detected among treatments, supplementation of carbohydrase significantly increased the ADC of NDF.

Growth Trial: in the subsequent eight-week growth evaluation, quintuplicate groups of 20 HSB juveniles (9g/fish) were fed one of seven randomly assigned diets: six plant-based diets containing different levels of supplemental carbohydrase; and a fish meal-based diet (FM-30). No differences in survival (95-100%) were found among groups. Supplementation of carbohydrase to the plant-based diets did not affect growth, feeding rate, and feed efficiency of HSB. When compared to FM-30-fed groups, HSB fed the plant-based diets consumed relatively more feed but displayed similar production performance. Additional results on whole-body proximate composition, and protein and energy retention efficiencies will be presented.

Based on the current results, supplementation of carbohydrase additive to plant-based diets may slightly improve carbohydrate digestibility by HSB, but supplementation of protease to these formulations does not seem beneficial.

PAIN IN AQUATIC INVERTEBRATES – THE QUESTIONING TRIAD

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The word "pain" was originally used to describe a human emotional negative experience. Pain is a clinical expression of tissue damage, an "unpleasant sensory and emotional experience" (International Association for the Study of Pain). While past research studies conducted on pain were focused on mammalians, current science is showing that also fish and shellfish species own functions and mechanisms potentially related to pain. Although there is insufficient research-based data required to answer questions related to the biology of pain in most of these species (i.e. existing receptors, pathways and specialized neurons to detect and recognize noxious stimuli), it may seem appropriate to give them the benefit of scientific doubt, at least. Here, the questioning triad and current approaches used in assessing pain in aquatic invertebrates shall be presented.

THE HUNT FOR WILD CAUGHT PROBIOTICS: COMPARISON OF MICROBIOMES FROM 569 VERTEBRATES INCLUDING 115 FISH SPECIES

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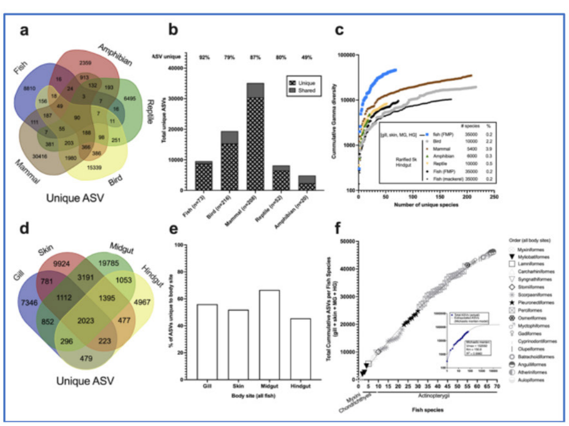
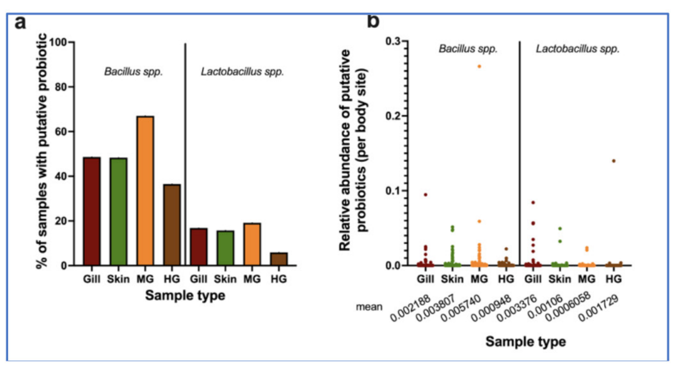
Most probiotics used in aquaculture are borrowed from terrestrial livestock systems and therefore may not be the most effective strains. We evaluate and explore a broad range of vertebrates contrasting differences in the microbiomes of fish with mammals. The host-associated gut microbiome in vertebrates is shaped by a variety of biological factors including phylogeny, diet, age along with environmental factors such as geography, habitat, and climate. Here we perform a meta-analysis, comparing the hindgut microbiomes of 569 unique species of vertebrates. We then compare factors which drive the mucosal microbiomes (gill, skin, midgut, and hindgut) across 115 species of wild marine fish. Gamma diversity metrics show that while mammals have the highest diversity amongst vertebrate classes when comparing hindgut, the lion's share of microbial diversity in fish exists in other body sites including the midgut, gill, and skin and that when included collectively have much higher microbial diversity than other vertebrate classes (Figure 1a-d). To quantify impacts of host phylogeny, trophic level, habitat type, swim performance, and body site we compare the gill, skin, midgut, and hindgut microbiomes from 25% of the total marine fish diversity from Southern California including 101 species.

Total microbial diversity across vertebrate hindguts and within multiple body sites of fish. a) Hindgut microbiome samples from 569 species of vertebrates were rarified to 5000 reads and unique or shared ASVs (amplified sequence variants) determined for each class. b) The percent of unique ASVs only found in a given class as compared. c) Rarefaction of cumulative gamma diversity as a function of unique vertebrate species. Included is a single fish species, *Scomber japonicus*, sampled over three years 'black dots' and the unrarified FMP samples which had detectable bacteria in all four body sites (gill, skin, midgut, and hindgut). d) Gamma diversity of 68 fish species across four body sites. e) Percentage of unique ASVs associated with a given body site across the 68 fish species. f) Rarefaction curve of increasing gamma diversity (inclusive of four body sites) as a function of increasing fish species.

Distribution of putative probiotic ASVs grouped by genera: *Bacillus* or *Lactobacillus*. a) The total percentage of samples within a given body site having either a Bacillus ASV or Lactobacillus ASV. b) The relative abundances of Bacillus and Lactobacillus ASVs within each body site (including samples with 0 counts).





REFLECTIONS OF A FULBRIGHT STUDENT 7 YEARS LATER: SUCCESS AND FAILURE OF INTEGRATED FARMING AND AQUACULTURE IN MALAWI

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Malawi is a small country in Sub-Sahara Africa bordering one of Africa's Great Lakes, Lake Malawi or Lake Nyasa. Malawi has a population of around 17 million with over 90% as subsistence farmers. Approximately 17% of children under 18, are either orphans or considered vulnerable due to the HIV epidemic and other challenges including malnutrition and stunting. While the lake is the primary source of protein for many, inland populations do not have the same access to seafood. In addition, one of the primarily challenges for village communities is seasonal hunger due to not having access to water during the dry season. In 2015 I traveled to Malawi as a Fulbright student to study ways of improving rural freshwater fish farming using integrated farming techniques (poultry and fish farming). Here I will discuss both the science opportunities to improve food security in this region along with the social aspects I learned as a US student conducting research and development work in a completely different culture. My long term goals are to continue to build research capacity and international exchange opportunities for both Americans and Malawians.

Construction of two types of fish ponds: concrete vs local plastic materials.

AN EVALUATION OF MICROPLASTICS FOUND IN THE EASTERN OYSTER (*Crassostrea virginica*) AND THE SURROUNDING ENVIRONMENT

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Microplastics (MP, < 5 mm) are found in coastal waters across various environmental compartments (biota, water, marine snow, sediment). These particles can be incorporated into marine snow (heteroaggregations) and sink to the benthos quickly where they interact with benthic organisms. The eastern oyster (*Crassostrea virginica*) is a commercially important species that has been shown to ingest MP; however, oysters are discriminant suspension feeders that do not consume all particles to which they are exposed.

This study focused on identification of the polymer compositions, shapes, and sizes of MP found in different environmental compartments on a recreational oyster bed in Norwalk, Connecticut. Oyster, water, marine snow, and sediment samples were collected and the quantity and types of MP in each were determined. Many precautions were taken to minimize and monitor MP contamination in the field and laboratory because quality control and assurance measures are essential for gathering reliable data. The MP were isolated from organics using previously validated digestion and density separation methods. Particles in all samples were viewed under a dissecting microscope and those suspected of being MP isolated and photographed. The size, shape, color, and aspect ratio were determined using microscopy and ImageJ software. The polymers were identified by micro-Fourier transform infrared spectroscopy (FTIR) and only MP with a quality library match (≥65%) were considered plastic (Figure 1). These data will aid in determining the types of MP (polymer composition, shape, size) to which oysters are exposed, and identify those they ingested. Such comparisons are important to determine if MP in the environment are problematic for the eastern oyster and if so, what MP types should be addressed in future environmental policies. Additionally, the dominant MP types and shapes identified in the environment are being used in laboratory exposure experiments to determine MP particle selection.

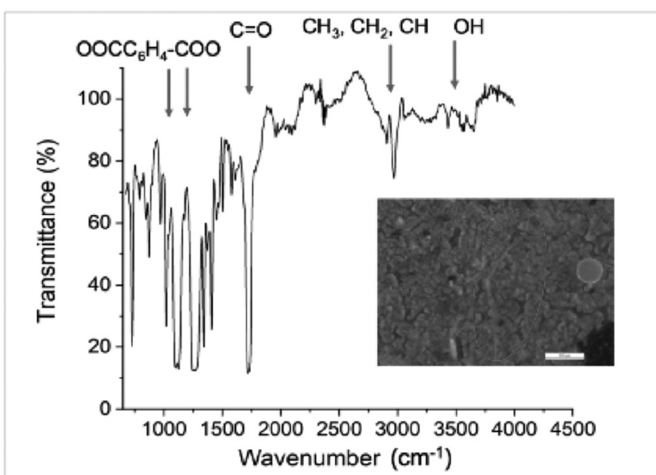

Figure 1. A FTIR spectrum collected from a polyethylene terephthalate (PET, polyester) microfiber in marine snow (96.02% match). The peak at 1730 cm^{-1} is indicative of C=O stretching and a characteristic hydroxyl peak can be seen at 3432 cm^{-1}. The terephthalate group peak is around 1240 and 1124 cm^{-1}.

DEVELOPMENT OF A GENE-CAPTURE PANEL FOR THE *Cichlid* SUBFAMILY *Pseudocrenilabrinae*

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Studies to detect introgressive hybridization within cichlid species in Southern Africa has been based on microsatellite and AFLP markers, but these markers have limited resolution as they represent a fraction of the whole genome diversity. With the technological advances made in molecular biology, there are already complete genomes available for four species of the subfamily Pseudocrenilabrinae, including *Oreochromis niloticus*. These genomes represent a precious resource to develop robust molecular tools to reveal the extent and effects of *O. niloticus* genetic invasion in *O. mossambicus* natural range. The objectives included (1) identifying candidate genes ontologies with orthologues, (2) obtaining exome coding sequence with SNPs variants from *O. niloticus* genome, and (3) designing an Ampliseq gene-capture custom panel for Pseudocrenilabrinae. I identified 2,040 candidate genes with 14 ontologies from the literature search and Genbank database mining. I retained a total of 247 coding sequences to design a panel on the Ampliseq website, after excluding 1224 genes that were either not annotated in the *O. niloticus* genome, or without orthologue in the Human genome. I also excluded 107 genes that were on scaffolds that failed to map to a chromosome, 449 genes with exons smaller than 300 bp and 13 genes that presented gaps in the alignment. Tests of transferability of the remaining amplicons across vertebrates using in-silico PCR testing confimed that there were successful across the cichlid subfamily Pseudocrenilabrinae with an average coverage of 99 %. This panel represent an important molecular conservation resource and opens new perspectives for accurate assessment of introgressive hybridisation between *O. niloticus* and *O. mossambicus*.

RAPID VIDEO SYSTEM DEVELOPMENT WITH OPALEYE, AN OPEN SOURCE PORTABLE VIDEO TOOLKIT

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The Suburban Marine Opaleye features multiple distributed nodes with globally time synchronized video capture and video stream. Designed from the start for flexible remote control with a Remote Procedure Call (RPC) Application Programming Interface (API) to ease integration into robotic systems.

Existing underwater camera systems tend to be very fixed function with limited ability to run custom code in the camera system. Suburban Marine is introducing a free, open source camera acquisition and processing framework that is portable to many different systems. The Opaleye allows running end user software in parallel with our camera acquisition code, and an efficient local-computer API permits fast transfer of camera data.

Allowing end-user code to run directly on the Opaleye package eases development of edge computing applications and increases portability as the edge computing applications can target a high level API rather than low level direct sensor access. The underlying libraries the Opaleye is built on are very portable and commonly supported on many different camera acquisition platforms.

Leveraging industry standard tools such as gstreamer, nginx, and Linux-for-Tegra, a flexible software development kit can be created with common operations abstracted into video processing pipeline units that can be combined like blocks as needed for a given application. Using common building blocks allows abstraction of hardware specific features (such as encoding acceleration) and eases portability between different systems such as Qualcomm Snapdragon and Nvidia Jetson.

Multiple Opaleye systems can be time synchronized with the IEEE 1588v2 PTP protocol. This reduces the level of effort as no digital camera sync lines need to be run in addition to the network. Globally correct (UTC) timestamped video is easy to create as the system can be synchronized with an external GPS driven PTP grandmaster at launch time, then run off a local PTP clock during the mission.

https://github.com/suburbanmarine/opaleye

THE EFFECT OF THE TRANSITION OF MOLECULAR WEIGHT PROFILE OF DIETARY PROTEIN ON LARVAL LARGEMOUTH BASS *Micropterus salmoides* DEVELOPMENT

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This study aimed to provide an efficient protein source for larval fish by using same-species muscle and endogenous enzymes to produce hydrolysates and, providing a series of diets with increasing molecular weight protein fragments through larval development. The objective of this study was to determine the effect of the degree of hydrolysis of Largemouth Bass (LMB) muscle protein obtained using LMB digestive tracts when used in first feeds for larval LMB on: 1) growth performance and survival; 2) the occurrence of skeletal deformities; 3) gene expression of intestinal peptide transporter PepT1; and 4) the muscle FAA composition.

Batches of fresh LMB muscle were hydrolyzed for 1.5, 3, and 6 hrs, respectively, in order to obtain hydrolysates containing protein profiles with differing molecular weights. The muscle was mixed with LMB endogenous enzymes obtained from whole fish digestive tracts at 25^{o}C and the pH was adjusted to 3-4 and later to 7-9 to mimic LMB stomach and intestinal digestion conditions, respectively. Five diets were produced for this study, the intact diet containing non-hydrolyzed LMB muscle and four diets in which the intact muscle protein was replaced in 50% with LMB hydrolysates. Those four diets were characterized by their level of each hydrolysate (presented as ratio of 1.5, 3, and 6 hrs hydrolysates, respectively): 1:1:1, 1:3:6, 1:3:1, 6:3:1 for diets A, B, C, and D, respectively. At 4 days post hatch (dph), larval LMB were randomly distributed into 21 (280 L) tanks, with ~2000 fish per tank. There were 7 treatment groups in this study, with 3 replicates, provided during 5-26 dph (Figure 1).

No significant differences were detected in growth performance between the intact protein and hydrolysate fed groups, regardless of molecular weight profile. However, the transition of hydrolysates with differing molecular weight profiles in the diets significantly reduced the occurrence of skeletal deformities in the larval LMB, compared to Hydro-A, which received the same hydrolysate diet throughout the entire experiment. These results provide support for the transition of the molecular weights of dietary protein throughout larval development, as a means of matching the absorption capacity of larval LMB. The results on PepT1 expression and FAA composition will be presented in the oral presentation.

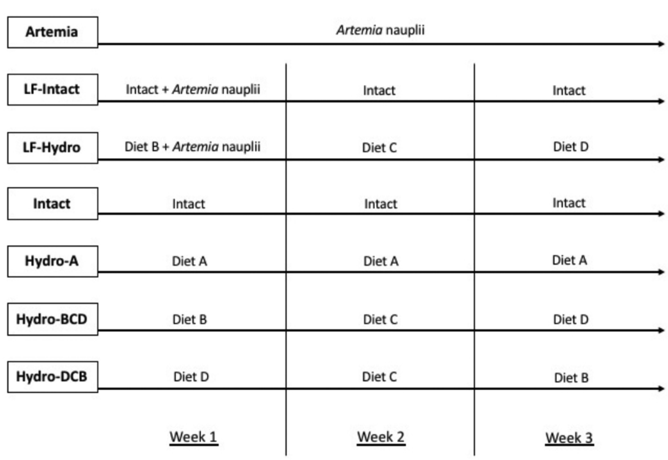

Figure 1. Feeding regimes for experimental groups

DOES THE ORIGIN OF THE MUSCLE AND/OR DIGESTIVE ENZYMES AFFECT THE QUALITY OF MUSCLE PROTEIN HYDROLYSATES USED AS FIRST FEED FOR LARVAL FISH?

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Fish protein hydrolysates have been used in the past as a source of protein in larval fish diets with various success. This study looked at utilizing same-species muscle and endogenous enzymes to produce a fish muscle hydrolysate for inclusion in larval dry diets. Specifically, the objective of this study was to determine the effect of the source of muscle and endogenous enzymes on the hydrolysis of fish muscle and its impact on; 1) growth performance and survival; 2) the occurrence of skeletal deformities; 3) gene expression of intestinal peptide transporter PepT1; and 4) the muscle FAA pool used as an indicator of dietary amino acid availability; in larval Walleye.

Four different hydrolysates were produced for this study, two obtained from Walleye muscle, one hydrolyzed with Walleye and another one with Tilapia endogenous enzymes. The other two hydrolysates were obtained from Tilapia muscle, hydrolyzed with either the Walleye or Tilapia endogenous enzymes. The muscle and enzymes were mixed continuously during the hydrolysis (at 22^{o}C and 28^{o}C for Walleye and Tilapia enzymes, respectively), and the pH was adjusted throughout the process to mimic stomach and intestinal digestion conditions. At 4 dph, larval Walleye were randomly distributed into 18 (280 L) tanks, with ~2000 fish per tank. There were 6 treatment groups in this study, with 3 replicates. Each group was fed to satiation for 3 weeks. The Artemia group was fed *Artemia* nauplii for the entire duration of the study. The rest of the groups received a dry diet supplemented with Artemia during the first 3 days. The Otohime group was fed with a commercial diet, and the other four groups were fed diets that contained a 50/50 level of intact protein and hydrolyzed protein produced from Walleye muscle, hydrolyzed with Walleye (W-W) or Tilapia (W-T) endogenous enzymes, or Tilapia muscle, hydrolyzed with Walleye (T-W) or Tilapia (T-T) endogenous enzymes.

The results showed that the group fed with the Walleye muscle hydrolysate, produced with Walleye endogenous enzymes, had a significantly higher average weight at the conclusion of the study, compared to all other groups (Figure 1). This result provides support for the use of protein hydrolysates produced with same-species muscle and endogenous enzymes, for inclusion in larval fish diets. The results on PepT1 expression, FAA composition, and skeletal deformities will be presented in the oral presentation.

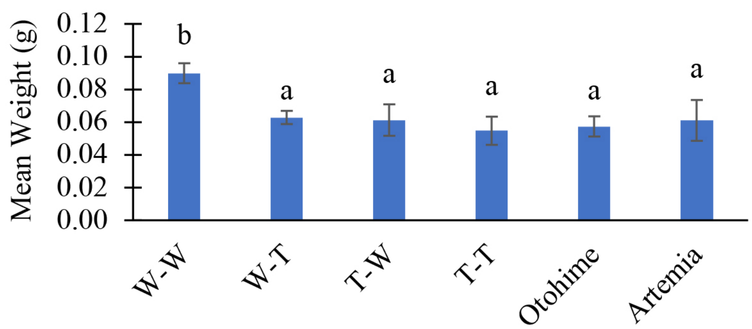

Figure 1. Mean weight at the conclusion of the study.

BIOECONOMIC MODELING OF OPTIMAL HARVEST TIME IN NILE TILAPIA (*Oreochromis niloticus*) CONSIDERING SIZE HETEROGENEITY AND MINIMUM MARKETABLE SIZE

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The size dispersion in farmed fish has a strong impact on the bioeconomic performance of production, directly affecting the net profit. This work aimed to develop a bioeconomic model based on data from an experimental to identify optimal harvest time (OHT), considering market restrictions based on minimum marketable sizes (MMS). The commercial sizes analyzed were 350, 400, 450 y 500 g for a 6-month growth period. Two treatments were performed by pre-selecting the distribution in sizes for the first organisms for a heterogeneous (HT) and a homogeneous (HM) distribution. Fish form HT system were in a range for size of 44-155 g with an initial variance of 604.19 and a coefficient of variation (CV) of 25.5 %. The size range in HM was 87-112 g, with an initial variance of 34.91 and a CV of 5.9 %. The net benefits were more significant in the HM distribution than in the HT. The maximum gain in HT was obtained in the MMS = 350 g with an OHT of 196 days and a net profit of US $ 5,551.61, while the gain generated in 500 g was negative. The maximization of the net gain in HM indicated a higher profit of US $ 3,326.97 in 350 g with an OHT of 181 days. The MMS = 500 g had the lowest benefit (US $ 615.43) with an OHT of 221 days. The marketable biomass was 99.30% for HT and 99.53% for HM. HM revealed higher revenues of 10.66%. Sowing fish with a homogeneous size reduces the dispersion in growth and obtains higher profits. This model is a practical tool for aquaculture and represents a guide line for technical decisions that result in a higher net profit for the producer.

Table 1. Sensitivity analysis considering the price and the main production costs.

MMS	Parameter	Concept	Variation (%)	System			
				HT		HM	
				OHT (%)	Net profit (%)	OHT (%)	Net profit (%)
350 g	p	Price	-10	-0.55	-161.42	-3.06	-158.0
			+10	11.05	169.46	8.67	163.17
	c_f	Feeding costs	-10	11.05	101.33	8.67	96.95
			+10	-0.55	-95.34	0	-94.12
	c_M	Maintenance cost	-10	1.10	6.85	4.08	-7.80
			+10	-0.55	-6.77	0	8.30
400 g	p	Price	-10	0	-187.48	-6.48	-218.46
			+10	12.57	189.40	7.41	225.46
	c_f	Feeding cost	-10	0.52	112.97	3.70	137.16
			+10	0	-112.96	-6.48	-133.78
	c_M	Maintenance cost	-10	0	8.44	0	12.49
			+10	0	-8.44	0	-12.49
450 g	p	Price	-10	0	-268.03	-3.06	-456.24
			+10	0	268.03	10.04	481.25
	c_f	Feeding cost	-10	0	165.66	10.04	326.85
			+10	0	-165.65	-3.06	-287.01
	c_M	Maintenance cost	-10	0	13.30	0	31.48
			+10	0	-13.30	10.04	-28.18
500 g	p	Price	-10	0	-937.57	-	-
			+10	0	937.57	-	-
	c_f	Feeding cost	-10	0	599.15	-	-
			+10	0	-599.15	-	-
	c_M	Maintenance cost	-10	0	52.76	-	-
			+10	0	-52.76	-	-

MARINE AQUACULTURE CAN MITIGATE IMPACTS OF CLIMATE CHANGE AND INCREASE SECURITY OF THE WORLD'S SEAFOOD SUPPLY

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The 2015 Paris Climate Agreement called for efforts to limit global warming to well below 2°C above pre-industrial levels, with an ideal target of no higher than 1.5°C. In 2018, the Intergovernmental Panel on Climate Change (IPCC) published a landmark report that detailed the emissions reductions necessary to meet this 1.5°C goal. According to this report, global net anthropogenic CO_2 emissions must reach net zero by 2050. Growing scientific evidence suggests that responsible marine aquaculture has the potential to mitigate climate change while feeding a growing population and increasing the resilience of the global food system, making it a valuable tool for the U.S. to meet its climate goals

This presentation explores the climate impacts of marine aquaculture against the backdrop of climate mitigation as a global and domestic priority. It is based on a series of interviews with scientists and marine aquaculture experts and a comprehensive review of existing scientific literature related to the climate impacts of farming finfish, shellfish, crustaceans, and seaweeds. The goal of this report is to present the state of the science, synthesize the existing literature, and summarize the key themes for consideration:

- Human population is projected to reach 9.7 billion by 2050 and the global demand for animal protein will rise by 73-88%
- One suggested mitigation measure is for future meat production to shift away from terrestrial livestock to more climate-friendly seafood
- Life cycle assessment (LCA) is used to quantify the environmental impacts of food systems by assessing impacts (e.g., energy use, greenhouse gas (GHG) emissions, land use, biodiversity impacts) per functional unit of protein produced and some studies suggest that the GHG emissions from aquaculture production are comparable to emissions from poultry and pork production and significantly lower than production of ruminants like beef, sheep, and goats
- Well-managed marine aquaculture development could increase the resiliency of our food system to future environmental, social, and economic shocks, including the impacts of climate change
- Seaweed farming can provide ecosystem services such as improving water quality, regulating ocean acidification, protecting coastlines, providing habitat for other species, and through carbon sequestration, be a tool to mitigate global warming by removing CO_2 from the atmosphere.

THE SPATIOTEMPORAL DEVELOPMENT OF TWO SHELLFISH POPULATIONS AND THEIR ASSOCIATED FILTRATION CAPACITY ON A LIVING SHORELINE, DELAWARE, USA

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The term "living shoreline" refers to a suite of tactics and techniques that aim to stabilize eroding shorelines while providing ecological benefits, such as habitat and water quality enhancement. Shellfish can play an important role in mediating water quality and clarity through their filter-feeding activities. As shellfish filter water, particulate matter and nutrients are removed from the water column and either transferred to the benthos or incorporated into the animals. As such, incorporating shellfish into living shorelines can facilitate ecological outcomes. This study tracked the spatiotemporal population development, and the associated filtration capacity, of oysters and ribbed mussels in three intertidal areas on a living shoreline between 2014 and 2020 across a range of intertidal positions and materials, including recycled oyster shell bags and Oyster Castles ®.

After 6 years, the Mispillion living shoreline successfully developed new ribbed mussel and oyster populations, which filtered nearly 2,500 kg of seston (Fig. 1). Population, and subsequent biomass, development were non-linear for both species across space and time and highlight the tandem synergistic and substitutive benefits of a multi-species approach across intertidal positions (Fig. 2). Although long-term monitoring across variable materials in multiple intertidal habitats created sampling challenges, the Mispillion living shoreline is a valuable case study for developing a greater understanding of how to maximize the potential contributions of shellfish to water quality goals, and the temporal expectations regarding their development.

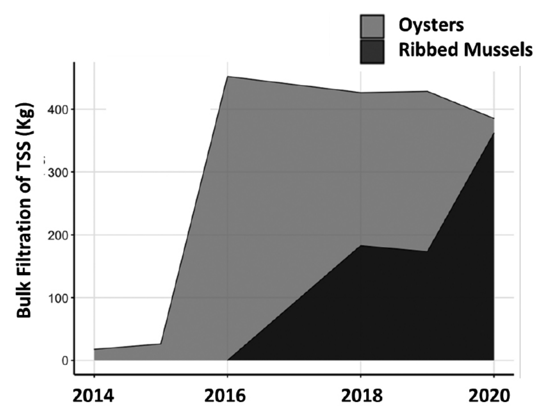

Figure 2. Bulk total suspended solids (TSS) filtration (Kg) by oysters (*Crassostrea virginica*, grey) and ribbed mussels (*Geukensia demissa*, black) between 2014 and 2020 in the low intertidal area of the Mispillion living shoreline.

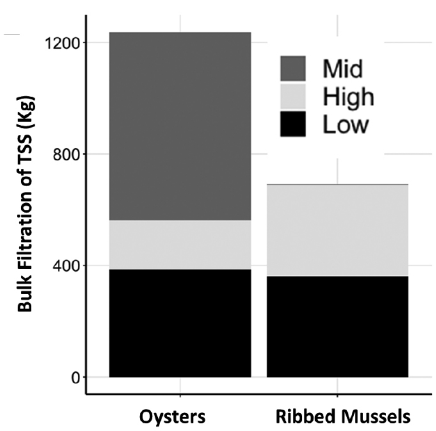

Figure 1. Bulk total suspended solid (TSS) filtration (Kg) by oysters and ribbed mussels in 2020 on the Mispillion living shoreline by area. Grey, white, and black coloration denotes the estimated filtration values of the mid, high and low intertidal intertidal positionss, respectively.

THE ATLANTIC SALMON MICROBIOTA DURING SMOLTIFICATION

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During smoltification process, Atlantic salmon (*Salmo salar*) displayed molecular and physiological changes allowing the parr-smolt transformation. In farmed fish this process can be modulated through controlled temperature and photoperiod conditions. Although fish changes during smoltification process have been studied in different levels, there are few investigations concerning the role of the fish microbiota. Herein, changes in the metabolic requirements, feeding behavior, and osmoregulation suggest a strong microbiota modulation. This study aimed to explore how the intestinal microbiota of Atlantic Salmon can be modulated during the parr-smolt transformation.

The experimental design evaluated a group of Atlantic salmon smolts exposed to gradual salinity change (GSC) and other exposed to salinity shock (SS). Intestinal samples were collected in freshwater (FW), 10, 32 PSU for GSC group and at 32 PSU for SS. DNA extraction was performed using the phenol: chloroform isoamyl alcohol protocol. Follow, the entire 16S rRNA gene were sequencing in the Nanopore MinION platform. Then reads analysis and BLASTN aligned were performed with EPI2ME software package. The results show that FW intestines have a greater presence of the Alteromonadaceae and Moraxellaceae families. Also, during GSC were observed high abundance of Moraxellaceae family. On the other hand, in salmon exposed to SS, the Vibrionaceae family shows evident predominance. To date, the composition of a healthy microbiota in fish has not been established. However, it has been suggested that high bacterial diversity is an indication of animal welfare. These results indicate that a greater diversity of families is observed in GSC fish compared to those subjected to SS.

Funding: ANID-Chile through the Postdoctoral grant FONDECYT (3190320), and FONDAP (#15110027).

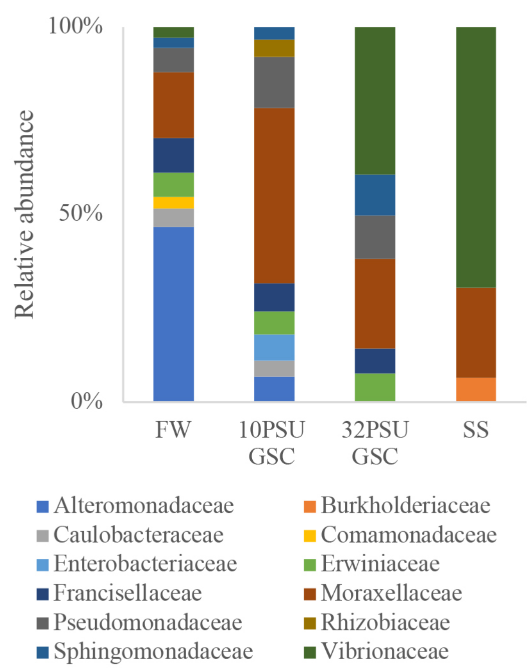

Figure: Relative abundance of intestinal microbiota at family level and experimental condition.

LARVAL SEA LICE (*Lepeophtheirus salmonis*) EXHIBIT BEHAVIORAL RESPONSES TO PRE-ADULT AND ADULT CONSPECIFIC CUES

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In the larval stage of the parasitic copepod *Lepeophtheirus salmonis*, the free living copepodid must locate and settle on a salmonid host. Chemosensory mechanisms play a role in determining whether a potential host is suitable for attachment, yet the full suite of chemical cues and resulting behavioral mechanisms used for host location and aggregation are unknown. After maturing, pre-adult females and adult male sea lice emit sex pheromones. Once mated, gravid females reduce the production of sex pheromones. The aim of this study was to investigate the potential that cues from pre-adult female and adult male sea lice influence copepodid behavior. Behavioral bioassays were conducted with copepodids exposed to water conditioned with three stages of conspecific lice (pre-adult female, adult male, and gravid female), and Atlantic salmon (*Salmo salar*, L.) conditioned water. Experiments demonstrated that copepodids exposed to water conditioned with the salmon host, pre-adult female or adult male sea lice elicited behaviors characteristic of arrestment, whereas sea lice exposed to gravid female conditioned water did not. These results suggest that *L. salmonis* larvae respond to the cues of lice stages known to produce sex pheromones, and we conjecture that they may serve to aggregate conspecifics and amplify infestations.

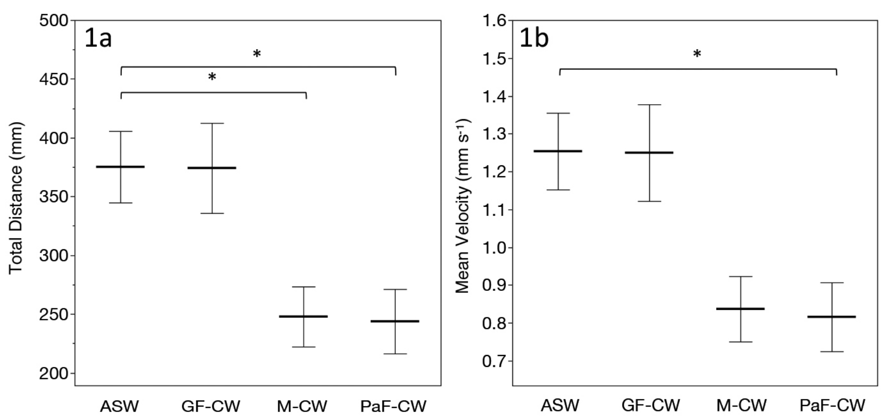

Figure 1. Behavioral response of copepodid sea lice (*Lepeophtheirus salmonis*) to seawater conditioned with conspecific cues in kinesis bioassay. ASW: Artificial seawater, GF-CW: Gravid female lice conditioned ASW, M-CW: Adult male lice conditioned ASW, PaF-CW: Pre-adult female lice conditioned ASW. **(1a)** Total distance travelled (mm), $p = 0.0056$. **(1b)** Velocity (mm s^{-1}), $p = 0.0065$. Data are presented as means ± SE. Connecting bars represent significant difference, * = < 0.05, ** = < 0.01. ASW control, n = 86; GF-CW, n = 42; M-CW, n = 43; PaF-CW, n = 41.

STATUS OF MANGROVE FORESTS IN HONDURAS: METALS AND PESTICIDES

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The ecological value of mangroves ecosystems includes carbon storage, coastal protection against extreme weather events and erosion, improvement of water quality and wildlife habitat. Although many conservation and restoration efforts have been made in the mangrove ecosystems of Honduras, they continue to disappear at a staggering rate. Shrimp aquaculture, port and urban development, solid waste and agrochemical contamination are some of the human activities that are endangering these ecosystems. In addition, extreme weather events and sea level rise are having devastating effects on mangrove forests.

The forestry and environmental departments of the government of Honduras, ICF (Instituto de Conservacion Forestal) and MiAmbiente+ (Secretaría de Energía, Recursos Naturales, Ambiente y Minas) are in charge of protecting and conserving these ecosystems, along with the non-governmental organizations that co-manage their protected areas.

A diagnostic study of Honduras' marine ecosystems was published in 2014 by ICF and USAID's ProParque project, estimating Honduras' mangrove coverage at 73,880 ha. 38,600 ha of mangroves are located in the Gulf of Fonseca and 35,280 are located in the Caribbean Sea. The historical extension of mangroves was estimated at 100,625 ha. Of the 26,745 ha of mangroves lost (more than a quarter), 22,800 ha were deforested for shrimp aquaculture and 2,000 ha for port and urban development. Historical and current data on mangroves extent were estimated based on bibliographic review and geographic information systems.

The dominant mangrove species found in the Gulf of Fonseca and the Caribbean Sea are *Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia germinans* and *Conocarpus erectus*. *Rhizophora racemosa* and *Avicennia bicolor* are also found in the Gulf of Fonseca. It was estimated that shrimp farms are responsible for destroying more than a third of mangroves in the Gulf of Fonseca. Moreover, Germanwatch ranked Honduras as one of the three countries most affected by the impacts of weather-related loss events (storms, floods, heat waves, etc.) from 1996 to 2015. In fact, according to the Honduran strategy for the integrated management of marine, coastal and freshwater ecosystems, shrimp aquaculture and the impacts of climate change are considered to be very high threats to the conservation of Honduran mangroves. Mangrove conservation, protection and restoration should be environmental priorities in Honduras, otherwise their ecological services are seriously threatened. Preliminary results on metals and pesticides in mangrove sediment and shellfish will be presented.

An in-depth review of the scientific literature regarding mangroves genomes and transcriptomes, as well as the epigenetic mechanisms associated with tolerance of mangroves to environmental stress and pollutants will be summarized.

COUPLING FRESHWATER MUSSEL AND SHORELINE RESTORATION PRACTICES IN THE DELAWARE ESTUARY, USA

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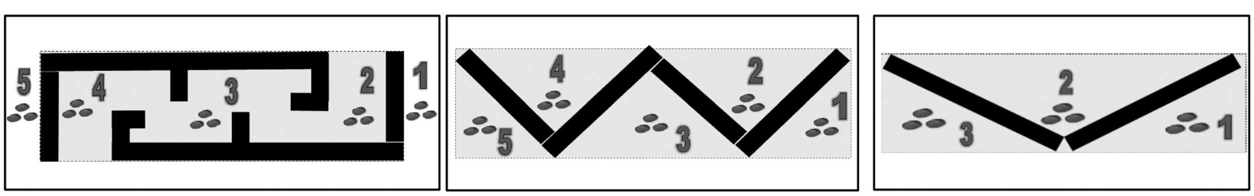
Tidal wetlands are a hallmark feature of the Delaware Estuary and span a salinity gradient from salt water at the mouth of the bay to fresh water in the upper reaches. Wetland loss and interest in conserving ecological function of shorelines has increased attention on nature-based solutions such as living shorelines. Living shorelines are techniques that incorporate natural materials to protect shorelines and provide ecological uplift while retaining connectivity between subtidal and higher elevation areas. Until recently, living shorelines have typically been applied in saltwater environments to help mitigate wave attenuation and erosion and to positively enhance environmental conditions. Additionally, some living shoreline structures have been shown to attract recruitment of sessile bivalves such as oysters and ribbed mussels, which promotes both structural resilience and diverse ecological benefits.

To date, living shoreline approaches have not been applied to freshwater habitats. In April, 2021, a pilot living shoreline project was installed along a degraded section of the lower Schuylkill River in southeast Pennsylvania, USA in freshwater tidal zone situated in an urban landscape with diverse anthropogenic stressors, with a goal to test whether native species of freshwater mussels could be incorporated into a multi-habitat mosaic type of living shoreline that will eventually include other targeted biota such as submerged aquatic vegetation. The approach was to install benthic stabilization structures to enhance habitat suitability for mussels, which are infaunal benthic animals sensitive to erosion. Structures of varying shapes (Fig. 1) were constructed using gabions filled with recycled and aged oyster shell, and the native freshwater mussel *Utterbackiana implicata* was stocked within and outside of structures in replicate cohorts of similar number and size.

The monitoring framework developed by the Delaware Estuary Living Shoreline Initiative was then adapted for this project, including changes in sediment grain size, bathymetry, and mussel retention, survival, and growth. Despite record flooding from the remnants of Hurricane Ida in August, 2021, the living shoreline structures and deployed mussels survived, and considerable sediment was trapped in and around the structures. Contingent on continued success, these results suggest that living shoreline approaches can be adapted for tidal freshwater conditions in degraded and vulnerable urban landscapes.

Figure 1. Three oyster gabion structure designs. Black shaded areas represent structures, gray areas indicate the install footprint. Mussel icons indicate locations of mussel deployment. Blue numbers indicate monitoring zones. Structures were referred to by shape: “V” (right), “W” (middle), and “G” (left).



TRADEOFFS IN SHRIMP BREEDING: NAVIGATING THE RELATIONSHIP BETWEEN GROWTH AND SURVIVAL USING GENOMIC TOOLS

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Shrimp farmers need animals that grow fast and survive the stress of farming. Animals that grow quickly enable more production cycles while survival is key to dependable and bountiful harvests. The combination of these traits is a holy grail for the industry but progress from breeding efforts has been lopsided.

Growth rate is highly heritable and simple to measure. Improving survival, however, is more challenging. Consequently, much of the germplasm released by breeding companies grow fast but lack tolerance to stressors prevalent in aquaculture. Genetic gains would be more dramatic if the relationship between growth and survival were better understood and utilized.

This presentation will discuss our use of genomics to study growth and survival of commercial *P. vannamei* families in production environments. After observing the impact of growth-focused selection on survival we have chosen to implement a breeding program that prioritizes survival before growth. Finally, we compare the genetic signatures of stocks selected specifically for survival to genetic diversity collected from around the world.

MAINE IS SIX MONTHS OF WINTER AND SIX MONTHS OF POOR SLEDDIN'. THE ONGOING SAGA OF SEA SCALLOPS (*Placopecten magellanicus*) AS A NEW SPECIES FOR AQUACULTURE

Dana L. Morse*, Hugh Cowperthwaite, Damian Brady, Christian Brayden, Marsden Brewer, Robert Brewer, Struan Coleman, Chris Davis, Alex de Koning, Phoebe Jekielek, Kohl Kanwit, Tom Kiffney, Nate Perry, Andrew Peters

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The seemingly-rapid advance of the nascent sea scallop farming industry in Maine belies the 20+ years that came before, where many distinct and smaller steps contributed to today's knowledge. The current state of technology and industry engagement can be traced perhaps as staring from a technology transfer trip to Aomori Prefecture in Japan in 1999, but ties between Aomori and Maine have existed for much longer, dating back to the wreck of the Maine-built ship *Chesbrough*, October 30th, 1889, off the coast of Shariki.

In the last 22 years, many steps have been taken to advance the possibilities for scallop stock enhancement and for intensive aquaculture. Optimizing spat collection, leasing and licensing changes to allow for easier experimentation with nursery and growout, adoption of proven equipment and trials of new gear and machinery, biotoxin testing, market development, and ongoing outreach have all played their role. Significant guidance, advice and relationships with Japanese counterparts and colleagues has been critical for the acquisition and adoption of husbandry techniques and equipment, as has been expertise gained from colleagues in the Atlantic provinces of Canada. A trip in 2016, led by Maine-based Coastal Enterprises, Inc. has proven to be a pivotal event, galvanizing interest and understanding, and leading to the first-ever aquaculture cooperative in the state, which is focused on scallop farming, and which is comprised of people having fishing and farming backgrounds alike.

More recently, scallop aquaculture has provided opportunities in a broadening suite of endeavor: training and educational opportunities for younger and graduate students; the use of aquaculture techniques such as spat collection as a platform to investigate larval drift, source-sink dynamics and coastal oceanography; methods development and approvals for new biotoxin detection processes, equipment and supplies to industry, sales and marketing of unusual products such as whole/live scallops, and business and financial services to producers. This once-discrete field of work is growing its own ecosystem, as part of Maine's working waterfront and the scientific/educational/services communities.

LESSONS LEARNED IN GROWING SEA SCALLOPS (*Placopecten magellanicus*) IN MAINE: EQUIPMENT, HUSBANDRY, OPERATIONS

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Sea scallops carry a high value per piece and as such are an attractive candidate for aquaculture. Scallop farmers must however contend with attributes of the species that present distinct challenges to acceptable growth, survival, harvest and marketing. High sensitivity to stocking density, short shelf life, very limited tolerance to heat/cold/dessication/low salinity, long larval duration, and limited catch-muscle capabilities all mean that there are novel ways to kill scallops that don't apply to other, more robust species such as oysters, hard clams and blue mussels.

Maine fishermen and producers have been experimenting with seed collection, stock enhancement and intensive aquaculture for over 20 years, and it's taken all of that to make the observations and trials necessary to raise scallops confidently. Most equipment and techniques have been adopted from Japan and Canada and modified to meet the requirements of the species and production along Maine's coast.

In this presentation, we will review the equipment, siting, husbandry, and biotoxin issues association with raising sea scallops.

AQUACULTURE PHYTOPLANKTON MONITORIN NETWORK: USE OF CITIZEN SCIENTISTS TO MONITOR HARMFUL ALGAL BLOOMS AND CHANGES IN ENVIRONMENTAL CONDITIONS

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Harmful algal blooms (HABs) occur when species of phytoplankton grow very quickly forming blooms resulting in water discolorations sometimes referred to as red tides. These blooms have been observed in every state resulting in over $1 billion in losses over the last serval decades to communities that rely on recreation, tourism and seafood harvesting. The direct economic impact of HABs does not include the socioeconomic impact from loss of subsistence harvest activities, disruption of cultural practices, water insecurity, food insecurity, and social interaction tied to coastal resource use. 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 since they are known to cause mortality of shellfish and finfish worldwide.

The National Phytoplankton Monitoring Network (PMN) is a community-based network of volunteers monitoring marine and freshwater phytoplankton and harmful algal blooms. Formed in 2001, PMN enhances the Nation's ability to respond to and manage the growing threat posed by HABs by collecting important data including phytoplankton species composition and distribution as well as environmental conditions. 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 are of paramount concern since they are known to cause mortality of shellfish and finfish worldwide. The goal of the AQPMN would provide aquaculture farms advanced warning of these shellfish toxic and ichthyotoxic blooms to empower growers to mitigate the effects of these blooms.

This citizen science approach to monitor HABs was able to grow into a national monitoring program by use of various web-based tools such as an interactive web site and a geographic information system tool for data visualization and searchable database. This presentation will outline the use of these technologies and highlight the use of volunteer data in aquaculture settings.

REDUCING RISK OF MARINE MAMMAL ENTANGLEMENT IN AQUACULTURE STRUCTURES BY REPLACING ROPE WITH SEMI-RIGID FIBERGLASS LINES

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Offshore aquaculture structures located in the Gulf of Maine are perceived to pose some risk to the critically endangered North Atlantic Right Whale, thereby making permits for such systems effectively unattainable. University of New Hampshire, in collaboration with Otherlab and Trophic LLC seeks to develop whale safe mooring and kelp cultivation technology. By replacing synthetic fiber ropes with composite materials, such as fiberglass rods, we believe that the chances of marine mammal entanglement can be significantly reduced, if not eliminated. Because they have rigidity and a minimum bending radius beyond which they break, composite lines cannot loop around whale appendages; the line would break or loosen before the formation of a wrap. Our project aims to demonstrate this technology in the context of a multi-tile kelp cultivation array fully exposed to the harsh winter conditions characteristic of the Gulf of Maine.

Project goals include evaluation of the technology as an entanglement prevention measure, development of operational and technological methods for enabling practical use of composite materials as structural or grow-lines in macroalgae aquaculture, and assessment of the durability of composite materials used as submerged load-bearing lines. We have designed and tested devices for terminating our composite lines, allowing for use as tension members. We have successfully deployed these lines as mooring and grow-lines on pilot scale kelp farms in the Gulf of Maine. And finally, we are continuing to evaluate changes in the mechanical properties of these materials after use on kelp cultivation systems.

ADDRESSING USER CONFLICTS ASSOCIATED WITH THE GROWTH OF THE SHELLFISH AQUACULTURE INDUSTRY IN NORTH CAROLINA

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The NC Division of Marine Fisheries (NCDMF) administers the Shellfish Lease and Aquaculture Program through the Habitat and Enhancement Section for the purposes of managing commercial shellfish aquaculture within the State. North Carolina has allowed for the private, commercial cultivation of shellfish in public trust waters for over 150 years. Over the past five years, the industry has been growing nearly exponentially in terms of leased acres. Within that general growth pattern, evolving technology has led to the proliferation of water column methods of cultivation (floating cages, bags, longlines, etc.), which are more obstructive to waterways. Shellfish leases are required by law to be compatible with other public trust uses, and as the industry has grown and changed, the benchmark of compatibility has become increasingly contentious. Additionally, in 2019, the State passed the Shellfish Aquaculture Bill, which was designed to further promote the growth of the aquaculture industry.

Navigation, recreational and commercial fishing, and conflicts with riparian landowners remain the largest challenges associated shellfish leases in North Carolina. These challenges are exacerbated by geographic constraints in the southern waters of the state and an increase in the density of shellfish leases around coastal population centers.

The NC Marine Fisheries Commission has adopted several new rules to address some of these user conflicts. A mandatory 250' buffer between new shellfish leases aims to improve navigability, and 'cumulative impact' language permits consideration of existing shellfish leases and the geography of a water body in making a final determination on a lease. The NCDMF has also adopted policies addressing user conflicts, including a mandatory notification of riparian landowners within 250' of proposed shellfish leases to increase public awareness and feedback in the public comment/public hearing process.

Collaboration with other state agencies has been extremely useful in forecasting potential user conflicts and developing innovative solutions. The State Marine Aquaculture Coordinator's Network (SMACN), founded in 2020, has served as a platform for state agencies and extension representatives to discuss regulatory frameworks and permitting requirements, logistics surrounding aquaculture enterprise areas, and strategies for addressing issues associated with growing aquaculture industries.

Addressing the rapid growth of the aquaculture industry has required a multifaceted approach that DMF continues to adapt to best facilitate the cohabitation of shellfish growers and public users in our coastal waterways.

THE NORTH CAROLINA SHELLFISH AQUACULTURE TOOL: AN INTERACTIVE PLATFORM FOR PUBLIC AWARENESS AND USER CONFLICT MITIGATION

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Due to the rapidly growing and changing shellfish aquaculture industry in North Carolina, the need for improved communication between shellfish lease applicants, the NC Division of Marine Fisheries (NCDMF), and the general public is more apparent than ever. In 2019, the NCDMF developed and launched a GIS based web tool that is publicly available with the goal of improving public awareness and transparency of the shellfish leasing process, and compiling resources for applicants pursuing a shellfish lease.

The Shellfish Aquaculture Tool (SAT) provides a single place for shellfish lease applicants to view existing leases, mapped submerged aquatic vegetation, shellfish closures, designated nursery areas, and a number of other designations that directly impact the suitability of an area for shellfish aquaculture. Additionally, as a shellfish lease application is processed, the SAT is updated with the status of the proposed lease so applicants can track their application as it is processed. This has helped decrease staff workload by making information accessible to the public without requiring staff assistance, and improved engagement at public hearings by increasing awareness of proposed shellfish leases.

Feedback has been very positive from staff and stakeholder groups, as the SAT is continually updated to improve functionality and ease of use. This SAT is one of the many ways in which the NCDMF is addressing user conflicts surrounding shellfish leases in North Carolina.

EFFECTS OF *Gynura procumbens* EXTRACT IN NILE TILAPIA, AS A STRESS REDUCING AND IMMUNE STIMULATING NUTRACEUTICALS

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Even though aquatic life may meet the world's demand for a significant amount of protein supply, it is not without shortcomings, the most serious of which is stress. The effects of different concentrations of *Gynura procumbens* on the hematological parameters of acutely stressed Nile tilapia (*Oreochromis niloticus*) were investigated in the present study, and the best concentration was determined, from experiments conducted in a recirculating aquaculture system. Stressed tilapia were fed with *Gynura pocumbens* extract (0, 0.0005, 0.001 and 0.0015% of feed weight) along with a hydrocortisone stress hormone (0.01% of fish body weight). In this experiment we evaluated blood glucose level, lysozyme activity, phagocytic activity, hematocrit, spleen somatic index and hepato somatic index of the fish. From the preliminary data, *Gynura* has shown to decrease the levels of blood glucose and brought the levels of serum lysozyme activity and phagocytic capacity in stressed fish to the levels of controlled fish. Chronic research will provide us with a better understanding of how to use *Gynura* to alleviate stress in fish.

POTENTIAL USE OF *Gynura procumbens* TO MODULATE STRESS AND IMMUNE RESPONSE IN FISH

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Fish aquaculture as an industry has developed significantly in order to offer human consumers with nutritious protein. Farmers, however, have been dealing with the impact of stress on fish growth, reproduction, and immunity as the industry has progressed. As a consequence, finding viable techniques to minimize stress in fish and enhance their capacity to resist disease, as well as lowering farmers' reliance on chemical treatments, is imperative. In Southeast Asia, *Gynura procumbens*, also known as longevity spinach, is a popular medicinal plant that has been used to lower blood glucose levels, which is major concern in stressed animals. Our previous investigation (on acute stress) was to see how *Gynura procumbens* extract benefited stressed tilapia and to find an effective concentration for a chronic study. In this experiment, we are investigating the effects of 0.0015% *Gynura procumbens* on stress physiology and immunology of Nile tilapia for 12 weeks. In this experiment we are using four treatment groups including control group (w/o stress and GPE), control treatment group (w/o stress but w/ GPE), stress group (w/ stress but w/o GPE) and stressed treatment group (w/ stress and w/ GPE). Results from this research will give us insight how effective *Gynura* extract is as stress mitigating substance. This research will be completed soon, and the findings will be presented at the World Aquaculture Society meeting.

FARMING ATLANTIC SURFCLAMS *Spisula solidissima* IN NEW JERSEY –EXPANDING GROWOUT SITES AND THE CAPACITY TO RESPOND TO CLIMATE STRESS

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Bivalve aquaculture along the Atlantic Coast is dominated by the eastern oyster (*Crassostrea virginica*) and hard clam (*Mercenaria mercenaria*). Continued success of bivalve aquaculture is threatened by diseases, weather fluctuations, unpredictable mortality, and the lack of crop diversity. The Atlantic surfclam (*Spisula solidissima*) is a promising aquaculture diversification species for high-salinity (25-32) coastal waters of NJ and the Northeast. This native species exhibits fast growth but faces challenges of temperature-driven mortalities. Over the past 5 years, experiments have been done to test for the potential to select for improved thermal tolerance in seed lines, and to test growth and survival of multiple cohorts at three shallow backbay farms. Results have demonstrated the capacity to select for thermal tolerance, and that the species can be grown at shallow water sites with a relatively short turnaround to market size (50mm clams in 12 – 15 months). In ongoing experiments, we are collaborating with fishing industry partners to deepen knowledge about the potential for this species to be cultivated at commercial scales in the open ocean, and to better understand the combined impact of coastal acidification and increasing temperature (multi-stressors)

DEVELOPING LARVAL CULTURE PROTOCOLS FOR THE X-RAY TETRA *Pristella maxillaris* TO MINIMIZE THE USE OF LIVE FEEDS

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The x-ray tetra (*Pristella maxillaris*) is an ornamental characid native to the Amazon River Basin. This species is highly desirable due to its unique transparent body and peaceful temperament in an aquarium setting. Although *P. maxillaris* has been successfully bred in captivity, peer-reviewed information on larval development, digestive physiology, and nutrition is scarce. This information would help improve the larval culture protocols of *P. maxillaris* by allowing for the transition of larvae from expensive live feeds to a cost-effective microdiet (MD) as early as possible while maintaining high growth and survival. To gain insight into the larval development of *P. maxillaris,* the digestive enzyme ontogeny and digestive tract morphology was examined at 20 timepoints throughout a 35-day trial. Pepsin, trypsin, and lipase activities were quantified using standard microplate assays and the morphology of the digestive tract was visualized using histology. The presence of a functional stomach was found at 22 DPH (days post hatch) indicated by the presence of gastric glands and pepsin activity. A 45-day dietetics trial was conducted to determine which of three commercially available MDs best promotes growth and survival in larval *P. maxillaris* compared to an *Artemia* reference diet. Survival was greatest for larvae fed *Artemia* compared to those fed MDs and standard length (SL) did not significantly differ among treatments (Fig. 1A). Lastly, a 35-day trial was conducted to identify the appropriate timing for weaning *P. maxillaris* larvae from *Artemia* to a MD. Larvae were subjected to one of five treatments: *Artemia* reference diet, exclusively MD, or MD introduction at 12 (W1), 18 (W2), or 24 (W3) DPH. Survival was greatest for larvae fed *Artemia* compared to groups weaned onto MDs and SL did not differ significantly between the *Artemia* treatment and the W2 and W3 treatments (Fig. 1B). The long-term effects of MD feeding will be examined to determine the economic benefits resulting from replacing *Artemia* in the larval production process to market size.

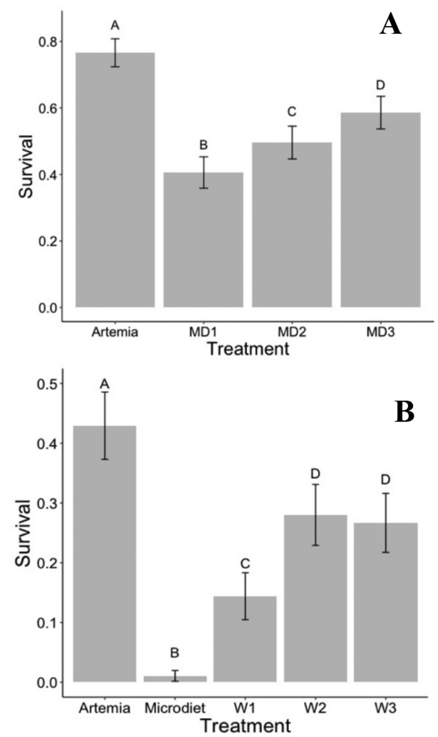

FIGURE 1. Proportion of *P. maxillaris* larvae (+/- SE) alive after a 45-day dietetics trial (A) and a 35-day weaning trial (B) (n=5, GLMM with Bonferroni distribution and estimated marginal means). Letters above bars denote significance ($p<0.05$).

EXAMINING THE DIGESTIVE PHYSIOLOGY OF LARVAL CHINESE ALGAE EATER *Gyrinocheilus aymonieri* TO IMPROVE LARVAL CULTURE PROTOCOLS

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The Chinese algae eater (*Gyrinocheilus aymonieri*) is a freshwater ornamental fish native to rivers of the Mekong Basin in Southeast Asia. This species is sold in high volumes in the freshwater aquarium industry due to its usefulness in controlling algae growth in home aquariums. Although *G. aymonieri* have been successfully cultured in farm ponds, challenges associated with larval survival and labor efficiency persist including the inability to grow this species to market size in a recirculating aquaculture system. To gain insight into the larval development of *G. aymoneiri*, developmental milestones, digestive enzyme ontogeny, and digestive tract morphology were examined. 7800 newly hatched *G. aymoneiri* were distributed equally among three 10 L tanks and fed a microdiet (MD) twice daily to satiation from two to 30 DPH (days post hatch). Three to five larvae were removed from each tank at each of 17 timepoints throughout the trial, photographed, measured, and assessed for developmental characteristics including eye and mouth formation, swim bladder inflation, and flexion. Larvae were preserved for digestive enzyme analysis and histological processing. The growth of larval *G. aymoneiri* followed a linear pattern with the notochord length increasing from 4.988 mm at 3 DPH to 8.99 mm at 30 DPH (Fig. 1). Eye pigmentation was seen at 1 DPH, with subsequent mouth formation and the onset of feeding at 2 DPH. Swim bladder inflation occurred soon after mouth opening at 3 DPH and with a single-lobed morphology. Larvae began to undergo flexion at 11 DPH and completed flexion at 19 DPH. At the completion of flexion, the swim bladder of *G. aymoneiri* became bi-lobed with a larger anterior lobe and a smaller, cylindrical posterior lobe. The activities of digestive enzymes including lipase, trypsin, and pepsin will be assessed at each of these timepoints via standard microplate assays. The changes in digestive tract morphology throughout the larval period will be visualized using histology. Together, these data will provide insight into the digestive capacity of this species throughout the larval period. This information will aid in improving larval diet and culture conditions to optimize growth, survival, and production efficiency.

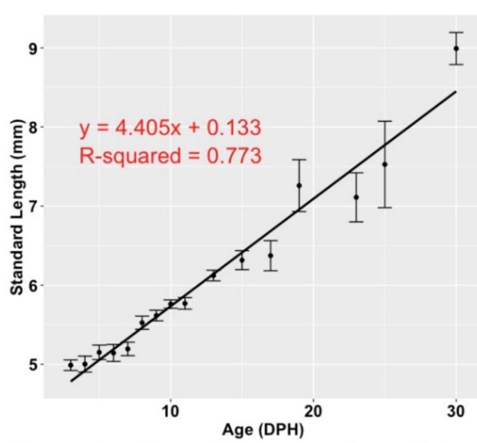

Figure 1. Growth progression of *G. aymonieri* fed exclusively MD from 3-30 DPH where data points represent notochord length (3-23 DPH) and standard length (19-30 DPH).

EARTHWORM, *eisenia fetida*, BEDDING MEAL AS POTENTIAL CHEAP FISHMEAL REPLACEMENT INGREDIENT FOR SEMI-INTENSIVE FARMING OF NILE TILAPIA, *oreochromis niloticus*

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The study amalgamated earthworm and agro-industrial wastes through vermicomposting and then evaluated the potential of the bedding (mixture of *Eisenia fetida* and vermicompost) to replace fishmeal in semi-intensive farming of *Oreochromis niloticus*. The bedding was used to substitute fishmeal at inclusion rates of 100, 60, 30 and 0% (D100, D60, D30 and control D0). In triplicates, the four homogeneous diets were fed to quadruplicate groups of 30 g *O. niloticus* for 112 days. There was no significant difference ($p > .05$) in mortalities, average length gain and FCR among all tests. Nevertheless, diet D0 had significantly ($p < .05$) superior amino acid profile, low fibre content and fish carcass crude protein (63.2 ± 0.72% dry matter). Subsequently, D30 and D0 produced fish with significantly higher ($p < .05$) mean weight gain (256.03 ± 0.4 g) and biomass (369,136 g) respectively. On to the contrary, diet D100 had significantly higher ($p < .05$) crude lipids content (9.4 ± 0.6% dry matter), economic returns and profit index than the control diet due to the comparatively low cost of producing the earthworm bedding. This simple biotechnology can commercially be upscaled to sustainably produce cheap and nutritious fish feed capable of increasing yields and maximizing profits.

Variables	Diet D100	Diet D60	Diet D30	Diet D0
Weight gain (g)	80.2 ± 0.4^{a}	81.9 ± 0.42^{b}	82.4 ± 0.27^{b}	83.4 ± 0.3^{c}
Survival (%)	83.3 ± 3.1^{a}	85.3 ± 6.4^{a}	90 ± 8.7^{a}	86.7 ± 3.1^{a}
Length gain (cm)	6.31 ± 0.04^{a}	6.35 ± 0.05^{a}	6.25 ± 0.03^{a}	6.28 ± 0.08^{a}
Specific growth rate	0.77 ± 0.0^{a}	0.78 ± 0.0^{b}	0.78 ± 0.0^{bc}	0.79 ± 0.0^{c}
Food conversion ratio	1.74 ± 0.01^{c}	1.7 ± 0.01^{b}	1.69 ± 0.01^{b}	1.67 ± 0.01^{a}
Protein efficiency ratio	0.33 ± 0.0^{a}	0.33 ± 0.0^{ab}	0.33 ± 0.0^{ab}	0.34 ± 0.0^{b}
Protein productive value	−0.12 ± 0.0^{a}	1.33 ± 0.1^{b}	1.42 ± 0.15^{b}	1.39 ± 0.04^{b}

Note: Values represent means ± standard deviation of triplicate tests $n = 50$. Different alphabets (a < b < c < d) in the same rows symbolize non-homogenous means ($p < .05$).

Variables	Diet D100	Diet D60	Diet D30	Diet 0
Total sales revenue	4,965.09^{a}	5,042.79^{b}	5,062.80^{b}	5,111.52^{c}
Price above variable cost	4,105.59^{a}	3,704.54^{b}	3,229.55^{c}	2,739.99^{d}
Price above fixed cost	2,832.93^{a}	2,910.63^{b}	2,930.64^{b}	2,979.36^{c}
Net returns	1,973.43^{d}	1,572.38^{c}	1,097.39^{b}	607.83^{a}
Break-even yields	0.52^{a} ± 0.0^{a}	0.58 ± 0.0^{b}	0.66 ± 0.0^{c}	0.78 ± 0.0^{d}
Profit index	5.78 ± 0.02^{d}	3.77 ± 0.1^{c}	2.76 ± 0.0^{b}	2.16 ± 0.0^{a}

Note: Values represent pooled data per triplicate except for the break-even yield and profit index which is means ± standard deviation of triplicate tests. Different alphabets (a < b < c < d) in the same rows symbolize non-homogenous means ($p < .05$).

BUILDING AN ECOSYSTEM-BASED MANAGEMENT COLLABORATIVE FOR SHELLFISH AQUACULTURE IN WILLAPA BAY AND GRAYS HARBOR, WASHINGTON

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The Washington Coast Shellfish Aquaculture Study (WCSAS) was a 3-year project funded by the Washington State Legislature to sustain shellfish aquaculture in the estuaries under changing environmental conditions by establishing a collaborative, ecosystem-based management framework. Ending in late 2019, the project emerged from a confluence of long-standing but continually evolving issues facing shellfish farmers and resource managers in Willapa Bay and Grays Harbor, specifically the challenges posed by (1) interactions between shellfish aquaculture and eelgrass and (2) burrowing shrimp management. In this talk, we will discuss project outputs and outcomes, the impacts of COVID on project objectives and working group process, and the status, goals, and next of the emerging EBM collaborative.

PERFORMANCE OF SANDFISH SEA CUCUMBER (*Holothuria scabra*) JUVENILES IN AN IMTA SYSTEM WITH FORKTAIL RABBIT FISH (*Siganus argenteus*) FINGERLINGS

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The College of Micronesia Land Grant Program has undertaken the development Hatchery based Sandfish sea cucumber , *Holothuria scabra* farming technology for local community based economic development, future commercialization and aiming at restocking the depleted stocks in the wild. In this regard, an experiment was conducted to find out the effect of polyculture on the growth, survival of Sandfish sea cucumber, juveniles in floating Ocean Nursery Hapa Net System – II with and without Forktail Rabbitfish (*Siganus argenteus*) fingerlings in an IMTA setting. The experiment aims to find out if any significant improvements in the growth and survival of juvenile Sandfish sea cucumbers can be achieved by polyculturing them with Rabbit fish fingerlings. Sandfish sea cucumber juveniles was stocked at 20 pieces per floating hapas in duplicates as controls and a second treatment batch of 20 juveniles were grown in poly culture with 100 Rabbitfish fingerlings in duplicates. The experiment was run for 30 days at the end of which time the growth (length and wet weight) and survival was tabulated for each treatment by measuring all surviving animals of fish and sea cucumbers. The Rabbitfish were fed daily a commercial feed in both the treatments while the control animals of sea cucumber juveniles just grazed on algae from the hapa net enclosures where they were held. The main physio chemical properties of the Ocean rearing water were monitored twice; in the morning and late afternoon. The results showed Sandfish sea cucumber juveniles and Rabbitfish figerlings performed significantly better in both length and wet weight when together when compared with control treatments. There was no significant difference in the survival between the different treatments.

DEVELOPMENT OF A MEDIUM DENSITY SNP-ARRAY FOR THE BLUE MUSSEL SPECIES-COMPLEX

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Blue-mussels from the Mytilus species-complex (*M. edulis* x *M. trossulus* x *M. galloprovincialis*) are an abundant component of the benthos community found in the high latitude habitats. Aside from their ecological value (Norling & Kautsky, 2007), these foundation species are of extreme relevance to the aquaculture industry, with 2 149 534 tonnes produced globally in 2019 (FAO, 2021). Mussels are able to withstand a wide range of environmental conditions and significant effort has been made to unravel the physiological consequences of environmental stress in this species (e.g Lesser, 2016; Melzner, 2011; Riisgard, 2013). As the development of genetic resources began to be emphasized in recent years, the genomic mechanisms underlying local adaptation as well as species distribution, remains poorly understood. To help overcome this, we developed a novel genomic tool, a medium-density SNP array for rapid, high-throughput genotyping of individuals in the *Mytilus* species complex.

For the creation of the array, 23 *Mytilus* spp populations were sampled from across their global distribution, incorporating 4 species in this complex (*M. edulis*, *M trossulus M. galloprovincialis* and *M. chilensis*), as well as hybrids. Samples were sequenced using a low coverage (4-6x) whole-genome resequencing approach, with sequences aligned to the *Mytilus galloprovincialis* genome prior to filtering and calling of SNPs. We have identified SNPs representative of global and within species diversity and explored levels of introgression in *Mytilus* spp populations distributed worldwide. The medium-density SNP-array (60K SNPs), developed in collaboration with Affymetrix, contains globally polymorphic SNPs, which capture the genetic diversity present in mussel populations thriving across a gradient of diverse environmental conditions. In addition, existing sets of published and validated SNPs were included on the array as informative for speciation, as well as diagnosis of transmissible cancer.

This tool will allow the consistent, fast and affordable genotyping of individuals, facilitating the investigation of ecological and evolutionary processes in these taxa. The applications of this array extend to shellfish aquaculture, contributing to the optimisation of this industry via (a) genomic selection of blue mussels; (b) parentage assignment; (c) inbreeding level assessment and (d) species/product identification and provenance. Besides, this tool will facilitate the development of genome wide association studies (GWAS) for key production traits as well as those related to environmental resilience. Such research is especially relevant to safeguard aquaculture production under climate change.

ASCERTAINING THE MARKET VALUE OF NORTH CAROLINA AQUACULTURED SEAFOOD

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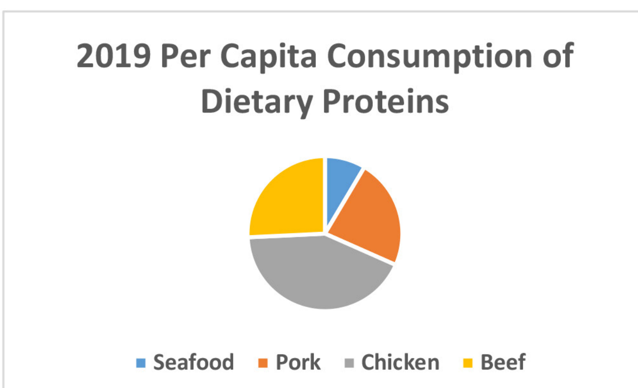
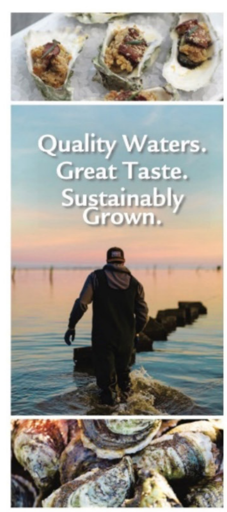
According to Seafood Health Facts, the per capita consumption of seafood (fin fish, crustaceans and shellfish) increased from 14.6 pounds per person in 1997 to 16.5 pounds per person in 2006, and then decreased to 14.9 pounds per person in 2018. In 2019, consumption rose to 19.2 pounds of seafood per capita, according to the National Fisheries Institute. Though seafood sales remained depressed in the foodservice sector as a result of the pandemic, purchases of fresh and frozen seafood at big-box retailers and traditional supermarkets rose through the latter half of 2020. Despite this positive development, the per capita consumption of seafood remains low relative to beef, pork and poultry because consumers have choices and apparent preferences for dietary protein.

Market research is a branch of social science that links the consumer to a marketer through information that identifies market opportunities and problems, evaluates marketing actions and monitors market performance. To help local seafood producers meet the needs and expectations of their customers, North Carolina Sea Grant has conducted qualitative (focus groups) and quantitative (online surveys) research to learn consumers' reasons for eating seafood, their perceptions of U.S. producers and what enhancements they want to facilitate preparing and consuming seafood in the home.

This paper will review the attributes North Carolina consumers say they value about local seafood, and it will show how research results were used to develop an approach to marketing cultivated oysters. While the demand for local seafood seems strong, this paper will also show that price remains a prime determinant of purchase intent.





INHIBITORY CAPACITY OF A NOVEL MICROBIAL ENHANCED PROTEIN AGAINST *Vibrio* spp. IN PACIFIC WHITE SHRIMP *Penaeus vannamei*

Sergio F. Nates*, Brandon White and Luke Fredrickson

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Recent studies on the development of practical diets for shrimp production systems using a novel microbial enhanced protein, ME-PRO®, have shown to be a promising solution to produce eco-friendly aquafeeds. The protein is processed at a state-of-the-art plant using non-GMO (non-genetically modified) soybean meal and a natural occurring, non-toxigenic, fungi, *Aureobasidium pullulans*. The fermented co-product also offers significant amounts of short-chain peptides and free amino acids that confer excellent attractability and palatability properties.

The objective of this study was to evaluate the inhibitory capacity of ME-PRO® at different concentrations (0.5%, 1% and 2%) against *Vibrios* spp. extracted from the midgut of Pacific white shrimp *Penaeus vannamei*. Shrimp post larvae (0.1 g) were weighed, macerated, and dissolved in 200ul sterile distilled water. A 50 uL sample was inoculated in TCBS agar and ChromAgar. All *Vibrio* and probiotic bacteria were resuspended in TSB medium. A 50 ul sample was inoculated in TSA agar plates in the case of probiotics and on ChromAgar for the *Vibrio* species. The results of bacterial growth count in agar were expressed in CFU/g for larvae. In addition, PCR analysis was conducted using the AP4 method with three sequences: F1 (ATGAGTAACAATATAAAACATGAAAC), R1 (ACGATTTCGACGTTCCCCAA) and F2 (TTG AGAATACGGGACGTGGG).

Results showed that inhibitory capacity of ME-PRO® was present at inclusion levels of 0.5%, 1% and 2% against *Vibrio* bacteria in macerates of shrimp larvae and inclusion in culture media ChromAgar *Vibrio* and TCBS. When the microbial enhanced protein was incorporated in TCBS and ChromAgar culture media, results indicated inhibitory activity for *Vibrio* type 1 (yellow colonies) between 38%, 48% and 57% respectively. For type 2 *Vibrio*, the inhibition rate was 78% and 100% for the 1 and 2% doses and for *V. parahaemolyticus*, 38%, 45% and 62% respectively. The evaluation of shrimp larvae macerates using TCBS agar indicated an inhibitory activity of *Vibrios* in the presence of the protein. Similarly, in ChromAgar a reduction in *V. parahaemolyticus* occurred when the 0.5% dose was used.

The assessment in this study indicated that an inclusion level as low as 1% ME-PRO® improves resistance to *V. vulnificus* and *V. parahaemolyticus* associated with high mortalities in shrimp aquaculture. Similar to previous studies, the results confirmed that supplementation into the diet of *P. vannamei* will confer a protective effect against the *Vibrio* species associated with Early Mortality syndrome (EMS).

HOW SUSTAINABLE IS AQUACULTURE?

Prof. Roz Naylor, Stanford University

Aquaculture 22: World Aquaculture Society Meeting
San Diego, CA: March 1, 2022

Prof. Roz Naylor will discuss her professional and personal journey in assessing the sustainability of aquaculture over the past two decades. Her comments will reflect insights from two reviews on aquaculture published in *Nature*, the first in 2000 ("The effect of aquaculture on world fish supplies") and the second in 2021 ("A 20-year retrospective review of global aquaculture). She will discuss the important role of professional collaborations as well as her work on aquatic foods as part of the global food system in shaping her views. The talk will explore major areas of achievement as well as persistent challenges along the path toward a sustainable aquaculture sector.

Rosamond (Roz) Naylor is the William Wrigley Professor of Earth System Science, Professor (by courtesy) in Economics, and founding Director of the Center on Food Security and the Environment (FSE) at Stanford University. She received her PhD from Stanford University in applied economics, her MSc in economics from the London School of Economics, and her dual BA degrees in economics and environmental science from the University of Colorado. Her research focuses on policies and practices to improve global food security and protect the environment on land and at sea. She has been involved in many field-level research projects around the world with her students and has published widely on issues related to aquaculture, global food systems, and food policy. She currently co-chairs The Blue Food Assessment, an international initiative aimed at providing a comprehensive scientific evaluation of the sustainability, nutrition, equity, and justice dimensions of aquatic foods cultured and captured in freshwater and marine environments within the global food system. She is a Fellow of the Ecological Society of America, a Pew Marine Fellow, and a Leopold Leadership Fellow. She is the President of the Board of Directors for Aspen Global Change Institute, a member of the Scientific Advisory Committee for Oceana, and a member of the Forest Protection Advisory Panel for Cargill. At Stanford, Naylor teaches courses on the World Food Economy, Human Society and Environmental Change, and Food and Security.

EVALUATION OF A HIGH PROTEIN DISTILLER'S DRIED GRAINS WITH YEAST AS A PROTEIN SOURCE IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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New technologies have been introduced by the ethanol industry to improve the efficiency of ethanol production, resulting in new types of distiller dried grain with different nutrient profiles. One of the new processing techniques removes fibrous corn components before fermentation and removing the soluble fraction after fermentation to produce a range of high protein distiller grain with yeast which contain from 40% (HP40Y) to 49% (HP50Y) protein. The current study was conducted to evaluate the efficacy of HP50Y and HP40Y as a replacement for corn protein concentrate (CPC) in diets of pacific white shrimps, *Litopenaeus vannamei*. In the growth trial, graded levels of HP50Y (0.0, 5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (13.1, 10.0, 6.6, 3.5 and 0.2%) In the second series of diet, graded levels of HP40Y (5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (10.5, 8.0, 5.5 and 2.5%) which was evaluated over a 40 days growth trial (initial weight 0.54 ± 0.01 g; n=4). At the conclusion, no significant differences were detected in growth, FCR, survival, food consumption and net protein retention of shrimp (P-value>0.05). However, results from regression analysis revealed that there was a significant increase (p-value=0.04; r^2= 0.20) (p-value=0.02; r^2= 0.25) in weight gain percentage of shrimp as the percentage inclusion level of HP50Y and HP40Y have increased in the diets of shrimp. Regarding net protein retention (NPR) there was no significant differences (P-value>0.05) among all treatments but from the linear regression analysis it came to know that NPR was going to be increase and there was a significant increase as the inclusion levels of HP50Y (p-value= 0.05; r^2= 0.19) and HP40Y (p-value= 0.03; r^2= 0.23) increased in the diets. The proximate whole-body composition of shrimp was indicated that there were no significant (P>0.05) differences between moisture, protein, fat, and ash of shrimp body. In summary, it was indicated that HP50Y and HP40Y both are good protein source and can be used up to 20% inclusion level in the diets of shrimp. In addition, the HP40Y and HP50Y also contains an elevated level of yeast, stimulating growth and could possibly enhance immune response of shrimp. Further studies should contemplate the supplementation of HP40Y and HP50Y as an effective protein source for the diets of carnivorous aquaculture fish species, for instance trout and other salmonids.

CREATING WORKFORCE PATHWAYS BY EDUCATING YOUTH THROUGH AQUACULTURE

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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.

In an effort to increase awareness for the next generation of seafood consumers and create defined pathways into the aquaculture workforce many extension educators and academics are creating K-12 aquaculture programs and working closely with K-12 schools to help integrate aquaculture curriculums into classrooms. A model initiated in Michigan that consists of three core initiatives geared toward creating a new aquaculture work force and an educated consumer base has arisen. The core tenants of this program include 1) K-12 Teacher trainings and assistance with teacher networking 2) K-12 curriculum development and a high school aquaculture competition and 3) Post-secondary programs identified and/or created.

The efforts that have been taking place in Michigan and the Midwest are centered around the Youth Education in Aquaculture Initiative (http://ncrac-yea.org/), an effort funded through a variety of funding partners including the North Central Regional Aquaculture Center, Michigan Sea Grant and the Center for Great Lakes Literacy. This program was started by Lake Superior State University Professor Dr. Barbara Evans, and is now co run by Dr. Evans and Elliot Nelson with Michigan Sea Grant. Through this effort a network of schools is being created to allow for cross school collaboration. In addition teacher trainings and curriculum resources are being compiled and offered. The keystone of the program is the Aquaculture Challenge, a competitive high school competition aimed towards engaging high schools in an integrative STEM and business competition. In addition new post-secondary programs have been created and are now seeing graduates who come through the high school programs and enter into post-secondary programs. This session will focus on these efforts and how they are leading to a clear and defined pathways into the aquaculture workforce.

Youth Education
in Aquaculture

ENGINEERING AND OPERATING LIFE SUPPORT SYSTEM APPLICATIONS FOR INTENSIVE AQUACULTURE

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Intensive aquaculture systems are generally regarded as controlled artificial aquatic environments (raceways, tanks, ponds) that attempt to maximize production of an aquatic organism with the use of flowing water to deliver the required amount of dissolved oxygen while also removing metabolic waste products. While the level of aquaculture intensity can vary significantly depending on such factors as species and facility type, water quality/chemistry, as well as health and physiological condition of the cultured organism, intensive aquaculture systems all share a common need for basic life support systems to mitigate for periods of operational "challenges" from one or more sources (water supply, power supply, mechanical failure, human error). Engineered life support systems can vary from simple to complex and are often tied to a specific life history stage or phase of the production cycle. Engineered solutions commonly address critical facility functions including power supply, water movement, gas control, temperature control, as well the proper design, maintenance and use of system controls, warning, and alarm systems. In addition to critical engineering life support systems, facility staffing, scheduling and operations can play a major role in the success (or failure) of an intensive aquaculture program. Common operational practices and procedures that can be applied in multiple intensive aquaculture systems are considered for the benefit of all aquaculture operators.

EVALUATING *Schizochytrium* sp. AS AN ALTERNATIVE LIPID SOURCE IN FISH-FREE FEEDS FOR SABLEFISH *Anoplopoma fimbria*

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Complete replacement of fish meal (FM) and fish oil (FO) in the diets of carnivorous farmed fish will likely be required to meet the projected growth of the aquaculture industry in a sustainable way. A number of viable ingredients have been found to replace FM, while suitable alternatives to FO have been more challenging to identify due to the essential long-chain omega-3 fatty acids that are missing from terrestrial alternatives. *Schizochytrium* sp. is a microalga that is high in docosahexaenoic acid (DHA), one of the aforementioned omega-3 fatty acids that are required by fish and important for human nutrition. This study was conducted to determine the effects of microalga-enriched fish-free feeds on sablefish growth, condition, and fatty acid profile.

A 20-week feed study was conducted in an outdoor tank array in Moss Landing, CA. Juvenile sablefish were distributed randomly into 18 tanks (1000L) with 15 fish per tank (average weight ± SD, 333g ± 2.4g). Six diets were formulated to contain 45% protein and 15% lipid. Diet 1 contained standard levels of FM and FO. Diet 2 contained FO, but no FM. Diet 3 was completely FM and FO free, made with terrestrial ingredients only. Diets 4, 5, and 6 were FM and FO free, and contained increasing levels of the microalga, *Schizochytrium* sp. (dried whole cell biomass), with flax oil making up the remaining lipid requirement. Treatments were assigned randomly to the tanks with three replicates per treatment.

Preliminary results suggest that sablefish growth increases with increasing inclusion of *Schizochytrium* sp. and is not significantly different in performance to the control diet containing standard FM/FO concentration. Fatty acid profiles of the fish fillets indicate that sablefish are able to incorporate DHA from the dried whole-cell *Schizochytrium* sp. into their muscle tissue commensurate with DHA inclusion in the diets.

GROWTH PERFORMANCE, SURVIVAL, BLOOD CHEMISTRY, AND GENE EXPRESSION OF CHANNEL CATFISH *Ictalurus punctatus* FED PROBIOTIC-SUPPLEMENTED DIETS

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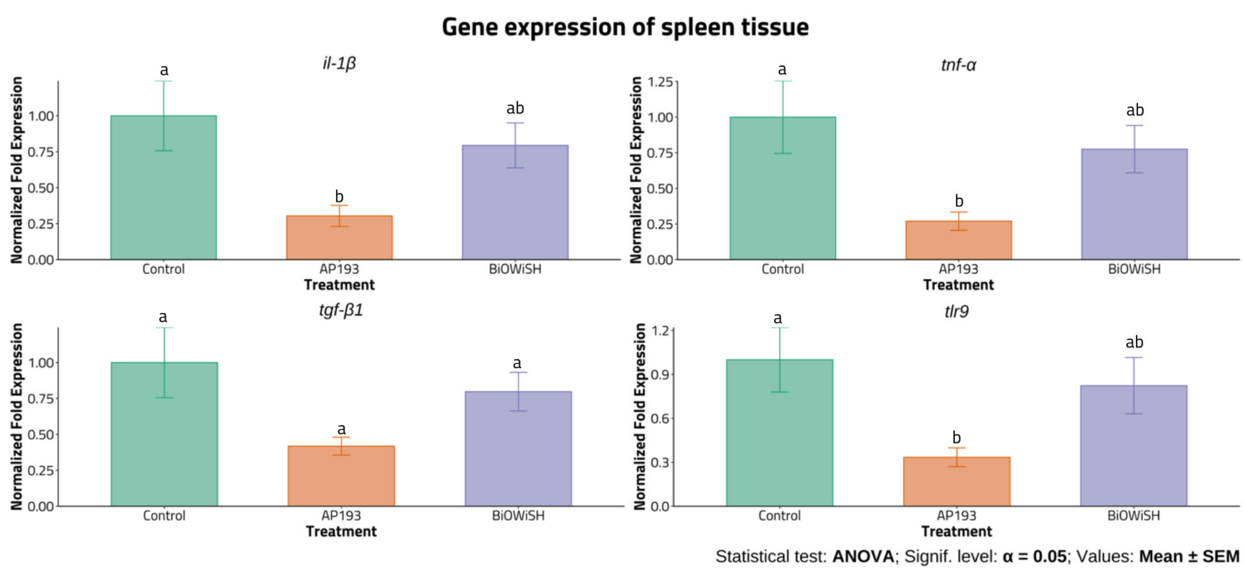
The channel catfish (*Ictalurus punctatus*) farming industry is the largest and one of the oldest aquaculture industries in the US. Despite being an established industry, production issues with disease outbreaks remain problematic for producers. Supplementing probiotics to enhance the immune system and growth potential is one of the approaches for mitigation. Although considerable laboratory data demonstrate efficacy, these results do not always translate within production-scale environments and involvement with more natural modes of disease transmission. Hence, the present work was conducted in the laboratory but incorporated flow-through water from the effluent of large catfish pond production systems. Two feeding trials were conducted in a series of aquaria system housing juvenile (34.8 ± 12.5 g) and fingerling (0.36 ± 0.03 g) channel catfish. Catfish in the first trial were fed with three experimental diets over eight weeks, using six replicate tanks. Commercial diets were top-coated with two selected probiotics, AP193 and BiOWiSH, at a concentration of 0.025g/kg and 0.5 g/kg of feed, respectively, and a control group (no additive) was included in the study. In the second trial, diets were top-coated with BiOWiSH at concentrations of 0, 0.25, 0.5, 1 g/kg. At the completion of these studies, growth performance, survival, hematocrit, blood chemistry, probiotic concentrations within the distal intestine, and immune expression of interleukin 1β (*il-1β*), tumor necrosis factor-alpha (*tnf*-a), Interleukin-8 (*il-8*), transforming-growth factor β1 (*tgf-β1*), and toll-like receptor 9 (*tlr9*) were evaluated using qPCR. Trial results revealed no differences (*p>0.05)* among treatments with respect to growth, survival, hematological parameters for both trials. With respect to gene expression, interesting trends were noted in the probiotic-fed fish with general down regulation, however, the results were not statistically significant differences except for *il-1β*, *tnf*-a, and *tlr9* for the first trial. The second trial is still under investigation for gene expression and the quantification of the probiotic within the posterior intestine. Based on these findings, more research utilizing probiotics under natural infection condition is warranted. This would provide additional insight for practical applications.



FINDING COMMON GROUND AMONG U.S. SHELLFISH PRODUCERS: A YEAR ON THE ROAD WITH THE SHELLFISH GROWERS CLIMATE COALITION

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Last year, with support from The Nature Conservancy, I hit the road in a converted cargo van and drove the entire coastline of the continental United States to recruit members for the Shellfish Growers Climate Coalition (SGCC). The SGCC is a national group of shellfish businesses united to advocate for federal climate policies that will secure a future for shellfish and the livelihoods and communities that depend on them. Through ethnographic methods like participant observation, interviews, and focus groups I built rapport in communities across the country, grew Coalition membership by over 70%, and learned about the challenges and opportunities of growing shellfish amidst a changing climate.

The shellfish industry in the U.S. is a diverse collection of individuals, communities, and organizations that span every imaginable cultural, geographic, and socioeconomic spectrum. The range of climate impacts on the industry is similarly immense- from changes in ocean chemistry and temperature in the Pacific Northwest to storm activity in the Southeast. Throughout my trip, however, significant patterns emerged out of apparent dissociation, which offer the industry a constructive way forward in the face of a largely distressing atmospheric outlook.

Despite having a variety of histories, personalities, and politics, shellfishers are bonded by a common value and a shared experience- dependence on shellfish resources and sea level rise. These facts provide a common experience upon which to build unconventional relationships and new alliances, allowing the shellfish industry to more effectively organize and advocate for itself in the face of an existential threat.

MONITORING TECHNOLOGIES FOR HABS IN OFFSHORE AQUACULTURE: A REVIEW

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As development of offshore aquaculture projects increases, daily monitoring of net pens becomes increasingly difficult and the need for remotely sensed monitoring tools becomes more of a priority. Increased dissolved nutrients resulting from offshore aquaculture pens could have an impact on phytoplankton composition and the potential to trigger the onset of harmful algal blooms (HABs). The impact of these blooms on the aquaculture industry amounts to approximately $8 billion/year globally due to mass mortalities in finfish, shellfish harvesting bans due to accumulation of phycotoxins, and human health costs. Currently, there are multiple commercial, academic, and government agencies working on remote sensing technologies to monitor offshore aquaculture facilities. This technology-based discussion aims to review current remote sensing technologies specifically used to monitor HABs and their applications to the offshore aquaculture industry. This review highlights current remote sensing HAB monitoring technologies along with their successes, limitations, knowledge gaps, and research needs. These technologies will be essential tools for early detection, tracking, and forecasting of HABs to improve fish health and operational efficiency of offshore aquaculture facilities.

DIETARY SUPPLEMENTATION OF XYLANASE (ECONASE XT) IMPROVES THE PLANT INGREDIENTS UTILIZATION IN NILE TILAPIA (*Oreochromis niloticus*)

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Currently, plant ingredients such as soybean meal and canola meal are commonly used feed ingredients in aqua feed industry. These ingredients contain high amount of anti-nutritional factors (ANFs) such as non-starch polysaccharide (NSP) and phytic acid. ANFs have significant effects on fish gut morphology, physiology, and mucus layer which influence the endogenous secretion of water, proteins, electrolytes, and lipids leads to reduce the nutrient and energy digestibility. Exogenous supplementation of enzymes can be used to overcome the problems, resulting in improved rate of digestion and nutrient absorption of plant protein in aquatic animals. Exogenous xylanase has the property of disrupting the plant cell wall integrity, thereby reducing the molecular size characteristics of NSPs. Consequently, this enhances rapid digestion by reducing the viscosity in the gut. Hence, the objective of this study was to evaluate the effects of dose dependent of xylanase supplementation on growth, apparent digestibility, and digestive enzymes activities in Nile tilapia diet.

Plant based four diets (isonitrogenous 35% crude protein and isolipidic 8% lipid) *viz* control diet and three different levels (16000, 32000 and 6400 BXU/kg) of three xylanase (Econase XT) supplemented in control diet. Diets fed in triplicate to tilapia juveniles (1452 fish, 126 ± 0.04 g initial weight) for 11 weeks. Fish were randomly distributed into 12 400-L tanks in recirculatory aquaculture system.

At the end of the feeding trial, supplementation of xylanase had significant effect of growth performance and feed utilization (Figure 1). Highest growth performance and protein efficiency ratio were observed in a group fed with diet 2 (16,000 BXU/kg) which was significantly similar to group 3 (32000 BXU/kg) whereas feed conversion ratio exhibited opposite trend. Hepatic histological changes were affected by dietary treatment. Data for nutrient retention and digestibility coefficient of nutrient and energy will be presented. Conclusively, dietary supplementation of xylanase (Econase XT) in the range of 16,000 – 32,000 BXU per kg is recommended for improving the plant protein utilization in Nile tilapia for sustainable aquaculture production.

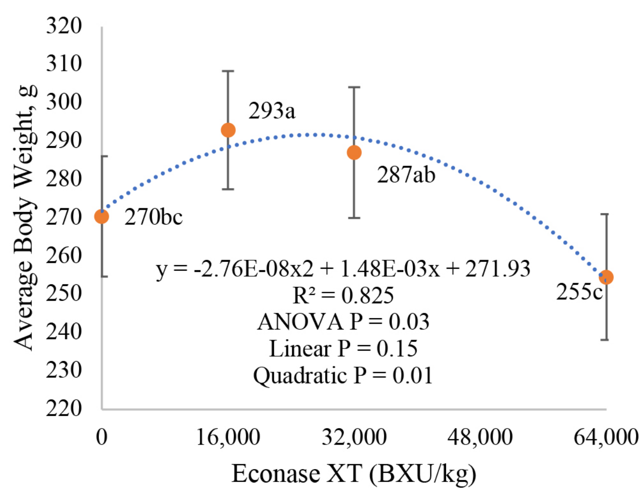

Figure 1: Effects of dietary xylanase on growth

FORMATION OF THE KENTUCKY STATE UNIVERSITY UNITED STATES AQUACULTURE SOCIETY STUDENT SUB-UNIT AND ACTIVITIES AMIDST THE COVID-19 PANDEMIC

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Pre-Pandemic Activities, Fall 2019. Kentucky State University (KSU) graduate students (under the leadership of David Fox (2021 graduate) and Faculty Advisor, Dr. Noel Novelo prepared application documents and held in-person meetings to establish the KSU United States Aquaculture Society (USAS) Student Sub-Unit. *Pandemic Beginnings.* The KSU USAS Sub-Unit was officially established amidst the growing pandemic. The USAS Board accepted KSU's application for formation of the USAS Student Sub-Unit in February 2020, and the Sub-Unit became an official Kentucky State University student organization in April 2021. *Activities.* Three Sub-Unit Presidents (coauthors) and executive leadership officials have been elected to serve since then. Student members held on-line video conference meetings during 2020, and a combination of on-line and in-person meetings in 2021 to coordinate and plan educational, social and community service activities.

Despite Covid-19 restrictions, KSU graduate students were involved in community service and local aquaculture in Scott County, Oldham County, and Franklin County in Kentucky. Highlights include: clean-up of pond and recreational areas at Yuko-En on the Elkhorn, a Kentucky-Japan Friendship Garden (Georgetown, KY); removal of thick filamentous algae and harvest of 1500 Large Mouth Bass at Crystal Bridge Fish Farm (Crestwood, KY); Oral and Poster presentations at Aquaculture America 2021; harvest of Fresh Water Prawns, and social activities at the KSU Aquaculture Research Center (Frankfort, KY), and design of the KSU USAS Student Sub-Unit's Logo (Figure 1) and T-shirts – some of which will be donated for the USAS silent auction at Aquaculture 2022 Conference. Future activities include increase of local Aquaculture farm visits, high school outreach and tours, liaison with other USAS Student Sub-Units to achieve common goals, and providing additional leadership, educational and networking opportunities as stated in Section II of the KSU USAS Student Sub-Unit By-Laws.

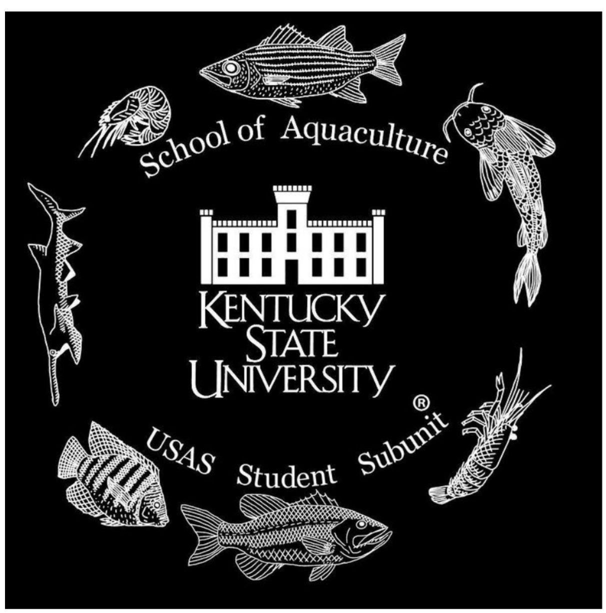

Figure 1. T-Shirt Logo Design.

NOVEL GENOMIC MARKERS ASSOCIATED WITH PHARMACOLOGICAL RESISTANCE IN THE SEA LICE IDENTIFIED THROUGH WHOLE-GENOME RESEQUENCING

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The sea louse *Caligus rogercresseyi* is a marine ectoparasite copepod species that impacts the salmon production in Chile, causing losses of more than 400 million dollars per year. This pathogen is mainly controlled by immersion baths with delousing drugs, which are directly applied in salmon cages covered by skirts or tarpaulins. The emergence of resistance has been suggested as the main cause of low efficacy of delousing drugs treatments against sea lice infection. In other arthropods, pharmacological resistance has been associated with duplicated genes at the genome of resistant individuals causing an amplification of the expression levels of critical genes related to the response to drugs, such as those involved in drug detoxification and excretion outside the cells. Pharmacogenomics approaches are now possible to conduct in *C. rogercresseyi*, having the draft of the full genome as a reference to infer these duplications as copy number variants (CNVs) in different resistant or susceptible strains. This study aimed to evaluate the presence of gene duplications, or gene-clusters duplications, related to genes with functions in delousing drug response, and its association with resistant phenotypes to azamethiphos in *C. rogercresseyi*.

The full genome of *C. rogercresseyi* was used as a reference to conduct whole-genome resequencing for known sea louse strains with divergent resistance to azamethiphos drug. Then, gene-clusters duplications in the novel specific whole-genome sequences for resistant and susceptible strains were identified and associated with resistant sea lice. Copy number variants (CNVs) in detoxication genes, such as *trypsins* were identified with differential p-value among resistant and susceptible strains (Table 1). Duplicated regions also implied expression changes in these strains (Fig. 1). The potential impact of this study for salmon aquaculture is the definition of novel resistant traits in families or populations of sea lice, and the identification of novel molecular markers based on CNVs, supporting the creation of monitoring programs for *C. rogercresseyi* resistance to delousing drugs. Funding: ANID-Chile through the grant FONDECYT (#11200813 and # 1210852), and FONDAP (#15110027).

Table 1. CNVs in *trypsin* genes found in *C. rogercresseyi*

Chromosome	Consequence	Fold change	p-value
Chr9	Loss	-91,48	2,2E-308
Chr12	Loss	-1,91	0,0085
Chr12	Gain	1,80	0,0189
Chr14	Loss	-2,14	0,0011
Chr17	Loss	-1,49	0,0002
Chr19	Loss	-1,81	0,0061

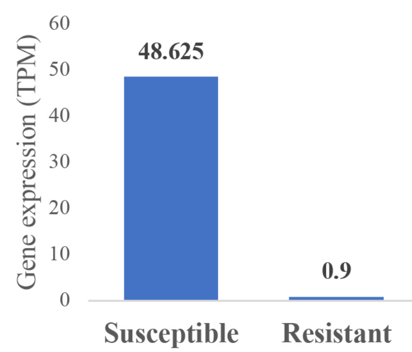

Figure 1. Gene expression of *trypsin* in sea lice strains

THE PATH TOWARDS A NATIONAL AQUACULTURE ECONOMIC DEVELOPMENT PLAN

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The Subcommittee on Aquaculture (SCA), previously known as the Interagency Working Group on Aquaculture (IWGA) and the Joint Subcommittee on Aquaculture (JSA), is a statutory subcommittee that operates under the Committee on Environment of the National Science and Technology Council (NSTC) under the Office of Science and Technology Policy in the Executive Office of the President. The SCA serves as the federal interagency coordinating group to increase the overall effectiveness and productivity of Federal aquaculture research, regulation, technology transfer, and assistance programs.

The Economic Development task force is one of three SCA task forces (Regulatory Efficiency and Science Planning) tasked with developing a strategic plan on its thematic subject matter. This task force is the third component of what will ultimately form a National Aquaculture Development Plan.

Over the past year, a group of technical experts across several federal government agencies sought stakeholder input numerous times to develop a draft outline for the Strategic Plan for Aquaculture Economic Development. The overarching vision is to support a robust, resilient, and environmentally sustainable domestic marine aquaculture sector. This Plan seeks to support the viability and expansion of existing operations, as well as encourage new entrants. The plan will help maximize the effectiveness of existing federal policies and programs while strengthening the public-private partnerships with our stakeholders.

This proposed session will provide an overview of the plan and public input received to date, as well as next steps for the Economic Development task force.

MARKETING U.S. FARM-RAISED SEAFOOD TO THE FOODSERVICE COMMUNITY

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With funding from the National Sea Grant Program, New York Sea Grant and the National Aquaculture Association (NAA) partnered to develop positive attitudes and perceptions about farm-raised seafood products in the foodservice community. To maximize the impact of those efforts, an effective and cost-efficient coalition of government, academia and industry, all focused on a common message is necessary. Extension educators play a pivotal role by sharing these resources with industry partners and others who can use these tools to grow market share.

Prior to the Pandemic, approximately two-thirds of all U.S. seafood consumption took place in restaurants and other foodservice establishments. Studies have shown that lack of knowledge and comfort with seafood products was a serious limitation to home use. As a result of lock-downs, restaurant closures, concerns about a healthy diet, and additional free time; more home cooks started sharpening their seafood skills. They experimented with new recipes and less-familiar species. But they also had lots of questions and concerns about farmed seafood. .

To help answer questions about U.S. farm-raised seafood, a set of talking points with on-line references has been developed. These materials were designed to help culinary and extension educators answer questions outside of their area of expertise.

Luring seafood diners back to foodservice will require that chefs focus on a wider range of species, more exciting presentations and enhanced plating techniques. To provide Post COVID strategies, a major article was published in *The National Journal of Foodservice Education*.

The project team has provided programs at a number of national conferences and trade shows, met with food writers individually and provided contacts and background information. A set of point of purchase materials that feature a QR code linking the product to a specific grower's website have been developed. Templates are available.

Food writers want exciting stories that follow production from the grower to the chef who ultimately uses the product. To tell that story, a collection of photos, videos, anecdotes, and recipes has been incorporated onto a flash drive for distribution to writers, culinary educators, and others who can tell our story to a larger audience. Multiple copies of the flash drive are available to Extension Agents. Routinely, writers ask for information about U.S. products. To take full advantage of those inquiries and develop story lines, we need additional recipes and chef contacts especially those that focus on a specific farm and its products.

At the suggestion of industry, an industry speakers' bureau is being developed to assist producers in taking advantage of opportunities to talk directly with chefs, restaurateurs, and others in the foodservice industry. Many groups such as local chapters of the American Culinary Federation regularly host dinner meetings and welcome guest speakers.

HOW THE PANDEMIC CHANGED THE U.S. MARKET FOR FARM-RAISED SEAFOOD

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Although the Pandemic seriously impacted the seafood industry, it also created opportunities for farm-raised seafood.

A More Seafood Savvy Consumer: Prior to the pandemic, most Americans viewed seafood as a luxurious dinner-out treat. In the U.S., approximately two-thirds of all seafood consumption took place away from the home in restaurants and other foodservice establishments. As a result of lock-downs, restaurant closures, concerns about a healthy diet, and additional free time; more home cooks started sharpening their seafood skills. They experimented with new recipes and less-familiar species. They also began sharing their new-found culinary skills with others on social media. Increasing familiarity with seafood preparation will alter consumer expectations and demands in both the retail and foodservice sectors.

A More Environmentally Conscious Consumer: Consumers have been bombarded with messages about climate change and dire warnings about availability of food. These ideas are resonating and attitudes toward farmed seafood are changing. In a recent survey of culinary educators, 88% percent felt that aquaculture was more sustainable in terms of land and water use than terrestrial animal production.

A Changing Supply Chain: It is estimated that between 85 and 90% of all the seafood consumed in the U.S. is imported. The loss in international trade as a result of border closures, changes in tariff rates, and disruptions to transportation meant a greater dependency on domestic production. This gap between supply and demand widened and prices rose. These economic disruptions forced seafood producers to restructure their standard business models and connect more directly with consumers and retail markets. The growing connection between producer and consumer can help to develop a positive awareness of aquaculture that builds on the widely accepted farm to table concept. These new market channels can be expected to grow as we move out of the Pandemic.

A More Competitive Price Structure: With limited supplies and the rising cost of other center of plate proteins, farm raised seafood is becoming more price-competitive. It is not subject to the drastic price swings experienced in wild harvest seafood. The question is which farmed species can be used in place of higher price ticket wild species and how best to position those products on the menu.

The challenge for growers is to respond to the needs of foodservice providers and consumers.

REGULATORY RENTS IN NORWEGIAN SALMON AQUACULTURE

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Command-and-control regulations such as licensed entry and production quotas can lead to regulatory rents. This can lead to production distortions, undesirable wealth redistribution, wasteful rent seeking behavior, and can alter incentives in ways that might compromise the objective of the regulation (Smith 2012; Squires 2016; Parry 2004)

We study rent formation and production distortions under maximum production regulations in Norwegian salmon aquaculture. In Norway, problems with sea lice (Lepeophtheirus salmonis) has led to a stop in the issuance of new production licenses. Consequently, Norwegian production is currently at maximum capacity. At the same time, demand for salmon has been strong, with record prices.

To analyze rent formation and production distortions we develop a general multiple-rotation optimal harvest and stocking model. The model is placed in two regulatory regimes: with and without a maximum production constraint on license production. With a maximum production constraint rents can be supported on the margin when licensing (entry) is fixed. This rent will mimic a resource rent creating potential confusion on the source of the rent in the industry. Furthermore, the regulatory rent incentivizes more intensive farming behavior with increased stocking of fish, a shortening of the production cycle and smaller harvested fish.

We parametrize the model and compare model predications to observed developments in the Norwegian industry. The behavior predicted by optimizing behavior under a maximum production regulation maps closely to actual industry developments since 2012. We endogenize the price of salmon and derive a counterfactual zero rent price to show how recent profitability developments can be explained by strong demand growth in the constrained production regime. Comparing the regulatory regime to the zero-rent unregulated counterfactual suggests large rent transfers to license owners. The environmentally motivated regulations has largely subsidized producers.

Understanding regulatory rents in aquaculture is important for the social license of the industry. Environmental regulations that leads to wealth transfers to the industry can threated the social license of the industry. An extractive rent tax can be justified on the basis that regulatory rents should accrue to the state. However, it should be noted that regulatory rents are not equivalent to resource rents, such as naturally emerge in fisheries, petroleum and hydro sectors. Regulatory rents are temporary, and tax regimes need to be flexible enough to account for this so as to not damage the future competitiveness of the industry. Regulatory rents are vulnerable to capture by competing markets, consumer substitution and industry cost escalations.

References:

Parry, Ian W. H. 2004. “Are emissions permits regressive?” *Journal of Environmental Economics and management* 47 (2): 364-387.
Smith, Martin D. 2012. “The New Fisheries Economics: Incentives Across Many Margins.” *Annu. Rev. Resour. Econ*. 4 (1): 379-402.
Squires, Dale. 2016. “Firm behavior under quantity controls: The theory of virtual quantities.” *Journal of Environmental Economics and Management* 79: 70-86.

BIOMARKER RESPONSES IN THE MUSCLE OF *Chrysichthys nigrodigitatus* CAUGHT FROM TRACE METALS AND PAHS POLLUTED RIVERS IN LAGOS STATE, SOUTHWEST NIGERIA

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The biological and chemical responses, such as the enzymatic activities of isocitrate dehydrogenase (IDH), acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) in the muscle of *Chrysichthys nigrodigitatus* collected from three trans-urban waterbodies of the Lagos lagoon system: Odo Iya-Alaro, Ibeshe and Agbowa-Ikosi rivers were studied. The sampling sites showed different types and degrees of pollution. The results demonstrated significant differences in muscle activities of IDH and AchE in the Odo Iya Alaro, Ibeshe and Agbowa-Ikosi rivers compared to control fish. LDH activity did not show any difference between sampling sites. Significant correlations were established between some biomarkers and trace metals: AChE was correlated with Pb, Cd and Cu concentrations in water; IDH activity was correlated with Cd and Cu concentrations in water and As, Pb and Cd concentrations in sediments; LDH activity was correlated with As and Zn concentration in water and Cd concentration in sediment. Only one correlation was established between the biomarkers analyzed and the concentrations of PAHs: benzo(b)fluoranthene concentration in sediment and IDH.

MULTISTRESSOR INTERACTIONS IN *Clarias gariepinus*: CONCURRENT PHENANTHRENE EXPOSURE AND *Mycobacterium marinum* INFECTION

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The simultaneous exposure of organisms to toxicants and disease causing agents poses a serious risk to fish stocks. Worldwide, aquatic animal disease outbreaks have been increasing in both frequency and severity, and many have been associated with anthropogenic environmental change. Little is known about the complex interactions of the immune system and biotransformational pathways of vertebrates; however, urbanization and coastal development create a scenario in which a wide range of species are exposed to chemical pollutants in conjunction with a wide spectrum of ubiquitous, opportunistic pathogens. These interactions can severely compromise organismal health. Potential effects include decreased fitness, increased predation, decreased fecundity, reduced metabolic activity, suppressed immune function and mortality. Recent attention has been paid to immunomodulation in toxicant exposed fishes. In this study we investigated the effects of the common polycyclic aromatic hydrocarbon phenanthrene in conjunction with *Mycobacterium marinum* infection in *Clarias gariepinus*. The goal of our study was to elucidate the interactions between stressors in the host organism. Fish were exposed to either a high or low dose of phenanthrene, infected with *M. marinum* or received a combination exposure of toxicant and bacteria. Results of our study were evaluated using survivorship analysis, toxicant body burden, and histology. Our data show an interaction between *M. marinum* infection and exposure to a high dose of phenanthrene in *Clarias gariepinus*. Survivorship was significantly reduced for fish only exposed to the high dose of phenanthrene as compared to all other experimental groups. The increased survivorship for fish exposed to both Mycobacterium and a high dose of phenanthrene suggests an antagonistic interaction between stressors. Body burden data, which show significant differences in the ratio of phenanthrene:metabolites between experimental groups, suggests a disruption of the biotransformational pathway. We postulate that the inflammatory response, initiated by bacterial infection, is impeding the ability of *Clarias gariepinus* to completely metabolize phenanthrene. In addition, the correlation between reduced metabolite production and increased survival indicates that phenanthrene metabolites are more toxic than the parent compound. Our study underscores the importance of investigating multiple stressor interactions as a way to better understand complex environmental interactions.

MOLECULAR PROFILING, GROWTH PERFORMANCE AND SELECTION TRAITS FOR DIFFERENT STRAINS OF C*larias gariepinus* BURCHELL IN MAJOR NIGERIAN FRESHWATER BODIES

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The African mud catfish (*Clarias gariepinus*) is well known for its nutritional and economic importance and it's the most important cultured fish species in Nigeria. Its sustainable production is being threatened by suspected inbreeding depression occasioned by the use and reuse of imported frys and fingerlings from exotic homogenous stocks. Morphological, genetic, and molecular studies were carried out to determine the residual genetic variability and assess the possibility of improving the fish quality through selective breeding of local strains. Three hundred and ninety two samples were collected with the aid of gill nets and analysed using multivariate tools of principal component analysis and Discriminate function analysis (DFA) for morphometric studies; Growth performance was examined by heterosis performance on Specific growth rate(SGR), Hatchability, survival and mean growth over a period of 26 weeks through reciprocal crossings of different strains, while the molecular analysis examined the selection traits through protein profiling using Sodium deodosulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, and genetic differentiation using random amplified polymorphic DNA(RAPD) assay and microsatellite analysis. Morphological comparisons revealed morphometric homogeneity between *C. gariepinus* fish population in River Benue and River Niger and their tributaries which clustered into five considerably distinct populations. The growth performance analysis shows significant difference in mean weight gain, specific growth rates and survival rate ($P<0.05$). Sokoto sourced strains (SKT) gave the best growth of 595.00±4.33g, closely followed by its reciprocal hybrid with South African strains (SA ♀ X SKT ♂) 554.00±6.22g, while the New Bussa strain had the lowest growth value (70.00±2.07g) at the grow-out level. The cross between the Dutch and Yola strains gave the highest positive mean heterosis of 28.54% ($P<0.05$). The data obtained from polypeptides of *C. gariepinus* revealed Sokoto (MW = 100kDa); Yola (MW = 40kDa, MW = 30kDa); Dutch (MW= 15kDa); SKT and YLA hybrid ((MW = 30kDa); Dutch and Yola (MW = 15kDa) as identifying negative marker bands. The DNA-RAPD analysis recorded an overall low polymorphism among and within the local and exotic populations of *C. gariepinus* in Nigerian freshwater bodies and their reciprocals (19.35%). However, considerably higher levels of polymorphism were detected between the Lokoja Ganija strains (29.67%) and Yola strains (12.09%). The nine microsatellite loci used to screen the wild *C. gariepinus* strains and their crosses germplasm revealed polymorphic information content (PIC) values that ranged from 0.077±0.231 to 0.395±0.399 with the local strains showing slightly higher allelic diversity than the three exotic strains. The among population heterozygosity deficit (F_{ST}) ranged from 0.0672 to 0.3171 with an average value of 0.1448. Estimation of effective population size (Ne) revealed that the Ne of the exotic strains was smaller than that of the local strains in Nigerian freshwater bodies. The low similarity coefficient (SC) value obtained in the phylogenetic dendograms indicates a high divergent of these strains from different water bodies.

VOLITIONAL SPAWNING PATTERNS OF THREE SPECIES OF CAPTIVELY HELD BUTTERFLYFISHES *Chaetodon ocellatus*, *Chaetodon striatus* and *Chelmon rostratus*

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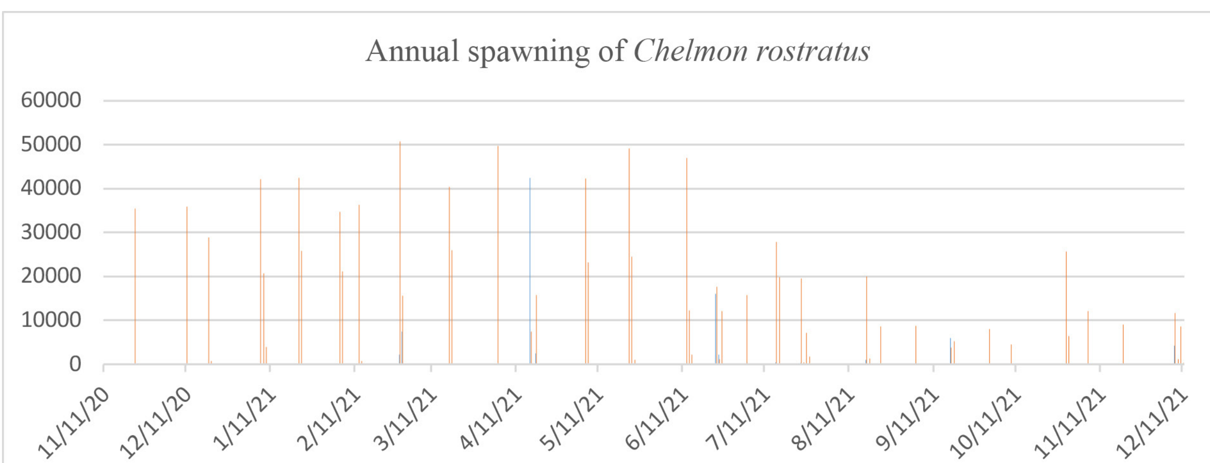
Established monogamous pairs of spotfish butterflyfish *Chaetodon ocellatus,* banded butterflyfish *Chaetodon striatus*, and copperbanded butterflyfish *Chelmon rostratus* were held in 1000 L covered fiberglass tanks each with a separate recirculating aquaculture system consisting of a trickle filter, bag filter, and UV sterilizer. Consistent lighting and temperature control were provided. Fish were fed diets consisting of frozen mysis shrimp, shaved squid, chopped krill, and Larry's Fertility Frenzy. Each system was equipped with an upwelling egg collector designed to remove both floating and sinking eggs. Daily for up to three years, spawned eggs were collected, volumetrically quantified, and percent fertilization was determined and recorded.

Chaetodon ocellatus spawned 203 times in 805 days with a total of 2,298,864 unfertilized and 80 fertilized eggs for a mean of 11,325 eggs/spawn. On 71 days when there was a spawn, a spawn occurred the next day. *Chaetodon striatus* spawned 172 times in 795 days with a total of 1,049,193 unfertilized eggs and 433 fertilized eggs for a mean of 6,100 eggs/spawn. On 26 days when there was a spawn, a spawn occurred the next day. *Chelmon rostratus* spawned 126 times in 851 days with a total of 1,714,357 unfertilized and 86,171 fertilized eggs. There were 19 spawns with fertilized eggs with a mean of 4,535 fertilized eggs per spawn. There were 126 spawns with unfertilized eggs with a mean of 13,606 unfertilized eggs. On 56 days when there was a spawn, a spawn occurred the next day, and on 10 more days a spawn occurred two days later. Further research is justified to identify causes of unfertilized eggs. This is the first reported spawning data for these three species of butterflyfishes held in captivity.



DEVELOPMENT OF AN ANTI-BURBOT IGM MONOCLONAL ANTIBODY AND OPTIMIZATION OF AN ELISA TO MEASURE ANTI-AEROMONAS SP. ANTIBODY TITERS IN BURBOT *Lota lota* FOLLOWING PATHOGEN CHALLENGE

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Burbot is the only freshwater member of the cod-like fish (Gadiformes) and are an ideal candidate for cool/cold-water aquaculture. Burbot are susceptible to disease outbreaks, and may become asymptomatic carriers, however they are refractory or even show limited mortality to most salmonid pathogens. However, larval and juvenile burbot have been shown to be susceptible to Aeromonas species, with mortality from ranging from 10 to 20% following laboratory challenges. Outbreaks of a species of Aeromonas most closely related to *A. veronii* have been observed to cause mortality up to 92% in juveniles. Development and optimization of a serological method using monoclonal antibodies to survey juvenile burbot for Aeromonas species, and aid in the generation of a vaccine, is paramount to the success of burbot aquaculture.

IgM proteins were isolated from burbot serum via an agarose affinity column containing immobilized mannan binding protein. The purified burbot IgM was injected into three mice for the generation of antibodies demonstrating specific affinity to burbot IgM, determined via ELISA (enzyme linked immunosorbent assay). Mice possessing antibodies for burbot IgM were culled and the spleens harvested to generate hybridoma cell lines. Using an ELISA the hybridoma cell lines were screened against the heavy and light chains of burbot IgM to identify a candidate for an anti-burbot IgM monoclonal antibody. Four hybridoma cell lines yielded specificity to burbot IgM heavy and or light chain. Three cell lines had affinity for the heavy chain, and two showed affinity to the light chain. The cell line showing the highest absorbance for burbot heavy chain is currently being used to develop and optimize an ELISA to measure immune response of burbot to Aeromonas infection. This optimized ELISA will serve as a primary tool to aid in the development of a vaccine to prevent Aeromonas species infections in burbot aquaculture.

FATTY ACIDS AS POSTPRANDIAL REGULATORS OF FEEDING IN RAINBOW TROUT *Oncorhynchus mykiss*

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Historically, the two most expensive components of salmonid aquaculture feeds have been fish meal and fish oil as the primary protein and lipid sources. As the feed industry moves toward the use of less expensive, alternative protein and lipid sources, the fatty acid profile of feeds can change significantly. Rainbow trout (*Onchorynchus mykiss*) are capable of fatty acid sensing and juveniles have been suggested to differentiate diets and consume more feed in the presence of docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). However, much remains unknown regarding the mechanisms behind feed consumption. The objective of this project was to better understand the effects of fatty acid signaling postprandially relative to feed consumption. This knowledge will be crucial toward maximizing the sustainability of aquaculture feeds by more effectively balancing lipid sources to increase fatty acids that stimulate feed intake, while reducing those that suppress feed intake.

The effects on feed consumption of six individual, purified fatty acids were observed. Oleic acid (OA), palmitic acid (PA), alpha-linolenic acid (ALA), linoleic acid (LA), EPA, and DHA were each individually gavaged into juvenile rainbow trout; a sham treatment for each fatty acid was also administered. After fish were gavaged, 30 minutes was allotted for fish to recover and generate a response to the treatment or sham. Then all fish were fed to satiation and sampled at 30 minutes and 60 minutes postprandial. At both timepoints, the hypothalamus was collected to measure gene expression of two anorexigenic neuropeptides, pro-opio melanocortin (POMC) and cocaine-and-amphetamine-related transcript (CART); as well as two orexigenic neuropeptides, neuropeptide Y (NPY) and agouti-related peptide (AgRP). Feed consumption for each treatment was also measured for correlation of feed consumption to gene expression.

Trends in preliminary data suggest decreased feed consumption relative to sham controls for PA, EPA, and DHA. When comparing across fatty acids, pairwise comparison showed PA treatment reduced feed intake relative to other treatments with the exception of DHA ($P<0.05$), and DHA treated fish consumed less feed than EPA treated fish ($P<0.05$). Therefore, early results suggest that individual fatty acids have the capacity to influence feed intake postprandially in rainbow trout. The impacts of these fatty acids on genes encoding hypothalamic regulators of appetite will also be discussed.

EFFECT OF HYPOXIA ON THE CHANNEL CATFISH TRANSCRIPTOME

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Channel catfish (*Ictalurus punctatus*) used in commercial aquaculture are raised in earthen ponds characterized by diel swings in dissolved oxygen concentration that may decrease to moderate or even severe levels of hypoxia. Low dissolved oxygen in catfish production ponds can suppress appetite, leading to suboptimal growth. To understand the underlying molecular framework that may govern appetite in channel catfish during hypoxia, the hypothalamus transcriptome was analyzed to identify differentially expressed genes (DEG) and patterns of genes responding to hypoxia. Channel catfish were subjected to 12 hours of hypoxia at 20% oxygen saturation (1.8 mg O_2/L at 27 °C) followed by 12 hours of recovery in normoxic water (100.1% oxygen saturation; 8.0 O_2/L) and fish were sampled at 0-, 6-, 12-, 18-, and 24-hour time points, with the hour 6 and 12 samplings occurring during hypoxia. Among all time points sampled, 190 genes were differentially expressed, with the greatest numbers found during the periods of hypoxia. The amount differentially expressed genes fell sharply during the normoxic recovery time points. Differentially expressed genes were grouped by function into Gene Ontology (GO) biological processes and were most overrepresented by the group "response to hypoxia" and GO molecular processes were predominated by "iron ion binding". Gene expression patterns in the hypothalamus suggests an attempt to increase vascularization coupled with a shift to anaerobic glycolysis provides tissue-level protection from hypoxic damage. This work identified several gene candidates that could be useful targets for future studies investigating appetite, in addition to other potential biomarkers for hypoxia.

Variable	Full model	Full model	Time point			
			6H	12H	18H	24H
padj	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.0
DEG (*n*)	317	190				
DEG > 1.0 $\log_2$ fold-increase (*n*)			58	54	20	10
DEG < -1.0 $\log_2$ fold-decrease (*n*)			1	4	0	1

LEGAL CHALLENGES AND OPPORTUNITIES RELATED TO CULTURE OF NEW AND EMERGING SPECIES

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Aquaculture is growing in the United States. Aquaculture production is expanding beyond the culture of traditional species (e.g., oyster, salmon) to culture of new and emerging species (e.g. seaweed, sea cucumbers, striped bass). The culture of new and emerging species often raises a host of legal and policy challenges. For example, as is the case with striped bass, there may be an existing legal framework designed to protect wild populations that prohibits the possession, transport, or sale of certain species. However, that is not always the case. Existing permitting regimes may present barriers if they do not apply to new species that growers wish to culture, creating uncertainty about the appropriate permitting path. New or emerging species may also raise food safety concerns if there is limited scientific data or literature about hazards associated with the products. For example, depending on the state, seaweed growers can face legal and policy issues with both permitting of operations and the food safety framework, especially for raw products. Further, growers may not always be the only ones questioning what the regulatory framework is. State regulators also face uncertainty with how to interpret and apply existing law to new and emerging species/products and what the desired legal framework should be. The National Sea Grant Law Center is involved in a number of aquaculture research projects related to shellfish, finfish, and seaweed culture. This paper will discuss the legal challenges surrounding the culture and production of new and emerging species, as well as opportunities to address such challenges through increased communication and coordination among state and federal aquaculture managers.

DEVELOPMENT OF *Aplysia californica* EMBRYOS OUTSIDE THE EGG STRAND TO PROVIDE NEW OPTIONS FOR CRYOPRESERVATION?

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The California Sea Hare (*Aplysia californica*) is a biomedical model used for studies in molecular neurobiology, electrophysiology, learning, and memory. As with other models, research communities often require maintenance of different genetic lines. Cryopreservation has been proposed as one method to preserve these lines. In many aquaculture species, sperm is cryopreserved, but because *A. californica* is an internal fertilizer, their sperm is difficult to extract. Thus, we are investigating if embryos and larvae can be cryopreserved instead. During spawning, multiple embryos are enclosed within egg capsules which are packaged within a strand (Figure 1A). The multiple barriers associated with the strand make it difficult to determine how well and how quickly cryoprotectants can penetrate embryos, information that is vital for developing a cryopreservation protocol. An alternative could be to freeze embryos or larvae free from the strand. Egg strands were cut into 1-cm pieces; six were added to 32 ppt artificial sea water (ASW), and six were cut open to release embryos into the ASW. Samples were observed for 16 days at 16°C or until >90% of the individuals died. Development was assessed and larval stage demographics were counted for each sample. Approximately 87% of embryos in strands developed normally into the early veliger stage, while only 8% of the free larvae developed into early veligers and of these, more than half exhibited abnormal development. The remaining free embryos arrested at an earlier developmental stage, trochophore larvae (Figure 1C). This suggests that the embryos depend on the environment provided within the strand to undergo normal development. Although some *A. californica* embryos can develop outside the strand and capsule, they developed slower and abnormally. Further research to develop ways to culture embryos outside the strand and capsule is needed to determine whether cryopreserving free larvae would be feasible.

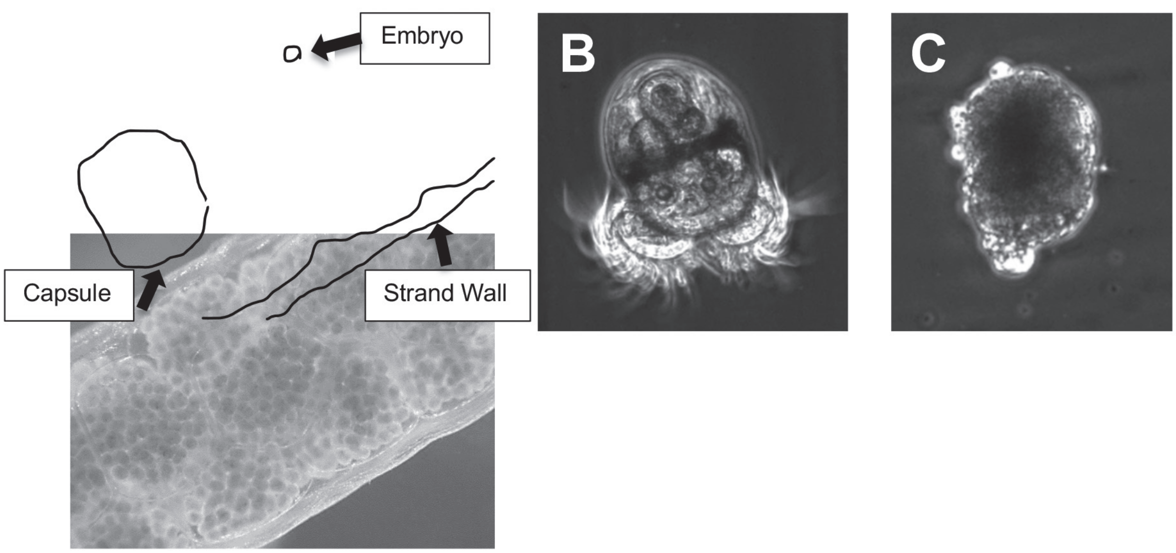

Figure 1: A. *Aplysia californica* egg strand structure. The outer strand walls encase numerous egg capsules which are each populated with 10-50 embryos. **B.** Stage-2 veligers were the most frequently observed form after 16 days within the strand. **C.** When embryos were removed from the strands and capsules, most larvae stopped development at the trochophore stage.

NUTRITIONAL EVALUATION OF BLACK SOLDIER FLY FRASS AS A NOVEL INGREDIENT IN FLORIDA POMPANO (*Trachinotus carolinus* L) DIET

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The rapidly growing aquaculture industry is dependent on efficient and sustainable aquaculture feeds. Furthermore, commercial aquaculture diets are more expensive compared to other animal feeds. Partly or complete substitute of expensive protein sources with cheap byproducts feed ingredients can drop diet costs and alleviate the environmental impact. Moreover, increasing competition for feed ingredients like soya, wheat, corn, and barley between aquaculture, poultry, and cattle industry and biofuel may threaten aquafeed production's sustainability. Frass is the waste product from growing black soldier fly larvae after harvesting the larvae. Recently, insect larvae meal has received significant attention as a novel alternative protein source in aquafeed. This byproduct ingredient contains around 200 g/kg protein and significant essential nutrients and normally made of biomass fed into the insect, dead larvae, and larvae excretion. Previous studies suggested that frass addition has a positive impact on vegetable growth and therefore suggested to be applied as a functional biofertilizer. Frass also contains chitin and beneficial microbes, which might have a positive influence on fish intestinal tract health. Recently published data on adding frass in catfish and tilapia diets showed that dietary frass can improve fish performance and the fish immune system.

This study evaluates the potential of dietary Black soldier fly frass on Florida pompano's growth, body composition, and the intestinal microbiome. Four experimental diets were formulated containing different levels of frass (0, 6, 12, and 18%). The frass was used to replace an equal mixture of corn, wheat, and soybean meal. Juvenile Florida pompano (initial weight of 31.4±0.7 g) were fed to 3% of biomass twice a day for eight weeks. Replacing carbohydrates sources with frass did not improve growth performance resulting in a lower specific growth rate (SGR) and higher feed conversion rate (FCR) ($p < 0.05$). Frass significantly increased visceral somatic index (VSI) compared to the control diet (4.1-4.6 versus 3.9%). However, the hepatic somatic index (HIS) was lower in fish that were fed with frass-containing diets (0.84-0.92 versus 1.28%) ($p < 0.05$). Adding frass to the diet did not change the body composition ($p < 0.05$).

Table 1: Growth performance of Pompano fed with Frass-based diets

	Control	Frass 6%	Frass 12%	Frass 18%
Weight (g)	56	55.1	53.1	52.2
FCR	1.27	1.32	1.32	1.43
SGR	2.36	2.29	2.29	2.15
VSI	3.87	4.09	4.78	4.58
HIS	1.28	0.84	0.95	0.82

INTEGRATED APPROACHES TO ENHANCE SUSTAINABILITY, RESILIENCY, AND ROBUSTNESS IN US AGRI-FOOD SYSTEMS-AN OVERVIEW OF NATIONAL INSTITUTE FOR CELLULAR AGRICULTURE

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As the world population increases to 10 billion by 2050, total food and meat production must rise by 70 and 100%, respectively, to satisfy global demand. The US food production system faces several issues in meeting this demand. Increasing water scarcity in major production regions and increasing vulnerability to disruptions from natural disasters due to climate change are just some of the growing issues that prompt the need for new technologies in meat production. Also, a critical challenge in food supply chains is food loss issues that present significant sustainability and security challenges, with 60 percent of meat becoming processing waste (1.4 billion tons for livestock; 800 million tons for seafood). New sources of sustainable and nutritional protein would help address these concerns and focus on the present proposal. Cultivated meat production is emerging as a feasible solution to address immediate societal problems by developing new sustainable agri-food systems to feed a rapidly growing global population. This industry will provide nutritious and safe foods for consumer options while reducing environmental impact (78-96% fewer greenhouse gas emissions, 99% less land use, and 82-96% less water use).

This proposed work aims to develop new adoptable techno-economically viable cultivated meat systems and develop new educational platforms for training future professionals. We have assembled a research team that uniquely combines a molecular biologist, a biomedical engineer, a food engineer, a biosystems engineer, a data scientist, a protein chemist, a flavor chemist, a sensory expert, a food safety specialist, an environmental scientist, and a consumer specialist, each of whom has long-standing research interest in sustainable food production systems. This proposal brings together six different academic institutions with Tufts University, Virginia Tech, MIT, University of California, Davis, Virginia State University (HBCU), and the University of Massachusetts, Boston.

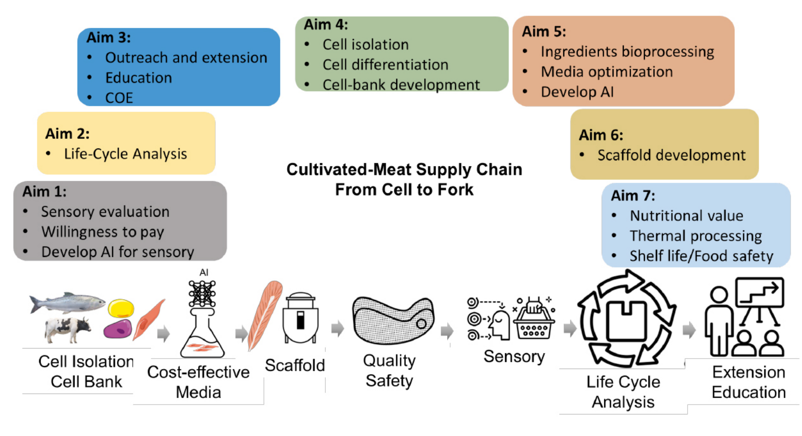

Fig. 1. Overview of the proposed integrated research, extension, and education efforts

ANALYSIS OF CARBON AND NITROGEN ISOTOPES TO ASSESS NUTRIENT LOADING IN REHOBOTH BAY, DE AND ITS POTENTIAL IMPACTS ON OYSTER (*Crassostrea virginica*) AQUACULTURE

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Since the 1950s, the Delaware Inland Bays (DIB) oyster (*Crassostrea virginica*) population has succumbed due to anthropogenic influences. These changes decreased tolerance of nutrient influxes and led to the decline of the DIB ecosystem, social, cultural, and economic loss. Established oyster aquaculture sites and pilot reefs are expected to aid in bay restoration but require monitoring to identify nutrient sources. Thus, this research focuses on nitrogen and carbon isotope data from terrestrial, estuarine, and biological sources within Rehoboth Bay, DE to identify possible nonpoint pollution sources through nutrient tracking. The primary objective is to identify non-point pollution sources through monitoring of field sites (Figure 1). It is hypothesized that the greatest difference in isotopic signatures may be found at the wastewater plant and marina near the agricultural site due to high nutrient loading; these findings may correlate with water quality and isotopic data analyses. Samples of soil, submerged sediment, water, and oyster tissues were collected across the bays and analyzed for stable isotopes. Water quality parameters were monitored weekly at aquaculture sites, artificial oyster reefs, and non-reef locations, and nutrient tests identified concentrations of nitrogen, nitrate, ammonia, calcium carbonate hardness, alkalinity, and orthophosphate. Total suspended solids, stable isotopes, and biological analyses were conducted following the standard protocols previously published. Results showed improved water quality at aquaculture sites and highlighted oyster effectiveness on ecosystem restoration. However, nutrient test analyses indicated a necessity for increased monitoring and data analysis at sites for clearer identification of non-point pollution sources. Isotopic analysis results are currently being conducted and is expected to provide further information about pollution sources and aid in the creation of best management practices for oyster aquaculture and bay health restoration.

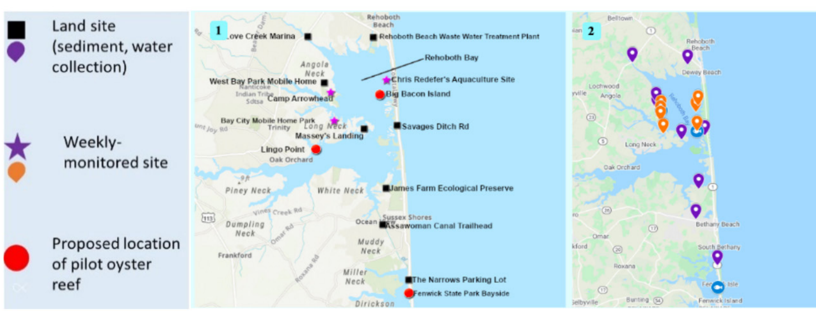

Figure 1. Map showing the study sites for water, land, sediment, and oyster sampling in Rehoboth Bay, Delaware. *Map by M. Gadde.*

WINTER BLOOM DYNAMICS AND MOLECULAR ANALYSIS OF BENTHIC SEDIMENTS FOR HAB, *Dinophysis acuminata,* AT TORQUAY CANAL, REHOBOTH BAY, DELAWARE

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The toxins produced by some members of the *Dinophysis* group lead to an illness known as Diarrhetic Shellfish Poisoning (DSP). Diarrhea along with other uncomfortable symptoms (i.e. pain, cramping, vomiting, chills, fever) occurs following consumption of filter feeding shellfish that have accumulate okadaic acid in their tissues. The risk this organism may pose to oyster aquaculture sites within Rehoboth Bay is unknown, so studying its bloom dynamics is essential to ensure a healthy oyster aquaculture industry within Rehoboth Bay.

D. acuminata has been historically identified at high concentrations (>20,000cells/L) in water samples from Rehoboth Bay, Delaware, but the reach of spring blooms and how far they extend to aquaculture sites had not been determined. Temperature, nutrients, and prey abundance can be drivers of *Dinophysis* blooms. Molecular techniques (PCR) and microscopy have been used to determine the presence or absence of *D. acuminata* within sediment and water samples from three sites within Rehoboth Bay. A transect was sampled three times in January and one-time early February during an unexpected winter bloom of *D. acuminata*. The bloom was not strictly isolated to the well monitored site at the Torquay Canal bulkhead (TQB; DNREC RB64) but did not extend into Rehoboth Bay or remain at bloom densities beyond site TQB (10,000-20,000 cells· L^{-1}) for more than 2 weeks. The bloom reached cell density of 191,000 cells· L^{-1} on January 6, 2020. On January 14, 2020 cell density at site TQB fell to 4,000 cells· L^{-1} but were at bloom levels (10,000-20,000 cells· L^{-1}) just outside of Torquay Canal, in Bald Eagle Creek at site TQ12 (15,666 cells· L^{-1}) and site TQ11 (11,666 cells· L^{-1}). At TQB site during the winter 2020 bloom event combined nitrate and nitrite levels did not approach the maximum level, but levels increased on January 7, January 14, and February 3. Orthophosphate increased at site TQB on December 23, 2019 and January 14, 2020. There were no significant relationships between cell density and water temperature, chlorophyll-*a* concentration, conductivity, dissolved oxygen (DO), combined nitrate and nitrite concentrations (NO_x), or orthophosphate concentrations (PO_4^{3-}) during 2020 winter bloom event.

Some dinoflagellates form a resting cyst life stage that allows them to overwinter within benthic surface sediments until spring when conditions become suitable for bloom development while others overwinter in the water column. Sediments were analyzed through PCR to determine presence of *Dinophysis* using genus specific primers to monitor cyst density or abundance within the sediments during winter months. Samples were taken at Torquay Canal every month from June 2018-February 2020. Samples were taken at Camp Arrowhead and James Farm in summer 2018 (July, August, September) and August 2019. *Dinophysis* was found in sediments through PCR analysis at Torquay Canal (DNREC Station Code: RB64), Camp Arrowhead (UDCMP Station Code: RB44), and James Farm (DNREC Station Code: IR36). Limited data exist on *Dinophysis acuminata* for Rehoboth Bay and the results of this study will strengthen resources for monitoring HABs species in Delaware.

Microscopic image of *Dinophysis acuminata* during the 2020 winter bloom at TQB January 6, 2020 Image by A. Pappas.

QUANTIFICATION OF THE GROWTH RATE DISPARITY OF *Enterocytozoon hepatopenaei* INFECTED *Penaeus vannamei* AND *Penaeus monodon* UNDER LABORATORY CHALLENGE CONDITIONS

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Enterocytozoon hepatopenaei (EHP), the etiologic agent of Hepatopancreatic Microsporidiosis (HPM), has emerged as one of the most problematic infectious diseases in shrimp aquaculture over the last ten years. To further investigate the disease biology and to evaluate efficacy of candidate therapeutics in controlling HPM, the University of Arizona has developed an EHP challenge method. The EHP challenge method involves amplifying EHP inoculum in live shrimp (*Penaeus vannamei)* by directly injecting EHP inoculum in the hepatopancreas of shrimp. Subsequently, EHP-injected animals can be sacrificed at 15 days post-injection, hepatopancreas dissected and used for an oral challenge of Specific pathogen Free (SPF) shrimp. Although an EHP challenge method is available, the effect of EHP infection on growth under laboratory challenge conditions was unproven. This study is aimed to quantify the effect of the disease progression on growth rate in Pacific white shrimp, *P. vannamei*, and black tiger shrimp, *P. monodon*.

The EHP experimental challenges involving *P. monodon* and *P. vannamei* were run side-by-side under the same environmental conditions. Animals were fed using automatic feeders throughout the day. The duration of the study was 45 days and the growth rate of EHP-infected animals and healthy animals were measured at Days 0, 15, 30 and 45 post-infection. At each time point, a subset of animals were sampled to quantify the EHP infection using H&E histology and qPCR. The results will reveal how under identical rearing conditions (e.g. feeding rates, stocking densities,rearing condition) in a laboratory experimental challenge, EHP infection affects the growth rate of *P. monodon* and *P. vannamei*.

USING FISHERIES TECHNIQUES TO ESTIMATE AGE AND GROWTH OF HYBRID CATFISH (*Ictalurus punctatus* ♀ x *Ictalurus furcatus* ♂) FROM COMMERCIAL CATFISH FARMS

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In west Alabama, catfish producers routinely face the challenge of fish that exceed market size (aka "big fish") in their commercial ponds. These fish are evading harvest and can increase their size significantly before the next harvest occurs. This is a problem because processing plants prefer catfish in the range of 1-4 lbs. Once over this range, processing plants are forced to hand-fillet fish versus using the automated methods that are more cost-effective and are stuck with the additional task of finding a market for these larger fillet products. This leaves the farmer receiving a lower price per pound or oftentimes no financial return for fish found above the premium size. The cost of resources that went into feeding and caring for that fish for an added amount of time, sometimes for several years, must also be factored in. Harvesting inefficiencies and ponds with uneven bottoms that allow catfish to escape seines are major drivers of the big fish problem. Due to their larger size and growth potential, hybrid catfish (*Ictalurus punctatus ♀ x Ictalurus furcatus ♂*) tend to be a greater big fish issue than channel catfish. Little is known regarding the age structure and growth rates of hybrid catfish that repeatedly evade capture and remain in commercial ponds for extended periods.

The objective of our study is to quantify the age structure and growth of hybrid catfish that evade capture and remain in ponds following commercial harvest. Twelve ponds that have recently been harvested will be sampled to collect 100 fish per pond using an electrofishing boat with dip nets. Study ponds will be sampled before being restocked with a subsequent crop of fingerlings. A very low pulse rate is documented as most effective for sampling large catfishes, with numerous studies backing up low-frequency electrofishing (LFE), in pulsed-DC as the best method for collecting blue catfish. Following collection, fish will be numbered, and total length (mm), weight (g), and sex of fish will be recorded. Diet contents will also be identified to check for cannibalism. We will extract otoliths from the brain cavity and use the cut method to estimate fish age. This method involves cleaning the otolith of any brain matter, embedding the otolith in a clear epoxy resin, and cutting into it with an IsoMet low-speed precision saw to reveal the core and annuli (annular growth "rings"). Once we can accurately observe the core, we will use Jenoptik Gryphax, a high-quality image analysis software program connected to a microscope, to count the rings surrounding the core. This counting will be conducted by two readers independently and compared. If there is any dispute regarding age, a third reader will be brought in to resolve the disagreement. Data collected on fish age, growth, and longevity of hybrid catfish found in ponds following harvest will help producers and researchers better understand the big fish problem and hopefully lead to future solutions to this issue. This study is currently in the data collection phase.

INTRODUCING THE AUSTRALIA NEW ZEALAND ASSOCIATION OF AQUARIUM PROFESSIONALS

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ANZAAP is a not-for-profit association that provides support and quality peer reviewed information to its membership of professional aquarists serving as a forum for dissemination of information to further the science of aquarium design, maintenance and aquatic animal husbandry and health, both in aquatic research model organisms and species of public aquarium significance.

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EVALUATION OF AQUEOUS MAGNESIUM CONCENTRATION ON PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN LOW SALINITY WATER OF WEST ALABAMA

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L. vannamei is a highly adaptable euryhaline species. It has been produced in different production systems over a wide range of environmental salinities. Compared to seawater, the ionic profile of inland low salinity sources can be dramatically different, varying markedly among sites and having low and variable concentrations of Mg^{2+} and K^{+}. Several approaches have been investigated to improve shrimp production in low salinity waters (LSW) in Alabama including modification of the waters ionic composition and dietary modifications. While Mg^{2+} is supplemented at the beginning of each production season by commercial shrimp producers, the target concentration used by Alabama farmers (>20 mg/L) may not be high enough, particularly for larger shrimp in the later phase of the production cycle as shrimp farmers have reported increased mortality during this specific stage in ontogeny. In order to investigate this issue, two studies were conducted on a commercial shrimp farm in west Alabama. In the first study, additional Mg^{2+}supplemented to production ponds was evaluated to determine if increases in survival, growth, and production of *L. vannamei* reared in LSW could be achieved. In this study, a total of 10 commercial production ponds (ranging in size from 0.4 to 3.2 hectares) were used. Shrimp were stocked into each pond at a density of 271,800 sperm/ha and offered a commercial feed twice a day. An initial amount of Mg^{2+}present in a fertilizer, so that sulfate of potash magnesia ($K_2SO_4MgSO_4$), was used as a source of both K^{+} and Mg^{2+} by the farm for each pond to attempt to raise Mg^{2+} levels in ponds to 20 mg/L. After stocking, an agricultural grade magnesium chloride ($MgCl_2$) was used to quadruple (~80 mg/L) the amount of Mg^{2+} in four commercial shrimp ponds while another six commercial ponds served as the experimental control. Pond water was sampled at the beginning of the trial, each month, and before harvest to determine pond ionic profiles (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}). Shrimp were sampled monthly for hemolymph osmolality, hemolymph ions, and whole-body ion composition. The second study evaluated survival and growth performance of larger shrimp (>15 g) reared in two on-levee flow through-tank systems. One tank system received water from a production pond containing the standard amount of Mg^{2+} (20 g/L) compared to a similar tank system with water sourced from a production pond with four times the amount of magnesium. Twelve tanks (800-L) were stocked with shrimp at three different densities (20, 25, 30 shrimp/tank). Following 8 weeks, tanks were harvested to determine production performance of shrimp and preliminary analysis indicated there were no differences in survival, growth, weight gain (%), or food conversion ratio (all $p < 0.05$) in shrimp reared at different densities in waters of varying Mg^{2+} composition. Analysis of hemolymph osmolality, hemolymph ions, and whole-body ion composition are ongoing. Data from these trials will help determine whether additional Mg^{2+} can increase survival, growth, and production of larger *L. vannamei* reared in LSW.

REPLACEMENT OF FISH OIL BY A HIGH-DHA MICROBIAL OIL IN SALMON DIETS: EFFECT ON GROWTH PERFORMANCE, LIPID COMPOSITION AND GENE EXPRESSION

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The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential nutrients for farmed fish and for their human consumers. Fish oil is one of the main sources of these long-chain polyunsaturated fatty acids; however, due to an increasing demand for EPA and DHA and with a limited supply from wild fisheries, there is a need for alternative sources that are sustainable and cost effective for aquaculture. Here we investigated replacement of fish oil with a high-DHA, low-EPA oil extracted from single-celled thraustochytrids. This provided a unique opportunity to determine how these dietary fatty acids independently influence lipid metabolism and physiological pathways in salmonids.

A 16-week feeding trial was conducted with Atlantic salmon fed diets with a complete or partial replacement of fish oil with microbial oil. There was no significant difference in growth performance among the dietary treatments but we observed differences in lipid composition and gene expression. We investigated total lipid class and fatty acid composition in liver and muscle tissues using thin-layer chromatography and gas chromatography with flame ionization detection and mass spectrometric detection. Our results showed no significant differences in total lipids and lipid class concentrations among the dietary treatments for both tissues; however, significant difference were observed in proportions of omega-3 and omega-6 total lipid fatty acids and phospholipid fatty acids. In addition, there were differences in the phytosterol composition and in triacylglycerol and phospholipid molecular species. Microbial oil diets increased levels of DHA in phospholipid structures phosphatidylcholine and phosphatidylserine, while bioactive prostaglandin E2 levels were related to precursor arachidonic acid levels. These results correlated with hepatic lipid metabolism biomarkers, although some levels were the same with high dietary DHA (high microbial oil) and high EPA (fish oil) indicating successful replacement of fish oil with microbial oil.

This research was undertaken thanks in part to funding from the Canada First Research Excellence Fund, through the Ocean Frontier Institute

PRESENCE OF CARP EDEMA VIRUS (CEV) IN CYPRINIDS: MONITORING IN NATURAL ENVIRONMENTS OR IN PRESENCE OF DISEASED CARP

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Carp Edema Virus (CEV) is a double-stranded deoxyribovirus belonging to the *Poxviridae* family. It is recognized as the causative agent of Koi Sleepy Disease (KSD) in common carp (*Cyprinus carpio*) and koi carp (*C. carpio* var. koi). KSD exhibits clinically with abdominal edema and severe branchial damage and can lead to high mortality rates in infected populations. Although the species showing clinical signs are mainly carp and koi carp, a recent study has shown the presence under experimental conditions of the viral nucleic acid in other cyprinid species, specifically in bleak (*Alburnus alburnus*), crucian carp (*Carassius carassius*), Prussian carp (*Carassius gibelio*), roach (*Rutilus rutilus*) and tench (*Tinca tinca*). Our study aims to investigate the presence of CEV in cyprinids in natural basins or kept in environments with positive carp.

ISOLATION AND IDENTIFICATION OF *Vibrio* COLONIES FROM CAPTIVE JUVENILES OF LESSER SPOTTED DOGFISH *Scyliorhinus canicula*

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Gram-negative curved rods bacteria also known as the Vibrio are spread globally. Skilled colonizers of any kind of aquatic ecosystems from shallow to high depths. Some vibrios (e.g., *V. anguillarum*, *V. ordalii*, *V. salmonicida*, *V. vulnificus*, *V. alginolyticus*, etc.) can have pathogenic effects on aquatic life as in marine vertebrates and invertebrates. However, there are currently few reports in the literature on infectious diseases affecting sharks. Therefore, the aim of this study was to clarify the cause of tank mortality occurred in juveniles of lesser spotted dogfish *Scyliorhinus canicula* (Linnaeus, 1758), a small demersal shark (Carcharhiniformes: Scyliorhinidae) reared in an Italian public aquarium.

Sharks were placed in interconnected tanks (1 m^3 each) with a recirculating aquaculture system (RAS) in accordance with the current legislation on animal welfare. A few months after hatching, elasmobranchs (Figure 1) showed the first symptoms, i.e., anomalies in swimming, fast breathing, and lack of appetite. Therefore, according to the Ethics Committee, 20 juvenile specimens (mean±SD: weighing 2.16±0.29 g, and total length 94.60±4.03 mm) have been subjected to various analyses including necropsy, parasitological and virological examination, and bacteriological and biochemical analysis. In order to exclude the presence of different pathogens of marine fish species (parasites, bacteria, Betanodavirus).

No external lesions were found, also the necropsy did not show macroscopic lesions of the viscera. Parasitological (internal and external examination) and virological (Betanodavirus RT-PCR) tests reported negative results. Regarding bacteriological analysis, positivity were retrieved in 12 out of 20 specimens (60%) from at least one matrix (i.e., head kidney, brain, or blood). However, the identification by VITEK-MS did not produce any valid results. All the 24 strains isolated shown a fragment of 730 bp from the *rpoB* gene amplification: 11 isolates (45.8%) were identified at species level as *V. cyclitrophicus*, whereas the remaining 13 (54.2%) as *V. crassostreae* (BLASTn nucleotide sequence identity value ranging from 98 to 100%).

Although the pathogenic role of these microorganisms has not been proved as well as the presence of other pathogens cannot be excluded, the study reported two *Vibrio* species in poorly studied aquatic animals. The weaknesses and strengths of the different diagnostic methods were also highlighted.

FIGURE 1. Captive juveniles of lesser spotted dogfish *Scyliorhinus canicula*.

AN INSIGHT TO AQUAPONICS PRODUCTION: PROBLEMS AND BENEFIT

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As the world's population grows, demands for increased food production expand and leads to finding alternative, sustainable, and reliable methods to provide food. In this context, the idea of aquaponic production was introduced by aquaculture research community in the mid-1970. An aquaponic system represents the perfect integration of aquaculture and hydroponics. However, little research has been conducted on commercial-scale aquaponics production especially from the sanitary point of view. Disease outbreak can compromise the harvest and resulting as a limiting factor, leading to the end of an operation.

Aquaponics combines the hydroponic production of plants and the aquaculture production of fish. Over the last years has gained increased interest due to its sustainability. Several fish species i.e., tilapia, goldfish, carp, koi, catfish, and barramundi are commonly used due to their features for aquaponic production of a wide range of edible plants. The selection of plant and fish species is a key point for aquaponics production. To note, there are inherent issues that need to be assessed in the near future. Water quality has a key role in this kind of systems and should be balanced in base of the needs of fish, plants, and bacteria. The asset of physicochemical water parameters (e.g., temperature, dissolved oxygen, ammonia) should be constantly monitored and balanced between the fish, plants, and bacteria. One of the main challenges to a successful aquaponics operation is related with disease prevention and control, thus pathogens can affect both fish and plants. Since recycling water is a perfect environment for pathogen amplification, a disease outbreak can compromise an entire crop and lead to the end of an operation. Generally, aquaponic systems are not compatible with the use of chemicals compounds both for fish disease and plant treatment. Antibiotics and antiparasitic products may affect the biofilter activity and plant survivor. Moreover, medicines used for treating fish diseases and pesticides cannot be used, cause plants and fish may absorb and accumulate them. To prevent or manage the impact of a certain disease, water disinfection can reduce or eliminate pathogens, however disinfection methods, can harm the health of the fish or plants; thus, they must be used carefully. The risk of pathogen introduction in an aquaponic system is very limited and generally linked to the introduction of infected fish. Pathogens are also introduced through further pathways as: make-up water, air, animal vectors, feed, dirty equipment and via staff or visitors. In literature there was only few studies that describe the occurrence of pathogens in aquaponics production. On the other hands, according to our recent studies, sanitary problems are frequently caused by bacteria and parasites. Among parasites species, *Ichtyophtirius multifils, Hexamita* sp., *Gyrodactylus* sp. and *Dactylogyrus* sp. are the most impactful. The expansion of aquaponics production all over the world highlight the need to elaborate sanitary methods and adequate protocols to prevent and control disease outbreak in this kind of aquaculture production.

MODULATION OF ANTIOXIDANT DEFENSE AGAINST OXIDATIVE STRESS EXERTED BY FISH DIETS SUPPLEMENTED WITH A BASIL SUPERCRITICAL EXTRACT

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The wide use of antimicrobial agents in modern food animal production has led to the emergence of antimicrobial resistance worldwide. In aquaculture this has resulted in the emergence of antibiotic-resistant bacteria in aquatic environments, increase of antibiotic resistance in fish pathogens, transfer of resistance determinants to bacteria of land animals and to human pathogens, and alterations of the bacterial flora both in sediments and in the water column. Phytotherapy is based on the use of plants for prevent or treat human or animal diseases. At the beginning of the 20th century, phytotherapy was in competition with modern medicine and in particular with antibiotic molecules.

Recently, the use of essential oils is also rapidly increasing in the aquaculture sector as a means of greater industrial and environmental sustainability. Previous studies showed beneficial effects of essential oils on growth, immunity antibacterial and antiparasitic activities in fish crops. In this study, the modulation of antioxidant defense against oxidative stress exerted by fish diets supplemented with a basil supercritical extract (BEO) was assessed in rainbow trout *Oncorhynchus mykiss*. The basil supercritical fluid extracts were obtained from dried clean basil leaves using a supercritical fluid extractor.

The BEO extracted was added to the commercial feed flour in the proportions of 0.5%, 1%, 2% and 3% (w/w) and subsequently mixed with fish oil to obtain a suitable compound for pellet preparation.

A 30-day trial was carried out using 20 square fiberglass tanks supplied by artesian well water (constant temperature of 13 ± 1 °C) in an open system (flow-through). The experimental diets (A: control; B: 0,5%; C: 1%; D: 2%; E: 3%) were randomly assigned to the tanks (four replicate tanks per diet).

The daily feed quantity was set at 1% of tank biomass. Eight fish from each experimental group (2 fish per tank; four replicates) were sampled at the middle (15 days; T1) and at the end (30 days; T2) of the experiment. Liver and kidney were sampled from each specimen and stored at -80 °C for biochemical analysis. The levels of stress biomarkers such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase, glyoxalase I, glyoxalase II, lactate dehydrogenase, glutathione and malondialdehyde showed a boost of antioxidant pathway in fish fed with 0.5% BEO supplemented diet. Higher BEO supplementation led to failure of activity of several enzymes and depletion of glutathione levels. Malondialdehyde concentration suggests a sufficient oxidative stress defense against lipid peroxidation in all experimental groups, except for 3% BEO supplemented diet. Our results suggest for a supplement of BEO in fish-diet up to 0.5% to avoid potential oxidative pressure in farmed trout.

SUPERTROUT: IMPROVING SUSTAINABILITY AND PERFORMANCE OF AQUACULTURE FARMING SYSTEM THROUGH BREEDING FOR LACTOCOCCOSIS RESISTANCE IN RAINBOW TROUT

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Lactococcosis is a well-known infectious disease affecting aquaculture farming systems, caused by the increase in water temperature associated to the global warming. *Lactococcus garvieae,* a warm-water pathogen, is the responsible of lactococcosis and significant economic losses in aquaculture worldwide, in particular for rainbow trout farming system. The loss due to this infection is around 10-60% of the total rainbow trout production; mortality increasing are reported when water temperature exceeds 15 °C. The overall objective of SUPERTROUT is to improve sustainability and profitability of small-scale farming system facing lactococcosis in rainbow trout applying innovative strategies.

This infectious disease is a critical issue for the countries bordering the Mediterranean Sea where the temperate climate, associated with the global warming, in recent years, favoured the durability and diffusion of lactococcosis outbreaks. This issue mainly concerns Italy, Turkey, Spain and Greece, top producers of rainbow trout (*Oncorhynchus mykiss*).

The increase in the demand for aquaculture products brings with it the need to make production systems more efficient and sustainable. This entails a management and technological improvement, including:

- selection of natural genetic resistance of trout by testing the mutation 140T on MHC II gene;
- development of recombinant vaccine to be administered by immersion;
- improvement of reproductive performances exploiting trout genetic features.

The use of marker assisted selection and the development of recombinant vaccine will enhance sustainability of the farming system, mainly due to the reduction of antibiotic treatment, and of the consequent environmental contamination with antimicrobials and of the risk of antibiotic-resistance development. This approach will contribute to increase profitability, reducing direct and indirect costs related to disease control.

Figure 1: Member countries participating to the project: Italy, Turkey, Spain and Greece.

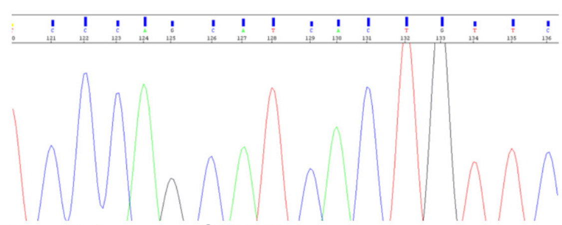

Figure 2: Sequence of MHC II gene.

Figure 3: Trout affected by lactococcosis.

IMPACT OF ARTIFICIAL SUBSTRATE TYPE ON OYSTER RECRUITMENT DENSITY AND SIZE IN THE YORK RIVER

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Restoration of eastern oyster (*Crassostrea virginica*) reefs is important because of their usefulness as shoreline protection and habitat for temperate estuarine communities in the Chesapeake Bay and its tributaries. Oysters prefer to settle in crevices such as those found on natural shell substrates to reduce predation, yet natural shell for use in restoration is becoming scarce in the Chesapeake Bay. Finding an alternative settlement substrate that is just as complex as natural shell and can mimic the benefits of shell substrates without the expense seems to be the best way to encourage oyster recruitment and survival, thus promoting restoration. The goal of this experiment is to determine which artificial substrate type promotes the highest oyster recruitment and growth. To answer this question, six replicates of six reef types were deployed in a randomized block design across the two experimental sites. The reef types included are oyster castles, oyster diamonds, x-reefs, c-domes, granite, and loose oyster shell (Figure 1). All of the reefs are roughly between 0.09 - 0.28 m^2 and stand between 0.30 – 0.46 m tall, except the oyster castle structure, which consists of five 0.028 m^3 blocks. Six months after deployment, oyster recruitment densities, and sizes were measured on each of the reefs. There were notable differences in oyster settlement densities and sizes among substrates. Structures with internal space showed the highest amount of recruitment, which may be because of shelter from predation and sedimentation during early life stages. All substrates had well-established oyster communities, which suggests that artificial substrates of varying shapes and compositions can be effectively used for oyster restoration.

Figure 1. *from left to right*: oyster castle structure, x-reef, granite in a Vexar basket, diamonds, c-dome, shell in a Vexar basket

BIOACTIVE PHENOLICS PROFILING OF AQUAPONIC LETTUCE

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Aquaponics, an integration of aquaculture and hydroponic systems, is expected to become a surging technology to provide fish and vegetable production benefits while reducing problems associated with freshwater and reactive chemicals usage. However, information available on bioactive compounds especially phenolic profiles in leafy lettuce is lacking in aquaponics production systems. Both red and green lettuce samples were collected from replicated aquaponics and hydroponic systems (as a control) and analyzed for their growth, yield, and antioxidant properties. All together nineteen phenolic compounds were analyzed in lettuce samples using LCMS technology. Results showed that aquaponics had a significantly higher (by 7%) leafy lettuce production when compared with hydroponics, coincided with a large accumulation of phenolic compounds. Red varieties of lettuce had a high phenolics level than that of the green one. Among the phenolic compounds, the quercetin-malonyl glucoside, quercetin glucuronide, di-caffeoyl tartaric acid, ferulic acid were consistently higher in aquaponics lettuce when compared to the hydroponic lettuce. Out results suggested that the anti-oxidant quality of aquaponic lettuce is much better in context of public health claims when compared with hydroponic lettuce.

SMART, SUSTAINABLE SHELLFISH AQUACULTURE MANAGEMENT PROGRAM

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New technologies have revolutionized the agricultural landscape over the past several decades, improving yield and efficiency while decreasing cost and labor. However, on-bottom oyster aquaculture practices have remained relatively unchanged since the 1800's. In this integrated project, an innovative smart sustainable shellfish aquaculture management (S3AM) framework is used to achieve long-term goals of enhancing nationwide shellfish farm production, preserving environmental heath, sustaining economic viability of shellfish farm operations, and enhancing quality of life for farmers and society as whole.

This program integrates an interdisciplinary team with expertise in Engineering, Computer Science, Biology, Environmental Science, Economics and Aquaculture from the Atlantic, Gulf, and Pacific regions to pursue five supporting objectives through research, education, and Extension activities. These include 1) Developing enabling technologies and management solutions for the S3AM framework to improve farm productivity and profitability in coastal regions nationwide. 2) Validating the S3AM in the laboratory and through farm trials in the three coastal regions. 3) Modeling and assessing the economic impacts of the proposed S3AM framework. 4) Integrating knowledge generated from the S3AM into education and outreach activities to prepare future generations of workforce to address globally pressing issues of sustainability. 5) Building a nationwide extension network to engage stakeholders, disseminate the S3AM, and assess its short, medium, and long-term outcomes.

The project entails a system-based approach to develop and implement new technologies and management practices that address the imperative shellfish aquaculture industry needs and to identify economic barriers and opportunities, leading to significantly improved farming efficiency, productivity, and profitability. Current program status and future outlook will be discussed.

Figure 1. (A) Underwater remotely operated vehicle (ROV) platform. (B) Computer vision identification and assessment of on-bottom oysters. (C) Oyster dredging operation on the Chesapeake Bay in Maryland.

FINANCIAL BOTTLENECKS FOR AQUAPONIC PRODUCERS

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The commercial aquaponics industry has struggled to become established globally to spite significant interest from the general public for sustainable and urban agriculture. Many of the issues that prevent aquaponics from gaining a foothold are financial. These specifically relate to 1) obtaining startup funds, 2) economic viability, and 3) marketing of products. This presentation will discuss where and how farmers obtain funds, the need for loan programs, and need for viable business plans that lead to successful businesses. The bottlenecks for economic viability to be discussed are cost of system infrastructure, operating cost in various climates, challenges of scaling up, diversity of income streams, and cash flow. Finally, the realities of marketing products with relation to consumer acceptance, sales strategies at volume, and product value in relation to local demands and cost of production will be discussed.

CAN WE EVER REALLY GROW SHRIMP IN AMERICA?

EVERYONE AGREES THAT WE NEED TO GROW MORE SHRIMP IN THE US. BUT IS THAT EVEN POSSIBLE?

Robin Pearl

President
American Mariculture, Inc.

The US loves shrimp. We consume over 1.6 billion pound per year. The domestic wild catch industry can supply approximately 10% of our annual requirement, so we have no choice but to import 90% of our shrimp.

How can we change that? Many aquaculture companies promising to reduce the US's dependence have come and gone over the past 20 year. Investing hundreds of millions to figure it out, yet NONE have ever been able to figure out how.

The hurdles in building a viable industry are well known, but ideas on how to overcome them have not worked. So, what is next? I will discuss potential game changing technologies that may finally make a real difference. I will share some of my personal experiences in building the largest fully integrated shrimp farming company in the US, and I will present my thoughts for bussing entrepreneurs who are attracted to the market, but who need to know the potential pitfalls.

ALLOMETRIC GROWTH DURING LARVAL DEVELOPMENT OF PACIFIC RED SNAPPER *Lutjanus peru* IN CULTURE CONDITIONS

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Body segments related to primary functions like feeding and predator avoidance show a higher relative growth rate and differentiation during larval development. This type of growth is called allometric growth and has been reported in many fish species during larval development and is considered as an adaptive response to increase survival. As a result, the early ontogeny of fishes is characterized by drastic changes in every stage of the larval period. The objective of the present study was to evaluate the allometric growth during the larval stages of the Pacific red snapper to test the hypothesis of a differential growth during early development which can be useful to evaluate growth under different experimental culture conditions.

Larvae were obtained by spontaneous spawning of Pacific red snapper broodstock held under controlled conditions. From hatching until day 30, random samples of 10 to15 larvae were sampled and digitally photographed. On each digital photograph, the TL and several body proportions associated with feeding and locomotion were measured to the nearest 0.01 mm (Table1). Allometric growth patterns during the developmental stages were modeled by a power function of TL and the patterns in allometry were described by the growth coefficient (i.e. power function exponent, b) in the equation $Y = aX^b$. Isometric growth occurred when $b = 1$. A positive allometric growth occurred when $b > 1$ and a negative allometric growth when $b < 1$.

The results showed that body segments have a different growth coefficient depending on the stage of the larval period (Table 1). Clear positive allometry was observed during the yolk-sac larvae in the TAL. During the preflexion stage, only the TRL showed a clear negative allometry. A tendency to isometry was observed in all the body segments during the postflexion stage. Our results showed that Pacific red snapper larvae have a differential growth suggesting priorities in development to increase survival during this critical period.

Table 1. Growth coefficients of body segments during the stages of the larval period of the Pacific red snapper *Lutjanus peru* larvae. - Not Measured.

Stage	Body segment								
	HL	TRL	TAL	HH	BH	MH	TH	ML	ED
Yolk-sac larvae	0.49	0.26	1.54	-	-	-	-	-	-
Preflexion	1.74	0.49	0.90	1.95	2.30	2.59	1.95	1.38	1.39
Flexion	1.04	0.81	1.02	1.11	1.26	1.16	2.06	0.97	1.16
Postflexion	0.94	1.14	0.96	0.77	0.94	1.25	0.93	0.68	0.94

BH = body height; TRL = trunk length; ED = eye diameter; HH = head height; HL = head length; MH = muscle height; ML = mouth (maxilla) length; TAL = tail (post anal) length; TH = tail height.

EFFECTS OF STARVATION AND POINT OF NO RETURN DURING THE EARLY DEVELOPMENT OF THE PACIFIC RED SNAPPER *Lutjanus peru*

Renato Pena*, Ivette Moguel-Hernández, Gretchen Haro-Ballesteros, Mauricio Contreras-Olguín, Silvie Dumas, Laura Flores-Montijo

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The larval period is a critical phase in fish development where larvae are particularly vulnerable to starvation and predation which are the main regulators of larval survival. Particularly at the onset of the exogenous feeding after yolk exhaustion when larvae must start feeding on their own. If no food is available, starvation may induce severe behavioral, developmental and nutritional problems, leading to a deterioration point known as the point of no return (PNR) in which the larvae will no recover even when food is present and dead is imminent. The time to reach the PNR is species-specific. In culture conditions, the determination of the PNR and the evaluation and description of the effects of early starvation can be a valuable tool to identify larvae with starvation symptoms and it could also be useful in the evaluation of new or different culture conditions or prey types. Hence, the objective of the present study was to establish the PNR and to use different methods to evaluate and characterize the effects of starvation at the time of first feeding of Pacific red snapper *Lutjanus peru* larvae.

To study the effect of starvation on the early development of Pacific red snapper, mature wild-captured *L. peru* broodstock were hormonally induced to spawn in the laboratory. The viable eggs were placed in a 100L cylinder-conical white tank for incubation. Larvae were randomly sampled directly from the incubation tank at 0 (hatching), 24, 48, 72, 96 and 120 hours after hatching (hah). The PNR was estimated using the feeding incidence as reference. Different body proportions were measured in sampled larvae and histological analysis was performed. Biochemical analyses included the estimation of the digestive enzymatic activity (trypsin, cathepsin-like, amylase and lipase), and the concentration of proteins and free amino acids (FAA).

The first 48 hah were characterized by an active differentiation and growth which corresponded to the yolk-sac stage. The PNR occurred between 96 and 120 hah when the feeding incidence was less than half of the maximum incidence registered. After the exhaustion of the yolk sac and oil globule, steady degradation of the larval body was evident since all body segments decreased until the end of the study. The digestive tract reflected the effects of starvation by a gradual shrinkage of the enterocytes of the anterior and posterior intestines. Furthermore, the digestive enzymatic activity diminished during the starvation period compromising the digestive and absorptive capacity of the larvae. The free amino acids showed important fluctuation during the starvation period although the total concentration of FAA increased at 120 hah.

The results suggest that the morphometric and histological features of the larvae could be a useful indicator of their starved condition under culture conditions. Further studies are required to evaluate the recovery capacity of the larvae to early starvation.

METAGENOMIC ANALYSIS OF MICROBIOTA IN *Hippocampus ingens* JUVENILES IN CULTURE CONDITIONS

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The Pacific seahorse *Hippocampus ingens* is an economically important species in the Pacific coast of Mexico. Aquaculture represents a viable alternative to reduce the impact of fisheries on the natural populations of *H. ingens*. However, in order to improve and optimize the culture protocols and ecological relationships, an important step is to describe their associated microbiota.

To achieve this goal is essential to determine which microbial organisms conform the microbiota in healthy sea horses and which bacterial organisms can harm them. We were able to access *H. ingens* juveniles from a commercial producer (INGENS Cultivos Marinos) in Mazatlán, Mexico, in three health statuses: i) healthy (normal swimming and feeding activity), ii) disease (lethargic, non-feeding, and mostly in the bottom of the tank), and iii) post-disease (recovered from the disease stage). Five individuals were selected for each condition and euthanized with an overdose of anesthetic solution (4% phenoxyethanol). Individuals in every heath status were dissected in two main sections: cephalic and body (in the body section the digestive tract was dissected and analysed).

Cephalic and body sections from each health status were pooled and labeled as: cephalic healthy individuals (HCH); body healthy individuals (HBH); cephalic disease individuals (HCD); body disease individuals (HBD); cephalic post-disease individuals (HCpD); and body post-disease individuals (HBpD). Every pool was processed for total DNA extraction and the resulting extracted nucleic acids were sequenced for 16S rDNA V3 region. From all samples sequenced we kept 443854 high-quality paired-end-assembled reads and analyzed only those 415860 reads assigned to bacteria, based on the 16S ribosomal RNA. We determine the bacterial diversity, richness, and evenness in the hippocampus bacterial communities related to each condition (healthy, disease, and post-disease) and body section through rarefaction (interpolation) and extrapolation (R/E) sampling curves analysis.

The results showed differences related with the health status in two main groups: healthy (HCH, HBD) and disease related (HCD, HBD, HCpD, and HBpD). The rarefaction (interpolation) and extrapolation (R/E) sampling curve analysis showed a possible effect of health status over the bacterial community structures of the juveniles. The dominant phylum in samples from healthy sea horses was *Cyanobacteria* (49.6% - 54.8%). The dominant phylum for disease-related samples was *Proteobacteria* exhibiting a distribution from 61.5% to 85.4%.

The structural differences in the bacterial community between healthy and disease-related seahorses strongly suggest a direct effect of health status over a differential bacterial proliferation. However, more studies are required to determine how bacterial organisms are directly related to the health status of the Pacific seahorse juveniles

ALLOMETRIC GROWTH DURING LARVAL DEVELOPMENT OF PACIFIC RED SNAPPER *Lutjanus peru* IN CULTURE CONDITIONS

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Body segments related to primary functions like feeding and predator avoidance show a higher relative growth rate and differentiation during larval development. This type of growth is called allometric growth and has been reported in many fish species during larval development and is considered as an adaptive response to increase survival. As a result, the early ontogeny of fishes is characterized by drastic changes in every stage of the larval period. The objective of the present study was to evaluate the allometric growth during the larval stages of the Pacific red snapper to test the hypothesis of a differential growth during early development which can be useful to evaluate growth under different experimental culture conditions.

Larvae were obtained by spontaneous spawning of Pacific red snapper broodstock held under controlled conditions. From hatching until day 30, random samples of 10 to15 larvae were sampled and digitally photographed. On each digital photograph, the TL and several body proportions associated with feeding and locomotion were measured to the nearest 0.01 mm (Table1). Allometric growth patterns during the developmental stages were modeled by a power function of TL and the patterns in allometry were described by the growth coefficient (i.e. power function exponent, b) in the equation $Y = aX^b$. Isometric growth occurred when $b = 1$. A positive allometric growth occurred when $b > 1$ and a negative allometric growth when $b < 1$.

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GROWTH AND DEVELOPMENT OF PACIFIC SEAHORSE *Hippocampus ingens* IN CULTURE CONDITIONS

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Seahorses *Hippocampus sp*. are fishes with unique morphological and ecological characteristics that make them particularly susceptible to overfishing, bycatch and anthropogenic disturbances. The seahorse trade worldwide has been increasing in recent years and aquaculture has been proposed as an alternative to address unsustainable trade of seahorses for traditional medicine and ornamental industry. However, the lack of basic information regarding growth patterns and developmental stages hinders the advances in stablishing the optimal culture conditions.

The Pacific seahorse *Hippocampus ingens* is the sole species on *Hippocampus* found in the Pacific coast, with a range extending from California to southern Peru. It is most commonly associated with algae or rocks, and strongly associated with soft corals and coral reefs. In the present study we report the growth and development of juvenile *H. ingens* kept under culture conditions during the first 30 days of development.

Pacific seahorse juveniles were obtained from one spawn of *H. ingens* broodstock held under culture conditions in the commercial farm INGENS Cultivos Marinos in Mazatlán, Mexico. Juveniles were maintained in 50L plastic tanks. Seawater was treated with mechanical (5µm), biological and UV filtration. Culture conditions in the aquaria were maintained at a temperature of 24-27ºC, salinity of 33-35 ups, gentle aeration, a 12:12 photoperiod and O_2 saturation of 100%. Juveniles were fed with *Artemia* nauplii, prey density fluctuated from 5-10 nauplii/ml.

Every day, starting with the newly born juveniles (day 1) and until day 30, three juveniles were sampled, anesthetized with a 4% phenoxyethanol solution, weighted at the nearest 0.0001g and photographed with a digital camera. Standard length was calculated in every photograph using an image analysis software. Weight gain (WG), specific growth rate (SGR) and condition factor (CF) during the studied period were estimated as follows WG(%)=(Wf−Wi)/Wi×100; SGR(%)=((lnWf−lnWi)/t)×100; CF=(W/L)×100 where, Wf is the final weight (g),W is the weight (g),Wi is the initial weight (g), SL is the standard length (cm), and t is the duration (d).

Newly born juveniles showed an initial SL of 7.502 ± 0.121 (average ± std dev) mm and an average weight of 0.0010 ± 0.0001g. In the end of the study at day 30, the maximum SL was 32.658 ± 3.58 mm and an average weight of 0.0723 ± 0.0254g. Our results suggest the presence of two developmental stages, the first one during the first 14 days with a low SGR, WG and CF and the second during the last 16 days with a significant increase of these parameters. The specific growth rate during the period studied was 0.15 gr/day.

DEVELOPING MATHEMATICAL AND STATISTICAL THINKING IN THE CONTEXT OF AQUACULTURE

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Developing mathematical and statistical critical-thinking, problem-solving and analytical skills is of outermost importance in today's world. These skills have been set and summarized for students from kindergarten through 12th grade in the Common Core State Standards, and it is necessary to define the methods, materials and different strategies to support them. The present research focused on the implementation of didactic materials to assist the teaching/learning process of a statistics topic in high school students using aquaculture data.

This study consisted of a virtual implementation of six lessons about the measures of central tendency, using data from artificial reproduction of the long whiskered catfish (*Pimelodus grosskopfii*). Sequential situations from broodstock feed management, broodstock selection, fish condition factor, fish oocyte size distribution, selecting females for hormone injection based on oocyte distribution, and stocking of fingerlings were linked to concepts of random sampling, types of variables, the mean, median and mode for non-grouped and grouped data.

The research was performed at a high school institution in Neiva (Huila, Colombia), with students from two courses of ninth grade, using a qualitative approach. The methodology was a multiple case study, selecting three students from each course. Data collection methods included questionnaires, interviews, documents, and direct and indirect observations. The implementation of the didactic material included animated videos that allowed the introduction to the lessons as initial motivation; power point presentations that included the contextualized aquaculture situation; diagnostic tests to determine previous knowledge, and post-evaluation test to determine acquired knowledge, using Google forms; and finally, homework that was submitted after each lesson.

Five (5) types of mathematical standards were evidenced in each of the sessions, including: 1) Number and operations thinking; 2) measurement and data systems; 3) operations and algebraic thinking; 4) Rations and proportional relationships; and 5) Statistics and probability. The virtual implementation of this statistics manual in the context of aquaculture was of interest to students, and it may highlight the importance of applying real context situations and the usefulness of mathematics in students' daily life.

SHELL COVER, RUGOSITY, AND TIDAL ELEVATION IMPACT NATIVE AND NON-INDIGENOUS OYSTER RECRUITMENT TO CONCRETE AND AFFECT REEF BALL DESIGN

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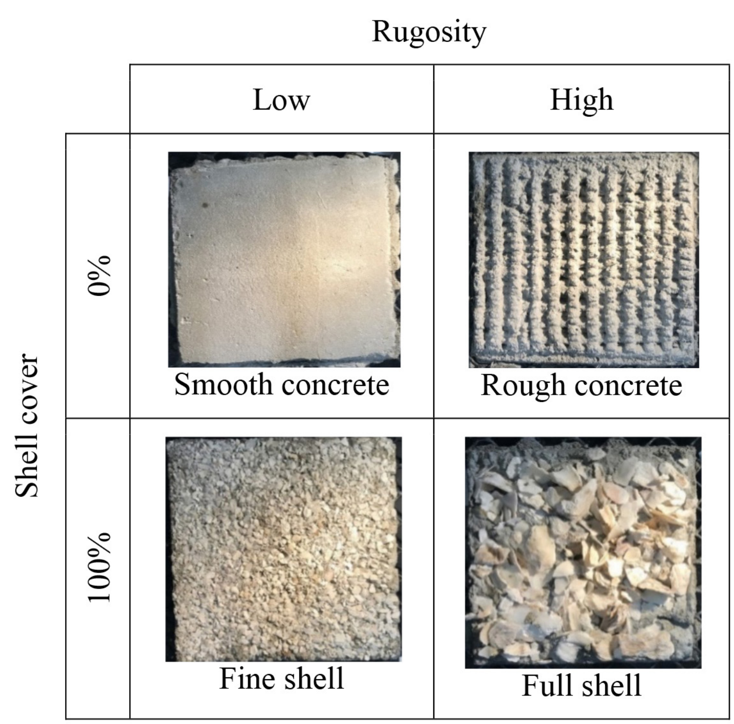
Estuaries have been armored with artificial habitat to protect coastal infrastructure from erosion, but armoring has negative ecological impacts. Other shoreline protection strategies, such as eco-engineered seawalls and living shorelines, offer more natural, rugose substrata to native species while limiting coastal erosion. Concerns about recruitment of non-indigenous species (NIS) call into question whether eco-engineered structures can be better designed to foster native communities. In southern California, USA, we explored whether concrete reef balls that recruit native Olympia oysters, *Ostrea lurida*, could be engineered to avoid recruitment of the NIS, *Magallana (*formerly *Crassostrea) gigas*.

We modified concrete tiles acting as proxies for reef balls with added shell cover and rugosity to determine if there was a treatment that favored native species recruitment. We deployed four treatment types (n=7 replicates per treatment) embedded into 15 x 15 cm concrete tiles: two with surface shell (100% cover of crushed or large, rugose shell fragments) and two without shell (smooth or rugose concrete) at two sites in San Diego Bay and one site in Newport Bay, California, at two tidal elevations (0 and 0.6 m MLLW) from May to September 2018.

O. lurida recruited in generally higher percent cover and abundance than *M. gigas* onto all treatments across all sites at 0 m MLLW and treatments that combined 100% shell cover with high rugosity at two of three sites at 0.6 m MLLW, a tidal elevation to which *O. lurida* rarely recruits. Recruitment strength of both native and non-indigenous oysters showed remarkable context-dependency, varying across sites and treatments, but a generalized recommendation emerged: projects that utilize reef balls across the range of the *O. lurida* should explore adding shell cover and rugosity to the concrete to favor native *O. lurida* and discourage non-indigenous *M. gigas*.



THE VELELLA EPSILON PROJECT: PIONEERING OFFSHORE AQUACULTURE IN THE SOUTHEASTERN GULF OF MEXICO; CHAPTER 4 – FINALIZING PROJECT PERMITTING AND PREPARING FOR DEPLOYMENT

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The Velella Epsilon Project is an extension of previous projects (Velella Beta-test and Velella Gamma Project) which demonstrated small-scale offshore marine fish culture in the waters of Kona, Hawaii. The Velella Epsilon (VE) Project will adapt these technologies to Gulf of Mexico (GOM) waters, while pursuing two simultaneous efforts: (a) permitting and deployment of a research-scale, demonstration net pen in Federal waters, and in tandem, (b) navigating the commercial permitting process to obtain a commercial offshore aquaculture permit in the GOM, while documenting this effort in a Manual for Aquaculture Permitting Pathway (MAPP).

The VE Project focuses on a small, pilot-scale (single net pen) aquaculture system where up to 20,000 almaco jack (kampachi; *Seriola rivoliana*) fingerlings would be reared for approximately 12 months in Federal waters approximately 40 miles west southwest of Sarasota, Florida. We expect to yield approximately 17,000 fish (85% survival rate) with a final fish size of approximately of 4.4 lbs/fish. An estimated final maximum harvest weight of 74,800 pounds [lbs] whole weight is anticipated. These fish will be landed in Florida, marketed, and sold to state- and Federally-licensed dealers, in accordance with state and Federal law.

The VE Project will lay the groundwork for wider acceptance of commercial aquaculture in the GOM region by: (1) Serving as a platform for the promotion of rational aquaculture policies and demystification of the industry, by providing a working net pen example to politicians, constituents, journalists, and other influencers of policy or public perceptions, as well as the local community; (2) Increasing public awareness of, and receptivity towards, offshore aquaculture and the need to culture more seafood in U.S. waters, by providing public tours of the offshore operation, including (possibly) snorkeling inside the net pen, and fee fishing; (3) Acting as a demonstration platform for data collection of water quality, potential benthic impacts, and marine mammal and fish stock interactions resulting from offshore aquaculture in the GOM; and (4) Providing local recreational, charter, and commercial fishing communities with evidence of the benefits of aquaculture, through the fish attraction device (FAD) effects of the project, and by documentation of fish aggregation and fishing boat activity around the VE Project.

Chapter 4 – Finalizing Project Permitting and Preparing For Deployment - will walk us through the fourth year's experiences and achievements of finalizing the permitting process while collaborating with net pen manufacturers and engineering firms to evaluate a demonstration size net pen system that is representative of, and scalable to, a commercial farm scale system.

IMPACTS OF POLYUNSATURATED ALDEHYDES ON LARVAL BIVALVES

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Recent water quality issues led to slowed growth, increased morphological abnormalities, altered feeding behaviors, and mortality among bivalve larvae being reared at an East Coast shellfish hatchery. The potential toxin was identified as a polyunsaturated aldehyde (PUA) which belongs to a class of compounds known as oxylipins. Oxylipins are bioactive secondary metabolites produced by a variety of organisms including plants, mosses, fungi, and algae. They are derived from a number of fatty acid precursors via lipoxygenase-based pathways. Oxylipins have been well characterized in marine diatoms and several studies have documented the pathological impact they have on zooplankton grazers and other phytoplankton. In contrast, relatively few studies have examined the effect of oxylipins on marine bivalves.

In this study, larvae of two bivalve species, *Crassostrea virginica* and *Mytilus edulis*, were exposed to two polyunsaturated aldehydes, 2,4-decadienal and 2,4-heptadienal. During the experiment, larvae were reared in filtered seawater containing a non-diatom diet composed of a mixture of T-Isochrysis and Pavlova. Decadienal and heptadienal treatments were applied shortly after the larvae had progressed to the D-stage with subsets of larvae being reared at 6 different concentrations of each chemical. The effect of these two PUAs upon larval feeding, morphological development, growth, and survival were monitored over a period of five days post-exposure. While previous studies have examined the effects of PUAs on bivalve hemocyte cell cultures, this is the first to investigate the range of concentrations over which these toxins impact larval bivalves.

ADVANCING TELEOST GENETIC ENGINEERING CAPABILITIES THROUGH THE DEVELOPMENT OF FISH VIRAL VECTOR TECHNOLOGy

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While new fish genetic engineering tools continue to be developed, several key technological roadblocks exist when working with fish species that are not found in mammalian organisms. This has limited some genetic engineering applications in fish that are commonplace in fields such as biomedical research. One of these technological obstacles is the lack of quality fish viral vector technology for which to transfer genetic material into fish cells and tissues. Mammalian viral vector systems based on lentivirus, adenovirus, and adeno-associated viruses (AAV) are highly developed and routinely used for basic biology and gene therapy by thousands of researchers around the globe. Toolkits for these viral vectors allow even inexperienced laboratories to perform complex genetic engineering experiments. Efforts to adopt viral vector technology has often focused on trying to adopt mammalian vector technology by using high viral titers to overcome otherwise poorly compatible tropism between the two distantly related vertebrate species. Fortunately, engineering fish specific viral vector technologies can utilize common viral genome engineering techniques that are common in both the virology and human gene-therapy fields.

In an effort to bring this powerful technology to common use in aquaculture we are developing fish specific viral gene delivery vectors that can naturally infect fish cells and tissues. We are utilizing two main approaches to developing these viral gene delivery systems. First, common mammalian viral vectors are being pseudotyped with fish viral glycoproteins to provide enhanced fish specific transduction capabilities for these widely used systems. The second approach involves re-engineer the genomes of potent fish viral pathogens to utilize them as gene delivery vectors. We are evaluating the potential applications of this technology for use on cultured cells, single cell embryos, gametes, or for tissue specific genetic engineering applications using salmonids (*Oncorhynchus spp.*) as a model system. The technology is being developed for both the long-term transfer of genetic material as well as for transient expression of CRISPR genome editing components to induce targeted genetic modifications. We believe that developing easy to use viral gene delivery vectors will significantly increase the pace of discovery in the aquaculture research field enabling new genetic engineering applications that are not possible today.

OHNOLOG SPECIFIC GENE EDITING REVEALS FUNCTIONALIZATION PATTERNS IN THE ACTIVIN/MYOSTATIN SIGNALING PATHWAY IN RAINBOW TROUT *Oncorhynchus mykiss*

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The growth and development of metazoan tissues is regulated by the activin receptor signaling pathway, a member of the larger transforming growth factor-beta superfamily. The activin receptor signaling pathway is especially important for embryonic development, reproductive development, and muscle growth. Myostatin, perhaps the most well-known activin receptor signaling pathway is ligand, is a major negative regulator of muscle growth in vertebrates. However, in mammals myostatin functions in coordination with other ligands such as activin A to regulate muscle size, representing less than half of the total activin receptor signaling pathway muscle inhibitory functions. While myostatin has shown similar function in fish the role of the broader activin receptor signaling pathway in regulating muscle mass is poorly understood. Defining the function of this pathway is complicated in fish since they retain many duplicated gene ohnologs, which are the result of one or more whole genome duplication events.

To gain insight into the potential function and diversification of the activin receptor signaling pathway in fish we used rainbow trout (RBT, *Oncorhynchus mykiss*) as a model system. Salmonids like RBT have genomes characterized by two whole genome duplication events, one ancestral teleost specific (Ts3R) event that is found in all teleost fish species, and a second more recent salmonid specific (Ss4R) whole genome duplication event that occurred in the salmonid lineage. We investigated the evolution of the activin receptor signaling pathway across these two whole genome duplication events by analyzing the molecular phylogeny and expression profile of 53 activin receptor signaling pathway genes across 23 adult tissues to develop a detailed expression atlas for the pathway. Our gene expression atlas includes all known duplicated gene ohnologs for ligands, receptors, inhibitors and signal transducers of the pathway. The gene expression atlas revealed unique insights into the evolution of duplicated genes in rainbow trout suggesting evidence of both subfunctionalization and neofunctionization of the genes after duplication.

The functional consequence of gene duplication was further examined by selectively targeting the four ohnologs of activin A using CRISPR genome editing technology and examining changes in signaling pathway dynamics in skeletal muscle. Our results identified key interactions between activin receptor signaling pathway members in response to perturbation of activin A signaling, which provides insights into the diverse functions adapted by activin A after gene duplication. The findings highlight how teleost whole genome duplication events facilitate diversification and redundancy of gene function and how the complicated genome structure of fish is an important consideration when designing genetic enhancement efforts.

THE WASTE MANAGEMENT OF LARGE-SCALE RECIRCULATING AQUACULTURE SYSTEMS AND POTENTIAL VALUE-ADDDED PRODUCTS FROM THE WASTE STREAM

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Recirculating Aquaculture Systems (RAS) is an emerging technology that is capturing the attention of the field of aquaculture due to its ability to produce high-quality seafood using sustainable models. This study explored the waste management of RAS, specifically further treatment technologies, and the potential value-added products from the RAS waste stream that can be reused. Advanced, tertiary, or down-stream treatment technologies available for RAS were investigated through a literature review. Utilizing the constant comparative method and semi-structured interviews with stakeholders in RAS, opinions and attitudes were collected about advanced treatment of RAS waste and reuse opportunities presented from value-added products generated from the waste stream. Collected data provided insight on the current state of waste management of RAS and how RAS waste management may develop over time.

This study confirmed that all RAS users intend to expand their waste management methods to incorporate reutilization of waste, whether by assimilating nutrients back into their respective system or by producing value-added products for other markets. RAS waste is a resource, holds economic value, and will be reutilized. Enhanced RAS waste management must be cost effective to the farmer or publicly subsidized if the public demands the result. The motivations behind further reuse of generated waste, typically, stems from the scale of the farm. The findings reflect that large-scale RAS will reutilize their waste in an effort to reduce costs of getting rid of the waste, whereas smaller scale farms will reutilize their waste to reincorporate valuable resources back into the farm. The major recommendation from this study is for RAS farms to work directly with agricultural and energy management groups to develop efficient waste management strategies and utilize useful synergies. With better defined best practices and increased co-management between aquaculture, energy, and agriculture, RAS will continue to increase its contribution to the seafood supply chain.

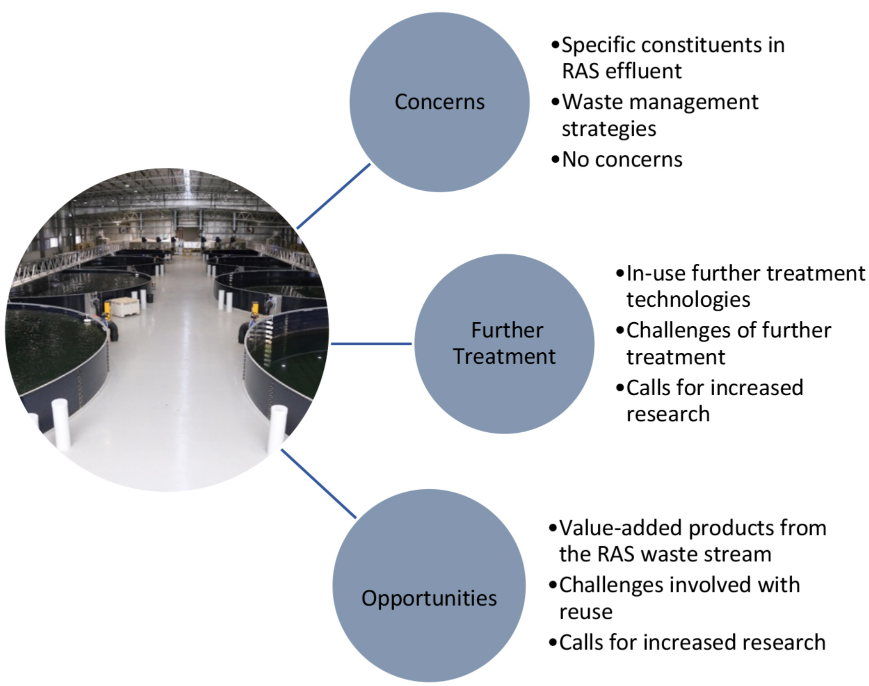

Figure - Overarching themes resulting from the semi-structured interviews on RAS waste management. The figure displays the three overarching themes: Concerns, Further Treatment, and Opportunities. It also briefly describes what each theme entails.

REPLACING SOY PROTEIN WITH INSECT MEAL IN A FISHMEAL FREE DIET FOR ATLANTIC SALMON (SALMO SALAR)

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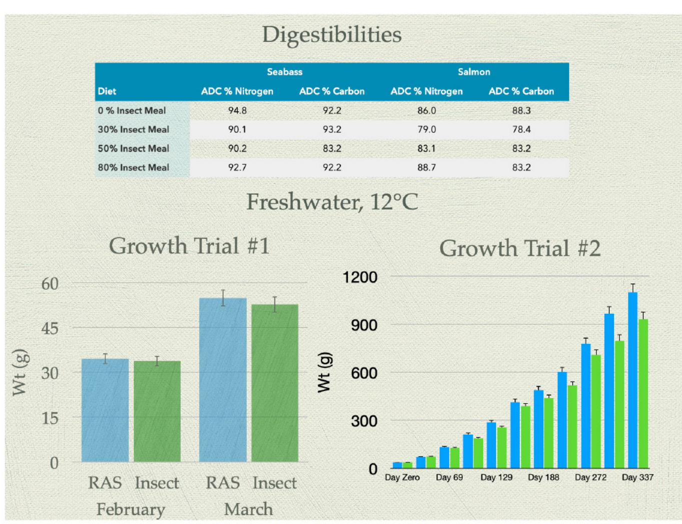
In the coming years, conventional agriculture will fall short of meeting global protein requirements. Today, fishmeal is considered a gold standard protein ingredient in pet food and livestock feed due to its amino acid composition and digestibility characteristics. However, the global supply of fishmeal is capped at 5-7M tons per year because there are limited fish in the sea. Volatile prices and environmental concerns have left many investors and consumers seeking alternatives to fishmeal, a market worth $9.5B annually. Insect farming could help fill growing gaps in agricultural supply chains. Many commonly farmed insects boast fast growth cycles, high fecundity, minimal water requirements, and an ability to consume a variety of agricultural byproducts and waste streams. Research trials have shown that the amino acid profiles and digestibility of these species could make them an ideal ingredient in the $25B pet food market and the $100B aquaculture feed industry.

Prior work from the Place laboratory (Watson et al. 2013a; Watson et al. 2013b, Watson et al. 2014) has shown complete fishmeal/fish oil replacement is possible through judicious choice of ingredients, most importantly with the inclusion of taurine when using plant protein sources. The Place laboratory has worked with **Instar Farms** to investigate the possibility of using mealworms (*Tenebrio molitor)* as replacement for fishmeal. This work has resulted in a shortening of the production schedule, an improvement in the amino acid content of the insect meal, and establishment of secondary products from the waste material (e.g. chitin). This collaboration has resulted in a clear feasibility in replacing all the soy protein in a commercial diet (25% by weight) for Atlantic salmon. Unfortunately, the current production level for *T. molitor* would severely restrict its wide acceptance as an aquaculture feed ingredient. A new commerical partner, **Ovipost,** grows superworms (*Zophobas morio)* which alleviates this bottle neck because of higher production yields. I will present data on using these insects in attaining a fishmeal/soybean free aquaculture diet for salmon.



Digestibilities

	Seabass		Salmon	
Diet	ADC % Nitrogen	ADC % Carbon	ADC % Nitrogen	ADC % Carbon
0 % Insect Meal	94.8	92.2	86.0	88.3
30% Insect Meal	90.1	93.2	79.0	78.4
50% Insect Meal	90.2	83.2	83.1	83.2
80% Insect Meal	92.7	92.2	88.7	83.2

AQUACULTURE PLANNING - DETERMINATION OF BIOLOGICAL AND PHYSICO-CHEMICAL PARAMETERS TO ASSESS THE POTENTIAL OF FISH AND SHELLFISH OFFSHORE AQUACULTURE

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Aquaculture is a rapidly growing sector and offshore production of marine species is essential to keep up with demand, increasing production and reduce imports in European countries. In this study we focus on Portuguese coast that has several geographical factors that allow the implementation of more production units. The physicochemical parameters (temperature, dissolved oxygen, salinity, pH, ammonia, nitrite, silica and chlorophyll *a*) along the Portuguese coast were analysed to evaluate the potential of increasing production of some species with economical interest as marine fishes already produced in the country: gilthead seabream *Sparus aurata*, sea bass *Dicentrarchus labrax,* meagre *Argyrosomus regius* and greater amberjack *Seriola dumerili*, or even initiate the rearing of additional species with high interest, such as the sardine *Sardina pilchardus* or salmon *Salmo salar*. The potential of increasing bivalves' production as mussel *Mytilus edulis*, scallop *Pecten maximus*, oyster *Crassostrea gigas, Crassostrea angulata* and clam *Ruditapes decussatus* was also analysed. The water samples were collected in 5 different areas of the Portuguese coast (area A is northernmost area, B in the centre-north, C in the centre, D the southernmost zone of the west coast and zone E in the south) in two oceanographic campaigns of the project Aquimar in 2018 and 2019. The currents data were collected between 2018 and 2020 and waves height data were collected from 1988 to 2019 by the Hydrographic Institute. According to the results, there is a wide variety of species that can be considered for production. Area A presented the lowest temperatures in both campaigns, which leads us to consider the potential of production of some species as salmon *Salmo salar*, mussel *Mytilus edulis* and scallop *Pecten maximus*, location B and C reveal some potential to implement salmon *Salmo salar* and oysters due to lower temperatures and the highest concentrations of silica. Location D (where gilthead seabream and sea bass are already produced) seems suitable for oyster production and location E for the implementation of more fish production as meagre *Argyrosomus regius*, sea bream *Sparus aurata*, sea bass *Dicentrarchus labrax,* sardine *Sardina pilchardus* and greater amberjack *Seriola dumerili* due to the highest temperatures from all locations. Although this study shows that areas A, B and C have the harshest oceanographic conditions and the areas D and E appear to be more suitable to implement aquaculture structures, site selection for aquaculture is a complex process and pilot studies are essential to analyse the feasibility of developing offshore aquaculture along more sites of the coast of Portugal.

Acknowledgements

This study had the financial support of Operational Programme MAR2020 through the project MAR–02.01.01–FEAMP-0107 "AQUIMAR - Marine knowledge supporting aquaculture", and the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UIDB/ 04292/2020 granted to MARE-Marine and Environmental Sciences Centre. AP was supported through the Scientific Employment Stimulus Programmes (CEECINST/00051/2018).

REPRODUCTIVE CYCLE OF THE INVASIVE ASCIDIAN *Styela plicata* IN MUSSEL *Mytilus edulis* AQUACULTURE

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Mussel farming has faced challenges such as the establishment of non-indigenous species that may become invasive (NIS) in the farming environment. These introductions are occurring at an accelerated rate and fouling NIS can have a relevant impact in mussel production by altering several variables related to the welfare of farmed animals, such as mechanical interference with shell function and competition for food and space. Some NIS represent a particularly significant impact for aquaculture, including tunicates that clearly can have a negative effect on mussel productivity by reducing the mussels' growth rates and causing severe mortality of the reared mussels. The tunicate *Styela plicata* is a biofouling ascidian widely distributed, which is associated with damages caused in aquaculture. This study aims to study the reproductive biology of the ascidian *S. plicata* present in mussel cultures in the Albufeira coastal lagoon in Portugal, thus contributing to a better understanding of how environmental variables such as temperature and salinity can influence its development and establishment. The reproductive cycle of the ascidian *S. plicata* was characterized through the evaluation of the gonadosomatic index (GI). The histological analysis of gonads was performed monthly (n=30) over a 14-month period to identify the gametogenic stages of the gonads of individuals collected from a mussel farm raft located in the Albufeira coastal lagoon. GI was highest in summer, when the highest temperatures and salinities were also recorded. No clear seasonal reproductive pattern was observed since mature gametes were present throughout the year. Spawning can occur continuously, although more pronounced in late summer and early autumn. The present study confirmed the occurrence of an established populations of *S. plicata* in the Albufeira coastal lagoon, since the individuals analysed can be fertile all year-round. A prolonged reproductive period can confer a competitive advantage for *S. plicata*, which guarantees the permanence in areas that have already been invaded and offers the opportunity to expand its area of occurrence. These results suggest that additional studies on the dynamics of this population are needed, especially to clearly identify the time of greatest settlement of larvae, so that actions to mitigate biofouling may occur. It can be concluded that the current environment of the lagoon offers advantages for *S. plicata*. The temperature and salinity of the lagoon do not exceed the tolerance limits for the reproduction of the species and the low hydrodynamics of the lagoon favours the survival and the successful establishment of this tunicate. Thus, mussel farming in the Albufeira coastal lagoon is threatened and that mitigation measures need to be implemented. The presence of biofouling in marine aquaculture represents one of the main barriers to efficient and sustainable production and its control is a challenge for which solutions are still being searched.

Acknowledgements
This study had the financial support of Operational Programme MAR2020 through the project MAR-02.02.01-FEAMP-0004 (ProtectInvad - Protection against Invasions in Coastal Aquaculture Systems) and the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UIDB/ 04292/2020 granted to MARE-Marine and Environmental Sciences Centre. AP and PC were supported through the Scientific Employment Stimulus Programmes (CEECINST/00051/2018 and 2020.01797.CEECIND, respectively).

EFFECTS OF DIFFERENT DIETS ON THE SURVIVAL AND GROWTH OF THE SEA CUCUMBER *Holothuria arguinensis* HATCHERY-REARED JUVENILES

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The growing demand for sea cucumber products constitutes a continuing threat to wild stocks and to the habitats in which they live in. A possible way to mitigate the detrimental effects economic demand has on ecosystems around the world is to increase supply, as such, aquaculture stands as an emerging alternative. A promising species for future European aquaculture production, with high commercial interest, is the sea cucumber *Holothuria arguinensis*. With a natural distribution along the North-Eastern Atlantic, including the coast of Portugal, this species is already showing a decrease in wild populations due to unregulated exploitation. With the implementation of successful larval rearing and settlement, more emphasis needs to be placed on the on-growing phase of juveniles. The optimisation of the diets and feeding of *Holothuria arguinensis* juveniles to reduce their mortality and increase growth performance has not yet been studied. This species feeds on the sediment and studies with other detritivorous species point to the inclusion of macroalgae as part of the diet. In this regard, the following study aimed to assess growth and survival rate of hatchery-reared *H. arguinensis* juveniles (n=324; mean weight: 20±7 mg; mean length: » 1 mm). Individuals were distributed in 3 RAS, each with three 50 L tanks with sandy substrate and were fed during 6 months with three diets (Diet 1 = *Rhodomonas baltica* + *Chatoceros calcitrans*; Diet 2 = *Rhodomonas baltica* + *Saccorhiza poliscides* and Diet 3 = *Rhodomonas baltica* + *Chaetoceros calcitrans* + *Saccorhiza poliscides*) administered 3 times a week. *H. arguinensis* fed with diet 2 showed the highest survival rate (84.26%) when compared with individuals fed with diet 1 (59.26%) and diet 3 (40.74%). Juveniles fed with diet 2 and diet 3 showed a significantly higher final weight (diet 2: 0.57±0.15 g and diet 3: 0.48±0.05 g) and a higher length (diet 2: 1.9±0.42 cm and diet 3: 1.7±0.09 cm) than individuals fed with diet 1 (0.27±0.08 g and 1.1±0.24 cm). Specific growth rate (SGR) was higher in juveniles fed with diet 2 (9.23 % month^{-1}) than in juveniles fed with the diet 1 (4.11 % month^{-1}) or with the diet 3 (7.60% month^{-1}). The addition of the macroalgae *S. poliscides* in the *H. arguinensis* juvenile diet shows potential benefits on growth. At the end of the trial juveniles whose diet was supplied with *S. poliscides* showed an increased growth observed with a higher final weight, length and SGR when compared to individuals without this macroalgae in their diet. Results were most promising particularly with diet 2 where there was a higher survival rate. To optimize sea cucumber *H. arguinensis* rearing more trials should be conducted with hatchery-reared juveniles comparing sediment enrichments, the efficacy between macroalgae diets, the production with fishfarm waste or the growth in integrated multi-trophic aquaculture systems (IMTA).

Acknowledgements
This work was financed by the Operational Program Mar2020 nº MAR-02.01.01-FEAMP-0052 "Newcumber - Advances for the sustainable rearing of sea cucumbers". It received further financial support from Fundação para a Ciência e a Tecnologia (project UIDB/04292/2020), A.C. Brito with the Scientific Stimulus Programme – CEECIND/00095/2017, A. Pombo through the Scientific Employment Stimulus Programme CEECINST/00051/2018 and F. Azevedo e Silva with the individual research grant 2020.09563.BD.

AQUACULTURE PLANNING - DETERMINATION OF BIOLOGICAL AND PHYSICO-CHEMICAL PARAMETERS TO ASSESS THE POTENTIAL OF FISH AND SHELLFISH OFFSHORE AQUACULTURE

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Aquaculture is a rapidly growing sector and offshore production of marine species is essential to keep up with demand, increasing production and reduce imports in European countries. In this study we focus on Portuguese coast that has several geographical factors that allow the implementation of more production units. The physicochemical parameters (temperature, dissolved oxygen, salinity, pH, ammonia, nitrite, silica and chlorophyll *a*) along the Portuguese coast were analysed to evaluate the potential of increasing production of some species with economical interest as marine fishes already produced in the country: gilthead seabream *Sparus aurata*, sea bass *Dicentrarchus labrax,* meagre *Argyrosomus regius* and greater amberjack *Seriola dumerili*, or even initiate the rearing of additional species with high interest, such as the sardine *Sardina pilchardus* or salmon *Salmo salar*. The potential of increasing bivalves' production as mussel *Mytilus edulis*, scallop *Pecten maximus*, oyster *Crassostrea gigas, Crassostrea angulata* and clam *Ruditapes decussatus* was also analysed. The water samples were collected in 5 different areas of the Portuguese coast (area A is northernmost area, B in the centre-north, C in the centre, D the southernmost zone of the west coast and zone E in the south) in two oceanographic campaigns of the project Aquimar in 2018 and 2019. The currents data were collected between 2018 and 2020 and waves height data were collected from 1988 to 2019 by the Hydrographic Institute. According to the results, there is a wide variety of species that can be considered for production. Area A presented the lowest temperatures in both campaigns, which leads us to consider the potential of production of some species as salmon *Salmo salar*, mussel *Mytilus edulis* and scallop *Pecten maximus*, location B and C reveal some potential to implement salmon *Salmo salar* and oysters due to lower temperatures and the highest concentrations of silica. Location D (where gilthead seabream and sea bass are already produced) seems suitable for oyster production and location E for the implementation of more fish production as meagre *Argyrosomus regius*, sea bream *Sparus aurata*, sea bass *Dicentrarchus labrax,* sardine *Sardina pilchardus* and greater amberjack *Seriola dumerili* due to the highest temperatures from all locations. Although this study shows that areas A, B and C have the harshest oceanographic conditions and the areas D and E appear to be more suitable to implement aquaculture structures, site selection for aquaculture is a complex process and pilot studies are essential to analyse the feasibility of developing offshore aquaculture along more sites of the coast of Portugal.

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IMPACT OF GONAD SIZE, STRUCTURE AND MATURITY LEVEL IN COLOR AND TOTAL CAROTENOIDS CONTENT OF SEA URCHIN ROE

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Sea urchin roe is a seasonal seafood highly appreciated worldwide as a gourmet product called *uni*. The overexploitation status of several wild stocks has been driving the development of echinoculture as an alternative to meet the growing market demand. The market value of the sea urchin roe depends of several quality standards, such as, size, color, texture, and flavor. Sea urchins fed with dry pelleted diets produce high gonad yield, but other quality standards such as the bright orange color have been difficult to achieve. The color of the gonads depends on its content of carotenoids which is influenced by several factors including sea urchin species, diet and gonad structure and size. However, the mechanisms associated to gonad color enhancement is still poorly known. The present study aimed to analyze the evolution over time of the total carotenoids (TC) content and color attributes of *Paracentrotus lividus* sea urchin gonads in relation to gonad somatic index and maturity level. The urchins were fed for 24 weeks with three experimental diets formulated with practical ingredients of different sources (fishmeal-based, algae-based, and plants-based sources) and equally supplemented with β-carotene. Gonad TC and color were analyzed using spectrophotometric and colorimetric (CIE L^*, a^* and b^* color parameters) methods and the color was additionally scored using a four levels visual scale. The correlation between gonad carotenoid content, gonad biometric parameters and color attributes was analyzed considering possible sex-related differences in gonad structure and carotenoid deposition mechanism. Our results showed that the carotenoids content and color attributes of sea urchins' gonads were mainly influenced by sex and gonad maturity level. While TC decreased with increasing gonad size for both females and males, the females presented always higher percentage of bright yellow and orange gonads. These results showed that carotenoids metabolism and storage mechanism in the females beneficiates the achievement of the color demand by the market, independently of dietary carotenoids source. The relatively high proportion of ovaries presenting grade I after 24 weeks trial also indicates that the residence time of carotenoids in the ovaries might be higher than in the testes. The reduction of total carotenoids content in the gonads through time, showed that the effectiveness of dietary β-carotene supplementation in dry pelleted diets is very important to increase the proportion of grade I gonads. Further studies in the β-carotene metabolism are necessary to improve the bioavailability of this pigment and its metabolization into echinenone to be stored in the gonads.

Acknowledgements

This study had the financial support of Operational Programme MAR2020 through the project 16-02-01-FMP-0004, and the support of FCT, through the strategic project UIDB/ 04292/2020. JP was supported by the Integrated Programme of SR&TD Centro-01-0145-FEDER-000018. AP was supported through the Scientific Employment Stimulus – Institutional Call - (CEECINST/00051/2018).

INSIGHT INTO INTERACTIONS BETWEEN THE MAJOR OYSTER PATHOGENS *Haplosporidum nelsoni* AND *Perkinsus marinus* FROM LONG-TERM DISEASE DIAGNOSTIC DATA FROM VIRGINIA

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Haplosporidium nelsoni (MSX) and *Perkinsus marinus* (dermo) co-occur in *Crassostrea virginica*, overlapping in seasonal patterns of infection and sharing host tissue space. We examined the prevalence and intensity of these two common microparasites over time to better understand multi-pathogen interactions and patterns of co-infection. Diagnostic data from 2007-2020 for individual oysters across the Virginia portion of the Chesapeake Bay were analyzed to determine whether any correlations occur between parasite prevalence and intensity. We found a weak positive association between advanced infections of dermo and higher intensity MSX infections in individual oysters in the Chesapeake Bay, but an opposite trend in co-infected oysters from the seaside Eastern Shore. Analyses of sample prevalences of both parasites from 1989-2019 revealed a positive correlation between MSX and dermo prevalences. Collectively, these data do not point to strong contemporary interactions between the two pathogens infecting oyster hosts. Interestingly, however, the sample prevalence analyses revealed eight outlying data points, all of which were high MSX-low dermo prevalence samples from before 2007 (marked by triangles in Fig. 1). Notably, these were all from reefs of low oyster abundance, a suggestion that MSX may have benefitted at times from the decrease in dermo caused by low host density unfavorable to directly transmissible *P. marinus*. While contemporary interactions between the two pathogens would appear to be modest at best, these data do suggest that the intensification of dermo since the 1980s may have historically suppressed MSX infections, and that this multiparasite-host system may have reached something of a stable state after earlier decades of more intense interaction and coevolution.

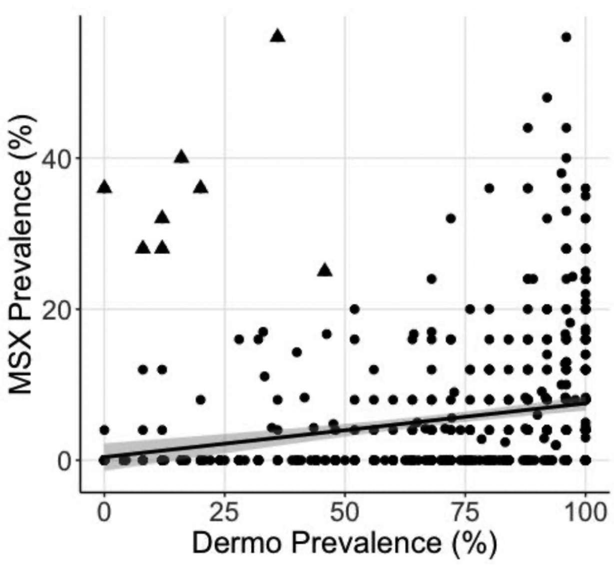

Figure 1. Total percent prevalence of parasites in oysters from 1989-2019. Data points represent the percent of oysters with MSX and/or dermo at a single site and year. Triangles indicate data points with high MSX and low dermo prevalence from low abundance oyster reefs. Adjusted R^2 = 0.04858.

PHENOTYPIC CHARACTERIZATION OF *Edwardsiella piscicida* ISOLATES FROM CATFISH AQUACULTURE

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Catfish industry is the largest aquaculture sector in the U. S. contributing 74% of the total finfish production. Hybrid catfish (♀ channel catfish (*Ictalurus punctatus*) × ♂ blue catfish (*I. furcatus*)) is preferred in intensive production systems due to its superior production traits. Edwardsiellosis in catfish (Figure 1) caused by the gram-negative bacteria, *Edwardsiella piscicida* is a major threat causing significant production losses. Food-sized hybrid catfish are more vulnerable to *E. piscicida*, causing significant economic burden as most of the production costs would have been incurred at this stage. Although the archived *E. piscicida* isolates (2013-2018) have been categorized into five genetic clades, these are not phenotypically profiled. Phenotypic characterization of these genetically and temporally distinct *E. piscicida* isolates will provide relevant insights on their adaptations to changing environments and resources. This study analyzed the growth characteristics of representative *E. piscicida* isolates at different incubation temperatures. In addition, the salt tolerance, nutrient preferences, motility, and aerotolerance of these isolates were evaluated. Growth of the bacterial strains was significantly low at 12ºC and 45ºC when compared to other tested temperatures. Trypticase soy agar (TSA) and Mueller-Hinton agar with 5% blood supplementation significantly augmented bacterial growth (Figure 2) compared to *Salmonella- Shigella* and MacConkey agar. Growth of *E. piscicida* isolates were significantly inhibited in media with >3% salt levels. The studied bacterial strains revealed a facultative anaerobic growth pattern in thioglycollate broth. No significant phenotypic variations among the studied isolates representing different genotypes were observed. Further studies of bacterial isolates (temporally and spatially distributed) covering a much wider geographical area and host species will provide pertinent information on these deleterious pathogens. A comprehensive phenotypic characterization will be beneficial during disease diagnosis and to develop efficient management strategies against these bacteria.

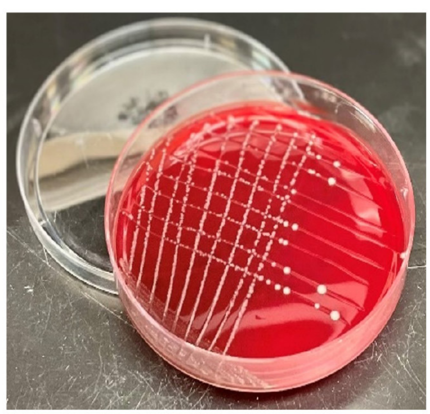

Figure 1. The characteristic "hole in the head" in hybrid catfish caused by *Edwardsiella piscicida* infection. Inset: Electron micrograph of *E. piscicida*. Figure 2. White, punctate, and slightly hemolytic *E. piscicida* colonies on TSA plate supplemented with 5% sheep blood.

ADVANCES IN UNDERSTANDING SOYBEAN MEAL INDUCED DISTAL ENTERITIS IN SALMONIDS

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Despite the promising amino-acid profile of soybean meal, inflammatory reactions arising from its inclusion in aquafeeds becomes a limiting factor to increased utilization of this alternative plant-based ingredient in salmonids. Numerous anti-nutritional factors commonly present in plant-based ingredients have been recognized and studied as potential causes for the inflammatory effects related to soy-induced distal enteritis. Efforts to address poor performance using plant-based diets include selection in a strain of rainbow trout (*Oncorhynchus mykiss*) on a 100% plant-meal based feed containing 19.6% SBM and 25.6% soy protein concentrate. In addition to improved growth performance, this selected strain has shown concomitant resistance to the development of distal enteritis and evidence of increased oral tolerance to SBM. Several recent collaborative studies between the Hagerman Fish Culture Experiment Station and Bozeman Fish Technology Center highlight work that has been done to characterize soybean meal induced distal enteritis and develop a clinical model for early detection, progression, and amelioration of adverse effects.

Resistance to distal enteritis observed in the Hagerman selected-strain rainbow trout provide an excellent contrast and model for further characterization of acute mucosal inflammatory responses involving IL-17 paralogs, other interleukins, growth factors, calcium-binding proteins and other immunogenic markers. Additional studies have also characterized induction of T helper17 cells and the repression of T regulatory cells involved in oral tolerance as well as changes to gut histology and microbiota.

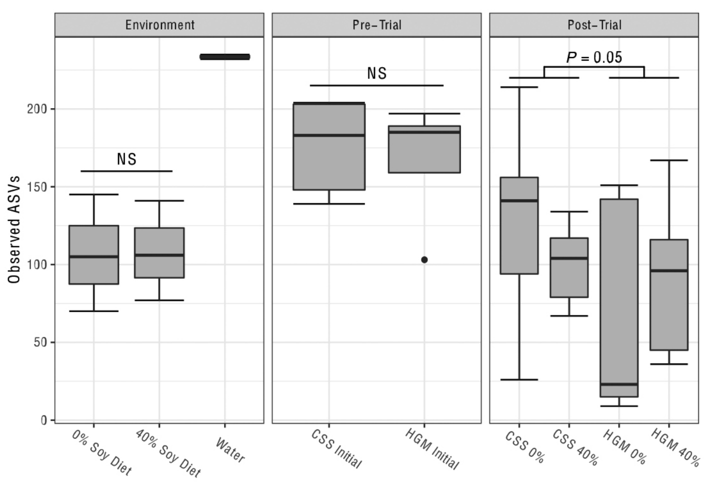

Figure 1. Microbial richness (observed ASVs) detected among environmental (diet and water) and fish gut samples. Pre-trial samples were collected to evaluate any differences in gut microbiota between the fish strains prior to the 12-week feeding trial. Differences in alpha diversity between the diet samples and the initial pre-trial gut samples were tested by a paired t-test. Post-trial gut microbiota samples collected after 12-weeks were compared by fitting a two-way linear model (Diet * Strain + Diet:Strain).

PHENOTYPIC AND TRANSCRIPTOMIC CHARACTERIZATION OF DERMO TOLERANT EASTERN OYSTER FAMILIES

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Disease tolerance occurs when pathogens and parasites have minimal effect on host fitness. The degree of tolerance is typically quantified by the relationship between pathogen load and host survival or reproduction. Dermo disease, caused by the protozoan parasite *Perkinsus marinus*, negatively impacts survival in both wild and cultured eastern oyster (*Crassostrea virginica*) populations. Several studies have demonstrated breeding oysters that survive in Dermo-endemic environments leads to higher population-level survival in subsequent generations; however, it is unclear whether the increased survival is due to evolved resistance or tolerance to the disease. Here we describe a disease challenge experiment designed to 1) test for evolved tolerance among selectively-bred eastern oyster families and 2) characterize phenotypic and transcriptomic responses to the parasite. Eastern oyster families exhibiting a range of field survival phenotypes were obtained from a well-established breeding program and each family was divided into four groups. The groups were exposed to distinct doses of *P. marinus* (0, 10^6, 10^7, and 10^8 parasite spores per gram wet weight) via injection in the adductor muscle and monitored for survival every day for 50 days. Individuals (n = 3 - 6) from each family/dose combination were censored seven days post exposure and mantle tissues were collected to confirm parasite load (via qPCR) and generate global gene expression profiles (via RNAseq). mRNA samples from two families at each extreme of the dose-response tolerance spectrum were ultimately selected for short-read, paired-end Illumina sequencing. On average, 93M reads per sample were generated and 93% of paired reads from each sample mapped to the eastern oyster genome. Counts of reads mapped to each feature (transcript) of the nuclear genome were used to quantify gene expression. Principal component analysis of gene expression profiles showed significant clustering of samples by treatment (control vs. injected) along PC1 and by phenotype (tolerant vs. susceptible) along PC4. To assess the effects of family, phenotype, and dose on the transcriptomic response to Dermo, comparisons between control and treated sample expression were made at multiple levels (e.g. each family and dose separately, families grouped by phenotype at each dose, and all doses combined for each family). Depending on the comparison, hundreds to thousands of differentially expressed transcripts were detected. Analysis of overlapping differentially expressed transcripts found a higher percentage of overlapping transcripts among doses within each family than among families within a single dose, suggesting that the host genotype/phenotype may be more important than parasite load in driving the transcriptomic response to Dermo. More detailed analyses of differentially expressed transcripts, functional annotation and enrichment are underway. Taken together, the results of this study should provide valuable insights to this host/parasite interaction and the mechanisms underlying Dermo tolerance in the eastern oyster.

CHARACTERIZING NORTH AMERICAN COMMERCIAL AQUAPONICS: AN INTERVIEW AND CASE STUDY ASSESSMENT OF EMERGENT PATTERNS IN TECHNOLOGY, INNOVATION, AND OPERATIONS

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The number of commercial aquaponics operations in the United States, Canada, and Mexico has expanded over the last decade in North America (Table 1). Due to its emergent nature, the exact size of the aquaponics industry is difficult to establish. The definition of what constitutes a commercial aquaponics operation varies, while some assessments focus on the production of produce and fish, most sources recognize that many aquaponics operations expand their offerings via other aquaponics related services. Survey-based investigations of commercial aquaponics have been conducted at a global scale, for Europe, for the Czech Republic, and for South Africa. Though not yet used commonly in aquaponics research, the well-established qualitative research methods of semi-structured interview and qualitative thematic coding offer the opportunity to delve deeper into emergent patterns in technology use, innovation, and operation of commercial aquaponics farms in North America. This approach holds potential to uncover patterns and connections between both qualitative and quantitative data within the experiences and stories of practitioners, aspects which play a key, and sometimes overlooked, role in use, choices, and innovation of technologies and operational practices impacting farm success. Along with case study analysis, semi-structured interviews (n=25) were conducted in 2021 with a representative sub-section of practitioners and qualitatively coded for emerging patterns and themes. This was paired with analysis of technical and operational data (general farm attributes, level of technology adaptation, business model and continuity) to find vital patterns, correlations, and connections within the characteristics and practices of commercial aquaponics in North America which crucially impact the economic and sustainability performance of the growing commercial aquaponics industry and farms within it.

USDA Census 2013	Love et al 2015	USDA Census 2018	Author's count 2021
71	72	83	94

Table 1- Count of aquaponics based on the USDA definition (farms that generated $1000 of produce and fish in the past year) in the United States

ASSESSING THE ABILITY OF THE CALIFORNIA SEA CUCUMBER (*Apostichopus californicus*) TO ASSIMILATE ORGANIC WASTE FROM MEDITERANEAN MUSSEL (*Mytilus galloprovincialis*) AQUACULTURE IN SOUTH PUGET SOUND, WA

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There has been recent interest in Washington State, USA in the culture of the California sea cucumber (*Apostichopus californicus*) for both wild-stock enhancement and as an aquaculture resource. The species is a good candidate for Integrated Multi-Trophic Aquaculture (IMTA), where the animals are supported entirely on the excess organic waste from existing floating aquaculture operations (e.g. bivalves or finfish). To determine how *A. californicus* would perform when held beneath a mussel aquaculture site, a field study was carried out in Totten Inlet, WA. Enclosures with different densities of sea cucumbers were deployed beneath existing *M. galloprovincialis* aquaculture rafts. The effects of *A. californicus* density on growth rate was measured, and the effect of cucumber presence on deposition of nitrogen, carbon, and total organic matter (TOM) within enclosures was determined.

During the summer and fall of 2020, sea cucumber were placed at two sites at two different densities in Totten Inlet, WA. At the first site, 12 plastic oyster cages 56 cm (L) × 56 cm (W) × 18 cm (H) were modified to hold treatments of 2 and 4 cucumber/.30 m² (6 and 12/m²). These cages were hung six meters below floating *M. galloprovincialis* rafts along with an equal number of empty cages. A second "Control" site was placed 250 m away from raft enclosures in a similar configuration. Bi weekly measurements were taken throughout a 3-month period.

At the end of the study there were no significant differences for sea cucumber growth (mass) between different density cages ($p < .05$). TOM was significantly higher under the raft, but was not predicted by cucumber density ($p<.05$). Carbon and Nitrogen values significantly decreased after out plant, and were higher under the raft site, but were not affected by sea cucumber density ($p<.05$) (Table 1, Fig. 1). These results suggest that while C and N values were comparatively higher under mussel aquaculture in late summer, the presence of sea cucumber at low density within cages had no effect on the quantity of C or N in accumulating sediments in Totten Inlet.

Table 1. Generalized Linear Mixed Model results for Nitrogen measured in cage sediments. Random effect included cage (which were repeatedly measured).

	Coefficient	DF	t-value	p-value
Intercept (Control Site, Empty)	0.5042	155.00	57.79	<.001
Density 2	-0.0113	18.00	-1.61	0.12
Density 4	-0.0032	18.00	-0.46	0.65
Raft Site	0.7120	18.00	38.09	<.001
Number of Days	-0.0004	155.00	-3.98	<.001
Density 2 : Raft Site	-0.0023	18.00	-0.17	0.87
Density 4 : Raft Site	-0.0152	18.00	-0.99	0.34
Raft Site : Number of Days	-0.0074	155.00	-35.42	<.001

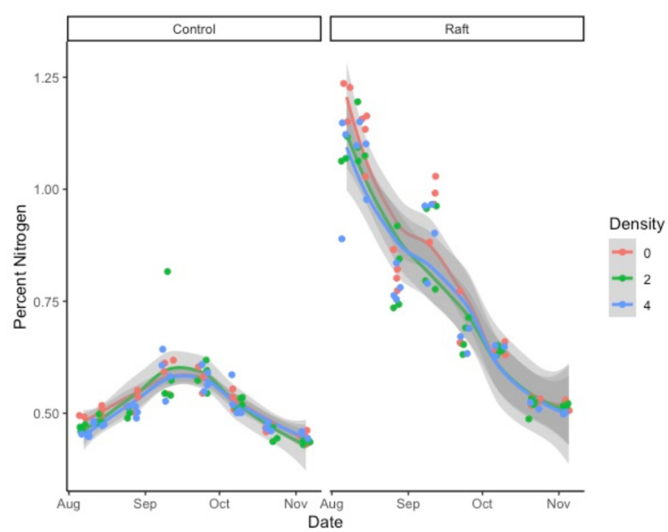

Figure 1. Percent Nitrogen measured from sea cucumber cage sediments. Elemental analysis was used to find % C and N.

EVALUATION OF PROCAP GOLD AS A REPLACEMENT FOR FISHMEAL AND SOYBEAN MEAL IN THE DIET OF HYBRID STRIPED BASS

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Aquaculture continues to be the most rapidly growing segment of agriculture, which increases the demand and, consequently, the cost of marine feedstuffs commonly utilized in aquafeeds. Thus, many research efforts have been made to find alternatives to fishmeal, thereby lessening the dependence on these nutritious but finite resources. ProCap Gold (PCG, Marquis ProCap, Hennepin, IL) is a high-protein distiller's dried grain with solubles (DDGS) product that has shown considerable potential to replace other protein feedstuffs in the diet of tilapia. Supplementation of up to 37.5% of total dietary protein with ProCap Gold DDGS had no negative impact on growth performance, survival, condition indices, or protein conversion efficiency of Nile tilapia. However, due to the relatively high lipid content of PCG, carnivorous species may be able to utilize higher inclusion levels in their formulations. As such, the current study was conducted to evaluate the nutritional value of PCG as a substitute for fishmeal and soybean meal in the diet of hybrid striped bass (HSB, *Morone chrysops* x *M. saxatilis*). The reference diet was formulated to contain 40% crude protein, equally contributed by Special Select menhaden fishmeal and soybean meal and 13% total lipid, while the test diets contained incremental levels (20, 40, 60, and 80%) of the protein provided by fishmeal and soybean meal replaced with protein from the PCG product. Each diet was fed twice daily at a rate approaching apparent satiation to four groups of 17 juvenile HSB (2.8 - 4.0 g/fish) stocked into 110-L aquaria operated as a recirculating system for 8 weeks. The HSB fed the basal diet and experimental diets containing up to 40% of total protein contributed by PCG grew rapidly and increased their initial body weight by over 800% with a feed efficiency approaching 1.0. However, as higher levels of PCG were included in the experimental diets, weight gain, feed efficiency, protein efficiency ratio, survival and muscle yield were progressively reduced, whereas hepatosomatic index and intraperitoneal ratio values progressively increased. Whole-body proximate composition of HSB fed the various diets showed a reduced moisture level as PCG inclusion increased while crude protein and ash were not significantly affected. Determination of digestibility coefficient values for PCG by substituting at 30% by weight in a nutritionally complete reference diet yielded reasonably high values for crude protein, gross energy, organic matter, and phosphorus (Table 1) compared to the reference diet (Fig. 1). Based on these various responses, up to 40% of dietary protein contributed by fishmeal and soybean meal could be replaced with ProCap Gold without significantly affecting growth performance, condition indices or body composition of HSB juveniles.

Table 1. Percent Apparent Digestibility Coefficients (ADCs) of the ProCap Gold ingredient (means ± standard deviation).

	Apparent Digestibility Coefficients (%)			
Ingredient	Crude Protein	Gross Energy	Organic Matter	Phosphorus
ProCap Gold	76.1 ± 0.56	82.9 ± 2.29	84.8 ± 2.56	100.0 ± 10.1

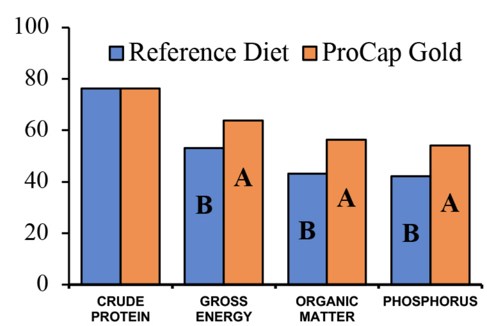

Figure 1. Dietary Percent Apparent Digestibility Coefficients of reference and experimental diets with ProCap Gold supplemented at 30%.

GENOMIC RESOURCE DEVELOPMENT TO SUPPORT COMMERCIAL AND RESTORATION CULTURE OF NORTH AMERCIAN ABALONE SPECIES

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Along the western coast of North America, seven species of abalone are distributed over a broad range from Baja California, Mexico to Alaska. Unfortunately, for all these abalone species, disease and fishing pressure has led to drastically declining natural stocks. These declines have resulted in the listing of white (*Haliotis sorenseni*) and black (*H. cracherodii)* abalone as endangered under the U.S. Endangered Species Act. The declines have also resulted in the closures of U.S. commercial and recreational fisheries for all abalone species, with the exception of a small subsistence/personal use fishery for pinto abalone (*H. kamtschatkana*) in Alaska. Due to these closures, abalone are one of the few groups of marine species where culture production dominates the commercial market. Commercial abalone production has greatly expanded over the past decade to become a thriving global industry due to high market value of abalone products. In the U.S., most commercial production operates in California and primarily utilizes red abalone (*Haliotis rufescens*), with green abalone (*H. fulgens*), and pink abalone (*H. corrugata*) as potential candidates for aquaculture development in warmer, more southern locations. Restoration culture is also a high priority in the U.S.; this practice aims to breed and release animals back into the wild to enhance natural populations. For abalone, restoration culture has resulted in outplantings of young pinto, green, and endangered white abalone back into coastal waters. Genomic resources have been used to greatly improve production for most livestock, agriculture, and aquaculture species (where developed); however, few genomic resources exist for West Coast abalone species. These genomic tools can enhance commercially important traits that are essential to improved production efficiency and abalone culture expansion in California (e.g., growth rate, disease resistance, thermal tolerance). Development of these genomic resources can also improve abalone restoration culture by helping to evaluate genomic diversity and culture-biased selection to guide breeding and outplanting efforts. To help advance abalone aquaculture practices for commercial and restoration culture in the U.S., we are in the process of developing a genomic toolkit for several of these species. This work includes sequencing the genomes of red, green, black, and white abalone, along with additional sequencing projects on other abalone species and hybrids. Results, to date, on these projects will be presented.

MODELING OFFSHORE AQUACULTURE ESCAPE RISK ASSESSMENTS USING OMEGA

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The 2020 Executive Order on Promoting American Seafood Competitiveness and Economic Growth and subsequent development of Aquaculture Opportunity Areas (AOAs) has accelerated interest and resulted in a renewed push to develop offshore marine aquaculture. Permitting of offshore aquaculture remains a challenge, and among the various components are analyses to determine the environmental and ecological impacts of farm operations, which need to rely on science-based assessments. One of the primary ecological concerns is the potential for negative ecological and genetic impacts when farmed fish escape and encounter wild stocks; this topic is the most publicized when large scale cage failures occur. NOAA Fisheries and ICF have developed a scientific decision-support tool called the Offshore Mariculture Escapes Genetics Assessment (OMEGA) model, to understand the potential impacts of farm escapes to marine resources (through risk assessment analyses), and to aid the industry and regulators in defining how offshore farms can operate to avoid impacts.

OMEGA is a mathematical model with inputs under three broad parameter categories: wild population biology and stock assessment, cultured population and aquaculture operations, and interactions between cultured and wild populations. We are using OMEGA to conduct primarily two types of analyses. The first type is risk assessment scenario modeling for a range of species, escape levels, escape categories, and production levels; this modeling helps set boundaries and conditions to avoid impacts from escaped fish. These analyses can be used to help inform environmental impact statements (EISs) and will be a component of the Programmatic EISs for the AOAs. The second type is project level assessments for specific farm (or planned farm) operations. In these assessments, anticipated farm parameters (e.g. target production levels, projected farm expansion, net pen infrastructure, harvesting schedules, brood source, and other characteristics) are explored to determine if the operation will avoid impacts from escaped fish, and help to identify alternatives for current or future plans, as needed. The presentation will go through examples of these two types of analyses and demonstrate how producers can benefit from OMEGA modeling analyses. In addition, we will briefly describe one of our current projects aimed at identifying and understanding how newer and improved net pen technologies will avoid potential impacts of escapes.

A NOVEL RESPIROMETRY METHOD TO ESTIMATE BIOFILM ACTIVITIES ON BIOFILTER CARRIERS FROM RECIRCULATING AQUACULTURE SYSTEM

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Biofilm, the aggregates of microbial layers that are attached on the surface of biofilter carriers, plays a central role in nutrients removal in recirculating aquaculture system (RAS) to achieve high degree water reuse for fish production. The bacterial activities in biofilm are of great importance for surveillance and management of RAS biofilter, but cannot easily be measured. Moreover, a biofilm detachment process and laboratory equipment are required for current methods to characterize the biofilm, which seem unsuitable for on-site application in a RAS facility. The present study was conducted with an aim to develop a simple and reproducible method that allows *in-situ* estimation of biofilm activities without destroying biofilm integrity.

Three different types of biocarriers, extruded polypropylene (EPP), injection molded polypropylene (IMPP) and polymeric foam (PF) from biofilters connected to the same RAS were tested in our tailor-made respirometry system. The oxygen uptake rates (OUR) of biofilm in respirometric chambers packed with biocarries were measured after intermittent spiking with either ammonium, nitrite or acetate. The standard substrate degradation batch kinetics tests for evaluating biofilter performance were also conducted to confirm the feasibility of our proposed method.

Results showed that the method allows estimation of endogenous respiration rates, as well as respiration of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and heterotrophic bacteria (HB) in biofilm. The highest activities, calculated and standardized as volumetric oxygen consumption rate (VOCR), were found in PF with values of 1441, 677 and 166 g $O_2 \cdot m^{-3} \cdot d^{-1}$ for AOB, NOB and HB, respectively (**Figure 1**). Substrate degradation batch tests matched respirometric tests well for all three tested carriers, confirming the feasibility and robustness of our proposed method to assess biofilter performance. Our respirometry-based method allows to compare and separate nitrification and heterotrophic processes, which will provide a valuable tool in biofilter performance tests and lead to a better understanding of the dynamic and relationship between biofiltration and water quality.

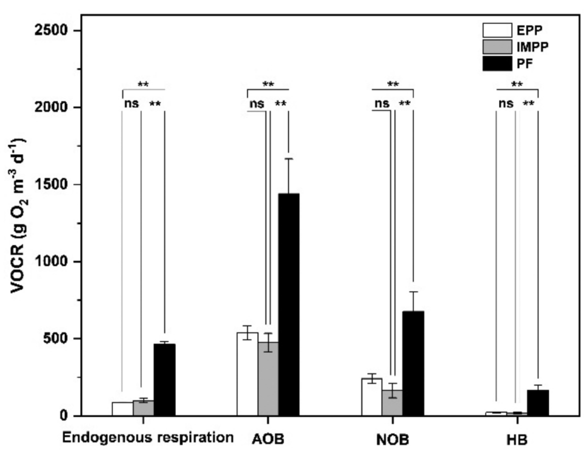

Figure 1. Bacterial activities based on volumetric oxygen consumption rate measured in three different types of biocarriers. Bars and error bars denote mean ± standard deviation of the mean (n=3; *P<0.05; **P<0.01; ns=not significant).

GHOSTS OF OCEANS PAST: WHAT CAN DATA ON HISTORICAL PARASITE BURDENS TELL US ABOUT THE FUTURE OF MARINE DISEASE?

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The abundances of free-living species have changed dramatically in recent decades, but little is known about change in the abundance of parasitic species; this is a major gap, given that changes in parasite transmission could be vitally important for fisheries and aquaculture production. We investigated whether populations of several parasites have shifted over time in two shore crab hosts, *Hemigrapsus oregonensis* and *Hemigrapsus nudus*, by comparing the prevalence and abundance of three parasite taxa in a historical dataset (1969–1970) to contemporary parasite abundance (2018–2020) for hosts collected from 11 intertidal sites located from Oregon, USA, to British Columbia, Canada.

Our data suggest that the abundance of the parasitic isopod *Portunion conformis* has varied around a stable mean for the past 50 years. No change over time was observed for larval acanthocephalans. However, larval microphallid trematodes increased in prevalence over time among *H. oregonensis* hosts, from a mean of 8.4–61.8% between the historical and contemporary time points. The substantial increase in the prevalence of larval microphallid trematodes could be due to increased abundances of their bird final hosts, increased production of parasite infective stages by snail intermediate hosts or both. Our study highlights the variability among parasite species in their temporal trajectories of change.

FRIEND OR FOE? EFFECT OF EELGRASS DENSITY ON FILTER FEEDER BIOMASS AND CONDITION INDEX IN A MULTI-HABITAT LIVING SHORELINE

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Over the past decades, estuarine ecosystems have suffered significant habitat loss. Implementation of multi-habitat living shorelines (MHLS) can restore lost habitat function. Researchers constructed a MHLS from 2016 to 2017 in Newport Bay, California by restoring eelgrass and oyster beds together and in isolation. Increased sedimentation from eelgrass could threaten oysters and other filter feeders. This study will investigate how eelgrass impacts filter feeder biomass and condition index. To compare short-term and longer-term success of oysters restored with varying densities of adjacent eelgrass, I will calculate the total biomass and per capita condition indices of native and non-native filter feeders on restored oyster beds one- and two-years post-restoration. I hypothesize that increased eelgrass density will increase sedimentation rates onto adjacent oyster beds, causing declines in filter feeder biomass and per capita condition indices. I excavated filter feeders from quadrats on oyster beds, identify by species, and weigh to determine wet and dry tissue and shell weight, and quantify condition index. I quantified sedimentation by measuring mud deposition on oyster beds using a periodontal probe and quantified filter feeder and eelgrass densities using quadrat surveys.

Mud deposition during each year from 2018 to 2020 was significantly higher on oyster beds restored adjacent to eelgrass vs oyster beds restored alone. In 2018, *O. lurida* had the highest biomass relative to other bivalves on oyster beds restored alone; nonnative *M. senhousia* dominated biomass on oyster beds restored adjacent to eelgrass *C. spinosum, M. galloprovincialis,* and *O. lurida* had 26, 6, and 7X higher biomass, respectively, on oyster beds restored alone vs. with eelgrass. In 2018, *O. lurida* had 2X higher condition index on oyster beds restored alone vs. with eelgrass. Processing of 2019 samples is in progress. Increasing understanding of the relationship filter feeders share with eelgrass will inform future management decisions about whether to restore MHLS together or in isolation.

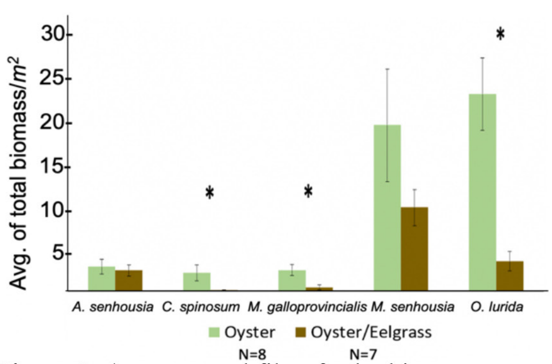

Figure 1. Average total filter feeder biomass as a function of filter feeder species at DeAnza during 2018 in Newport Bay, CA.

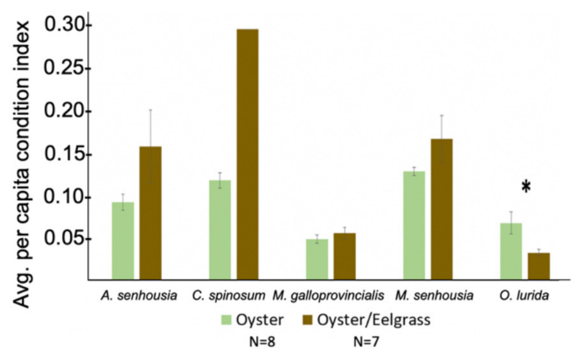

Figure 2. Average per capita condition index of filter feeder species at DeAnza during 2018 in Newport Bay, CA.

UNIVERSITY OF THE VIRGIN ISLANDS (UVI) – HISTORICAL PERSPECTIVE AND OPPORTUNITIES IN AQUAPONICS AND AQUACULTURE

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The University of the Virgin Islands (UVI) is a public, co-ed, 1862 land grant and Historically Black College and University (HBCU) and the only institution of higher learning in the territory of the US Virgin Islands that awards certificates, associates, masters, and doctorate degrees. Until recently UVI was lacking an academic program in Agriculture, but on June 20, 2020 UVI Board of Trustees approved the creation of a new School of Agriculture, to combine agricultural research programs, extension programs, and academic programs in one unit.

The research component has a long tradition developed by the Agriculture Experiment Station (AES), which conducts basic and applied research in animal science, aquaculture, aquaponics, biotechnology, forage agronomy, fruit and ornamental crops and vegetable crops. The Extension programs of the Cooperative Extension Services (CES) consist of Agricultural and Natural Resources, 4-H/Family and Consumer Sciences, and Communications, Technology and Distance Learning.

The Academic unit has already six certificate programs (General Agriculture, Agricultural Business, Agrotourism, Horticulture, Forestry and Nursery Management, and Aquaculture), and two Associate degrees (Agroecology, and Horticulture) and it is developing some more certificates, associate of applied science degrees, and bachelor's degree programs in the areas of Animal Science, Regulatory Science, Cannabis Social Science, Cannabis Biotechnology, and Agricultural Business/Economics.

UVI is worldwide recognized due to the Aquaponics short course developed at the AES which provided training in plant and tilapia production to people in mainland U.S., the Caribbean and around the world. The new Agriculture programs and the Aquaculture certificate will provide opportunities for research collaboration in Aquaponics, Maraponics, and Marine aquaculture, among others, due to the privileged geographical situation with access to coastline and tropical temperatures all year around. Also, the Aquaculture program will explore biological and technical principles for microalgae, bivalves, echinoderms, holothuroids, and fish production in the Caribbean. Theoretical and practical knowledge as well as hands-on operational experience is emphasized, using laboratory and field equipment.

CALIFORNIA SEAFOOD CONSUMPTION SHIFTS DURING THE COVID-19 PANDEMIC

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The pandemic has sent shockwaves through seafood consumption, with food service providers--the source of 75% of U.S. seafood sales[1]--shuttered or forced to adapt by widespread shelter-in-place orders. Seafood consumers already navigate a heavily consolidated market: just five species comprise 70% of seafood consumed, and seafood consumption in the U.S. is the lowest per capita of any industrialized nation[2]. Given the heterogeneous impacts of the COVID-19 pandemic, how will seafood sourcing and consumption change? In order to understand the COVID-19 pandemic's effect on seafood sourcing and consumption, we used Facebook quota sampling to recruit a diverse cross-section of California residents to take a series of surveys over the course of a year (n=640 remained in study for the full year).

Due to California's diverse population and varied geography, we will use fixed effects models to analyze how consumption frequency, point of sale, and diversity of species consumed was affected by the COVID-19 pandemic. We will be able to explain consumption patterns by a variety of drivers, including: population density, distance from coast, and a variety of demographics (e.g. wealth, education, race, gender, household size). Our final survey just wrapped up this fall, by the triennial meeting we should have near-final results to share.

1 Love, D.C., Asche, F., Conrad, Z., Young, R., Harding, J., Nussbaumer, E.M., Thorne-Lyman, A.L. & Neff, R. (2020). Food Sources and Expenditures for Seafood in the United States. *Nutrients*, 12, 1810.
2 Shamshak, Gina L. et al. 2019. US seafood consumption. *Journal of the World Aquaculture Society* 50.4, pp. 715-727.

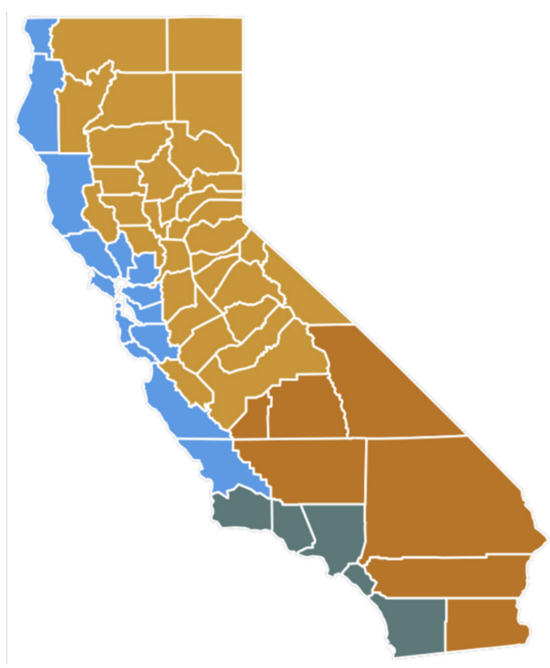

***Figure 1**: Facebook ads recruited survey participants across 4 distinct parts of California as part of our effort to get a cross-section of California seafood consumers.*

CHARACTERIZATION AND SIGNIFICANCE OF CLCA GENE FAMILY IN FISH HEALTH

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Bacterial diseases cause significant economic loss to the aquaculture industry. Antibiotic resistance calls for new solutions alternative to antibiotics. CLCA genes are well conserved across species and are of interest due to their physiological and pathological significance. CLCAs are expressed in various tissues, including mucosa, epithelium, and endothelium. Mucus is the first line of defense for a fish living in a complex environment. Fish mucus has metalloproteases that act as antibacterial peptides. Certain CLCA family members are stress-inducible zinc metalloproteases in higher organisms. We characterized the CLCA gene family members in various fish species and identified the metal-binding motif HEXXH in most fish CLCAs. We also found significant CLCAs induction in response to bacterial infection in channel catfish and zebrafish. Interestingly, we find that CLCAs are secreted into the mucus and other tissues along with other stress-inducible factors suggesting an essential role in fish immunity. Our other data indicate that the soluble isoform of fish CLCA acts as a potential antibacterial peptide or an immune activator.

FEASIBILITY OF USING POMEGRANATE *Punica granatum* SEED OIL, RICH IN CONJUGATED LINOLENIC ACID, AS A LIPID SOURCE IN THE DIET OF COMMON CARP *Cyprinus carpio* JUVENILES

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In this study, the feasibility of using pomegranate seed oil (PSO), rich in conjugated linolenic acid and its partial replacement for fish oil in fish diet were investigated. Common carp, *Cyprinus carpio*, juveniles (1.8 ± 0.1 g) were fed four isonitrogenous and isolipidic diets with similar basal composition but different oil mixture containing 100% fish oil (A), 50% fish oil +50% sunflower oil (B), 50% fish oil +25% sunflower oil +25% pomegranate seed oil (C) and 50% fish oil +50% pomegranate seed oil (D) for 8 weeks. The highest weight gain was observed in fish fed diet D ($p < 0.05$). Test diets had no significant effect ($p > 0.05$) on saturated and monounsaturated fatty acid contents of fish muscle. Docosahexaenoic acid (22:6n-3; DHA) was significantly lower in the muscle of fish fed diet B ($p < 0.05$) compared to those fed diet A. However, there was no significant difference in the muscle DHA content of fish fed diets A, C, or D. No specific hepatocyte damage associated to dietary pomegranate seed oil was found in this study.

Results of this study revealed that the partial substitution of fish oil with PSO (50%) in the *Cyprinus carpio* diet results in an improved growth performance. However, there was no significant difference between the body proximate composition and fatty acids profile (except for conjugated linoleic acids and n-3/n-6 ratio) as compared to the control group. Moreover, it also causes the transfer and accumulation of punicic acid in the muscle of fish and thus increases the nutritional value of fish. This study showed PSO can be introduced as a sustainable novel aquafeed ingredient for further supplementary studies, for example, expression of genes associated with peroxisome proliferator-responsive enzymes and liver health indexes plus additional prolonged experiments, which can shed light on the findings of this study and verify whether PSO is a practical novel aquafeed ingredient.

IMPACT OF IMAGE DATASET SIZE AND QUALITY ON A CONVOLUTIONAL NEURAL NETWORK MODEL ACCURACY FOR IN-TANK FISH DETECTION IN RECIRCULATING AQUACULTURE SYSTEMS

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Recirculating aquaculture systems (RAS), a land based intensive aquaculture technology, are being adapted globally as a sustainable alternative to wild fishing. Since RAS grow the fish in a controlled environment, precision technologies can be conveniently adapted to improve system performance and reliability and assist growers with important fish management decisions. Recent advancements in computer vision and artificial intelligence (AI) have significantly improved the reliability, repeatability, and accuracy of the models and drawn interest of the aquaculture industry and research community. The convolutional neural network (CNN) assisted image classification and object detection models are being developed in the aquaculture industry for fish management including feed optimization, biomass and yield estimation, fish health and waste management. However, machine learning approaches are data-intensive and model precision and accuracy primarily depend on the data quality. When imaging underwater, challenges including turbidity, fish density, and distortions caused by the underwater environment are expected to impede feature identification. Therefore, this study was conducted to investigate the effect of number and quality of images, imaging conditions and pre-processing operations on the fish detection accuracy of the object detection model. An underwater sensing platform was developed with four commercially available imaging sensors [Raspberry Pi camera (model: Pi 4 HQ, Raspberry Pi foundation, Cambridge, UK); GoPro (model: HERO9, GoPro, Inc., California, USA); Oak-D (model: Oak-D Depth AI, Luxonis, Colorado, USA); Ubiquiti security camera (model: G3, Ubiquiti Inc., New York, USA)] customized and deployed in a RAS tank with Rainbow trout. The images from all the sensors were first collected with ambient LED lighting at an interval of 5 sec. Later, supplemental LED lighting was added above the tank to acquire the imagery data for comparison with ambient lighting. The acquired images from various imaging sensors in different light conditions were divided in batches of 100 images and annotated as partial and whole fish. The annotated images were segregated into training, validation and test datasets in a ratio of 70:20:10, respectively and utilized to train a custom Yolo V5 model in Roboflow software (Roboflow, Inc., Des Moines, Iowa, USA) for fish detection. The effect of sensor specific image quality, number of images, light conditions, and image augmentation on fish detection accuracy is being investigated and pertaining results will be presented in terms of precision, mean average precision, and recall.

THE PROMISOR ROLE OF SKIN MUCUS TO SCREEN FOR FISH STRESS BIOMARKERS: A PROTEOMICS APPROACH

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Managing fish stress is crucial to ensure a sustainable aquaculture production and the standardization of stress biomarkers would be an important contribute to the existing species-specific stress management protocols. Proteomics was employed in this study as a tool to discover more robust fish stress biomarkers, since proteins are ubiquitously affected by abiotic and biotic stimuli in a slower timescale when compared with endocrine responses. The analysis of proteome changes in different tissues, e.g. liver and mucus may offer not only tissue-specific protein fingerprints, but can be also highly advantageous in the context of fish welfare, as skin mucus allows for sampling in a non-invasive way. Moreover, this combined proteomic analysis provides a more detailed insight into the molecular mechanisms, as a complementary picture of the animal´s physiological state under stress.

Sparus aurata was exposed to different suboptimal rearing conditions in three separated trials: overcrowding (OC30 and OC45), net handling (NET2 and NET4), and hypoxia (HYP30 and HYP15), using fish reared under optimal conditions for the species, as control. By the end of the trials, fish were sampled and protein extracts from liver and mucus samples were prepared for further analysis by reverse phase nano liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Proteins identified with high confidence (protein FDR <0.5%; peptide FDR <0.1%) within each trial were analyzed by One-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Proteins were screened for enriched KEGG and REACTOME pathways. Pairwise comparisons between control and stressed fish samples were also established within each trial (T-test, q <0.05) and up- and down-regulated proteins were further classified according to Gene Ontology Enrichment (Fisher's Exact test, FDR <0.05). The discriminative power of specific differential abundant mucus proteins was assessed through ROC curve analysis after binary logistic regression.

Label-free shotgun proteomics reproducibly identified a mean of 1300 proteins in at least 4 out of 6 fish per treatment, either in the liver or in the skin mucus of gilthead seabream. A total of 297 (liver) and 250 (skin mucus) differentially regulated proteins were identified between challenged and control fish across the three trials. A tissue-specific stress regulation was observed, although 40 common proteins, mostly implicated in translation, protein folding processes and immune system, were found to be differentially regulated in both tissues, suggesting that the skin mucus can be an accurate reflection of the stress-induced adaptations occurring in the fish liver after an environmental disturbance. The ROC curve analysis showed that the mucus proteins haptoglobin and alpha-2-macroglobulin presented a high predictive power (AUC > 0.8) and could thus be further investigated as challenge-independent biomarkers. Still, further verification and validation steps are required. This integrated approach provides a starting point for the validation of lab-based welfare indicators for this species and a development for the fish welfare assessment measures, to further improve aquaculture sustainability.

ABUNDANCE OF SHELL-BORING POLYCHAETE WORMS IN AQUACULTURED OYSTERS FROM MAINE USED FOR REEF RESTORATION IN GREAT BAY, NH

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Natural populations of eastern oysters (*Crassostrea virginica*) have been decimated because of disease and overharvesting. In response, restoration programs have been initiated to rebuild substrate and reintroduce breeding oysters to reestablish reefs, thereby restoring ecosystem services. Since 2009, The Nature Conservancy (TNC) has teamed up with several agencies to restore degraded oyster reefs in New Hampshire's Great Bay estuary. Recently, the TNC Supporting Oyster Aquaculture and Restoration (SOAR) program was established to buy up to 5 million eastern oysters from farms in multiple northeastern states and move them to nearby restoration grounds. The intention was to support oyster farmers who have had difficulty selling oysters during the COVID-19 pandemic. Unlike other states in the program, there are no restoration grounds in Maine to serve as "nearby" reefs. Instead, SOAR sought to move oysters from Maine farms to the Great Bay estuary.

Given biosecurity considerations and the recent growth of oyster aquaculture in the Great Bay estuary, there was concern regarding the planting of oysters from distant farms on the restoration reefs and the likelihood those oysters would introduce new pest species. Despite the routine movement of seed from Maine to New Hampshire farms, New Hampshire oyster farmers were worried about introduction of novel species of blister worms, in the polychaete genus *Polydora*. To support the SOAR program and Maine oyster farmers, samples of oysters from each participating Maine farm were inspected for shell-boring polychaete infestations. *Polydora websteri*, a common shell-boring species with a worldwide distribution, was in high abundance in reference samples from oyster farms in Great Bay and in samples from the restoration reefs. A second shell-boring species, provisionally identified as *P. onagawaensis*, is also present on oyster farms in Maine but has not been observed previously in Great Bay. We used burrow shape, microscopic analysis of morphological features, and molecular analysis of the mitochondrial CO1 (mtCO1) gene to identify worms extracted from the oyster samples from Maine farms to species. We found that the abundance of shell-boring polychaetes was variable along the coast of Maine, with farms in close geographical proximity having very different pest loads. Both *P. websteri* and *P. onagawaensis* were identified by molecular analysis on some Maine farms while only *P. websteri* was found in the samples from New Hampshire farms and restoration sites. The resulting data were used to reduce the likelihood that *P. onagawaensis* was introduced to New Hampshire waters. This project represents a successful collaboration between The Nature Conservancy and University of Maine undergraduate students to prevent the transfer of non-native species from oyster farms to the reefs, which in turn would have increased the likelihood of the pest species recruiting to Great Bay oyster farms.

INTRASPECIFIC VARIATION IN TOLERANCE TO ACUTE TEMPERATURE STRESS IN THE BLUE MUSSEL *Mytilus edulis*: A TEACHING MODEL

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Physiological adaptations play a key role in biogeographic patterning. Understanding how variation in physiological tolerances structures species distributions is critical to predicting species shifts and community resilience in the face of climate change. As part of a series of “Integrated Marine Science” courses, students at the University of Maine explore how to measure the physiological tolerances of the blue mussel (*Mytilus edulis*) a foundational species whose presence often influences the diversity and productivity of temperate zone intertidal communities.

In previous research, Braby and Somero used a method known as impendance pneumography to measure how heart rate changes under acute stress among three species in the genus *Mytilus* and demonstrated that the patterns of distribution for these congeners is correlated with tolerance to temperature and salinity stress. Our students use the same approach to estimate variation in physiological tolerance to acute temperature stress among mussels sampled from populations of *M. edulis* north and south of Cape Cod, MA, USA. They specifically focus on the determination of the high critical temperature (H_{crit}) or Arrhenius break temperature, which is the point where heart activity falls sharply with increasing temperature. The class results suggest that mussels from southern populations show higher tolerance to acute thermal stress when compared to their northern conspecifics. We will discuss some ramifications of their findings and how these research activities enhance our undergraduate curriculum in marine science and further students’ understanding of how climate change research is conducted.

BIOENERGETIC PROFILING TO IMPROVE STRESS RESPONSE IN EASTERN OYSTERS

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The Extracellular Flux Analyzer system (EFA; Agilent Technologies) enables the high-resolution measurement of changes in oxygen levels in a 96-well plate based assay and is designed to measure the mitochondrial profile of live cell cultures. By selective application of pharmaceuticals during analysis, the EFA essentially performs a mitochondrial stress test and indicates the degree to which cells have the capacity to respond to stressors. This system has been adapted to measure fish mitochondrial profiles and has shown that reserve capacity can be predictive of survival, hatching, and growth rates.

As part of an on-going Northeast Regional Aquaculture Center (NRAC)-supported project, we have recently constructed a series of genetically related diploid and triploid oyster lines derived by crossing diploid and tetraploid NEH males with female oysters from three regional lines. The performance of resulting lines were assessed throughout their early developmental stages and are being deployed in field trials at multiple grow-out sites to support the comparison of dynamic energy budgets between diploid and triploid oysters. We have also developed the protocols to use the EFA to measure and compare the bioenergetic profiles of oyster spat from a subset of the diploid and triploid NRAC lines: NEH diploid, NEH triploid, NEH x Islip NY hybrid diploids and NEH x Islip NY hybrid triploids. Initial estimates indicate there are line specific differences in basal metabolism and mitochondrial reserve capacity. Similar differences were also noted in oyster resistance to *Vibrio* infections. The grow-out performance of these lines is currently being followed and we will correlate mitochondrial profiles for each line with their response to field conditions and stress-response in additional lab-based experiments.

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 are released into the circulatory system where they are taken up specifically by growing oocytes via receptor-mediated endocytosis within clathrin-coated pits. Two vitellogenin receptors (LR8 and Lrp13) 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. 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 also 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.

STRIPERHUB: STRIPED BASS (*Morone saxatilis*) AQUACULTURE

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StriperHub is a Sea Grant (NOAA) consortium of diverse academic, government, and private sector partners and stakeholders that aims to overcome barriers to develop and expand striped bass (*Morone saxatilis*) aquaculture in the U.S. through demonstration and promotion of commercial-level culture, economics, and marketing. By value, 90% of seafood products consumed in the U.S. are imported, totaling a $16 billion seafood trade deficit, and half of these imported fish are reared in aquaculture operations. The recently funded Sea Grant StriperHub centered in North Carolina will address this deficit by developing striped bass as a candidate aquaculture species to strengthen the domestic seafood industry and boost the economies of coastal and rural communities of the U.S.

Currently, there is no appreciable aquaculture of white-fleshed marine fishes in the country--a candidate species in this regard would command a premium price, have high consumer demand, and adapt well to localized production environments. Research conducted over several years shows that striped bass meets all of these criteria. While hybrid striped bass (striped bass x white bass, *Morone chrysops*) is a successful freshwater aquaculture species, particularly in the South and Midwest ($50 million farm gate value 2018), there is an untapped demand for (pure-strain) marine striped bass by consumers in coastal states. However, both striped bass and hybrid striped bass see high demand from lucrative ethnic markets, seafood restaurants, and sushi bars. Culturing striped bass allows for diversification of the industry to "open" systems (coastal areas) as the fish can live in fresh or saltwater, unlike hybrid striped bass.

The StriperHub is coordinated by North Carolina Sea Grant and integrates with other Sea Grant programs, industry partners, government researchers, policymakers, and university scientists to consolidate and streamline striped bass commercialization efforts. 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 will be available in markets along the Eastern U.S. Coast. Specific program goals are to:

1) Identify domestic producers for commercial production and provide an adequate supply of fish to consistently supply markets;
2) Demonstrate profitability through production, marketing and economics;
3) Clarify regulatory permitting and licensing procedures; and
4) Promote comprehensive extension, marketing, training, and educational visibility to consumers and stakeholders.

ENRICHMENT OF FISH WASTE WITH VODKA AND OTHER CARBON SOURCES TO INCREASE MINERALIZATION OF NUTRIENTS IN AQUAPONICS AND RAS

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Inadequate nutrient levels are one of the drawbacks to achieving success with aquaponics on a commercial scale. In fact, for many plants with higher nutrient demands such as tomatoes and hemp, nutrient supplementation must occur to maximize production. When solid wastes are removed from an aquaponics system, many potential plant nutrients are also removed in their non-bioavailable forms. Important nutrients such as potassium and calcium as well as micronutrients such as iron, boron and zinc remain unavailable to plants outside of their ionic forms. Therefore, mineralization of solid fish wastes is an essential process to maximize plant production in aquaponics. It allows for nutrients that are initially removed from an aquaculture system a way to be added back to the system without compromising water quality or producing waste. In essence, it allows for the existence of a truly zero-discharge aquaculture system. In order for this to work, there has to be adequate resources for bacteria to digest solid wastes during the mineralization process. While previous studies have documented significant increases in nutrient levels after aerobic mineralization, it is unclear if nutrient levels are being maximized or if there are factors limiting aerobic degradation of solids. While the focus is generally to provide enough oxygen for the bacteria through aeration, it remains a bit of a mystery whether carbon availability is limiting. Therefore, we proposed an experiment to examine nutrient levels after the addition of two carbon sources, ethanol (vodka) and citric acid to aerobic mineralization systems. Initial results were intriguing and analysis of results is underway.

THE ROLE OF ECO-CERTIFICATION IN MODERATING PUBLIC PERCEPTIONS AND ATTITUDES TOWARDS MARINE SALMON AQUACULTURE

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Eco-certification programs reward producers that meet a set of sustainability criteria with eco-certified status. This status can lead to improved access to markets and price premiums. Producers also cite improved reputation and social acceptance as a motivation to seek eco-certification; however, the impact of eco-certification on producer and industry reputation is unclear.

In Canada, marine salmon farming takes place in rural coastal communities on both the Atlantic and Pacific coasts. Although aquaculture eco-certification criteria emphasize reducing local environmental impacts, eco-certification addresses social legitimacy (or social licence) at a global scale by providing consumers who may be distant from the site of production with assurance that a product was farmed sustainably. Therefore, eco-certification might be expected to interact with social acceptance or marine salmon aquaculture differently in urban and rural communities.

A public survey circulated through Facebook community groups was used to explore how eco-certification status affects public perceptions and attitudes towards marine salmon aquaculture in Nova Scotia (Atlantic) and British Columbia (Pacific). Participants were asked a series of questions about their (1) attitude towards salmon farming and the importance of potential outcomes and impacts to them, (2) knowledge of and perceptions of aquaculture eco-certification, and (3) place of residence including population and proximity to the coast. Responses were used to explore the relationship between eco-certification and public attitudes towards salmon aquaculture in rural and urban communities.

IMPACTS OF LATE SUMMER BLOOMS IN THE LOWER CHESAPEAKE BAY

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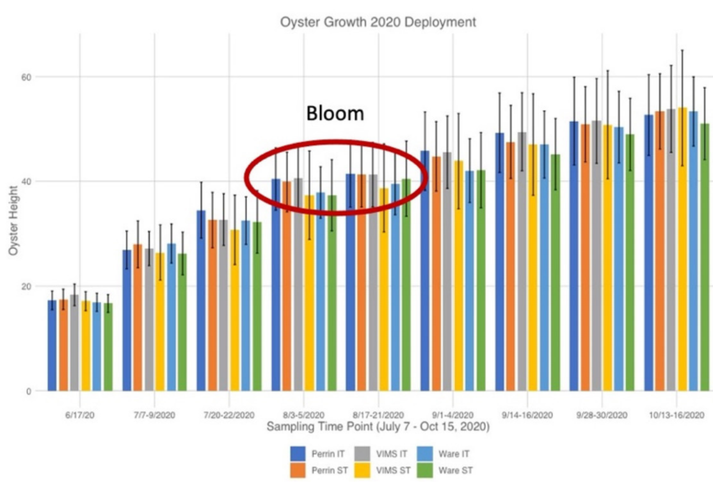
Blooms of *Margalefidinium* (formerly *Cochlodinium*) *polykrikoides* and *Alexandrium monilatum* occur most years in the late summer in lower Chesapeake Bay. Annual variations in rainfall, temperature, wind, salinity, nutrient levels, cyst densities and other environmental parameters determine whether there will be blooms and affect the timing, density and distribution of blooms when they occur. Reported impacts of *M. polykrikoides* and *A. monilatum* blooms in the lower Chesapeake Bay have varied with year and location. Local shellfish aquaculturists have reported oyster mortalities during and immediately following these blooms during some years and this prompted us to conduct field studies with juvenile oysters examining the impacts of blooms and other stressors using different grow-out strategies at aquaculture sites with differing physical characteristics with an aim toward identifying mitigation strategies. Generally, mortality was higher for oysters grown intertidally than for those grown subtidally and higher at bloom-impacted low energy sites compared to sites with little or no bloom activity. In addition, during two bloom seasons growth rates slowed during the blooms (Figure).

Ecosystem level impacts of these late summer blooms were also observed. Water samples were collected weekly in and outside of bloom patches to examine impacts on nutrient cycling and microbial community composition. The bloom of *M. polykrikoides* was accompanied by high production of dissolved organic carbon (DOC), while DOC was drawn down to low concentrations during the *A. monilatum* bloom. The DOC produced by *M. polykrikoides* may have supported the *A. monilatum* bloom, which occurred after the M. polykrikoides bloom, either via osmotrophy or by release of nutrients via microbial decomposition. Bloom samples of both species had altered microbiomes compared to non-blooms samples. In addition, There was a selective group of prokaryotes found in the particle-associated portion of the microbiome that was only found when *A. monilatum* was present. Blooms of these two algal species impacted the estuarine microbiome in different ways, likely leading to shifts in estuarine carbon and nutrient cycling, with *M. polykrikoides* having a greater potential impact on the overall functioning of the estuarine ecosystem than *A. monilatum*.



EXPANSION OF GENE FAMILIES THROUGHOUT BIVALVE MOLLUSC EVOLUTION

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Bivalves play vital roles in ocean conservation and food security by acting as ecosystem engineers and underlying >20% of global aquaculture production. Robust stocks are required for restoration or aquaculture breeding programmes, however, our understanding of bivalve biology and evolution is limited. Due to high levels of heterozygosity and repeated regions, generating genome assemblies for bivalves was hindered until advances in long read sequencing technology. This has led to a great increase in the number of bivalve assemblies in recent years. By analysing the genomes of 32 species representing each molluscan class, we identify gene families that have undergone expansion during bivalve evolution. These included ancestrally-retained expansions in redox, chaperone and protein recycling gene families and more recent expansions in innate immune response gene families. This mirrors adaptation strategies of other sessile organisms such as plants and reflects the high level of tolerance bivalves require during constant pathogen exposure.

POPULATION GENETIC AND STOCK ENHANCEMENT TOOLS FOR CONSERVATION OF THE OVERFISHED WHITE SEABASS *Atractoscion nobilis*

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White seabass (*Atractoscion nobilis*) is a commercially and recreationally important Sciaenid species off the coast of California, with fisheries dating back over a hundred years. Beginning in the 1920s-1930s, periods of population decline have been noted in this species, prompting the establishment of management plans. In addition to harvest regulation changes to combat declining stocks, the California state legislature established the Ocean Resources Enhancement and Hatchery Program to aid in the conservation of white seabass through stocking of cultured juveniles in southern California. Aquaculture-based stock enhancement is a powerful tool to promote conservation and increase population abundance of stocks of marine species.

White seabass have been released by Hubbs-SeaWorld Research Institute since the 1980s, with almost 2.7 million juveniles released to date. Although assessments of population structure have been conducted, a fine-scale assessment of genetic health, genetic diversity, and contribution to the wild population is lacking for this species. Our project has developed a highly polymorphic microsatellite marker panel for white seabass which is able to differentiate individuals with a high level of confidence. Using this, biological samples from fin clips and otoliths of over 400 adult white seabass broodstock used in the stocking programs (dating back to 1995) have been genotyped. The resulting genetic data were subsequently used to develop a genetic parentage model in a likelihood-based program (Cervus) to determine the contribution of stocked fish. Following genotyping of samples of unknown parentage, the model provides genetic assignment of the most likely candidate hatchery parents if a stocked fish. Archived white seabass otoliths (>1400) from field collections spanning 1992-2019 (Figure 1) have been genotyped to evaluate comparisons of traditional coded wire tag and genetic-based tagging methods and assess the contribution of cultured white seabass to the wild sub-adult and adult population. The combined dataset will provide the opportunity to characterize fine-scale patterns of gene flow and genetic health of the wild population(s). To responsibly conduct a stocking program which prioritizes the maintenance of diversity in the wild population, a genetic component is a powerful tracking tool to evaluate contribution to the population and provide critical information to minimize any potential negative impacts to the wild population.

Figure 1. Archived white seabass otoliths which range from the Channel Islands, USA to Baja California, Mexico.

CLIMATE CHANGE VULNERABILITY ASSESSMENTS AND AQUACULTURE

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Increases in global population and seafood demand are occurring simultaneously with fisheries decline in an era of rapid climate change. Aquaculture is well positioned to help meet the world's future seafood needs, but heavy reliance of most global aquaculture systems on the ambient environment and ecosystem services suggests an inherent vulnerability to climate change. In order to design and implement a comprehensive and effective response, policy makers and farmers must have an informed understanding of how climate change will affect aquaculture sectors and reliant communities. Climate change vulnerability assessments are an internationally recognized assessment process that can aid the advanced planning of adaptation measures by identifying which sectors, regions, and species are the most threatened.

Vulnerability assessments are generally based on three components: 'exposure', 'adaptive capacity', and 'sensitivity'. Vulnerability assessments can be challenging for several reasons. Firstly, data inputs are often combinations of quantitative and qualitative data, and the resultant model is typically described as semi-quantitative. Some quantitative inputs such as wind and wave exposure may have magnitude and frequency data generated from climatic models. Other inputs, such as farmers' perception of risk, are equally crucial, but are subjective and qualitative. This means that a vulnerability assessment must stitch together very different types of information and translate these into a single understandable value. Another challenge is differences in data availability or scale. For example, high resolution storm surge models, capable of projecting flooding risk under different emissions scenarios, may be available for some coastal regions but not others. In this case, investigators cannot simply exclude storm surge risk, so instead they must pursue a more qualitative approach such as relying on expert opinion. This raises a third challenge: the difficulty of comparing vulnerability across regions that have been assessed using different data and vulnerability methods.

Notwithstanding these limitations, climate change vulnerability assessments are an important tool for guiding decision making. The increasing rate of climate-driven changes does not provide the luxury of waiting until all uncertainties and methodologies have been sorted. Here we present current practices and limitations of climate change vulnerability assessments in aquaculture and suggest possible solutions to practically address these challenges and uncertainties.

MEANS VERSUS MEDIANS: DOES IT MAKE A DIFFERENCE FOR AQUACULTURE REGULATORY COMPLIANCE OF BENTHIC SULFIDES?

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Benthic sulfide concentration is a common proxy for dissolved oxygen. It is often used to classify benthic health as Oxic, Hypoxic, or Anoxic for regulatory compliance of marine finfish aquaculture. This site classification can dictate if mitigative action is required, such as changes in farm management practices or production levels.

Extensive variability is common with environmental physicochemical metrics such as sulfides. Accurate measurements necessitate replicate sample collection to determine measures of central tendency (*i.e.*, mean or median). Scientific research typically aims to confirm or refute a hypothesis through inferential statistics based on an acceptable level of confidence. However, most regulatory environmental monitoring does not achieve the statistical power necessary for valid inferential comparisons. This is often because determining if there is an impact is not in question and measures are used to scale an effect. It could be that a small number of samples are collected in an exploratory nature with results used to trigger a larger sampling regimen, if needed. It may also be that the ideal number of replicates is cost prohibitive or impractical to achieve. Sulfide measurements for benthic classification arguably meet all these criteria, being notoriously difficult to measure, highly variable, and expensive. Consequently, descriptive statistical measures such as means are common sulfide reporting metrics applied for regulatory compliance of marine finfish aquaculture in many jurisdictions. However, sulfide data distributions are also often skewed. This begs the question: would sulfide medians be a more accurate measure for regulatory compliance given the potential for skewness? If so, would medians favour more stringent or more lenient regulatory compliance?

In Nova Scotia, Canada, benthic sulfide data has been collected at marine finfish aquaculture sites annually since 2002. In this jurisdiction, means are applied to replicates to determine sulfide values at individual sample locations, and the mean of sample locations are used to classify farm site condition. A total of 193 historical sulfide site assessments were reanalyzed, applying medians to sample values and sample locations. An R dashboard was created to enable ease of plot generation for individual years and sites, and to track changes in classification upon the application of medians. If medians were applied to only sample values alone, 3% of sites transitioned to a less impacted classification. If medians were applied to both sample values and sample locations, almost 10% of site classifications changed, with most transitioning to a less impacted classification. Implications on environmental monitoring programs of finfish aquaculture are discussed in the context of traditional sulfide monitoring and new more accurate methods being proposed.

EXPLORING AQUACULTURE POTENTIAL OF TWO SPECIES OF SURF CLAMS AT MASSACHUSETTS SHELLFISH FARM SITES

Josh Reitsma[1,2], Harriet Booth, Abigail Archer, Paul Wittenstein, Emma Green-Beach, Matt Weeks, Patrick Ross, Mark Begley, Mike Dunbar, Steve Wright, Craig Poosikian, Iris Pickard, Alex Brown, Dan Martino, Greg Martino, Brandon Small, Roxanne Smolowitz

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Shellfish aquaculture production at the hundreds of farms in Massachusetts is roughly 95% oyster sold by the piece. There has been much interest in diversifying but many challenges. Previous work has shown that Atlantic surf clams have potential as an emerging aquaculture species in the Northeastern US. While clam growth has been relatively rapid in all studies, survival has been challenged by warm waters and intertidal conditions typical of many aquaculture areas in MA. Most aquaculture work in the region has been done with the more common and well-known Atlantic surf clam, *Spisula solidissima solidissima*, though the Southern surf clam, *Spisula solidissima similis* also populates the mouth of many estuaries on the southern side of Cape Cod. The natural history of Southern surf clams is poorly understood in the New England region, but aquaculture potential has been suggested for other parts of the species range.

To determine and compare the aquaculture potential of the two species, seed from both species were obtained using local parent stock in summer of 2020 and verified for genetic identity. The seed of both species were planted at nine different shellfish aquaculture sites representing a range of conditions including subtidal and intertidal. Seven of the sites were monitored closely for growth and survival comparison between the two species using mesh-covered trays buried in the sediment and sampled at roughly bimonthly intervals. Data are being collected into fall of 2021, but early results suggest some clear advantages in survival and sometimes with growth using Southern surf clams, *Spisula solidissima similis*, over Atlantic surf clams, with the most pronounced differences in intertidal conditions.

DEVELOPMENT AND OPTIMIZATION OF A qPCR ASSSAY FOR THREE COMMON FISH PARASITES *Cryptocaryon irritans Uronema marinum* AND *Amyloodinium ocellatum* IN AQUARIUM SYSTEMS

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The goal of this research is to develop a multiplex qPCR assay to detect these three protozoans in the water column of aquarium environments. The ciliate, *Cryptocaryon irritans,* is a common fish parasite that causes marine white spot disease, otherwise known as "Marine Ich", in both natural and aquarium environments. The life cycle of *C. irritans* involves three separate stages that allow for quick infection and mortality of aquarium fish. The most notable stage involves the trophont, which produces the signature "white spot" on infected fish. The theront, a free-swimming stage of *C. irritans*, can survive for approximately 48 hours in the water column. After this time, it either dies or infects a host fish. *Uronema marinum* is an opportunistic parasite that causes infection and mortality in both natural and aquarium environments. Infection of *U. marinum* causes ulceration of the skin and gills, and eventually infiltration of the musculature, skin, and central nervous system. Infection with the dinoflagellate *Amyloodinium ocellatum* is visible by brown spots on the skin and gills of affected fish. This parasite has caused significant losses of aquarium fish in both public and home aquaria. Amyloodiniosis (the disease caused by *A. ocellatum)* is commonly referred to as "Marine Velvet". This protozoan has a similar life cycle to that of *C. irritans*, differing only with the length of time that the dinospores (equivalent to theronts) can remain free-swimming (can survive for 15 days without a host).

All three protozoans have a stage in which they are free-swimming in the water column. Identifying increases in abundance of these parasites in the water column of aquarium tanks would provide time for preventative/treatment methods to occur before morbidity and mortality occurs. Currently, a successful TaqMan qPCR assay has been developed for *C. irritans* using previously designed primers from Taniguchi et al (2011) and a newly designed fluorescent probe. This specific assay has been able to produce an estimated organism number for each sample tested. A TaqMan qPCR assay for *U. marinum* is in development using newly designed, validated primers and a fluorescent probe. Primers are currently in development for *A. ocellatum* for use in the multiplex TaqMan qPCR assay with the other two protozoans. These primers and probes will be added to the *C. irritans* qPCR for simultaneous detection of the three parasites in the water column of aquarium systems.

EFFECTS OF SULFADIAZINE AND PLUMBUM ON NITROGEN CYCLING OF THE SEA CUCUMBER *Apstichopus japonicus* CULTURE POND

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Antibiotics and heavy metals are potential pollutants that threaten the healthy development of coastal aquaculture. In this study, we conducted an indoor experiment using sediment column to evaluate the effects of sulfadiazine (SDZ) and plumbum (Pb^{2+}) on nitrogen cycling of the sea cucumber *Apstichopus japonicus* culture pond system. Three treatments were designed *i.e.* SDZ treatment (SDZ), ion Pb^{2+} treatment (Pb) and the mixture of SDZ and Pb^{2+} (SP) were added to the overlying water of the sediment column. The fluxes of ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) in sediment-water interface; the gene abundance of ammonia monooxygenase (amoA), nitrite reductase (nirK, nirS) and nitrous oxide reductase (nosZ) in the sediments were evaluated; the sediment microbiota was analyzed by 16S rRNA. The results showed that three treatments all promoted NH_4^+-N emission; the emission of NO_3-N and NO_2^-N were first decreased then increased in the three treatments($P<0.05$). The sediment genes abundance were all significantly decreased in the three treatments, except that amoA in the SDZ treatment and nirS in the SP treatment were significantly increased($P<0.05$). A total of 110 phyla in sediment microbiota were identified. The number of operational taxonomic units (OUTs) and Shannon index were significantly increased in the Pb and SP treatments($P<0.05$). The functions of nitrous oxide reduction, nitrate reduction and nitrite denitrification microbial community acted as biomarkers which may influence nitrogen cycling process in sea cucumber culture pond. Difference abundance of microflora *Proteobacteria* and *Firmicutes* may influence the abundance of nitrogen cycling. In conclusion, the SDZ and Pb had an influence on nitrogen cycling emission of the sea cucumber culture pond system by changing the abundance of sediment microbial community and the nitrogen cycling genes. This study provide theoretical basis for optimizing the culture environment of sea cucumber from the aspect of nitrogen cycling.

FISH HEALTH CERTIFICATION PROGRAM AT ARKANSAS AGRICULTURE DEPARTMENT, LITTLE ROCK

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The aquaculture industry is rapidly growing in supplying a protein food source for human consumption. As aquaculture continues to grow, so do the fish health regulations. As a result, the need for reliable fish health testing for export purposes and certification has grown exponentially. Arkansas Veterinary Diagnostic laboratory (AVDL) uses various testing methods to meet the multiple requirements of state, regional, and international authorities. The Arkansas Agriculture Department started a new fish certification program in 2021 to inspect fish for the presence of diseases prior to export to other states and countries. A significant part of this program has been driven by the Arkansas Bait and Ornamental Fish Certification Program. Annual and semi-annual farm-level fish health inspections for specific pathogens according to OIE standards are conducted by the laboratory using National Animal Health Laboratory Network (NAHLN), Animal and Plant Health Inspection Service (APHIS) approved protocols. This program inspects nearly 22,000 fish annually from Arkansas state as well as several other states.

AQUACULTURE AT THE CROSSROADS OF GLOBAL WARMING AND ANTIMICROBIAL RESISTANCE AND THE USE OF BIOACTIVE PLANTS AND ALGAE AS A SUSTAINABLE ALTERNATIVE

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Considering aquaculture's importance in global food security, it is key to fully understand the risks this sector might face in the light of global change. Aquatic animal diseases are one of the major limiting factors in aquaculture development, yet it is poorly understood how their emergence and severity might change with global warming. Furthermore, the extensive use of antibiotics to prevent and treat such diseases has been associated with the emergence and spread of antibiotic resistance (AMR) bacteria, posing a serious threat to global health. Our research has focused on trying to understand how the aquaculture sector will be impacted by these global threats and we found that aquaculture-derived Multi-Antibiotic Resistance (MAR) indices correlate with MAR indices from human clinical bacteria, temperature, and countries' climate vulnerability (Figure 1). We also observed that infected aquatic animals present higher mortalities at warmer temperatures. These results suggest that countries most vulnerable to climate change, will probably face the highest losses in aquatic animals together with the highest AMR risks, highlighting the need for urgent action. In this regard, sustainable solutions to minimize antibiotic use and increase system and animal resilience (i.e. OneHealth approaches) like using functional feeds (e.g. bioactive plants) are urgently needed. We previously found that the red algae *Asparagopsis taxiformis* increased the expression of two immune-related genes in the orbicular batfish and displayed antibacterial properties against fish pathogens such as *Tenacibaculum* bacteria. More recently, we performed a meta-analysis and observed that plant-enriched diets significantly increased fish growth, immunity parameters and disease resistance. Interestingly, these effects were overall conserved regardless of the fish trophic level, treatment duration and type of plant material used (i.e. powder or extract).

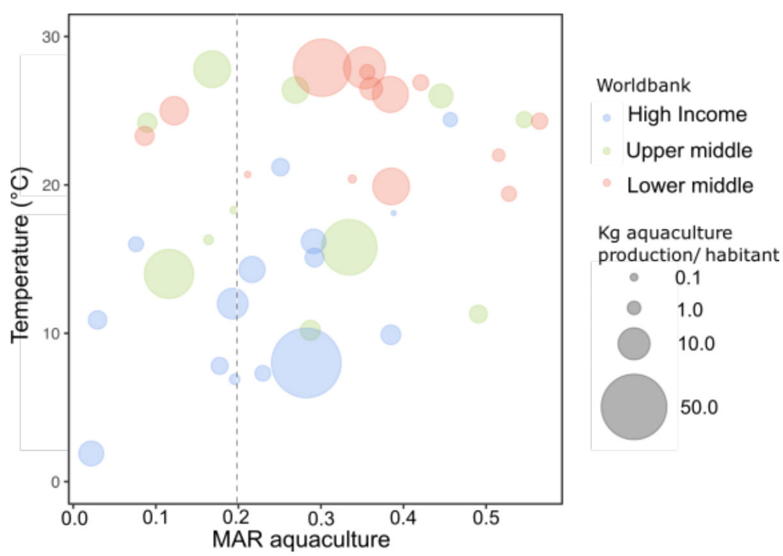

Figure 1. Correlations between the MAR from aquaculture-related bacteria and environmental temperature.

MARKET ANALYSIS OF HAWAIIAN GROWN SEA CUCUMBERS *Stichopus horrens, Holothuria whitmaei* AND *Actinopyga varians*

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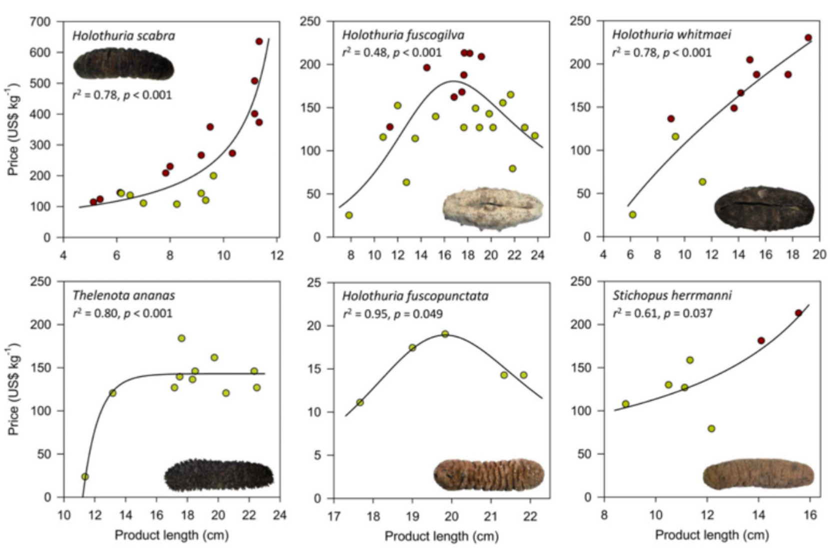
The use of fishponds was integrated into the socio-economic culture of Hawai'i for hundreds of years. Prior to contact with the Europeans in 1778, there were estimates of 360 fishponds producing over 900,000 kg fish/year. Since contact with the westerners, the use of fishponds has decreased but a restoration and cultural revival is underway. Hawaiian fishponds are known to produce many species of finfish (mullet, milkfish, gobies, perch, etc.), seaweeds, shellfish, and terrestrial crops. Kaua'i Sea Farm (KSF) will be the first to culture sea cucumbers in their fishpond.

KSF operates the Nomilio fishpond, an 18-acre pond formed in the caldera of an extinct volcano that filled with water. Two channels (*auwai*) connect the pond to the ocean allowing fresh and salt water to mix. Nomilio is ideal for growing a wide range of species. Currently, KSF is growing northern quahogs (*Mercenaria mercenaria*), Pacific oysters (*Crassostrea gigas*) and eastern oysters (*Crassostrea virginica*). In 2022, KSF will be introducing three native species of sea cucumbers *Stichopus horrens*, *Holothuria whitmaei* and *Actinopyga varians* to their fishpond.

Sea cucumbers are a highly valued species and have been consumed, primarily in Asia, since ancient times as both foods and traditional medicines (Mohsen et al., 2021). A high market demand still exists today, and wild stocks are often overfished and overexploited (Eggertsen et al., 2020). Sea cucumber aquaculture exists throughout the world especially in India, Madagascar, and Australia. With the high demand most sea cucumbers, wild caught or cultured, are exported to Asia for final sale. The aim of this study is to: (1) investigate a domestic market for three species of native Hawaiian sea cucumbers grown in Nomilio in the United States, and (2) survey other Hawaiian fishpond owners about incorporating sea cucumbers into their fishpond systems. If we find there is a lucrative market for them maybe sea cucumbers can help further revitalize fishponds and add a new source of revenue for Hawai'i.



OPTIMIZING ECOSYSTEM SERVICES ASSOCIATED WITH SHELLFISH AQUACULTURE

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A panel of experts will discuss the ecosystem services associated with shellfish aquaculture (habitat provision, nutrient removal, eutrophication mitigation, benthic stabilization, etc.) and encourage workshop participants to brainstorm and propose ways in which growers might tweak their farming methods to maximize the various services and improve the restorative aspects of shellfish farming.

Panelists:
Lisa Kellogg, VIMS
Daphne Munroe, Rutgers University
Heidi Alleway, The Nature Conservancy
Brett Dumbauld, Oregon State
Seth Theuerkauf, NOAA

DEVELOPMENT OF AN INNOVATIVE HATCHERY SYSTEM FOR BLACK GROUPER *Mycteroperca bonaci* AND RED GROUPER *Epinephelus morio* AT THE COLLEGE OF THE FLORIDA KEYS

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Traditional marine finfish aquaculture utilizes captive male and female broodstock to provide fertilized eggs for nursery and growout operations. This presents challenges for grouper aquaculture, especially in the United States where there are no hatcheries for any grouper species. Grouper are protogynous hermaphrodites (i.e. mature first as female and then transition to males) with a single large male controlling a harem of smaller females. This is problematic when trying to capture male broodstock because males typically live in deeper water (e.g. > 30m depth) and experience barrow trauma as the gas bladder expands during capture. Females are much easier to capture because they are more abundant and live in shallower water. However, expecting females to transition into male broodstock in captivity is unrealistic and could take many years, if ever. Therefore, finding alternative grouper broodstock management strategies for hatchery production could benefit the industry, especially in the US.

An alternative method for grouper broodstock management and fertilized egg production for black grouper (*Mycteroperca bonaci*) and red grouper (*Epinephelus morio*) is currently being developed at the Southernmost Marine Aquaculture Research & Training (SMART) Center at the College of the Florida Keys. The concept focuses on the collection of grouper sperm from a variety of sources including: (1) visceral discards from commercial and recreational fishers, (2) collection of sperm during catch-and-release fishing, and (3) captive grouper in public aquaria. Viable sperm are then cryopreserved in liquid nitrogen and stored long-term in a -86°C freezer.

Female grouper are easier to bring into captivity, so special recirculating aquaculture systems are being developed at the SMART Center to control temperature, photoperiod, and lunar cycle, mimicking spawning conditions for *M. bonaci* and *E. mori*. Other factors introduced into the broodstock systems include: (1) recordings of spawning behavior, (2) small amounts of grouper sperm from cryopreserved samples, and (3) a combination of both treatments. Fish behavior will be recorded using submersible infra-red cameras and periodic sampling for egg development. If natural egg production is unsuccessful, female grouper will be implanted with Oviplant™ (i.e. Salmon Gonadotropin – Releasing Hormone analogue; sGnRHa). Eggs produced will then be mixed with thawed sperm from cryopreserved samples and fertilization rates will be recorded. If successful, the systems and protocols can be applied to other commercially or ecologically important marine species that: (1) exhibit complicated life history strategies, (2) are difficult to spawn in captivity using traditional broodstock management techniques, and/or (3) have eggs, sperm, or gonads that can be easily obtained during commercial or recreational fishing.

NATIVE OYSTER RESTORATION FROM CALIFORNIA TO BRITISH COLUMBIA: LESSONS FOR THE CONSERVATION OF MARINE FOUNDATION SPECIES

April D. Ridlon*, Althea Marks, Chela J. Zabin, Danielle Zacherl, Brian Allen, Jeffrey Crooks, Gary Fleener, Edwin Grosholz, Betsy Peabody, Jodie Toft & Kerstin Wasson

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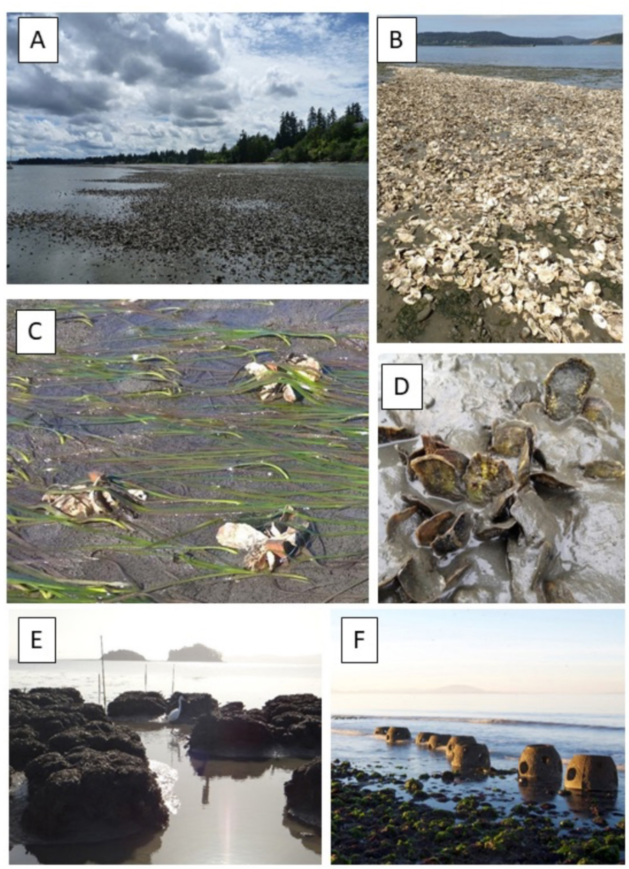
Marine foundation species (e.g. mangroves, kelp, corals, oysters) are critical to the structure and resilience of coastal ecosystems and provide key ecosystem services. Like many other foundation species, Olympia oysters (*Ostrea lurida*) have suffered severe population declines, to the point of local extinction in some estuaries. The restoration of these populations is a priority for maintaining ecosystem function of estuaries along the west coast of North America. In the first synthesis of all native oyster restoration projects conducted from California, USA, to British Columbia, Canada, we analyzed project motivations, goals, methods, and outcomes to provide a picture of the status of restoration for this species.

We found that restoration projects are currently spread unevenly across the species' range, driven by local goals and implemented with differing, sometimes contrasting approaches. We highlight the value of regional strategic planning and decision support tools to evaluate project design and methods for restoration, including the selection of substrates and the targeted use of aquaculture. We recommend future investment in larger projects, which our analysis found were more cost-effective, but which have been relatively rare for this species. We also recommend that funders support monitoring over broader temporal and spatial scales than in the past to better characterize long-term effects of restoration on oyster populations and the services they provide beyond the project footprint. We found that most projects successfully supported native oysters and engaged local communities, and recommended similar efforts to continue to enhance understanding of Olympia oysters, which remain unfamiliar to many coastal residents. We believe that the results of this synthesis are broadly applicable to marine foundation species generally, and can inform restoration and conservation efforts worldwide.



CONSERVATION AQUACULTURE OF OLYMPIA OYSTERS *Ostrea lurida:* A FRAMEWORK FOR EVALUATING THE BENEFITS AND RISKS OF THIS INTERVENTION FOR MARINE SPECIES RECOVERY

April D. Ridlon*, Kerstin Wasson, Tiffany Waters, John Adams, Jamie Donatuto, Gary Fleener, Halley Froehlich, Rhona Govender, Aaron Kornbluth, Julio Lorda, Betsy Peabody, Gifford Pinchot IV, Steven S. Rumrill, Elizabeth Tobin, Chela J. Zabin, Danielle Zacherl, Edwin D. Grosholz

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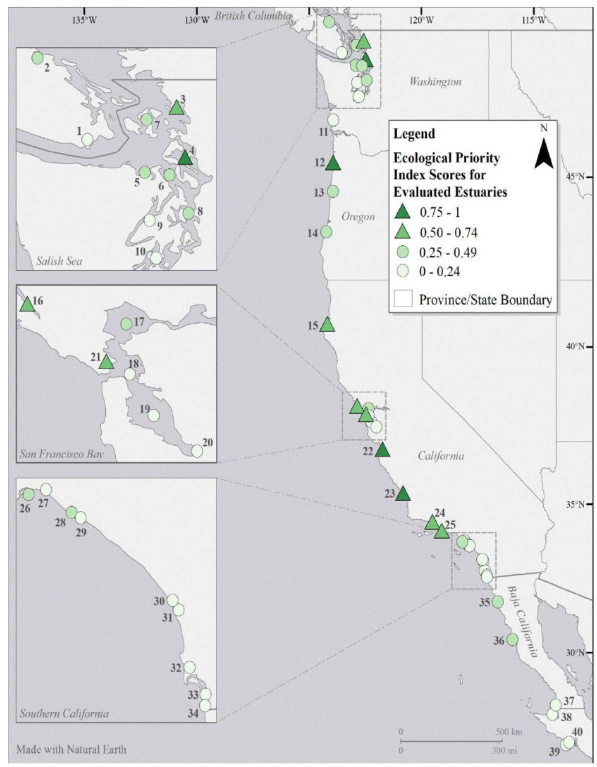
Conservation aquaculture - culturing a species to support conservation goals - is becoming an important tool to support marine species recovery and meet human needs. However, this tool comes with risks as well as rewards, which must be assessed to guide aquaculture activities and recovery efforts. Here, we present a range-wide approach to strategically planning the use of aquaculture to promote recovery of the most severely declined Olympia oyster (*Ostrea lurida*) populations, while also benefiting people.

We identified 12 benefits and 11 risks of culturing Olympia oysters. Benefits included identifying climate-resilient phenotypes that add diversity to growers' portfolios, while risks included potential negative ecological and genetic consequences of transferring hatchery-raised oysters into wild populations. Informed by these trade-offs, we identified ten priority estuaries along the range of the Olympia oyster where aquaculture is most likely to benefit the recovery of wild populations and where the benefits clearly outweigh the risks.



By integrating social criteria, we also evaluated which project types would likely meet the goals of local stakeholders in each estuary. Community restoration was most broadly suited to the priority areas, with limited commercial aquaculture and no current community harvest of the species, although this is a future stakeholder goal.

The framework we developed to evaluate aquaculture as a conservation intervention can be used with marine species globally; we provide a guide to prioritizing local knowledge and developing recommendations for implementation via transparent criteria. Our collaborative process engaging diverse stakeholders including managers, scientists, Indigenous Tribal representatives, and shellfish growers can be used elsewhere to seek win-win opportunities to expand conservation aquaculture where benefits are maximized for both people and imperiled species.

ALTERNATIVE PROTEINS: AN EVALUATION OF THE FISHMEAL FUTURE

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As aquaculture continues to grow worldwide, the demand for nutritional fish meal (FM) is increasing. Fish meal production increased 6.5% in the first eight months of 2021, yet this is not the sustainable choice for commercial aquaculture. The nutritional breakdown of commercial FM can be supplemented with alternative choices that have a more sustainable production path. It is estimated that 90% of the fish used for FM meets the standards for human consumption, so why are we using it to feed the fish we intend to eat? Global feed fish stocks are declining and disrupting ocean ecosystems. The potential of these fisheries collapsing could be devastating for maintaining the seafood industry. The current movement to continue adding soy-based protein to FM leads to an increasingly unsustainable future. Additionally, there are alternative proteins, such as seaweed and microbial meals that have yet to hit the commercial scale but are promising nutritional supplements. These emerging protein alternatives have significantly less carbon footprints when compared to soy and fish products, while maintaining the necessary nutrients. In short, this paper will review current fishmeal ingredients and alternative, novel fishmeal substitutes for their nutrient profiles and sustainability. As we continue to keep our planet's health in mind, it is imperative to prioritize sustainability and efficiency while considering FM alternative proteins. Although many of these products are in the infancy of production we will discuss their potential to reach the commercial aquaculture market. While also considering anti-nutritional factors and potential environmental externalities. Further research investigating the success of these products in a large-scale setting still needs to be addressed.

Possible Articles

Soy https://onlinelibrary.wiley.com/doi/10.1111/anu.13274
Brine https://www.globalseafood.org/advocate/brine-shrimp-bottleneck-expansion-part-1/

THE MAINE OYSTER TRAIL: A CASE STUDY FOR AQUATOURISM IN MAINE

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The Maine Oyster Trail is the first interactive and digital oyster trail in the U.S. and features 79 Maine oyster businesses which each offer one-of-a-kind experiences for visitors, like farm tours and direct sales. In 2020, the coronavirus pandemic had a major impact on Maine's growing oyster industry. To adapt to market changes, many oyster farmers began to diversify their businesses by incorporating direct-to-consumer sales, farm tours, and shucking events. In response, Maine Sea Grant and the Maine Aquaculture Association began to rebuild the Maine Oyster Trail to drive coordinated tourism activity to these new markets and opportunities. With culinary activities and seafood remaining the most popular interest for Maine visitors, the Maine Oyster Trail presents an opportunity to introduce visitors to the craft of oyster farming, while building community engagement and social license for aquaculture operations.

In rebuilding the Maine Oyster Trail, we aimed to create a user-friendly interactive website that allows visitors to find oyster farms and businesses, based on desired type of experience offered and location in the state. We also endeavored to design a mobile, digital passport feature that encourages users to track their farm visits, log their experiences on the trail, and earn rewards for visiting farms.

We began this project by surveying Maine oyster farmers to learn what visitor experiences they were offering or interested in. We made partnerships with other related trail initiatives, like the Maine Beer Trail, to understand how successful incentive-based trails function. In addition to web design, we worked directly with the farms and businesses to understand their unique operations and the details that would need to be included for the virtual tools to meet their needs. We created partnerships with other incentive-based trail initiatives, like the Maine Beer Trail, and web design professionals. To generate interest and begin marketing the trail ahead of the web launch, we created social media accounts and generated photo assets of farms.

The new Maine Oyster Trail launched in June 2021, with a website (maineoystertrail.com) that includes a customizable trip planner and a mobile digital passport. At the end of the first season (2021), the new Maine Oyster Trail has 79 participating businesses, over 1500 registered users, and nearly 2000 passport check-ins at oyster businesses. The website and social media analytics allow us to track user engagements, like accounts created, farm check-ins completed, and rewards earned. Additionally, the user-generated data allows us to report back to participating farms and businesses to directly demonstrate value of the trail and inform business practices around new oyster tourism ventures. The re-launched Maine Oyster Trail balances the needs of farmers and visitors, creating new and engaging tourism opportunities for visitors, while increasing value and business opportunities for Maine oyster farms.

SEAWEED AQUACULTURE IN MAINE: A DECADE OF DEVELOPMENT AND INNOVATION

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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. Seaweed cultivation has been extensively explored as a zero-input agricultural crop (growing entirely from ambient nutrients and the marine environment); as well as an aquaculture product with environmental co-benefits to cultivation including improvement of local water quality by way of increasing dissolved oxygen, uptake of excess nutrients, and sequestration of carbon dioxide. Furthermore, marine resource infrastructure and markets in Maine and the U.S. are well positioned to incorporate these emerging species and products into revenue streams alongside traditional fisheries.

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. A decade later, Maine has over 30 commercial kelp farms, 3 native kelp species under cultivation, exponential growth in acreage and landings (Figures 1, 2), diverse and expanding processing operations, and a viable supply chain. The rapid growth in Maine's emerging kelp sector has been enabled by an organized permitting process, a well-established maritime workforce and infrastructure, and coupled processor-product producer businesses that contract kelp from farmers and work with wholesalers and retailers to reach end consumers. Continued research and investments in seaweed production systems, processing infrastructure and technology, and new markets are critical to advancing the emerging seaweed sector in Maine and the U.S.

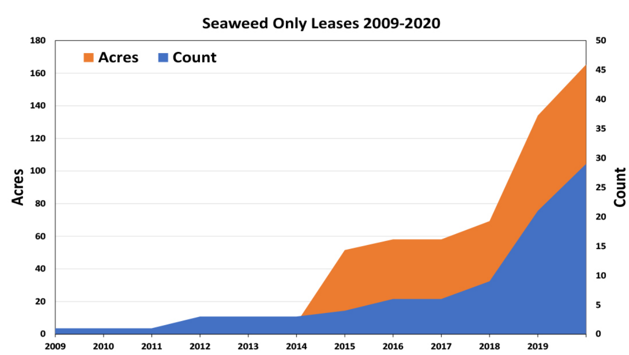

Figure 1. Seaweed leases and acreage under cultivation in Maine, 2009-2019. Source: Maine Department of Marine Resources, 2021.

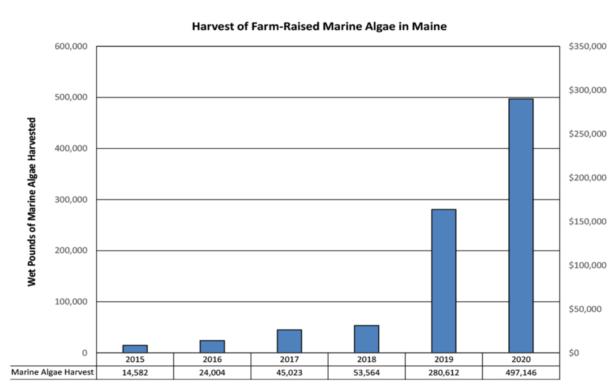

Figure 2. Growth in farmed seaweed landings, 2015-2020. Source: Maine Department of Marine Resources, 2021.

ECONOMIC CHALLENGES FACING THE EXPANSION OF THE US AQUACULTURE INDUSTRY

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In the United States, most seafood consumed is imported, with over more than half these imports coming from aquaculture. Domestic wild capture fisheries are limited in their productive capacity of US fishing grounds. However, U.S. consumers demand for seafood is continuing to increase with consumers also trying a wider range of seafood and more than 70% of seafood consumed outside of the home. With aquaculture products usually being sold directly to restaurants, wholesalers, and for non-food consumption to processors, the US aquaculture industry has to look beyond production efficiencies and integrate marketing opportunities along with other social benefits to truly emerge as a domestic leader in aquaculture. This paper will examine the extent to which the US aquaculture industry has the capacity to expand, along with the alternatives that could provide implicit value to the industry and supporting communities associated with the industry.

EFFECTS OF A NON-INDIGENOUS BRYOZOAN ON THE RECRUITMENT OF THE NATIVE OLYMPIA OYSTER, *Ostrea lurida*

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Non-indigenous fouling organisms settling onto artificial and natural hard substrata in estuaries can negatively impact native species via space competition, predation, or other mechanisms. The effects of *Amathia verticillatum,* and other fouling organisms on the recruitment of *Ostrea lurida,* were studied to determine whether their presence, biomass, and abundance affect oyster recruitment.

Terracotta tiles, proxies for available hard substrata, were deployed in Newport Bay, California at tidal elevations between -0.4 and +0.1 feet MLLW, April-October 2020, during the oyster's spawning and recruitment season. Five treatment groups (n=5 replicates per tile) were established to examine the effects of *A. verticillata* and other fouling organisms on recruitment of *O. lurida*: unmanipulated controls, *A. verticillata* removals, *A. verticillata* plus other fouler removals, other fouler removals with *A. verticillatum* additions, and other fouler removals with 2X *A. verticillata* additions. The treatment groups were maintained by adding or removing *A. verticillata* foulers as appropriate per treatment; all removals were quantified via volume displacement as a proxy for biomass. During tile retrieval, the volume displacement and wet weight of *A. verticillata* and other foulers were recorded. Oysters recruiting to the tiles were measured for length and width, identified, and counted. Percent cover of all species recruiting to the tiles using point contact techniques was recorded.

Results suggest that *A. verticillata* is facilitating *O. lurida* recruitment as *A. verticillata* removals had lower oyster recruitment. Some non-indigenous fouling organisms may not be as problematic as predicted when restoring native species in estuarine communities.

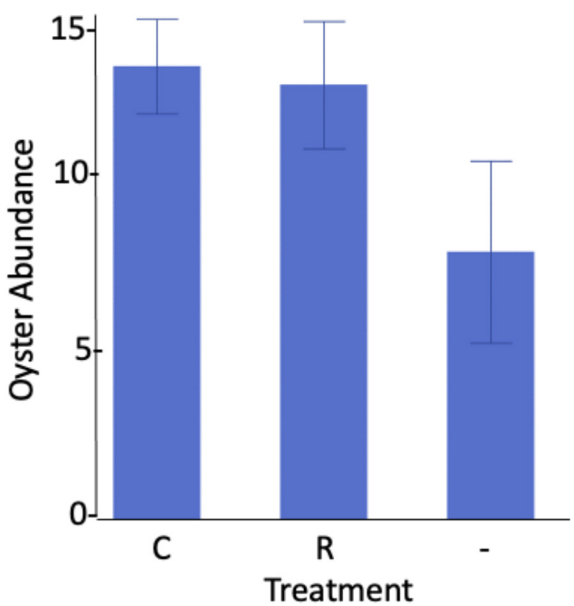

Figure 8. Average number of *O.lurida* on under side of tile per control (C), removal (R), and *A. verticiillata* (-) treatment (ANOVA, p<0.05). Error bars = 1 SE.

EPIGENETIC REGULATION IN AQUATIC MICRO-INVERTEBRATES: A NON-CANONICAL SYSTEM OF BACTERIAL ORIGIN

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Epigenetics is the study of mechanisms that have the capacity to direct regional and local activation or silencing of genes and transposons. The interest in epigenetic mechanisms has added an additional layer in the eukaryote genome complexity, which include covalent modification of histones, DNA methylation, and non-coding RNA. The genome contributes to epigenetic processes by encoding the necessary enzymatic machinery, such as methyltransferases, demethylases, or RNA-mediated silencing proteins. Altogether, with the inclusion of new sequencing techniques, it is becoming more feasible to characterize new molecular-level epigenetic factors even in non-model organisms.

Bdelloid rotifers are microscopic freshwater invertebrates able to survive desiccation at any life stage and to reproduce asexually. The genome of the bdelloid rotifer *Adineta vaga* is unusual in having over 8% of its genes originated from non-metazoan (predominantly bacterial) sources. Nevertheless, transposable elements (TEs) span only about 3.5% of the *A. vaga* genome, an unusually low fraction for a eukaryote. TE content is apparently being kept at a very low level by an expanded machinery for RNA-mediated silencing, which ensures production of pi-like RNAs leading to TE repression. Recently, we found that bdelloid rotifers lack the typical eukaryotic Dnmt methyltransferases responsible for 5mC DNA modifications, but instead encode an amino-methyltransferase of bacterial origin, which is fused to a eukaryotic chromodomain. We propose that the *A. vaga* amino-MTase may form a new layer in a genome defense system against invading TEs, which do not proliferate efficiently in bdelloid genomes and could be subject to unusual forms of epigenetic regulation.

CHROMOSOME LOCATIONS OF *RTE-3_LVa* NON-LTR RETROTRANSPOSON FROM THE FIRST SPECIFIC PATHOGEN-FREE (SPF) *Penaeus vannamei* PRODUCED IN THE UNITED STATES – A POTENTIAL SEX MARKER FOR SHRIMP

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Infectious hypodermal and hematopoietic necrosis virus (IHHNV), now called *Decapod penstylhamaparvovirus 1,* is one of the major viral pathogens of penaeid shrimps. Infection with this virus resulted in severe mortalities of up to 90% in *Penaeus stylirostris*. It is less virulent in *P. vannamei* and *P. monodon* and does not typically cause mortality; but it 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 shrimp containing DQ228358 do not cause infection in laboratory infection studies. 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 using DNA from adult SPF *P. vannamei* and primers from two microsatellites similar to *RTE-3_LVa* showed sex-specific bands, suggesting that *RTE-3_LVa* is a potential sex marker for shrimp. To 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 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.

AQUACULTURE, EXTERNALITIES, AND POLICY SOLUTIONS

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Global seafood supply is increasing, and seafood prices are steady despite plateauing global capture fishery harvests and reports of collapsing fish stocks. The reason is that aquaculture (farming seafood) is a rapidly growing food production technology that now accounts for roughly half of global seafood supply. Aquaculture is a key contributor to food security, but fish farming interacts closely with the surrounding ecosystem, and its rapid growth globally raises many environmental concerns. Potential negative externalities include water quality, disease spillovers, wild-domesticated genetic interactions, overuse of antibiotics, and the effects of reliance on wild-caught fish for feed on fish stocks. We show that the environmental externalities can be positive as well as negative, some are not true externalities because firms have incentives to internalize them, some perceived externalities do not exist, and the remaining ones can be addressed primarily through spatial management. Because outcomes are strongly influenced by governance of space, management challenges include both commons and anti-commons problems. We conclude that governance should focus on spatial management, adaptation to climate change, and facilitating technological innovation to address externalities and encourage sustainable development of the sector.

THE HURDLES OF DELIVERY CRISPR-Cas9 COMPONENTS FOR GENE EDITING IN PENAEID SHRIMPS

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CRISPR-Cas9 is often described as a revolutionary tool that unlocked the great potential of genetic edition due to the efficiency and feasibility of the method, allowing great advances in reverse and forward genetics studies. CRISPR-Cas platforms have expanded the toolbox in aquaculture, leading to great advances from the description of gene function to the establishment of new breeds with desirable phenotypes related to economic interest, and ultimately to food security and sustainability. Gene edition using the CRISPR-Cas9 system has been achieved in some crustacean species, however in Penaeid shrimps is particularly challenging, and several hurdles have halted the potential application of CRISPR-Cas9 technology in shrimp Aquaculture. In this work, the challenges to overcome during each step of the in vivo CRISPR-Cas gene edition process will be discussed. A particular focus on experimental approaches based on microinjection-free protocols for delivery of CRISPR-Cas components will be presented.

Physical (electroporation) and chemical (polyethylenimine and cationic lipids) transfection methods were applied in *P. vannamei* zygotes. Three different cargoes were prepared: DNA plasmid, mRNA, and a recombinant protein. Different ratios of sgRNA designed to recognize the PvCatL gene were used to prepare the CRISPR-Cas9 complexes. Treated shrimp zygotes were genotyped by HRM analysis and Sanger sequencing. Although high hatching rates were observed for most treatments, no irrefutable evidence of typical CRISPR-Cas9-induced gene edition was found; instead, an enrichment of gene variants was observed in treated organisms, which was detectable by HRM. The results are of interest to Aquaculture researchers working on this challenging topic, helping to improve their experimental design or as a reference to evaluate additional conditions to achieve the gene editing in Penaeid shrimps.

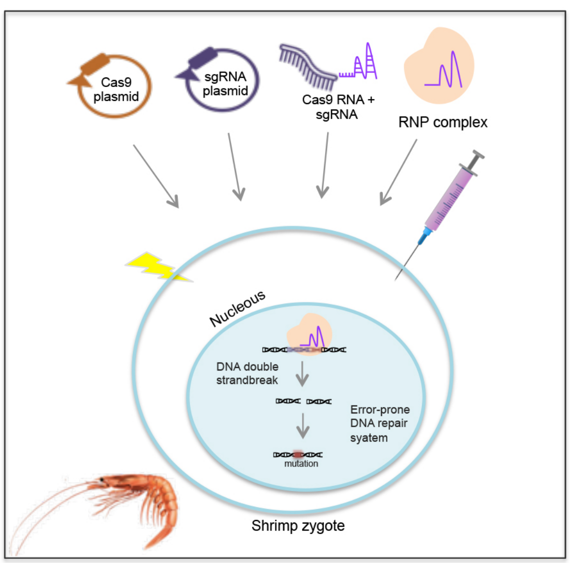

Figure 1. CRISPR-Cas9 gene edition system and its potential for Penaeid shrimp gene edition.

UNDERSTANDING HOW GROWTH AND YIELD VARY WITH RESPECT TO DENSITY FOR THE ATLATNIC SEA SCALLOP

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A two-year study was conducted, beginning in 2018, to understand the effect of density on the biological processes of the Atlantic sea scallop, Placopecten magellanicus. Quarterly sampling was conducted in two study areas of the resource, referred to as the Elephant Trunk Flex and Nantucket Lightship, where extreme recruitment events were observed in 2012 and 2013. Data collected included total scallop catch, as well as individual sea scallop length measurements, adductor muscle, gonad, and viscera weights, sex, reproductive stage, and shell samples for ageing. Data were analyzed to assess the impact of density on growth and yield using several methods. Generalized additive models were developed to model the relationship between catch-at-length and density (Figure 1). Growth data collected from shell samples were analyzed with a von Bertalanffy growth model. Finally, shell height versus meat weight relationships were estimated that incorporated several variables including density, study area, and depth (Figure 2). All analyses indicated density in combination with settlement at depth in potentially marginal habitat contributed to reductions in growth and yield.

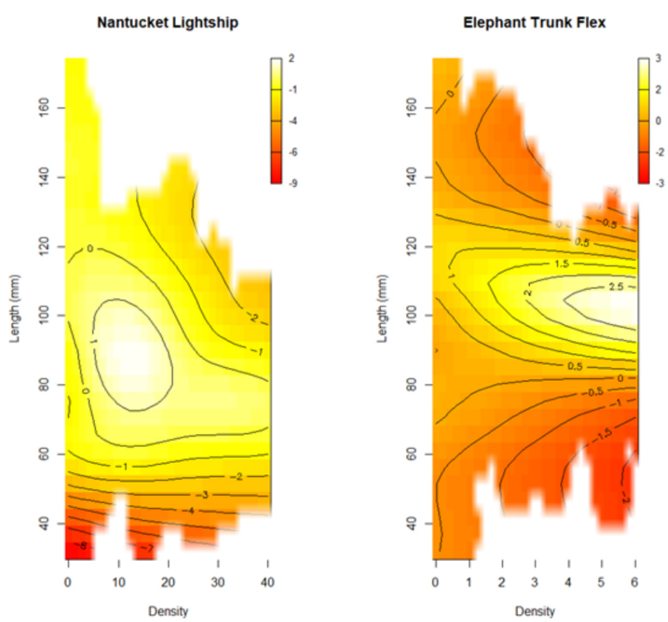

Figure 1. Predicted scallop catch-at-length as a function of density by study area.

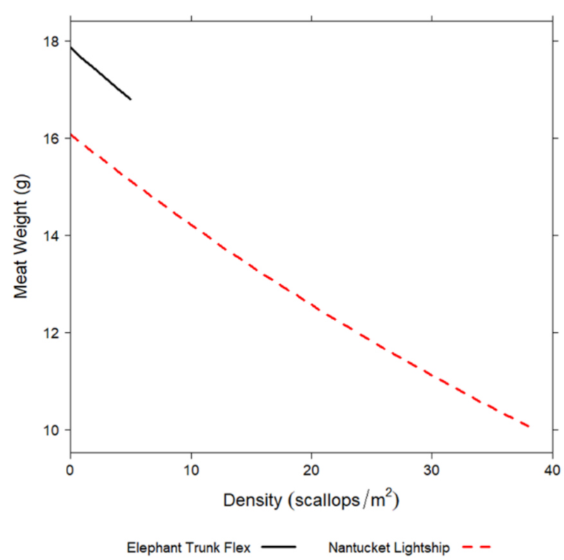

Figure 2. Predicted adductor muscle weight as a function of density by study area.

REPRODUCTIVE STATUS OF DIPLOID AND TRIPLOID MANILA CLAMS *Ruditapes philippinarum* REARED IN THORNDYKE BAY, WASHINGTON, USA

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The Manila clam has the second-highest aquaculture production, after Pacific oysters, on the U.S. West Coast. Post-harvest spawning by Manila clams in summer, however, results in substantial losses of product and reputation. Inducing triploidy (three sets of chromosomes) ought to solve this problem, since triploid (3n) bivalves are typically effectively sterile. We investigated the reproductive status of 3n and 2n Manila clams to confirm this assumption.

We produced a 1:1 mixture of 3n and 2n Manila clams on October 9, 2020. Ploidy was assessed by flow cytometry at 48 h, and the 1:1 ploidy ratio was confirmed in seed of 2 mm shell length. Larvae were reared in the hatchery for 23 days and then transferred to a downwelling tank and over-wintered in a temperature-controlled system with steady food. On May 28, 2021, we sorted clams into size cohorts and planted 400 clams, holding on an 850 µm screen, in Thorndyke Bay, WA, USA. Clams were planted at +0.3 m tidal height, in 15.2 mm diameter tubes covered with predator-exclusion netting, at 10 clams per tube. On August 31, 2021, we harvested 40 clams (confirmed: 18, 3n; 21, 2n) in order to analyze size (length, live weight) and reproductive status.

Although 3n were smaller than 2n, the differences were not statistically significant. All 2n appeared to have normal gonad development, with most having nearly fully or fully developed follicles or tubules (2+). All but one 3n had abnormal reproductive development. Shown below are a normal, 2n female (left) and an abnormal, 3n female (right). 3n Manila clams are sterile.

			Shell length (mm)		Live weight (g)		n, ≥ 2+	n, abnormal
Sex	Ploidy	n	Mean	Std Dev	Mean	Std Dev	gonad state	gonad state
f	2n	12	20.6	2.4	1.64	0.51	8	0
m	2n	9	20.4	2.3	1.69	0.56	9	0
f	3n	17	19.3	2.0	1.30	0.42	1	16
m	3n	1	20.2	-	1.37	-	0	1

ESTIMATING THE ECONOMIC IMPACTS TO THE FLORIDA SHELLFISH AQUACULTURE INDUSTRY OF THE 2021 RED TIDE EVENT

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Harmful algal blooms (HABs) occur when natural algae occurring in marine or freshwater environments grow uncontrollably, due to natural processes or elevated nutrient levels, and produce toxins harmful to plants, animals, humans, and ecosystems. Red tide is a HAB caused by the marine dinoflagellate, *Karenia brevis*, that has occurred multiple times along Florida's Gulf Coast over the past 16 years with major outbreaks occurring in 2005-2006, 2014, and 2017-2019. When red tides occur filter-feeding bivalves, like clams and oysters, can accumulate toxins that can cause neurotoxic shellfish poisoning. Because of this threat, Florida red tides lead to regulatory harvest closures of both aquaculture and wild harvest shellfish sites impacted by the blooms. While red tide events have little to no impact on shellfish survival, extended red tide events and associated shellfish aquaculture area closures can lead to significant economic losses for growers and wholesalers. Delayed harvests associated with red tide events can lead to losses associated with increased levels of natural mortality, an inability to market an increased supply of product when harvest restrictions are lifted, and inability to market clams and oysters that have grown past generally accepted market size.

Florida's Gulf Coast is currently experiencing a red tide event that started in December 2020 and is still active. This event started in Southwest Florida and has moved up the coast to impact Florida's Big Bend and Panhandle regions. Our analysis uses Florida Department of Agricultural and Consumer Services (FDACS) data on shellfish aquaculture production and closures by county along with price data gathered through surveys of Florida shellfish wholesalers to estimate the economic impacts associated with the 2021 red tide event. IMPLAN © software and Florida state/county databases from 2019 are used to estimate direct, indirect, and induced effects of the red tide on employment, labor income, value added, and output associated with both clam and oyster farming along Florida's Gulf Coast.

HIGH DENSITY CULTIVATION AND CO_2 UPTAKE BY PANEL ARRAYS OF THE MACROPHYTIC RED ALGA *Gracilaria vermiculophylla* IN A 100 L RACEWAY POND

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New approaches are needed for land-based cultivation of macrophytic red algae that reduce costly aeration requirements for biomass suspension and enable process intensification. The goal of this study was to demonstrate the high-density cultivation of the carbohydrate-rich macrophytic red alga *Gracilaria vermiculophylla* on vertical arrays of panels deployed in an open channel raceway configuration similar to raceway ponds that have been developed for mass cultivation of microalgae.

A clonal culture of *G. vermiculophylla*, consisting of branched, cylindrical thallus tissues of 8-10 cm length, was mechanically blended into 2-3 cm fragments and then fluidically injected onto a 3 mm polypropylene mesh support. Immobilized *G. vermiculophylla* mesh panels were aligned parallel to flowing seawater medium at nominal bulk velocity of 20 cm s^{-1} in a 100 L raceway pond of 20 cm liquid depth (FIG. 1). This raceway was equipped with real-time measurement of CO_2 concentration in the inlet and outlet gas for determination of CO_2 uptake dynamics. Specific rates for CO_2 uptake became saturated at 8000 ppm CO_2.

nutrient-replete conditions at 22 ^oC, row spacing was minimized at 6.5 cm, and the inlet gas CO_2 was increased from 1000 to 4000 ppm (day 7-14), and then to 8000 ppm (day 14-23) at 0.010 L gas L^{-1} liquid min^{-1} gas flow. Over the 23 day cultivation, biomass on the panel increased by a factor of 48, with final biomass loading exceeding 10 kg FW m^{-2} panel area, and cumulative CO_2 capture of 65%. The cumulative average areal productivity within the panel zone of the raceway exceeded 60 g AFDW m^{-2} day^{-1}, and final biomass density nearing 7.2 g AFDW L^{-1} (47 g FW L^{-1}) was achieved after 23 days (FIG. 2). Overall, these outcomes demonstrate the potential for land-based raceway cultivation of clonal red macroalgae of present and future commercial significance.

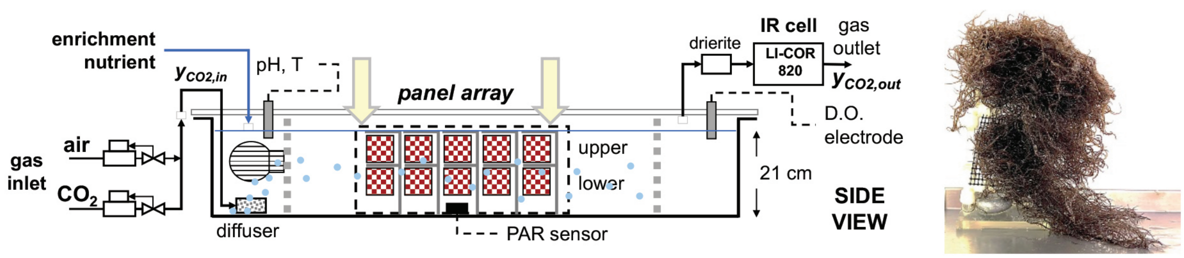

FIG 1. (a) 100 L raceway with red macroalgae panels. (b) Panel after 21 days

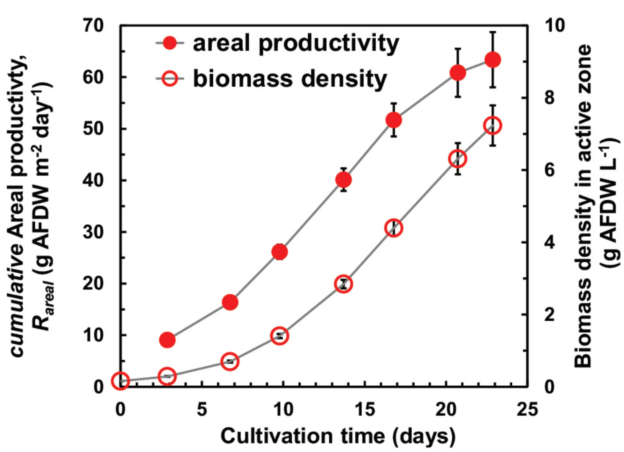

FIG. 2. Biomass productivity.

DEVELOPING SHELLFISH AQUACULTURE BEST PRACTICES TO ENHANCE HABITAT PROVISIONING

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Shellfish aquaculture gear creates complex structure that can increase the abundance and diversity of wild fish relative to reference habitats, and occurrence of habitat-related behaviors such as foraging, shelter, and reproduction have been documented on gear. Ongoing research programs in Connecticut, Massachusetts, and New Jersey seek to quantify fish interactions with aquaculture gear using underwater action cameras, and explore cultivation practices that may increase habitat services provided to wild fish assemblages. A variety of gear types were examined, including multiple styles of bottom cages and floating gear. Video was collected on subtidal and intertidal leases, and across a wide range in density of fouling organisms attached to gear. Video was recorded across the typical shellfish summer growing season, and into the fall and winter months, to assess potential impacts of winter gear removal on local fish assemblages. The speed at which fish return to shellfish gear post-disturbance was quantified through the use of continuous video. Results from these field programs will be synthesized, practices that favor habitat enhancement identified, and areas in need of additional research will be discussed. Information on best practices can aid resource managers in developing a permitting framework that includes ecosystem services provided by aquaculture in the regulatory process and may improve farm planning by identifying those practices undertaken by growers that are of greatest benefit to fish communities. A better understanding of how shellfish farming positively influences fish communities may increase social license for aquaculture among coastal communities and stakeholders.

THE MAINE AQUACULTURE HUB: A NETWORK TO STRENGTHEN AQUACULTURE IN THE STATE

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The Maine Aquaculture Hub is a network for strengthening aquaculture in Maine. It was founded in 2019 by six organizations that make up the Steering Committee: Maine Sea Grant, Maine Aquaculture Association, Maine Aquaculture Innovation Center, University of Maine Aquaculture Research Institute, and Coastal Enterprises, Inc.

Maine's seafood industry is a cornerstone of our state's economy and identity. Valued at more than half a billion dollars, the industry supports working waterfronts, coastal communities, and families. While the Gulf of Maine is a productive marine habitat, it is also one of the most quickly changing. Rapid development along the coast, impacts from climate change, and overharvesting are putting stress on wild caught fisheries, meanwhile appetite for fish protein is on the rise. Aquaculture plays a crucial role in supporting and fortifying seafood systems.

To address barriers to aquaculture in Maine and sustainably build the sector, the Hub is:
1. Building a 10-year Roadmap for aquaculture in the state
2. Training new and startup sea farmers through the Aquaculture in Shared Waters program
3. Funding projects that will address barriers to the industry at large

In developing the 10-year aquaculture roadmap, the Maine Aquaculture Hub held ten Focus Group meetings with a variety of stakeholders, in addition to numerous one-on-one phone calls. Ultimately 140 individuals representing 92 organizations provided feedback. Input was synthesized and a plan created that features four overarching goals, each with a number of specific action items as well as responsible organizations and resources needed to achieve them.

In building on the existing Aquaculture in Shared Waters training program, a second level of the course was developed, to assist new farmers in getting past the startup phase. That pilot program was turned into a workshop series for the winter of 2021-22, to reach beyond aquaculture producers and build connections needed to strengthen the sector.

As a result of its first call for proposals, the Maine Aquaculture Hub funded five industry-led projects to address barriers to aquaculture in the state. For the second round of funding, awarded proposals will be announced in early 2022.

Looking ahead, partner organizations of the Hub are being purposeful about the future of the described activities relative to the needs of the sector in the State. As aquaculture develops in Maine, there is opportunity and a responsibility to collaborate for a sustainable industry.

NOVEL MOLECULAR MARKERS IN THE SEA LOUSE *Caligus rogercresseyi* ASSOCIATED WITH HYDROGEN PEROXIDE TREATMENT

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The sea louse *Caligus rogercresseyi* is the main ectoparasite species affecting Chilean salmon industry. Hydrogen peroxide (H_2O_2) has been introduced as a delousing treatment to control lice infestations. The suggested mechanism is based on the formation of bubbles in the copepod hemolymph inducing a mechanical paralysis, detaching the parasite from the host. However, the molecular mechanisms underlying the response and potential resistance to H_2O_2 are not yet fully elucidated. This study aimed to expand the molecular knowledge through the single nucleotide polymorphisms (SNPs) discovery involved in the response of *C. rogercresseyi* against H_2O_2.

First, transcription expression of immune-related, antioxidant system, chemosensory receptors, secretome and cuticle formation genes were evaluated in individuals exposed to 3 concentrations of H_2O_2 (360, 744 and 1080 mg L^{-1}). Then, novel SNPs in *catalase, superoxide dismutase, serpin, cuticle 7, cuticle 19* and *trypsin 5* genes were validated in individuals from 3 populations differing in H_2O_2 sensitivity according to bioassays results.

Upregulation of genes related to antioxidant system, secretome and cuticle formation in exposed individuals were observed. Moreover, results from SNPs allele frequencies suggest that particular alleles of *catalase, superoxide dismutase* and *serpin* genes might be involved in the reduced susceptibility to H_2O_2 in the Potentially-Resistant (P-R) population (Table 1).

This study contributes to a better understanding of *C. rogercresseyi* responses to H_2O_2, providing new insights into the molecular mechanisms involved in drug resistance. Moreover, the novel SNPs found here could be a potential useful tool for H_2O_2 sensitivity evaluation in lice populations. Further studies will be needed to validate these polymorphisms as a molecular complementary tool for H_2O_2 sensitivity status evaluation. Nevertheless, our investigation will have important implications for H_2O_2 treatment strategies for sea lice.

TABLE 1. SNPs allele frequencies of seven genes in three *C. rogercresseyi* populations classified as Susceptible (S), Unknown (U) and Potentially-resistant (P-R) according to bioassays results

Gene	Allele	Frequency (%)		
		S	U	P-R
Catalase	CC	65%	80%	80%
	CA	30%	10%	20%
	AA	5%	10%	0%
Superoxide dismutase	TT	10%	10%	10%
	TC	60%	90%	90%
	CC	30%	0%	0%
Serpin	GG	80%	70%	100%
	GA	10%	10%	0%
	AA	10%	20%	0%
Cuticle 7	CC	50%	60%	50%
	CT	50%	30%	40%
	TT	0%	10%	10%
Cuticle 19	GG	65%	70%	60%
	GA	5%	20%	0%
	AA	30%	10%	40%
Trypsin 5	TT	15%	0%	10%
	TA	35%	60%	50%
	AA	50%	40%	40%

Funding: ANID-Chile through the grant FONDECYT (#11200813), and FONDAP (#15110027).

PROTOZOAL PARASITES IN ORNAMENTAL FISH

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Ornamental fish are often raised on fish farms where they receive limited treatment for parasitic diseases. Some aquarium fish are also still collected from the wild, where they may have naturally occurring parasites. The fish often arrive to the fish retailer with external parasites and should be treated with appropriate medications to reduce parasites before being sold to customers. Appropriate diagnostic tests need to be conducted to determine which parasites are present; often more than one type can be found on the fish. Each type of parasite needs a different medication to treat it effectively.

The most common protozoal parasites seen on freshwater aquarium fish include the Ciliates: *Chilodonella*, *Epistylis*, *Ichthyophthirius* (Ich), *Tetrahymena*, *Trichodina*; Flagellates: *Hexamita/Spironucleus*, *Ichthyobodo* (Costia), Trypanosome; Sporozoa: *Eimeria, Goussia;* Myxozoa: *Dicauda*, *Henneguya*; *Myxobolus*; Microspora: *Glugea*, *Microsporidium*, *Pleistophora*.

Treating the fish involves using medicated dips or by adding medication into the aquarium water. Some medications can also be supplied in the food. Medicated food containing metronidazole can be used for treatment of intestinal protozoal parasite infestations and should be fed for a minimum of 3 consecutive days. Repeated treatments may be necessary to effectively treat some parasites.

External protozoa are best treated using formalin or formalin/malachite green solution. Formalin effectively kills protozoan parasites on gills, skin, and fins. Formalin is not effective against internal infestations. Hydrogen peroxide, copper sulfate and potassium permanganate are also useful in certain species for treating external protozoa. The fish should be checked after treatment to ensure that the parasites have been completely cleared. Retreat as necessary prior to selling fish from the stores.

External crustacean parasites (anchor worms, fish lice) can be treated by adding diflubenzuron to the tanks of affected fish.

Monogenean trematodes (gill and skin flukes) are treated with fenbendazole. Since fenbendazole powder is not water soluble, it must first be dissolved in a small volume of alcohol, and then added to the water. Digenean trematodes (encysted flukes) live in the intestines, gills, and muscles of the fish, which acts as the intermediate host. Oral fenbendazole can be used to treat the fish for Digenean flukes. Intestinal nematodes (Camallanus, Capillaria) can also be treated by feeding the fish a fenbendazole or mebendazole medicated fish food.

PARASITIC WORMS AND CRUSTACEANS OF ORNAMENTAL FISH

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Ornamental fish are often raised on fish farms where they receive limited treatment for parasitic diseases. Some aquarium fish are also still collected from the wild, where they may have naturally occurring parasites. The fish often arrive to the fish retailer with external parasites and should be treated with appropriate medications to reduce parasites before being sold to customers. Appropriate diagnostic tests need to be conducted to determine which parasites are present; often more than one type can be found on the fish. Each type of parasite needs a different medication to treat it effectively.

The most common metazoan parasites of fishes include Monogenean trematodes: *Dactylogyrus* (gill flukes), *Gyrodactylus* (skin flukes), *Cleidodiscus, Diplozoon*; Digenean trematodes: *Sanguinicola, Clinostomum, Neascus*; Cestodes (tapeworms): *Bothriocephalus*, *Caryophyllaeus, Diphyllobothrium. Ligula*; Nematodes (roundworms): *Anisakis*, *Capillaria*, *Camallanus*; Annelids (segmented worms): *Piscicola* (leeches); Crustacea: *Lernaea* (anchor worms), *Ergasilus* (gill maggots), *Argulus* (fish lice)

Treating the fish involves using medicated dips or by adding medication into the aquarium water. Some medications can also be supplied in the food. Medicated food containing fenbendazole can be used for treatment of intestinal nematode parasite infestations and should be fed for a minimum of 3 consecutive days. Repeated treatments may be necessary to effectively treat some parasites.

External monogenean flukes are best treated using Praziquantel, usually dissolved into a solution of formalin or alcohol. Praziquantel kills monogenean parasites on gills, skin, and fins. Hydrogen peroxide and peracetic acid combination (Minn Finn) has shown some effectiveness in reducing flukes. Crustacean parasites have historically been treated with organophosphates such as Trichlorfon, but safer treatment such as diflubenzuron and lufenuron are commonly used now. The fish should be checked after treatment to ensure that the parasites have been completely cleared. Retreat as necessary prior to selling fish from the stores.

Preventing occurrence of some parasites, such as digenean flukes, involves removal of the intermediate hosts, such as snails or crustacea, and keeping birds and other wildlife out of the farm ponds.

EFFECT OF DIETARY CURCUMIN OR CURCUMIN NANOSPHERE ON GROWTH, IMMUNITY, HEAT STRESS RESISTANCE AND STRESS INDICATORS OF NILE TILAPIA (*Oreochromis niloticus*)

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Heat stress is one of the consequences of climate change which has devastating effects on fish farming especially in intensive systems, resulting in huge economic losses. The aim of this study was to evaluate whether dietary supplementation with free- or nano-form of curcumin improves growth performance, immune status, and heat stress resistance in Nile tilapia (*Oreochromis niloticus*).

Seven diets were prepared, six of which contained 3 levels of curcumin nano-sphere (50 (CN50), 100 (CN100) or 200 (CN200) mg kg^{-1}) or free curcumin (50 (C50), 100 (C100) or 200 (C200) mg kg^{-1}), and one diet contained no addition (CON). Fish (13.54±0.32 g) fed the experimental diets for 65 days and then they subjected to the acute heat stress by gradually raising the water temperature from 25 to 40□ within 3 h. Results revealed that dietary curcumin has enhanced growth performance with superiority to nano-curcumin over the free form. The optimum growth performance was obtained at CN100 (Fig.1). Heat stress rather than diets increased platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), leukocytes and neutrophils counts while lymphocytes decreased. CN50 and CN100 reduced the activity of liver enzymes (Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) more efficiently than the other diets and C200 gave the highest activity of these enzymes. CN100, CN200, C100 and C200 had higher immunoglobulin (IgM) levels than CN50 while C50 and CON gave the lowest values. C200 showed a higher level of complement C3 and C4 than the other treatments while C50 and CON gave the lowest values (Table 1). CN50 and CN100 have efficiently eliminated cortisol levels compared to the other treatments. All curcumin-supplemented diets, except for C200, produced lower glucose values compared to CON. After heat stress, ALT, AST, IgM, C3, C4, cortisol and glucose have been increased. Thus, curcumin nano-sphere has better ability than its free form to increase the ability of farmed fish to withstand heat stress, enhance growth rates and thus increase fish productivity.

Table 1. Effects of experimental diets on IgM, C3 and C4 of Nile tilapia

		CON	CN50	CN100	CN200	C50	C100	C200	SDM
25°C	IgM	0.82	1.30	1.88	1.87	1.11	1.84	1.72	0.43
	C3	1.30	2.16	2.72	2.49	1.26	2.20	2.28	0.59
	C4	2.47	3.56	3.66	4.02	3.23	3.61	3.80	0.59
40°C	IgM	1.88	2.34	2.92	2.89	1.95	2.87	2.79	0.46
	C3	3.42	4.65	4.80	4.45	4.13	5.47	5.59	0.52
	C4	5.23	6.83	6.20	7.13	4.83	5.70	7.71	1.05

SDM, mean standard deviation

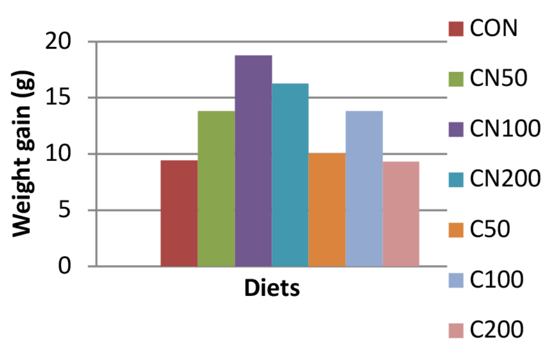

Fig 1. Effect of experimental diets on WG of Nile tilapia

ACTIVITY OF A NOVEL LIPOPEPTIDE BIOSURFACTANT AGAINST WATER-BORNE PATHOGENS

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Water-borne disease is a huge burden to society and economy, affecting aquaculture and ornamental fish farming as well as human health. Aquaculture alone suffers c. $10B in annual losses from diseases. A ciliated protozoan, *Ichthyophthirius multifiliis* (Ich), causing “white spot disease” is one of the most devastating and commercially important parasites in aquaculture worldwide. Existing remedies to counter Ich and other parasites in aquaculture are typically long-established or even historical and often hazardous, toxic, and difficult to use. Sundew has developed a new natural solution, called Biokos, with a considerable efficacy to control Ich and other water-borne diseases. Biokos is non-toxic to fish, environmentally friendly, and it is manufactured by simple fermentation. Here we present an efficacy study of Biokos on Ich parasite in an ornamental fish model.

An *in vivo* experiment was conducted to evaluate the efficacy of Biokos against Ich infection in a common goldfish (*Carassius auratus*) model. Fish were divided to three groups;

A) Negative control (not infected, not treated fish)
B) Infection control (Ich infected, not treated fish)
C) Treatment group (Ich infected, treated with Biokos at the concentration 6.3 mg/l).

The latter received Biokos every 48 hours. Goldfish in infection control reached 100% mortality within 14 days (Figure 1) with a significant increase in number of Ich trophonts on the body surface (Table 1), while all treated fish survived and no Ich trophonts were observed on the skin, fins, and gills after 14 days, i.e. 7 dosing of Biokos. Moreover, no behavioral changes (in terms of breathing and swimming patterns, and appetite) was recorded in treated group.

These results demonstrate the great efficacy of this natural anti-parasitic product against Ich, one of the most common parasites in aquaculture and ornamental fish, at a concentration as low as 6.3 mg/l.

Early data shows that Biokos is also effective against other fish pathogens including parasites, bacteria, and oomycetes. Further toxicity and environmental studies are underway as well as testing on further distinct fish parasite.

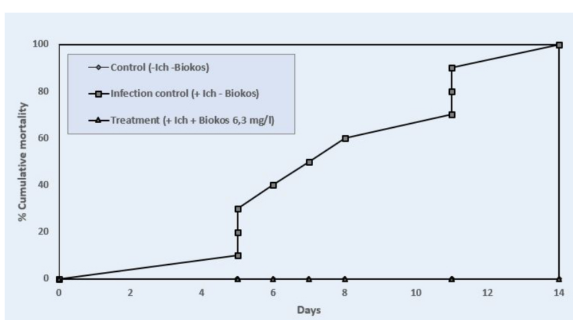

FIGURE 1. Cumulative mortality of experimental goldfish within 14 days

At initiation of experiment	At termination of experiment	
All fish	Infection control fish	Biokos-treated fish
248	518	0

TABLE 1. Average number of white spots (trophonts) on goldfish skin and tail fin

A BIOTECHNOLOGY PLATFORM TO MEET THE NEEDS OF RAS AQUACULTURE

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As aquaculture production must double in the next 30 years to meet the increased demand in fish consumption, our industry needs to offer innovative technologies to unlock key issues while being sustainable, environmentally conscious and appealing to both the feed manufacturer and the farmer. The aquaculture industry is currently experiencing a turning point, particularly in the US, with the booming of recirculating aquaculture systems (RAS). Creating functional products for aquafeed that support healthy fish while at the same time offering the quality products that consumers are expecting is one of the challenges that KnipBio is tackling.

KnipBio's single cell protein (SCP) technology platform enables a versatile, functional protein effective in practical formulations for RAS-based settings. Application examples include off-flavor remediation, juvenile health, and pigmentation. The lack of antinutrient factors as well as the presence of antioxidant carotenoids and prebiotics with immune-enhancing properties have the potential to lead to healthier fish and shrimp. By targeting specific issues encountered in RAS and other production systems, KnipBio Meal (KBM) products significantly decrease production costs while increasing the survival and quality of aquatic animals on the farm, which make operations more profitable.

KnipBio will present an update on its technical progress including recent strides in its R&D platform and animal trials, its main product characteristics and the status of its production and commercialization phases.

PREVENTION OF STRESS-DRIVEN MORTALITIES IN RAINBOW TROUT USING A SINGLE-CELL MEAL IN FEED

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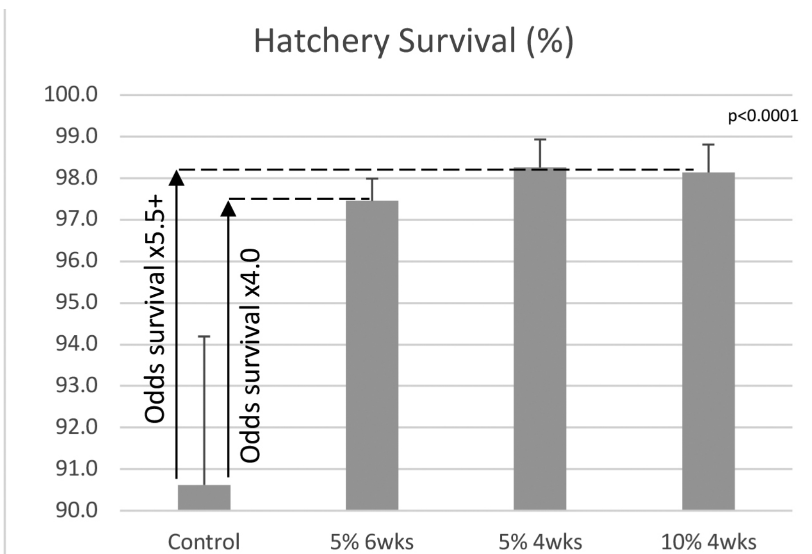
Aquaculture production must double in the next 30 years to meet the increased seafood demand. To meet this challenge, our industry needs to address long-standing bottlenecks in production and implement innovative approaches to increase the environmental, economic, and ethical sustainability of aquaculture. Morbidity and mortality observed during early life stages, when aquatic animals are particularly susceptible to stress, is a key limiting factor for most aquaculture operations.

KnipBio has partnered with Riverence in an on-farm trial to evaluate the effectiveness of KnipBio's single cell protein (KBM) to reduce mortalities incurred during out-planting of fingerlings from indoor hatcheries to outdoor raceways. KBM provides important molecules like antioxidant carotenoids and prebiotics with immune-enhancing properties. The indoor hatchery phase of the trial was designed as an incomplete 2x3 factorial (KBM inclusion and feeding duration) and included a commercial feed benchmark treatment. After out-planting, all fish were fed the commercial feed and were monitored for 4 weeks. In the hatchery phase, we observed that fish fed diets containing 5 or 10% KBM were 4 to 5.5 times as likely to survive than those fed an unsupplemented control diet. After out-planting, fish that had received a feed containing 5% KBM for 6 weeks were 2.2 times as likely to survive than fish that had received the commercial benchmark feed.

At the farm level, these significant improvements in survival rates suggest that dietary inclusion of KBM can yield improvements in fish welfare and production efficiency, as well as a reduction in the number of eggs necessary to reach production targets.

At the farm level, these significant improvements in survival rates suggest that dietary inclusion of KBM can yield improvements in fish welfare and production efficiency, as well as a reduction in the number of eggs necessary to reach production targets.



LUMPFISH (*Cyclopterus lumpus*) IMMUNITY AGAINST MARINE BACTERIAL INFECTIOUS DISEASES

Javier Santander*, Trung Cao, My Dang, Ignacio Vasquez, Setu Chakraborty, Katherinne Valderrama, Hajarooba Gnanagobal, Ahemd Hossain, Joy Chukwu, Oluwatoyin Onireti, Steve Hill, Zihyou Chen, Jennifer R. Hall, Jennifer Monk, Surendra Kumar, Vimbai I. Machimbirike, Cristopher Segovia, Nicole O´Brian, Andrew K. Swanson, Danny Boyce.

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Lumpfish (*Cyclopterus lumpus*) has become one of the most utilized cleaner fish for Atlantic salmon (*Salmo salar*) aquaculture in the north Atlantic region. Lumpfish in aquaculture sea-cages are known to prey upon ectoparasitic sea lice (e.g., *Lepeophtheirus salmonis*), which is a major threat to salmon farming around the world. Lumpfish perform well in cold environments, and are routinely commercially utilized as a form of pest biocontrol, since 2013 in Norway and 2017 in Atlantic Canada. Although healthy populations of lumpfish could theoretically be utilized over two salmon production cycles, biosecurity concerns and maintaining the long-term health of domesticated lumpfish remains challenging. Bacterial infectious diseases are the most frequent health issues of lumpfish impacting their performance and extended utilization. We have developed several bacterial infectious disease models in lumpfish for vaccines and bacterial pathogenesis studies. Here, advances in lumpfish immunity and infection of *Vibrio anguillarum*, *Aeromonas salmonicida*, *Pseudomonas* nov., sp. J380, *Moritella viscosa*, *Renibacterium salmoninarum*, and *Piscirickettsia salmonis* are examined and discussed. Bacterial infection and immune transcriptomics studies indicated that marine Gram-negative pathogens generally cause a more acute infection than Gram-positives. Among the tested pathogens in lumpfish, *A. salmonicida* and *V. anguillarum* are the most virulent. *M. viscosa* caused a distinctive gill inflammation and acute death. *Pseudomonas* nov., sp. J380 is an endemic pathogen that could infect lumpfish. *P. salmonis* is fully lethal in lumpfish, independent of the infectious dose, and causes slow morbidity and mortality rates with distinctive clinical signs. *R. salmoninarum* causes a chronic type of infection with characteristic clinical signs of bacterial kidney disease. These infectious models are a contribution to marine teleost immunity knowledge and vaccine development, towards a more efficient utilization of lumpfish in aquaculture sea lice biocontrol.

LUMPFISH (*Cyclopterus lumpus*) IMMUNITY TO *Vibrio anguillarum* VACCINES

Javier Santander*, Trung Cao, Joy Chukwu, Oluwatoyin Onireti, My Dang, Ignacio Vasquez, Setu Chakraborty, Katherinne Valderrama, Hajarooba Gnanagobal, Ahmed Hossain, Steve Hill, Zihyou Chen, Jennifer R. Hall, Jennifer Monk, Surendra Kumar, Vimbai I. Machimbirike, Cristopher Segovia, Andre Khoury, Yenney Hernandez-Reyes, Antoine Khoury, Nicole O´Brian, Andrew K. Swanson, Danny Boyce.

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Lumpfish (*Cyclopterus lumpus*) has become the predominant cleaner fish species used in North American salmon aquaculture for sea lice (e.g., *Lepeophtheirus salmonis*) biocontrol. Lumpfish utilization has contributed significantly towards eliminating the utilization of chemotherapeutants by effectively controlling the abundance of this damaging pest of Atlantic salmon (*Salmo salar*) aquaculture. *Vibrio anguillarum* is a frequent pathogen of lumpfish in Atlantic Canada. Here, several vaccine trials against *V. anguillarum* were conducted. We determined that the *V. anguillarum* growth conditions are essential for expressing protective antigens in the vaccine formulation. Generic commercial vaccines provide between 2-55% protection against *V. anguillarum,* which does not adequately protect the lumpfish. Generic or autogenous vaccines delivered by mucosal routes (e.g., dip or bath) stimulates the naïve fish. However, only systemic immunization regimes (e.g., intraperitoneal injection) induced significant protective immunity against the lethal *V. anguillarum* systemic or mucosal challenge. Mucosal immunization conferred an evident immune stimulation, but not immune protection. A combination of dip immune stimulation and intraperitoneal boost immunization may confer a longer-lasting immune protection, which could be optimal for lumpfish health management, and important to the continued success of cleaner fish biocontrol in salmon aquaculture.

MULTI-TROPHIC OFFSHORE AQUACULTURE INTEGRAL WITH IDLE OFFSHORE OIL AND GAS PLATFORMS IN THE GULF OF MEXICO

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Retired (or idle) offshore oil and gas platforms have been proposed for marine research and monitoring, offshore aquaculture, and offshore renewable energy (Satterlee, et al. 2018, Satterlee, et al. 2021). These platforms have several advantages that can be used to prove up and scale up offshore aquaculture, such as platform cranes, electrical supply, stable operating deck, and housing. They are also designed and built to survive hurricane conditions. Co-location of macroalgae farming with finfish aquaculture may provide nutrient synergy.

GORI's research also includes repurposing the offshore platforms for a variety of marine-related uses, including offshore renewable energy and climate mitigation. The hypoxic zone in the Gulf of Mexico caused by nutrient loading from the Mississippi River and mitigation with macroalgae is also looked at. GORI in collaboration with the Harte Research Institute will present research results on the ecosystem value of a standing offshore platform and to natural fish stocks which provides synergy with the aquaculture functions.

Satterlee, K., Watson, S., & Danenberger, E. (2018) New Opportunities for Offshore Oil and Gas Platforms - Efficient, Effective, and Adaptable Facilities for Offshore Research, Monitoring, and Technology Testing. OCEANS 2018 MTS/IEEE Charleston.
Satterlee, K., Bockus, A., Riley, K., Sclodnick, T., Snyder, B., (2021) MMEERSET Phase One: developing platform-based offshore aquaculture using a multi-use approach at Station Padre. Final Report to Gulf States Marine Fisheries Commission.

USE OF METAGENOMIC SEQUENCING TO IDENTIFY MICROBIAL DIVERSITY IN DIFFERENT COMPONENTS IN THE STARTUP OF AN AQUAPONICS SYSTEM RAISING WALLEYE *Sander vitreus*

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Aquaponics Systems (AS) utilize recirculating aquaculture technology and plant grow-out systems to diversify production of both fish and vegetables. These systems rely on biological nitrification to convert fish metabolites to nutrients available for plant growth. The successful operation of AS requires ammonia-oxidizing organisms to convert fish waste to plant food but little is known about the microorganisms actually present. Both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are believed to be responsible for this process. We utilized 16S Amplicon Metagenomic Sequencing to identify AOB and AOA species in the AS at Concordia University Wisconsin. This is a Deep-Water-Culture AS system and samples were taken from three components: biofilter, mineralization tank and raft tank. At startup a commercially available culture of nitrifying bacteria was used to inoculate the system; ammonium chloride was dosed prior to stocking walleye (*Sander vitreus*). Microbial samples were collected over a period of 6 months. To isolate DNA from the samples a Qiagen prep kit was used followed by “DNeasy® PowerBiofilm® **Kit.** Amplicon metagenomic sequencing classifies the target genes of 16S ribosomal RNA (rRNA) by universal primers and our results will describe and compare the phylogeny and taxonomy of bacteria, archaea and fungi in our samples over time. These results will provide valuable insight into key dynamics in the microbial community during the startup of AS and promote optimization of future microbial inoculants that include the most beneficial microbial species.

Note: microbial DNA samples will be sent to Retrogen, Inc. (San Diego, CA) for processing in December of 2021.

DEVELOPMENT OF AN IN VIVO *Enterocytozoon hepatopenaei* (EHP) EXPERIMENTAL CHALLENGE MODEL IN *Penaeus vannamei*

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Enterocytozoon hepatopenaei (EHP), one of the etiologic agents of Hepatopancreatic Microsporidiosis (HPM), has emerged as one of the most problematic infectious diseases in shrimp aquaculture over the last ten years. As the disease continues to spread across the world, there has been a surge in the development of preventatives, treatments, and genetically resistant lines of shrimp.

EHP is an obligate parasite and currently there is no *in vitro* culture method to propagate the parasite in a robust and reproducible manner. The inability to rapidly produce a large quantity of inoculum to conduct experimental challenges has been an impediment when screening for EHP resistance in shrimp and in experiments in developing oral treatments.

We describe here a simple yet robust challenge method to generate large quantities of EHP inoculum which can be used to conduct in vivo EHP challenges with *P. vannamei*. The method involves injection of EHP inoculum directly into the hepatopancreas of Specific Pathogen Free shrimp (*Penaeus vannamei*). In less than 21 days following injection, an EHP infection can successfully be established in the injected animals as determined by both H&E histology and real-time PCR detection in the hepatopancreas samples. Hepatopancreata dissected from the EHP-injected animals can then serve as inoculum for challenging several replicate tanks of SPF shrimp. As this EHP challenge model has been repeated in experimental assays, both the limitations and the advantages of this challenge model have been illustrated. The data accumulated from studies in which SPF *P. vannamei* are fed tissue derived from EHP-injected animals demonstrates a reproducible challenge model with predictable results.

FACILITY DESIGN ELEMENTS THAT ENHANCE BIOSECURITY & FOOD SAFETY WITHIN LAND-BASED AQUACULTURE FACILITIES

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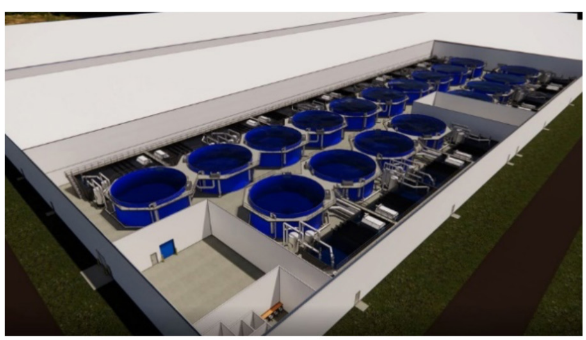
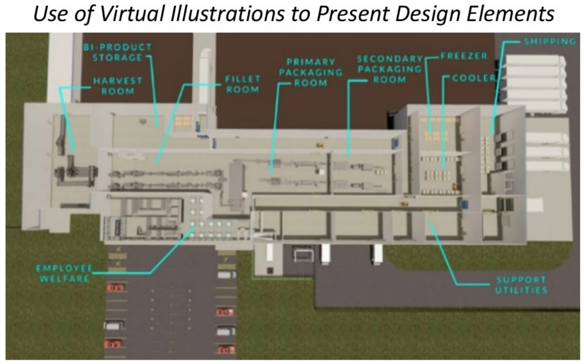
Food Safety and Biosecurity are two critical design elements widely addressed within seafood processing and land-based aquaculture facilities and equipment. According to recent data from the Centers for Disease Control and Prevention, about 48 million people get sick, 128,000 are hospitalized, and 3,000 die each year from foodborne diseases. Beginning with the American Meat Institute's (AMI) Design Task Force in 2002 and later supported by the 2011 Food Safety Modernization Act (FSMA), the Principles of Sanitary Design were developed to prevent problems rather than react after they occur. These best practices provide strong motivation to implement sanitary design and lower operating costs. Facilities utilizing the best hygienic design standards can be cleaned faster, with fewer chemicals, less labor, and lower wastewater treatment costs. In many similar operations, yields improve while product and waste reduce, so higher asset utilization results and product safety is enhanced.

These critical design elements apply to the design and construction of any land-based aquaculture facility, especially those considering integrating a downstream seafood processing operation. These elements are summarized as:

1. Design the property to facilitate biosecurity, sanitary conditions, and defense against the intentional adulteration of products.
2. Create a linear product flow in the facility.
3. Create hygienic zones of risk with secure entry requiring disinfection.
4. Control the movement of personnel and material flows to reduce cross-contamination.
5. Control water accumulation.
6. Control the room temperature and humidity.
7. Control the room airflow and air quality.
8. Building envelope supports sanitary conditions.
9. Integrate RAS and other equipment into a spatial design that provides accessible maintenance, cleaning, and sanitation to microbiological levels.
10. Utilize construction methods that facilitate sanitary conditions and building materials made of compatible materials.
11. Design utility systems that prevent contamination and harbourage areas for bacteria and moisture to collect.



Use of Virtual Illustrations to Present Design Elements



DIETARY SUPPLEMENTATION WITH CREATINE AND EDTA IN EUROPEAN SEABASS (*Dicentrarchus labrax*) MAINTAINS FISH QUALITY

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Introduction

Fish is a known healthy alternative to meat due to its high-quality proteins and protective omega-3 fatty acids. Also, the benefit of vitamins and minerals loads, increases the importance of fish in our diet. As aquaculture needed to keep up with the high demand for fish, tailored diets are paramount for a robust, healthy fish, that grows fast to fulfill consumers' expectations. It is common to add trace amounts of specific additives to fish diets to improve the quality of feed aiming mainly to increase health performance, fish feeding efficiency and flesh quality. Fish feed additives may also be an alternative to fulfill the consumer's health needs such as decreasing the allergenicity of fish products through modulation of specific proteins.

Allergies to fish are a growing health concern worldwide and avoiding fish (products) is still the only option to prevent severe reactions such as rash and anaphylaxis. It is known that 95% of allergic reactions are due to a white muscle protein called β-parvalbumin. This calcium-binding protein might be modulated with the inclusion of specific molecules in fish diets. Creatine and EDTA are known to be involved in reducing the intracellular calcium content and in chelating calcium ions, respectively. Creatine might then act as a modulator of β-parvalbumin expression while EDTA might induce a rearrangement in the 3D-structure of this protein, both aiming the reduction of its allergenicity. Previous work by our group showed a biological reduction in fish allergenicity upon a 3% EDTA supplemented diet. The present work aimed to contribute to a better understanding of the effects of dietary creatine or EDTA supplementation levels, aiming in reducing fish allergenicity, on European seabass flesh quality that would meet consumer's expectations.

Methodology

Two independent feeding trials with juvenile European seabass were performed at the Ramalhete experimental station (CCMAR/University of Algarve, Portugal). Fish kept in 500L tanks were fed with a commercial feed vs supplemented diets with Creatine (2% vs 5% vs 8%) or EDTA (1.5%, vs 3% vs 4.5%) under natural environmental conditions (dissolved oxygen above 5 mg L^{-1}). Initial body weight of the fish were 186 ± 0.83 g and 174 ± 1.29 g, for the creatine and EDTA trial, respectively. After three months of *ad libitum* feeding (twice a day), fish were killed by ice slurry and sampled for flesh quality through inspection of texture and sensorial analysis.

Table 1. Texture parameters of European seabass muscle fed Creatine supplemented diets

	CTRL	Creat2	Creat5	Creat8
Hardness (N)	50.4 ± 16.4	47.2 ± 12.0	49.1 ± 16.1	46.9 ± 12.1
Springiness	0.65 ± 0.05	0.65 ± 0.04	0.66 ± 0.05	0.64 ± 0.06
Chewiness	14.9 ± 5.0	13.7 ± 3.9	13.9 ± 3.9	13.2 ± 2.6

(Continued on next page)

Results

At the end of each trial, the edibility of fish was determined by a trained sensorial panel that performed tests on steamed fillets attributing points to different parameters (i.e., taste, firmness, succulence). Also, texture properties (i.e., hardness, springiness, and chewiness, between others) was determined mechanically and shown in Table 1. Both quality parameters (sensorial and texture) showed no significant differences between diets in both trials ($p>0.05$).

Conclusion

Dietary supplementation with Creatine (up to 8%) and EDTA (up to 4.5%) preserved European seabass quality and edibility. Nutritional strategies like diet supplementation with specific traced molecules amounts, are a promising avenue to tailor fish for Human consumption without jeopardizing consumer's expectations.

Acknowledgements

This work received Portuguese national funds from FCT- Foundation for Science and Technology through project UID/04326/2020. This work integrates project 16-02-01-FMP-0014- Allyfish co-financed by Mar2020 (4107IDNAD50308.18). DS, CRM and RC acknowledge FCT for PhD scholarships (SFRH/BD/136319/2018, SFRH/BD/138884/2018 and 2021.06786. BD).

THE GREAT LAKES AQUACULTURE COLLABORATIVE'S (GLAC) ROLE IN SUPPORTING SUSTAINABLE AQUACULTURE IN THE GREAT LAKES REGION

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Aquaculture production in the Great Lakes region is small and not keeping pace with increases in consumer demand for fish and seafood. The Great Lakes Aquaculture Collaborative (GLAC), one of the National Sea Grant supported Aquaculture Hubs, was formed to address potential barriers and develop opportunities for sustainable, land-based aquaculture in the region. GLAC is composed of Sea Grant extension educators and university researchers from Minnesota, Wisconsin, Illinois, Indiana, Ohio, Michigan, Pennsylvania, and New York. The project's primary goal is to provide relevant, science-based initiatives that support an environmentally responsible, competitive, and sustainable aquaculture industry in the region.

The GLAC has formed advisory groups in each of the eight Great Lakes states and an additional regional advisory group with a representative from each state group to help prioritize GLAC activities. Each advisory group is composed of aquaculture producers, distributors, and researchers. The ideas and priorities that arise from advisory group meetings and discussions drive the majority of GLAC activities. For example, we have held four informational webinars and two region-wide virtual events on topics chosen by our advisory groups. GLAC also has a robust research component and projects that are ongoing include 1) understanding consumers' willingness to pay for aquaculture products, 2) identifying what producers perceive as barriers to expanding and diversifying their businesses, and 3) identifying policy and regulatory hurdles for aquaculture producers in the region. In order to distribute our activities and research as widely as possible, we have developed a GLAC website (https://greatlakesseagrant.com/aquaculture/) that hosts our webinars, events, outreach materials, and research updates.

Through direct interaction with our advisory groups and among Sea Grant programs, a number of collaborations have grown out of the GLAC. Examples of new projects include a website (the Great Lakes Fresh Fish Finder at freshfishfinder.org) with the goal of connecting fish producers directly to consumers, and a funded project to develop consumer education materials with information about aquaculture and seafood for both youth and adult audiences. Finally, our GLAC team is working together to develop the next iteration of GLAC by filling additional gaps in the aquaculture industry in networking, workforce development, and consumer preference and education.

A GOLDEN OPPORTUNITY: STRATEGIES TO ACCELERATE GROWTH OF GOLDEN SHINER *Notemigonus crysoleucas* IN MINNESOTA AND OTHER NORTHERN CLIMATES

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The 2018 United States Department of Agriculture (USDA) Aquaculture Census reported that Golden Shiner were the most valuable baitfish produced in the United States (U.S.) with $16.4 million in total sales and over 3.9 million pounds sold. 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 by Minnesota bait dealers estimate a deficit of more than 10,000 gallons of Golden Shiner annually. Consequently, there is pressure from anglers, bait dealers, and legislators to import Golden Shiner from other states, though this is currently prohibited by law in Minnesota. The primary concerns are that 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 value of $2.4 billion. Our study aims to increase production of Golden Shiner in Minnesota as a preferred alternative to importation.

One barrier to increasing production of Golden Shiner in Minnesota is that growth rates are slow, it takes two years for Golden Shiner to reach market size in natural ponds. We propose to overcome this bottleneck by exploring four different strategies to grow Golden Shiner to market size (10-15 cm) 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 systems, 3) stocking outdoor, constructed ponds with newly hatched Golden Shiner sac-fry (0.5 cm), and 4) producing feed trained Golden Shiner indoors to a size of 3-5 cm, then stocking them into outdoor constructed ponds.

We are working closely with partners from the Minnesota bait industry and preliminary results are encouraging. If successful, this project could become a model for production of other minnows used as bait in Minnesota and other northern climates. A production manual and workshops highlighting project results will be developed upon project completion. Results will be shared with the bait industry, the aquaculture industry, legislators and interested citizens.

OFFSHORE AQUACULTURE DEVELOPMENT MOROCCO

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Since 2019, Pan Ocean Aquaculture B.V. (POA) has been further developing its propriety technology (SSFF - Semi Submersible Fish Farm) for offshore aquaculture installations based on the prototype installed early 2020 offshore Fujian Province, China, the SSFF150.

Apart from working with an array of customers worldwide for the sale or lease of SSFF offshore aquaculture installations, POA started its own offshore aquaculture development in 2019 offshore Morocco. Improvements developed such as wider application of advanced composite materials, automation and remote-control with ABS certification, on-board underwater feed system, environmental data collection and forecasting and adoption of Oil & Gas offshore safety standard practices, will be implemented on the SSFF200, a slightly larger version of the SSFF150 prototype.

Following a two-year license offshore aquaculture application period, and successfully cooperating with various Moroccan Government Authorities, the approval for a license for two areas each of 2.6 square Nautical Miles (about 9 km2 each) is in its final stage and expected to be awarded in Q1 2022. Phase I of the development will entail two SSFF200's (200,000 m3 cage volume, 110 m diameter) for grow-out of Seriola or, optionally, European Seabass, in water depths ranging from 110-130 meter.

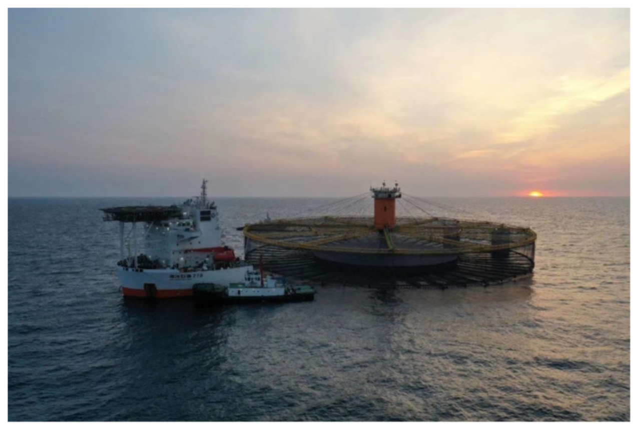

Figure 1 -SSFF150 offshore float-off at site in China

POA tailored technology and explored strategies and execution plans for a variety of business cases for grow out for both species. The SSFF200's will feature 8 sections each with an average total capacity of 2000 mT (depending on stocking density).

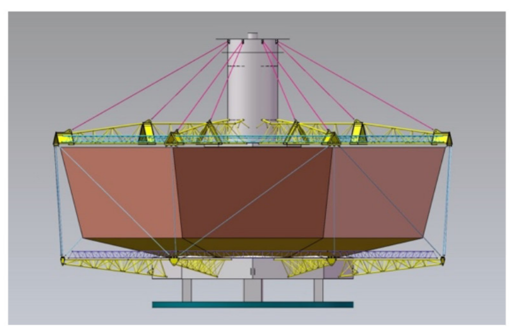

Figure 2 -- 3D model of SSFF200

Modern operational practices as can be observed in the salmon industry are even more so required, and to be built on, when developing offshore aquaculture. In addition to development of the SSFF200, a tailored well-boat for fingerling supply and service vessel for feed/general supply and harvesting operations are being designed to support the overall offshore development and ensure that operations can continue in a safe and efficient manner.

USING RNAi TO VALIDATE THE ROLE OF PERLUCIN GENE IN *Crassostrea virginica* LARVAE RESILIENCE TO OCEAN ACIDIFICATION

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Calcifying marine organisms, including the eastern oyster (*Crassostrea virginica*), are vulnerable to ocean acidification (OA) because it is more difficult to precipitate calcium carbonate ($CaCO_3$) under acidic conditions. We previously investigated the molecular mechanisms associated with resilience to OA in *C. virginica.* There were significant differences in SNP and gene expression profiles among oysters reared under normal and OA conditions. Converged evidence generated by both of these approaches highlighted the role of genes related to biomineralization, including perlucin, in resilience to OA. Perlucin is important for the nucleation of $CaCO_3$ crystals during bivalve shell formation, which has been shown to be negatively impacted by OA in *C. virginica*.

In this study, we used RNAi or gene silencing to validate findings and confirm the protective role of perlucin associated with resilience to OA. Larvae were exposed to short dicer-substrate small interfering RNA (DsiRNA) to silence perlucin or to one of two control treatments (control DsiRNA or seawater), before cultivation under OA conditions (pH ~7.3) or control (pH ~8) conditions. Two different transfection methods were performed in parallel, one during fertilization and one during early larval development (6 hours post fertilization). Both transfection methods were successful at significantly reducing the expression of perlucin; however, the transfection method did influence results. Larval viability, size, development, and biomineralization were monitored daily. Silenced oysters under acidification stress were the smallest, had shell abnormalities, and had significantly reduced shell mineralization, thereby indicating that perlucin does help larvae mitigate the effect of OA.

TRANSCRIPTOMIC, PROTEOMIC, AND FUNCTIONAL ASSAYS UNDERLINE THE DUAL ROLE OF EXTRAPALLIAL HEMOCYTES IN IMMUNITY AND BIOMINERALIZATION IN THE HARD CLAM *Mercenaria mercenaria*

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Circulating hemocytes in the hemolymph constitute the backbone of innate immunity in the hard clam *Mercenaria mercnearia*. Hemocytes are also present in the extrapallial fluid (EPF), the space demarcated between the shell and the mantle tissue, which is the site of shell biomineralization. This study investigated the transcriptome, proteome, and function of hemocytes found in the EPF and hemolymph in the hard clam. Total and viable hemocyte counts were similar between EPF and hemolymph. Overexpressed genes in the EPF were found to have domains previously identified as being part of the "biomineralization toolkit" and involved in bivalve shell formation. Biomineralization related genes included chitin-metabolism genes, carbonic anhydrase, perlucin, and insoluble shell matrix protein genes. Overexpressed genes in the EPF encoded proteins present at higher abundances in the EPF proteome, specifically those related to shell formation such as carbonic anhydrases and insoluble shell matrix proteins. Genes coding for bicarbonate and ion transporters were also overexpressed, suggesting that EPF hemocytes are involved in regulating the availability of ions critical for biomineralization. Calcium content of hemocytes in the EPF were significantly higher than those in hemolymph, supporting the idea that hemocytes serve as a source of calcium during biomineralization. Overexpressed genes also contained domains such as C1q that have dual functions in biomineralization and immune response. The percent of phagocytic hemocytes was not significantly different between EPF and hemolymph. Together, these findings support the idea that hemocytes in EPF have dual functions of biomineralization and immunity.

NUTRITIONAL PROGRAMMING OF FIRST FEEDING YELLOW PERCH *Perca flavescens* WITH SOYBEAN MEAL ENRICHED LIVE FOOD

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Effective utilization of dietary plant protein (PP) by fish is limited in some species. Nutritional Programming (NP) is a technique used to improve the acceptance of PP by fish wherein the fish is introduced to PP early in life, causing a nutritional adaptation to the same PP in later life stages. While NP is typically done with formulated feed, the objective of this study was to examine how NP via live food enriched with soybean meal (SBM) affects growth performance and intestinal health of Yellow perch *Perca flavescens* during its later stages.

Yellow perch larvae at 6 dph were distributed into 12 (280 L) tanks at 48 larvae per L. Four treatment groups were used: (1) a positive control group that received unenriched live food followed by fishmeal (FM)-based diet throughout the experiment; (2) a negative control group which received unenriched live food followed by SBM-based diet throughout the experiment; (3) a nutritionally programmed (PRO) group that received live food enriched with SBM, followed by FM diet in the juvenile stage and then a SBM-based diet during a SBM challenge; and (4) a non-programmed (Non-PRO) group that received unenriched live food followed by FM diet in the juvenile stage and then a SBM-based diet during a SBM challenge.

At the end of the SBM challenge at 145 dph the PRO group and positive control achieved a similar average weight (g), both of which were significantly higher ($P < 0.05$) compared to Non-PRO (Figure 1). PRO also achieved a similar weight gain (%) to positive control (Figure 1), and a weight gain (g) numerically higher than Non-PRO. This study showed that enriched live food is an effective vehicle for NP in Yellow perch given that growth performance of the programmed fish fed SBM was similar to the positive control and improved over the non-programmed fish. Intestinal health results will be presented during the oral presentation.

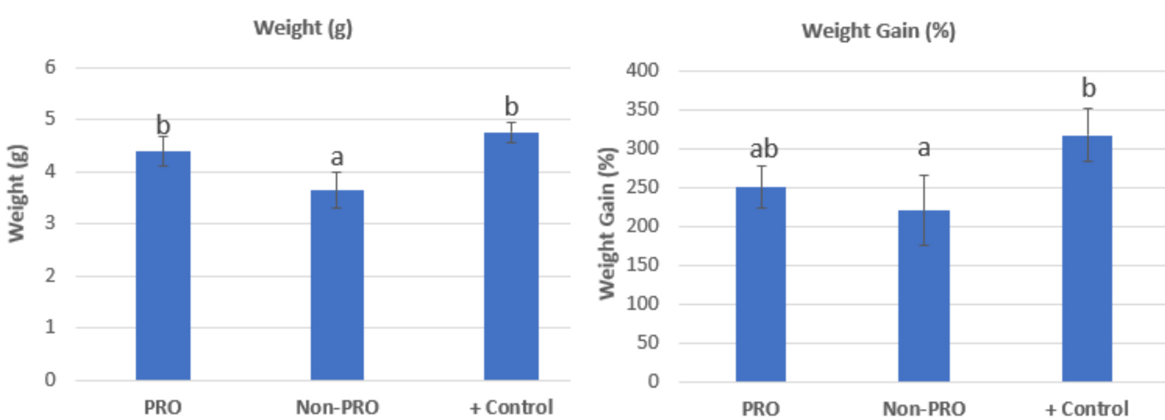

Figure 1. The average body weight and the weight gain of Yellow perch at the end of the SBM challenge at 145 dph. Different letters indicate statistical differences at $P < 0.05$. Negative control removed from experiment at 80 dph

LETTING THE ENVIRONMENT DO THE WORK – THE HIDDEN VALUE IN OPEN OCEAN FARMS

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The optimal density in net pens is a critical factor in farm profitability. Getting more fish out of every volume of growing space increases throughput in the same way that increasing a machine's capacity increases a factory's productivity. Stocking density can be limited by several factors including restrictions from legislation or certification bodies, density-dependent stress responses, or exceeding the carrying capacity of the environment. Usually, density is limited by the environment. With too many fish in too small of a water volume, oxygen can deplete, waste will accumulate in the sediments, and parasites can transmit too readily.

Open ocean environments offer some valuable characteristics that can dramatically increase a farm's bottom line by increasing the safe stocking density. Ocean currents are often stronger and do not show the tidal back-and-forth pattern typical of bays or fjords. The stronger water exchange increases the effective oxygen availability and disperses wastes over a wider area which, combined with oligotrophic conditions (low background nutrients), creates a very high potential to assimilate farm wastes into the natural food chain.

These effects are explored using a deposition model (TROPOMOD) and a farm-scale financial model to compare the financial returns of two potential farms, one in a high energy environment, and one in protected waters. The results are supported using two empirical examples from commercial farms and discussed in the context of farm planning.

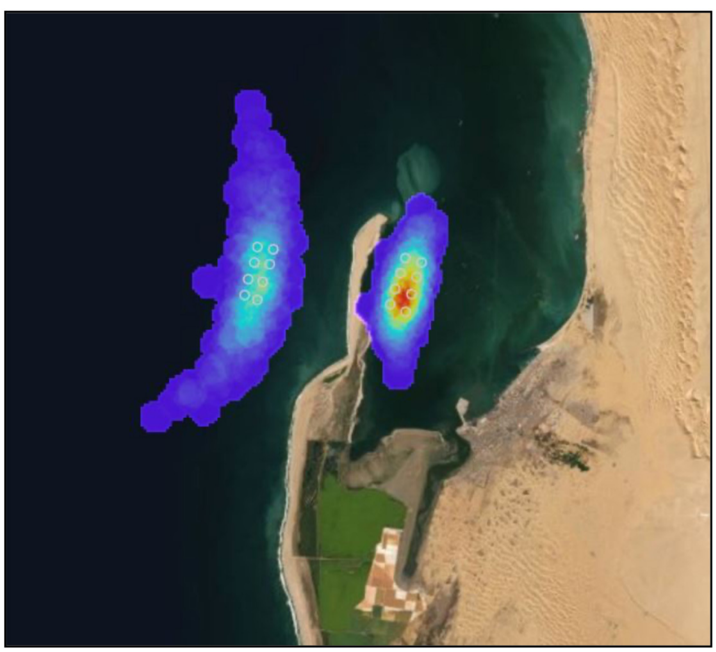

Figure 1: The effect of stronger currents on the grid outside the bay can be seen through the results of a deposition model. The grid on the left has a large area of effect but lower area of significant impact (area in red).

MAKING THE MARKET FOR SUSTAINABLY FARMED SEAWEED: A UNIQUE BIOREFINERY PROCESS TO DEVELOP HIGH-DEMAND, HIGH-QUALITY APPLICATIONS FOR ENVIRONMENTAL, ECONOMIC AND SOCIAL BENEFIT

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Seaweed aquaculture is a nascent industry in the western hemisphere which offers significant environmental, social and economic benefits. There is increasing demand for all natural, plant-based products which prioritise traceability and provenance, and importantly contribute to climate change mitigation through the use of lower carbon materials which offer a viable long-term alternative to resource intensive inputs. In addition, seaweed farms provide nursery grounds for sea life and protect seabeds below. Seaweed absorbs carbon and excess nitrogen which helps reduce ocean eutrophication. Seaweed as a food source or feedstock does not require cleared land, fresh water, insecticide or fertiliser.

With its innovative and highly technical biorefinery process that is being scaled up with support from key stakeholders including WWF and the European Maritime and Fisheries Fund, Oceanium extracts maximum value from the seaweed by developing a suite of applications that utilises the growing supply of farmed seaweed in the western hemisphere and meets consumer and corporate demand for all-natural products with provenance. Oceanium's products range from food ingredients and nutraceutical/cosmeceuticals as well as innovative seaweed-based materials for packaging. Oceanium works closely with seaweed farmers and stakeholders across the value chain to ensure the sustainability and longevity of the emerging seaweed farming industry.

Oceanium's mission is to develop and make seaweed-based products for People Health and Planet Health.

DEVELOPMENT OF RT-qPCR FOR MOLECULAR DIAGNOSIS OF HEMOCYTIC NEOPLASIA IN THE HARD CLAM *Mercenaria mercenaria*

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Over the last few years, the hard clam, *Mercenaria mercenaria*, aquaculture industry in Wellfleet, Massachusetts has been threatened by disease. This new disease is similar to a disseminated neoplasia previously described in other bivalves including the soft-shell clam, *Mya arenaria*. It is identified by abundant, atypical hemocytes filling the vascular system. The disease affects both sub-market and market sized animals, and heavily infected clams do not remain burrowed in the sediment, eventually dying on the surface. Current diagnostic methods rely on histopathology to identify and quantify the neoplastic cells within the clam. This method is not only costly, but time consuming. A new molecular diagnostic method that would use an easily obtained, even non-lethal, sample of hemolymph is being developed for the detection of hemocytic neoplasia in hard clams.

For transcriptome comparison, hemolymph was collected from the adductor muscle sinus from 5 diseased and 3 healthy clams using a 3 ml syringe with a 20-gauge needle. The hemolymph was stored in RNAlater at -80°C for RNA extraction and RNASeq. Transcriptome analysis showed upregulation of a DNA replication licensing factor MCM3-like (Minichromosome Maintenance Complex Component 3) in all sick individuals, but no production in healthy individuals. This protein was selected to create a species-specific reverse transcription quantitative real-time PCR (RT-qPCR) assay. Using archived hemolymph samples, molecular data will be correlated with histological diagnoses from the same individuals. This new diagnostic can be used to diagnose and quantify the severity of hemocytic neoplasia in hard clams.

HEMOCYTIC NEOPLASIA IN THE HARD CLAM *Mercenaria mercenaria* – CELL ORIGIN AND DIFFERENTIAL GENE EXPRESSION

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Over the past several years a disease similar to disseminated neoplasia (DN) previously described in other bivalves has been observed in the hard clam, *Mercenaria mercenaria*, in Wellfleet, Massachusetts. Work on DN in other bivalves has indicated that the disease is not only transmissible (Metzger et al. 2015) but that the neoplastic cell lineages tend to be specific for different bivalve species (Metzger et al. 2016). A retroelement (*Steamer*) has been determined to show increased expression in diseased soft shell clams (Arriagada et al. 2014), but whether it is responsible for the disease is still unknown. To better define and understand this disease in hard clams, we set out to identify the origin of the neoplastic cells and to examine whether similar retroelements were present and showed increased expression in infected individuals.

To determine the origin of the neoplastic cells, we acquired and compared the mitochondrial COI (mtCOI) gene sequences of hemocyte cells from normal and infected clams, and from tissues collected from a subset of normal and infected clams. This showed that haplotypes of the hemocyte cells from infected individuals were different than the corresponding tissue haplotypes and confirmed that the neoplastic cells originated from *Mercenaria mercenaria*. It was also noted that the mtCOI haplotypes of most transformed cells arose from previously identified haptophyte lineages that were likely uninfected, suggesting that more than one possible transformation event had occurred.

Transcriptome sequencing was accomplished on hemolymph from 5 infected hard clams and 3 uninfected hard clams collected from the adductor muscle sinus using sterile needles and preserved in RNALater at -80°C. The transcriptome was assembled using all 8 samples (529,795 assembled transcripts), and eukaryote BUSCO analysis indicated very good completeness (96.1% complete, 3.1% fragmented, 0.8% missing). Differential expression analysis indicated the up-regulation of 1,303 transcripts in the infected hemolymph, and 73% of these were annotated. Most of these transcripts were associated with cellular growth and cancer pathways, as expected for cells undergoing rapid replication. While there were no retroelements similar to the *Steamer* element from soft shell clam, there were oncogene-related sequences, RNA-directed DNA polymerases and Jockey-like retroelements upregulated in the infected hard clam transcripts. It is still not known whether this expression is a consequence or a cause of the transformation, but this data has revealed several potential targets for diagnostic methods.

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SUITABILITY OF A PORCINE HYDROLYSED INTESTINAL MUCOSA PROTEIN SOURCE FOR AQUACULTURE SPECIES BASED ON AMINO ACID PROFILES AND FEED REQUIREMENTS

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Aquaculture ongoing growth requires new efficient, safe, and sustainable protein sources. The use of plant-based protein sources, especially from soy, as partial replacement for fish meal is common but limited due to the presence of antinutrients. Palbio is a high-quality, sustainable, protein obtained from porcine intestinal mucosa as a byproduct of the heparin manufacturing process following a circular economy approach. It contains bioactive peptides and leads to health benefits in animal species, especially in swine. Its use in aquaculture might allow lower inclusion levels of plant-based protein sources. The essential amino acids profile is a key parameter to evaluate the suitability of a protein source for animal feed, and protein quality can be estimated by calculating the contribution of each amino acid to the ideal pattern. We evaluated the suitability of Palbio by analyzing its amino acid profile and comparing it with that of soy and with the requirements of Atlantic salmon (*Salmo salar*) and pig (*Sus scrofa*) using the ideal protein approach.

The amino acid content in three different Palbio HP industrial batches (20/0001, 21/0001, 21/0072; Bioiberica SAU, Palafolls, Spain) was measured with high-performance liquid chromatography with fluorescence detection (HPLC/FLD). Pig and salmon muscle amino acid profile were obtained from Bahelka et al., 2020 and Wilson & Cowey et al., 1985, respectively; soybean meal data from AmiPig (2000); and nutritional requirements from publicly available defined nutritional requirements (NRC of swine 2012, and NRC of fish and shrimp 2011).

Palbio HP results are shown in Table 1, while Figure 1 depicts a comparison and degree of overlap between the different amino acid profiles and nutritional requirements. Our analyses showed a high degree of overlap between Palbio HP and salmon muscle and nutritional requirements, and similar to those of pig. Conversely, soybean meal, as a reference plant-based protein source, shows a lower degree of overlap representing lower theoretical nutritional adequacy for salmon diets.

Given the suitability and the reported health benefits of Palbio as protein source for pigs and based on the herein reported suitability also for salmon, Palbio could serve as an adequate protein source to maximize growth and feed utilization in salmon and provide health benefits as well.

SUCCESSFUL EASTERN OYSTER (*Crassostrea virginica*) RECRUITMENT ON TWO ALTERNATIVE SETTLEMENT SUBSTRATES IN VIRGINIA TRIBUTARIES

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Eastern oyster (*Crassostrea virginica*) populations have been declining, and their preferred settlement substrate, natural oyster shell, is becoming scarce for use in restoration. Alternative substrates are increasingly used for oyster reef restoration, prompting a need to examine the effectiveness of various alternative oyster settlement substrates. Natural spat settlement is abundant in lower Chesapeake Bay, and settlement substrate is a liming factor. Oyster Castles have been commonly used as alternative settlement substrate, and a new scalable, concrete, reef-building substrate, Grow Oyster Reef Tiles, have recently become available. Our objective was to assess eastern oyster recruitment and size on two types of alternative substrates, Oyster Castles (OC) and Grow Tiles (GT), over a 3-year period. We examined four sites throughout the Chesapeake Bay in Virginia: Cherrystone Inlet, Mockhorn Bay, Lynnhaven Bay, and Elizabeth River. At each site, ten OC and ten GT were randomly interspersed parallel to shore in the intertidal zone. All sites were sampled in October 2019, July 2020, and June 2021. Combined data (2019-2021) were analyzed using various response variables and the factors year, site, and substrate type. Overall, both substrate types (OC and GT) had high oyster recruitment, above 50 individuals/m^2, which has been designated as the target density to demonstrate successful oyster restoration in Chesapeake Bay. Survival was high on both substrate types, and there were differences in densities by site; highest oyster densities were in Mockhorn Bay. Based on size-frequency histograms, two cohorts were evident for populations on both substrates, and both populations displayed an approximate 60-70 mm growth over the three years. Recruitment was greater on OC for the combined data over three years, but mean oyster size was typically greater on GT. Overall, both substrate types were successful in recruiting high densities of oysters (see Fig. 1), allowing survival of multiple cohorts, and promoting growth to adult size. This suggests that both substrates could be used effectively for restoration. Further research on mechanisms producing site differences or on the performance of additional artificial substrates could aid in future oyster restoration efforts, particularly in Virginia, where natural oyster settlement is prominent.

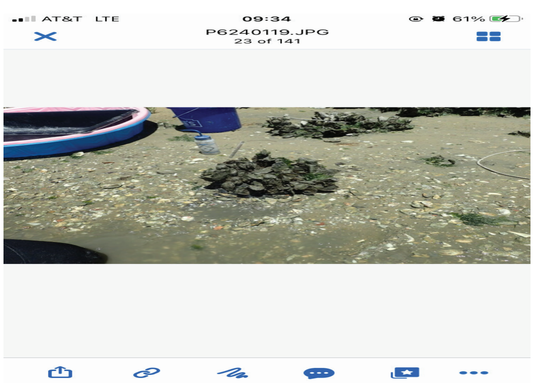

Figure 1. Dense oyster populations on a Grow Oyster Reef Tile, a new artificial settlement substrate.

STOCKING DENSITY INFLUENCE ON GROWTH PERFORMANCE AND WELFARE OF JUVENILE OLIVE FLOUNDER *Paralichthys olivaceus* IN A RECIRCULATING AQUACULTURE SYSTEM

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Currently, the recirculating aquaculture system (RAS) is considered an alternative system to mitigate the potential environmental deterioration occurred by traditional culture systems. The RAS is a closed system that can secure the independency from the external environments and can efficiently control the internal environments, thus enhancing productivity by intensification. However, intensification during the production process has often drawn concerns in terms of animal welfare. Good welfare is reflected in the culture animal's conditions and tolerance to the infectious matters or stressors. So, in this study, we evaluated the effects of stocking density on the growth performance and haematological changes of olive flounder.

An 8-week growth experiment was conducted with initial stocking densities of 3.29, 4.84, 7.14, and 8.56 kg/m^2 using juvenile olive flounder (59.7±3.5 g) in a RAS. An experimental RAS consisted of 12 square tanks (95 x 95 x 85 cm), two square tanks of moving-bead biofilter (100 x 100 x 100 cm), an UV sterilizer (80 W), a down-flow oxygen contactor, a centrifugal pump (290 L/min), and a drum filter (80 µm sieve). Water temperature was maintained at 25°C using two chillers (1 HP), and a submersible heater. Water turnover rate was set at 24 times/day and 10% of water were exchanged daily. Fish were fed a commercial feed (55% protein and 8% lipid) to apparent satiation twice a day.

Table 1. Results of growth performance and haematological analysis of olive flounder cultured in four different initial socking densities for 8-weeks

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4	*P* value
Initial stocking density (kg/m^2)	3.29±0.13^d	4.84±0.12^c	7.14±0.06^b	8.56±0.19^a	0.000
Final individual fish weight (g)	170.00±21.75^b	178.31±28.79^b	215.20±20.03^a	210.55±17.39^a	0.000
Final stocking density (kg/m^2)	7.68±0.61^d	13.28±0.44^c	20.17±1.23^b	23.14±0.68^a	0.000
Individual fish weight gain (g)	110.29±21.75^a	118.62±28.79^a	150.85±20.03^b	155.50±17.39^b	0.000
Feed conversion ratio	0.94±0.14	0.90±0.07	0.98±0.02	0.98±0.01	0.622
Specific growth rate (%/day)	1.86±0.23^a	1.93±0.28^a	2.28±0.17^b	2.25±0.15^b	0.000
Growth hormone (pg/mL)	551.2±97.13	525.3±52.02	544.62±76.38	506.79±38.79	0.304
Insulin like growth factor-1 (pg/mL)	147.27±23.04^a	122.01±3.95^b	112.75±3.59^b	94.39±8.47^c	0.001
Glucose (mg/dL)	49.67±17.06	39.6±8.48	50.4±15.17	40.6±15.85	0.082
Glutamic oxaloacetic transminase (U/L)	23.13±5.41	18.00±4.76	19.2±6.16	16.54±4.31	0.239
Glutamic pyruvic transminase (U/L)	9.50±2.41	7.67±1.68	7.93±2.46	7.00±1.75	0.121
Cortisol (ng/mL)	3.24±0.61^b	3.25±0.52^b	3.35±0.69^b	3.92±0.40^a	0.004

A PLACE IN THE SUN: EFFECTS OF SOLAR EXPOSURE ON *Ostrea lurida* AND *Magallana gigas* DENSITIES ON EXPOSED VERSUS PROTECTED PIER PILINGS IN SAN DIEGO BAY, CALIFORNIA

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Solar exposure and desiccation stress limit the distributions of intertidal organisms; these stressors will increase with climate change. In southern California estuaries, a zonation pattern exists between oyster species. *Ostrea lurida*, the only oyster native to the North American west coast, occurs at lower elevations compared to *Magallana gigas,* a non-indigenous species (NIS). *M. gigas* withstands warmer immersed temperatures than *O. lurida;* less is known about their emersed thermal limits. Solar exposure and desiccation stress may limit oyster densities. We hypothesized that *O. lurida* densities are more suppressed by increasing solar exposure than *M. gigas* densities. During low tides in June 2021, we randomly placed quadrats at tidal elevations between 0 and +1.0 m mean lower low water and at various solar aspects to survey oyster density on pier pilings shaded under docks at three sites and exposed above floating docks at two sites. *O. lurida* densities averaged 13 times higher on pier pilings under docks versus on exposed docks, while *M. gigas* densities did not differ (Two-way ANOVA, Species $p=0.001$, Above/Under $p<0.0001$, Species*Under/Above interaction $p<0.0001$). On exposed docks, *M. gigas* densities were 4 times higher on south-facing pier piles versus west-facing; *O. lurida* densities were not affected by solar aspect (Two-way ANOVA, Species $p=0.1514$, Aspect $p=0.0950$, Species*Aspect interaction, $p=0.0197$). *O. lurida* cover averaged 21 times higher on pier pilings under docks versus on exposed docks, while *M. gigas* cover did not differ; *M. gigas* cover under docks was similar to *O. lurida*, but above docks was 13 times greater than *O. lurida* (Two-way ANOVA, Species $p<0.0001$, Above/Under $p<0.0001$, Species*Under/Above interaction $p=0.0346$). Solar exposure affects oyster species differently; *O. lurida* achieves highest densities when shaded under docks, while *M. gigas* densities increase at southern solar aspects and is unaffected on exposed docks, positioning this NIS favorably with climate change.

A BACTERIOPHAGE COCKTAIL AS AN ALTERNATIVE FOR THE CONTROL OF *Vibrio parahaemolyticus* RESPONSIBLE FOR AHPND IN *Penaeus vannamei*

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Acute hepatopancreas necrosis disease (AHPND), caused by specific strains of *Vibrio parahaemolyticus*, has generated high mortalities in the shrimp cultures around world, and among the failed strategies to control the disease has been the use of chemotherapeutic agents, which may cause antibiotic resistance and detrimental effects in the environment. A friendly environment alternative is the use of the phage therapy. The aim of the present study is the formulation of a bacteriophage cocktail with a wide range of *Vibrio* genus hosts and able to lyse *V. parahaemolyticus* AHPND strains. The application of phages produced a significant inhibition (p<0.05) on the growth of both *Vibrio* strains (Fig. 1); however, there were differences on the effectiveness for lysing the target strain among single phages or cocktails. A cocktail of 12 bacteriophages showed 72.02% inhibition of *Vibrio campbellii* and 66.88% inhibition of *V. parahaemolyticus* with a cocktail of 3 bacteriophages.

Bacteriophages were also characterized to different physicochemical conditions, in this study all the bacteriophages demonstrated viability in chloroform and at different salinities between 5 and 40 g/L. The selection of bacteriophages for the formulation of a cocktail will be by the plating efficiency test (EOP) and tests to find resistant bacteria to bacteriophages. Afterwards, an experimental infection with *P. vannamei* will be done to evaluate the effectiveness of the bacteriophage cocktail to control *V. parahaemolyticus* AHPND. Additionally, samples will be taken for histology and metagenomics analysis.

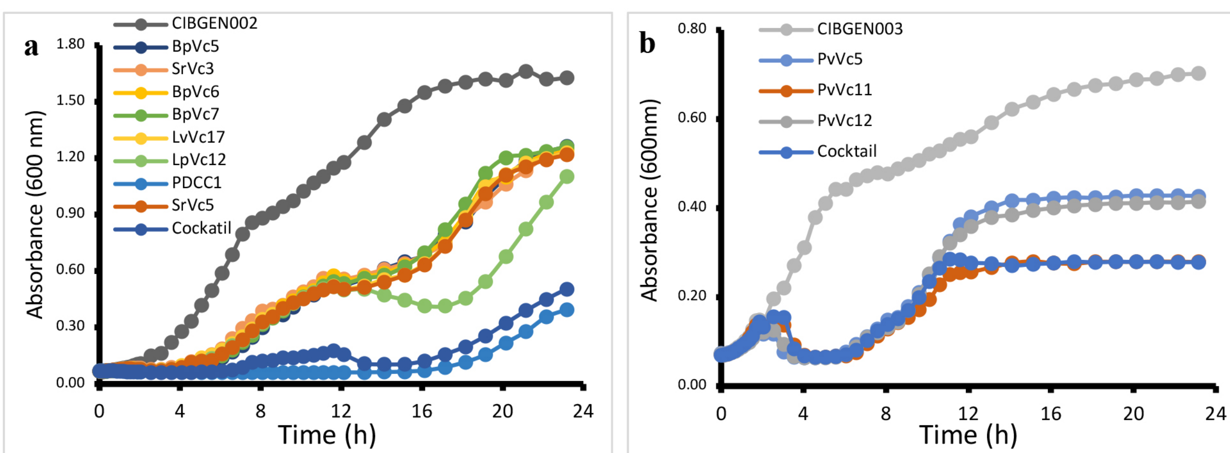

Figure 1. Killing curves of *Vibrio campbellii* growth and *V. campbellii* with 9 bacteriophages (**a**); *Vibrio parahaemolyticus* growth, *V. parahaemolyticus* with 3 bacteriophages (**b**), and their respective cocktails.

SEABASS IN THE CLASSROOM: AN AQUACULTURE EDUCATION PROGRAM IN SOUTHERN CALIFORNIA

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The Seabass in the Classroom (SITC) education program was developed and initiated by Hubbs-SeaWorld Research Institute in 2010 in partnership with, the California Department of Fish and Wildlife (DFW), and Get Inspired Inc. The program provides students in southern California the opportunity to learn about aquaculture, fisheries, and seafood by growing juvenile white seabass (WSB) in their classrooms for release into the ocean. Through this novel, hands-on, STEM classroom program, students are learning about the science and associated technological aspects required to support the growth and sustainability of aquaculture. A few important aspects of this program include:

- The on-site installation of a purpose-built recirculating marine aquaculture system for culture of white seabass, which requires student participation with system maintenance, water quality monitoring, feeding, food conversion efficiency and basic fish husbandry as a supplement to classroom instruction about the science of aquaculture.
- Modeling the program after California's white seabass replenishment program, whereby the students measure, weigh and tag the fish prior to getting a health inspection by the DFW after which they are released into the ocean.
- Discussions of the health of the oceans off southern California and globally; especially as it relates to fisheries resources.
- Understanding the historical trends and relationships of per capita consumption of seafood, global population increases, and production capacities from fishing and aquaculture.
- Discussions of the health value of eating fish that are high in omega-3 fatty acids.

Currently the program is exposing over 1000 students each year in 11 schools that have conducted 124 classroom growout cycles and released over 3100 fish.

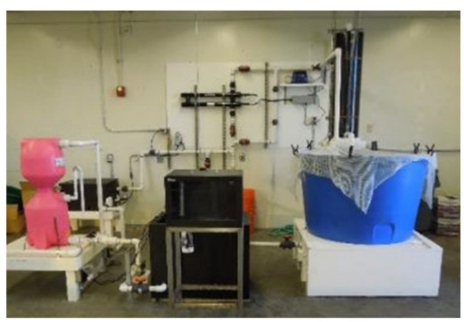

FIGURE 1. A purpose built SITC tank system installed in a high school classroom.

FIGURE 2. Students successfully releasing white seabass grown in their classroom.

TRAINING AQUAPONIC PRODUCERS THROUGH CONTROLLED ENVIRONMENT AGRICULTURE PROGRAMMING AT SANTA FE COMMUNITY COLLEGE

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The Controlled Environment Agriculture (CEA) program at Santa Fe Community College (SFCC) offers a certificate or an Associate of Applied Science Degree (AAS) option to a diverse mix of students, through online and on-campus instruction. The program specializes in water conservative farming methods like hydroponics and aquaponics. An Algae Cultivation certificate is embedded within the CEA program for students who want to specialize in that area of aquaculture production. Associated courses in Distributed Energy, Biofuels, Alternative Fuels, Water Treatment, Plumbing and HVAC create a broad selection of related courses students take as electives. Faculty and Staff in the SFCC-CEA have decades of experience in the field of aquaponics and manage state-of-the-art facilities including an off-grid production greenhouse, complete with battery storage and a variety of energy harnessing assets. New upgrades are being planned for Spring and Summer of 2021. The hands-on approach to learning has recently spawned three new aquaponic producers around Santa Fe, each with unique goals and each with a unique production model. These farms and the SFCC-CEA program will be discussed during this presentation.

TRANSCRIPTOMIC RESPONSES TO LOW SALINITY IN WILD SPAT OF THE EASTERN OYSTER *Crassostrea virginica*

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The Eastern oyster *Crassostrea virginica* is an ecological keystone species, and a critical fisheries resource for some coastal U.S. economies. Genetic and functional variation persisting in highly fragmented populations constitutes a critical adaptive resource in the face of anthropogenic environmental change. Regional genetic homogeneity is well documented along the Atlantic U.S. coast, suggesting high gene flow within and among estuaries. While strong gene flow makes adaptation to local salinities unlikely, developmental plasticity and responses to within-generation selection are two mechanisms that can structure phenotypic variation across the estuary. Specifically, gene expression responses to osmotic stress represent many molecular phenotypes potentially influenced by both mechanisms. Following on studies of gene expression plasticity and evolution in adult Delaware Bay oysters, here wild spat stage oysters were collected from moderate and low salinity regions in Delaware Bay and exposed to moderate (22 ppt) and low (6 ppt) salinity conditions in the lab. After seven weeks, the number and ontology of differentially expressed genes under these contrasting conditions was quantified in gill and mantle tissue to assess phenotypic plasticity in response to osmotic stress (comparing each source across treatments) and test for a significant effect of spat source region. Using environmental data, we link observed patterns of differential gene expression to conditions likely experienced by spat since settlement, influencing their development and the potential for within-generation selection. This research aims to elucidate the life-stage at which patterns of differential gene expression emerge across an estuarine salinity gradient.

PEOPLE WHO WORK IN FRESHWATER FISHPONDS SHOULDN'T THROW MUD: OFFSHORE AQUACULTURE CAN ADDRESS GLOBAL ENVIRONMENTAL AND SOCIAL IMPERATIVES

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This presentation responds to Belton, et al.'s 2020 besmirching of offshore aquaculture as supported by "crisis narratives" that "combine Malthusian anxiety with cornucopian techno-futurism, but disregard who would have access to the food produced".

Despite deniers, the planet is indeed in crisis. We truly should be anxious. We desperately need novel technologies and systems; if that's "techno-futurism", then let's embrace it. And rather than "disregard" consumers, we should have the highest regard for the power of the marketplace to drive these essential disruptions, and improve people's lives. Those who would stand knee-deep in a freshwater fishpond and defend the status quo, or who would blithely and blindly wish the world worked differently, put our planet, and all people, in peril.

Expansion of offshore aquaculture offers potential to significantly reduce greenhouse gas emissions by supplanting terrestrial livestock production; as well as lowering pressure for land-conversion and limited freshwater resources; mitigating ocean acidification; providing food for a growing planet, as well as feed, fertilizers and fuels; and possibly providing potential for long-term carbon capture and storage.

The challenges that we face – as a global community – cannot be resolved through promoting only 'one pure' form of aquaculture. Salinity should not be the sole rubric: production efficiencies and global ecological impacts should guide our work. We need to counter consumer predilections for land animal proteins by harnessing the power of their preferences. As an alternative to terrestrial meats, offering the umami of marine-sourced products is more likely to succeed than compelling commoners to only eat carp.

This presentation will also review Ocean Era's recent R&D work, in the context of the defining environmental imperatives of our time. We are developing a demonstration array for offshore macroalgae culture, and refining culture methods and diets for kyphosids (*Kyphosus vaigiensis*), mahimahi (*Coryphaena hippurus*), and kampachi (*Seriola rivoliana*). We are pioneering the permitting process in U.S. Federal waters for a demonstration net pen array off Sarasota, FL, in the Gulf of Mexico. We are also applying for the permits in Hawai'i State waters for a commercial fish and macroalgae co-culture operation offshore of 'Ewa Beach, Oahu.

The world desperately needs more seafood, be it from offshore aquaculture, marine RAS, or freshwater systems. We do ourselves, our profession and our planet a grave disservice by slinging mud at each other's efforts.

NUTRITIONAL EVALUATION OF A BYPRODUCT (FRASS) OF INSECT INDUSTRY AS AN ALTERNATIVE INGREDIENT IN NILE TILAPIA FEED

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Aquaculture is the fastest-growing food-producing sector in the world, and for this industry to continue expanding, there must be improved sustainability. One of the ways to achieve this goal is to use higher amounts of terrestrially based ingredients. Traditionally this has relied on using plant-based proteins and lipids, however, there is increasing interest in the use of insect meals. Recently, interest arose to evaluate a byproduct of the insect industry (EnviroFlight, LLC.) called "frass". It is composed of spent feed and waste products from the black soldier fly (*Hermetia illucens*) larvae and contains 38% carbohydrate and 22% crude protein. Thus, it could be new ingredient for fish. The aim of this study was to evaluate the nutritional potential of frass in Nile tilapia (*Oreochromis niloticus*).

Six diets (isonitrogenous: 300 g/kg crude protein and isoenergetic: 4300 kcal/kg) were formulated to trade the carbohydrate, lipid and protein nutrients with increasing levels of frass to replace the blend of plant ingredients that included soybean meal, corn-DDGS and wheat flour. Diets were as follows: Diet 1: Control (fish meal: 8%, corn DDGS: 30%, SBM: 33% and wheat flour: 23%); and Diets 2 – 6: 10, 20, 30, 40 and 50% frass was used to replace the blend of plant ingredients in the diets. Nile tilapia fingerlings (ave. weight: 2.0 g) were randomly distributed in 18 tanks (25 L, each tank contains 20 fish) in a recirculatory aquaculture system. Fish were fed 3 times per day at satiation level for nine weeks.

At the end of the trial, the weight gain (%) was higher in diet 6 (50% frass) than other groups (Fig.1), however, feed utilization showed no differences (P<0.05). The gluconeogenic gene mRNA expression indicates that the fish fed 30% frass had the lowest expression of glucose-6-phosphatase-2 (G6pca2) gene compared to other groups, but no significant variation was noticed in the G6pca1. The histological evaluation indicates that the fish fed the frass-based diets showed some hepatic inflammation and vacuolization (Fig.2). Conclusively, highest growth performance was observed at 50% inclusion of frass in the tilapia diet, however, it does appear that the nutritive value of frass could be further improved based on the observed hepatic alteration. Nevertheless, this frass product replaced 33, 54 and 64% of the protein, lipid and carbohydrate fraction in Nile tilapia diet, respectively, and could be an additional ingredient in tilapia diets to improve aquaculture sustainability.

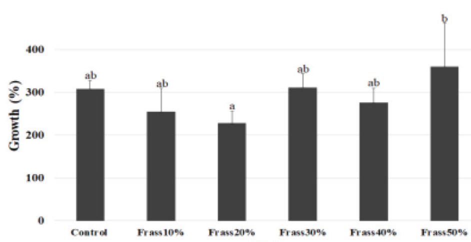

Fig 1: Growth (%) of fish fed frass-based diet. Mean ± SD, n=3. Mean values with different letters differ significantly (P<0.05).

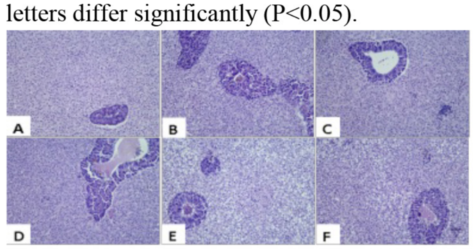

Fig 2: Liver histology of fish fed frass at 0% (A), 10% (B), 20% (C), 30% (D), 40% (E); 50% (F).

DISPENSABLE AMINO ACID L-ALANINE CAN LOWER THE LIPID LOAD IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIET

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Protein nutrition is always a prime area of research in aquaculture to develop sustainable aquafeeds. However, unlike the essential amino acids (EAAs), the non-essential amino acids (NEAAs) did not get much importance in fish nutrition. Many studies support that NEAAs play multiple roles such as growth, stress response, cell signaling, larval metamorphosis, gut health, feeding stimulant and so on. Among NEAAs, L-alanine plays an important role in energy-budgeting and acts a major source of gluconeogenesis. In this study, L-alanine is used as promising additive to reduce the dietary fish oil inclusion.

A 2 × 6 factorial designs containing 12 diets (isonitrogenous: 42% crude protein) with two levels of lipid (L: 20 and 14%) and six levels of L-alanine (A: 0, 0.5, 1.5, 2.5, 3.5, and 4.5%). A total of 576 rainbow trout juveniles (average weight: 12.4 ± 0.12 g) were randomly distributed in triplicates in 36 tanks and reared for five weeks in a recirculatory aquaculture system.

Dietary lipid level has significant effects on growth performance (Table 1). Fish fed low dietary lipid (14%) with 1.5-2.5% L-alanine ($L_{14}A_{1.5}$ and $L_{14}A_{2.5}$) exhibited similar ($p>0.05$) growth performance as control group ($L_{20}A_0$: 20% lipid without L-alanine). There was no significant effects of different level of lipid and dose dependent L-alanine on feed conversion ratio (FCR) and protein efficiency ratio (PER). Neither dietary lipid level nor dose dependent L-alanine has significant effects on FCR and PER (Table 1). Final data for growth, feed utilization and gene expression pattern for amino acids transporters and lipid metabolism will be presented after nine weeks of feeding trial.

This is the first report where we have found that dietary dispensable amino acid L-alanine can be successfully used to reduce significant amount of oil in rainbow trout diet without affecting the growth performance. Outcome of this study will reduce the feed cost of salmonid aquaculture.

Table 1: Growth and feed utilization of rainbow trout fed with different dietary lipid and L-alanine levels

Treatments	WG%	FCR	PER
$L_{20}A_0$	332.82^{bc}	0.83	2.87
$L_{20}A_{0.5}$	345.45^{c}	0.80	2.98
$L_{20}A_{1.5}$	331.4b^{c}	0.84	2.84
$L_{20}A_{2.5}$	344.33^{c}	0.81	2.94
$L_{20}A_{3.5}$	344.33^{c}	0.79	3.01
$L_{20}A_{4.5}$	318.64^{abc}	0.84	2.84
$L_{14}A_0$	294.87^{a}	0.85	2.81
$L_{14}A_{0.5}$	295.31^{a}	0.85	2.82
$L_{14}A_{1.5}$	300.02^{ab}	0.84	2.83
$L_{14}A_{2.5}$	319.79^{abc}	0.80	2.97
$L_{14}A_{3.5}$	293.89^{a}	0.84	2.84
$L_{14}A_{4.5}$	306.8^{ab}	0.81	2.95
SEM	4.09	0.006	0.02
p value	0.002	0.38	0.38
Two-way ANOVA			
Lipid	<0.001	0.31	0.29
L-alanine	0.91	0.26	0.24
Lipid*L-alanine	0.27	0.30	0.30

GENOMIC STUDY OF DOPAMINE RECEPTOR LIGAND BINDING SITES OF THE BIVALVE *Crassostrea virginica*

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Gill lateral cells of *Crassostrea virginica* are innervated by dopamine (DA) and serotonin nerves. DA slows down lateral cell cilia beating rates and serotonin accelerates them. DA receptors are classified as D1R and D2R. Physiology and cell biology work of our lab found the DA receptors involved in gill lateral cell cilia inhibition are D2R-like in the gill cells and D1R-like in the cerebral and visceral ganglia. Our HPLC studies found DA in various tissues, including gill, cerebral and visceral ganglia of *Crassostrea virginica*. Using immunofluorescence techniques, we showed the presence of DA neurons in cerebral and visceral ganglia as well as D2R-like postsynaptic receptors in gill lateral cells and D1R-like postsynaptic receptors in cerebral and visceral ganglia. Recently the genomes of *C. virginica* and other bivalves have begun to be mapped. By conducting searches of the NCBI (National Center for Biotechnology Information) database using DNA and protein sequences of *C. virginica* and other invertebrate and mammalian species we found matches for D1R genes on chromosomes 4 and 5, and D2R genes on chromosomes 3 and 5 of *C. virginica*. BLASTS of the receptors found matches with very low Expect Values (E values) and high Percent Identity of the D1R and D2R receptors to those in other bivalves, gastropods, insects, mice, rats and humans. Various invertebrates had Percent Identity above 60%, while humans and mice had Percent Identity of 30 - 40%. We hypothesize that the ligand binding sites (LBS) for D1R and D2R receptors in *C. virginica* are evolutionarily conserved and will closely match those of other animals. To study this, we searched the NCBI database for D1R and D2R LBS of *C. virginica* and compared them to other animals. We found D2R LBS contained 17 amino acids (W, D, V, S, F, T, L, S, S, S, W, F, F, N, F, T, Y) with very highly conserved (70 - 100%) to LBS of other bivalves, gastropods, insects, mice, rats and humans. D1R LBS have not yet been identified in *C. virginica*, nor in the other animals we searched for, except for humans where it contained 17 amino acids (W, D, I, S, T, S, A, S, S, S, W, F, F, N, F, V, W). The study complements our physiology and cell biology studies demonstrating the presence and function for DA in *C. virginica*, and shows the genome of *C. virginica* contains genes to produce DA receptor LBS that are similar to those of other animals. This new information is valuable as it shows that the simple nervous system of *C. virginica* can be used to expand studies on DA neurotransmission.

This work was supported in part by grant 2R25GM06003 of the Bridge Program of NIGMS, NIH grant K12GM09385407A1 IRACDA Program of Rutgers University and PSCCUNY grants 6234400 50 and 6343400 51.

SCALING UP RESTORATIVE AQUACULTURE OF ENDANGERED WHITE ABALONE *Haliotis sorenseni*

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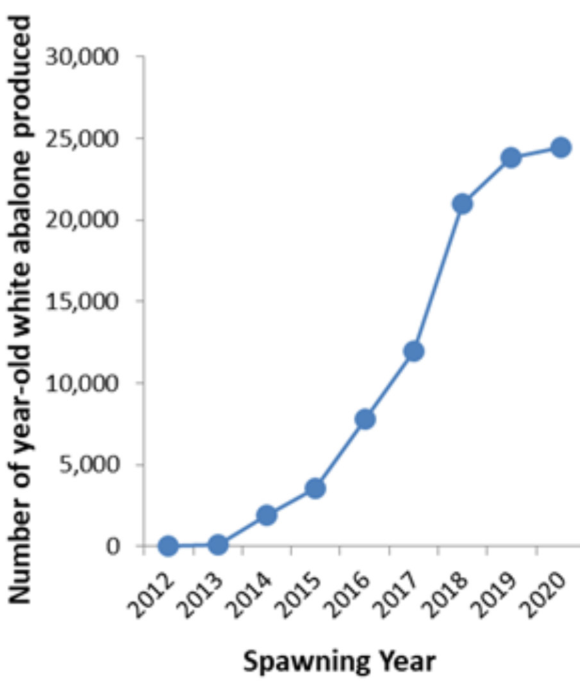
White abalone (*Haliotis sorenseni*) are an economically, culturally, and ecologically important species that was once abundant along the coast of southern California and Baja California, Mexico. Commercial overfishing severely reduced populations in the late 1900s, leading to fishery closure in 1996 and listing under the Endangered Species Act in 2001. It is estimated that there are fewer than 4100 white abalone in the wild, giving great urgency to recovery efforts. Restorative aquaculture and outplanting were identified as the primary action for species recovery. With early aquaculture setbacks due to disease and other challenges largely overcome, aquaculture efforts now focus on ways to scale up production in the face of limited genetic diversity among broodstock, limited gamete production, high post-settlement mortality, and climate change. To address the unique challenges of spawning, rearing, and outplanting white abalone, captive production protocols incorporate results of research on reproductive conditioning, settlement, disease, optimal diets, and climate change, including multi-stressor experiments that examine potential interactions among these factors. We hope this work will accelerate production from the approximately 25,000 one-year-old white abalone currently produced annually to the approximately 100,000 white abalone that likely need to be outplanted annually to restore the species. The work we present is led by the University of California, Davis, Bodega Marine Laboratory (UCD-BML) in partnership with state and federal agencies, universities, commercial aquaculture farms, and non-profit organizations.



THE GUT-BRAIN AXIS IN FISH AND POTENTIAL TO DEVELOP TARGETED APPROACHES TO FISH NUTRITION FOR AQUACULTURE

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The endocrine regulation of growth and appetite in fish has been partially characterized in a handful of species, most notably, goldfish, salmonids and catfish. Endocrine modulators, such as growth hormone (GH) and insulin-like growth factor-I (IGF-I) have been best characterized and often correlated to changes in feeding or nutrient intake. The regulatory functions of neuropeptide modulators of growth and appetite, with special emphasis on hypothalamic and gastrointestinal peptides, have also been well published for these species. Perhaps not surprisingly, salmonids and catfishes are among the most studied nutritionally as well, with most of their nutrient requirements being defined. More recently, nutrient sensing studies have shed light on chemosensory detection by fish of water born nutrients and the effect on feeding and metabolism. Even more recently, a focus gut microbiota has led to an explosion of publications on the impacts of genetics, diet and other environmental factors on gut function. Over the past two decades, my lab, like many others, has chipped away at the surface of the interactions between the endocrine system, the gut and even the microbiome. Yet, there is so much left unknown. The very complexity of the gut-brain axis suggests that we have only begun to identify the mechanisms involved in humans and terrestrial lab and farm animals, let alone in fish. While we can learn much from this literature, species-specific research must be a focus for aquaculture species. Only then can we truly develop targeted feeds and feeding protocols to maximize fish performance.

MODELING OFFSHORE TROPICAL MACROALGAE FARMS WITH EFDC

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Offshore farms of macroalgae have the potential to be important sources of food and biofuels in the future. Tropical waters offer benefits such as a year-round growing season, but in oligotrophic waters, additional nutrient input is required to support large biomass growth. As part of the ARPA-E MARINER program, the EPA-approved Environmental Fluid Dynamics Code (EFDC) was used to model a cluster of large (1 km x 2 km) macroalgae farms suspended at 10 m below the surface and supplied by artificially upwelled nitrogen (N)-rich deep seawater (DSW) west of the island of Hawai‘i. The EFDC domain was nested in a local ROMS model for hydrodynamic boundary conditions, and ambient concentrations of water quality variables, including nitrate, ammonium, carbon dioxide, and three phytoplankton types, were taken from Station ALOHA, Hawai‘i. Phytoplankton and the biogeochemical cycles were included as important contributors to nutrient competition and regeneration for regional-scale designs.

Simulations were run for 14 days for three farm layouts (Fig. 1) under multiple conditions, including 1 gallon per minute (gpm) of DSW provided per m^2 of farm; 2 gpm of the same; no DSW provided; no DSW, but higher surface N present; 1 gpm of DSW per m^2 with modified values for macroalgae growth rate and N half-saturation constant; 1 gpm of deeper (600 m) DSW per m^2; and 1 gpm of DSW per m^2 and no phytoplankton modeled. Default DSW was drawn from 250 m with 150 µg N L^{-1} of nitrate; 600 m DSW had 520 µg N L^{-1} nitrate. Default surface nitrate was 0.24 µg N L^{-1}; higher surface nitrate was 5.0 µg N L^{-1}. Default maximum daily growth rate (DGR) and N half-saturation constant were 22.5% per day and 5.0 µg N L^{-1}, respectively, while modified values for the same were 11% per day and 1.4 µg N L^{-1}.

Results were analyzed to provide average net DGR for the farm systems as a function of layout and design assumptions, showing the impact of these factors on macroalgae growth at regional scales (Fig. 2). In all scenarios, DGR was primarily controlled by nitrogen limitation. Sparser clusters of farms were advantageous when no DSW was provided, as they experienced less competition, while denser clusters fared better when DSW was provided on a per-farm basis, as nutrients advected away from one farm were more likely to benefit neighbors. Phytoplankton was a critical factor in determining macroalgae farm success and should not be neglected when modeling offshore macroalgae systems.

AN UPDATE CONCERNING THE FIELD RESEARCH AND MONITORING ON THE OCCURRENCE OF NEOPLASIA IN THE NORTHERN QUAHOG (= HARD CLAM) IN WELLFLEET, MASSACHUSETTS, USA

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Hematopoietic Neoplasia (HN) has been a significant cause of disease and death in the northern quahog (=hard clam) (*Mercenaria mercenaria*) in Wellfleet, Massachusetts (MA). Since the disease was first detected, it has continued to increase in severity and occurrence in aquaculture leases and has decreased the profitability of cultured hard clams. Research by others has determined that a similar disease in *Mya arenaria* (soft shell clam) and in species of cockles is spread directly between members of those species by a neoplastic cell that enters the water column, infects a naïve animal, proliferates in the vascular system of the infected bivalve and eventually causes its death. A similar scenario appears to be occurring in HN infections of hard clams. Data from recent field research and annual monitoring of hard clams in Wellfleet will be presented confirming that hard clams from different parentages and from different hatcheries are vulnerable to the infectious, tumorous disease. Findings also show that possible resistance to the disease may be present in a subset of hard clams. These survivors could potentially be used to develop resistant hard clam lines.

DEVELOPMENT OF IMPROVED COMMERCIAL FEEDS FOR RAINBOW TROUT *Oncorhynchus mykiss*. A CASE STUDY ON BALANCING COST, PERFORMANCE, AND SUSTAINABILITY

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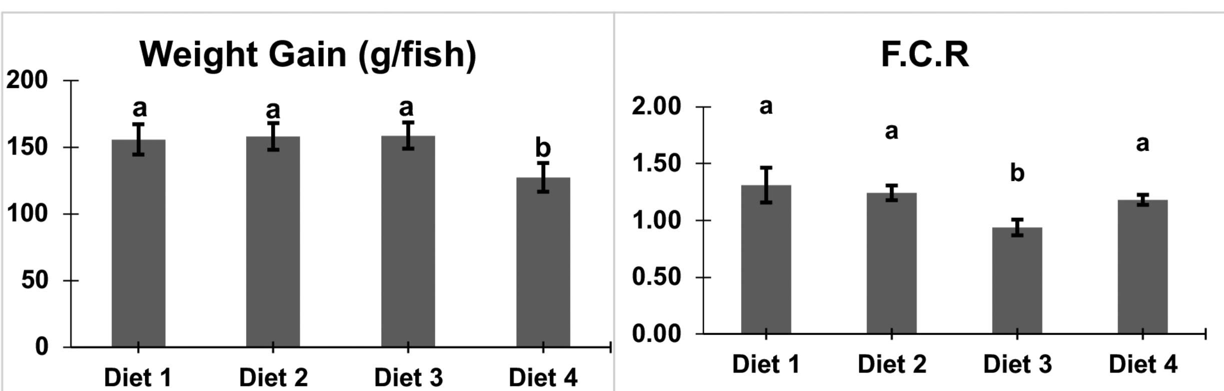
The state of static production volume and highly volatile market conditions of rainbow trout (*Oncorhynchus mykiss*) production in the US leaves farmers with few options for increasing the profitability of their businesses. It is well established that feed costs are the largest single variable cost, lowering trout feed costs as profits shrink is an industry priority. Four experiments were conducted at the Hagerman Fish Culture Experiment Station evaluating 16 commercially-produced trout feed formulas with the objective of optimizing the feed cost – fish performance balance. The primary objectives of experiments 1-4 were to measure the effects of feeding alternative formulas to rainbow trout on intestinal morphology and innate immunity, fecal turgidity, followed by performance during the juvenile lifestage, and growout lifestage, respectively.

Experiment 1 resulted in no statistical differences in all measurements except for higher oxidative radical production in fish fed Diet 4. Experiment 2 resulted in statistical differences in weight gain and FCR; and numerical differences in fecal durability. Experiment three resulted in significantly improved nutrient digestibility in Diet 4 while maintain statistically similar fish performance and fecal particle size distribution as Diet 1. Although the growth performance, feed utilization, fillet yield and whole-body composition results were not statistically different (P>0.05), practical numerical differences were observed in Experiment 4. Taken together, these results suggest that based on growth and feed utilization, fish fed diet groups 1 and 3 tended to perform better than fish fed the diet group 4.

Figure1. Weight gain (g/fish) and F.C.R of rainbow trout juveniles fed experimental diets for 12 weeks (Experiment 4).



WATER EXCHANGE METHOD AFFECTS FEED CONSUMPTION AND FEED CONVERSION IN *Clarias gariepinus* RAISED IN SMALL PLASTIC TANKS

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Catfish produced in Nigeria is often grown in tanks or earthen ponds with frequent water exchanges in order to evacuate wastes. However, much of the information on production rates and water exchange are based on anecdotal information and rarely backed up by measurements. Furthermore, only a few farmers record water use because many farmers move fish several times in a single production cycle. Therefore, we decided to test the mode of water exchange to see if there would be any difference in feed consumption or feed conversion so we could better design future trials comparing feeds and comparing different rates of water exchange. Twelve plastic tanks with capacity 1m^3 were each stocked with 25 subadult catfish, *Clarias gariepinus*, averaging 151±2g. Water exchange was designed to use the same total amount of water (500 liters per day), but on three different schedules: treatment 1 was a one-time 500L water exchange per day, treatment 2 was a one-time 1000L single exchange every two days and treatment 3 was a continuous (flow through) slow rate of exchange at a rate of 500L of water per day. The water source was a borehole located on the farm with temperature of ~27.4^{O}C. Water was first pumped into a header tank before discharging into rearing tank during water exchange. All rearing tanks drained their water from the bottom. Fish were fed every afternoon to satiation for 87 days beginning with a feed of 42% crude protein, followed by 33% crude protein feed for a total of 115 days. Treatments 1 and 3 resulted in significantly higher fish growth and better feed conversion than treatment 2. Even though the same amount of water exchange was applied in all treatments, a steady rate or smaller, more frequent exchanges worked better for intensive clarias production. It is recommended to avoid abrupt changes in water quality with *Clarias gariepinus* even if the replacement water is of better quality for the fish and since the method of water exchange significantly affects some parameters of the fish, it should be noted when reporting trials involving the species.

Table 1: Growth indices and food utilization parameters of *Clarias gariepinus* produced under different frequencies of water renewal.

Parameters	Treatment 1	Treatment 2	Treatment 3
Initial Weight (g)	149.50±1.72$_b$	146.70±1.63$_b$	158.20±3.81$_a$
Final Weight (g)	541.87±33.49$_a$	394.63±22.37$_b$	601.52±13.50$_a$
Survival (%)	93.00±3.42	99.00±1.00	95.00±2.52
Feed Conversion Ratio	1.55±0.05$_b$	1.82±0.07$_a$	1.40±0.03$_b$
Specific Growth Rate	1.11±0.05$_a$	0.86±0.04$_b$	1.16±0.02$_a$
Feed cost per kg gain (FCR*feed price in Naira)	662.05±23.45$_b$	776.20±31.74$_a$	596.68±13.99$_b$
Volume of water use per weight gain (L/Kg)	5710±609.9$_b$	8406±693.5$_a$	4822±229.0$_b$

Means±S.E on the same row with different subscript differ significantly ($p<0.05$).

USE OF NON-NATIVE COLUMBIA RIVER AMERICAN SHAD *Alosa sapidissima* AS AN ALTERNATIVE FISHMEAL SOURCE FOR JUVENILE SABLEFISH *Anoplopoma fimbria*

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In this study, we are investigating the use of American shad *(Alosa sapidissima)* as an alternative fish protein/oil for aquaculture. American shad is a non-native species of the marine and inland waters of the Pacific Northwest. The population of shad have increased over the years since introduction to the West Coast in the late 19th century, and currently is the highest fish population in the Columbia River. Shad are believed to have a negative impact on native fish populations; and the population of shad is predicted to continue to increase as a result of global climate change. There is a limited commercial harvest and sport fishery for shad, but a large percentage of the population remains underutilized. Future global food security depends on innovations to meet the forecasted 50% growth in demand for protein, with a 50 million ton shortfall for seafood expected by 2030. Aquaculture is the fastest-growing food producing sector globally and could help meet this demand, but aquaculture feeds are currently derived from ingredients like fishmeal and fish oil from industrial fisheries which are limited in availability and subject to price volatility. The nutritional composition of shad (high in protein and fat) make them a good species for fishmeal and fish oil production, and their use in aquaculture feeds would consequently reduce pressures on industrial fisheries and increase the environmental sustainability of domestic aquaculture. Reduction in the shad population could also be an effective tool for enhancing the survival of native fish populations, especially listed ESA species.

Experimental sablefish feeds were prepared in house with shad meal replacing industrial sardine meal at 0%, 50% and 100% replacement. Juvenile sablefish were fed experimental feeds to apparent satiation for 10 weeks to evaluate the effect of shad meal on fish growth, feed intake, feed efficiency, whole body nutrient composition, and liver condition. Fish grew well during the study with excellent survival. Results indicate shad may be a promising alternative feed ingredient for cold water marine fish feeds.

ONE HEALTH EPIGENOMICS, WASTEWATER-BASED EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE (AMR): A ROLE FOR GLYPHOSATE-BASED HERBICIDES, *Bacillus thuringiensis, Vibrio sp.,* METALS CHELATED BY GLYPHOSATE, ORGANOPHOSPHATES, DISINFECTANTS, AND PERSISTENT ORGANIC POLLUTANTS (PCBs, PAHs) IN EMERGING RESISTANT PATHOGENS OF PUBLIC HEALTH CONCERN

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Programa ‘UNA SALUD / ONE HEALTH Epigenetics and Microbiomes:
Somos lo que comemos / We are what we eat’
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Pathogens impact growth, reproduction, immunity, and behavior. Some environmental contaminants like metals, the herbicide glyphosate (GLY), organophosphates, and persistent organic pollutants (POPs).are considered endocrine disrupting chemicals (EDCs). Some metals are chelated by GLY, and metal concentrations vary in different tissues and species depending on environmental factors. Little is known about the interactions in wastewater of COVID disinfectants, antimicrobial resistance (AMR), and changes in the host epigenome, particularly in the presence of the antimicrobial herbicide GLY, pathogens like *Bacillus thuringiensis* (Bt), *Vibrio sp.,* GLY-based herbicides (GBH), metals chelated by GLY, organophosphates (chlorpyrifos, malathion), and POPs like PCBs and PAHs. GBHs can modify toxicity and epigenetic marks in animals and people.

Recent findings of portions of the SARS-Cov-2 coronavirus RNA in bivalve mollusks and in wild carp of Wuhan, China, as well as the potential association of parasite co-infection with a reduced risk of severe COVID-19 in African patients, suggest the need for a more holistic One Health approach to studying interactions of pathogens, AMR, and their host’s genomes and epigenomes.

A review of the molecular and epigenetic mechanisms involved in these interactions will be presented using metals chelated by GLY, Bt and AHPND-causing Vibrios, GLY-based herbicides, and COVID-19 disinfectants in wastewater as examples.

Research is needed to (a) monitor multiple contaminants in wastewater, estuarine seawater, shellfish, fish, and people living in the mangroves, and (b) study transgenerational epigenetic inheritance of health effects caused by exposure to the above-mentioned chemicals. Studies on the interactions of host’s (epi)genome, environmental contaminants, and emerging resistant pathogens should be a priority.

EVALUATION OF INTRACOELOMIC AND IMMERSION VACCINE BOOST FOR INACTIVATED IMMERSION VACCINE AGAINST *Lactococcus garvieae* INFECTION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Lactococcus garvieae is an important emergent bacterial pathogen of farmed fish in the USA. This study aimed to evaluate the safety and efficacy of an autogenous immersion (IM) vaccine against *L. garvieae* in rainbow trout (*Oncorhynchus mykiss*) as well as enhanced protection afforded by booster IM versus injectable (IC) vaccination.

Initial IM vaccination using a formalin-killed vaccine augmented by the adjuvant Montanide™ was followed by booster vaccination via IM or IC injection routes 273 degree days (dd) post-immunization along with appropriate PBS controls. The various vaccination protocol efficacies were evaluated by challenging fish with *L. garvieae* by cohabitation with diseased fish 399 dd post-booster administration.

Rainbow trout tolerated the vaccines well with no mortalities during vaccination, boost and challenge. A relative percent survival (RPS) of 98%, 14%, 3% and -8% was recorded in the IM immunized + IC injection boosted, IM immunized + mock IC injection boosted, IM immunized + IM boosted, and IM immunized + mock IM boosted treatments, respectively. Only the IM immunized + IC injection boosted treatments provided significant protection when compared to positive control treatments ($p<0.05$). Approximately 0%, 50%, 20% and 30% bacterial persistence was recorded in the IM immunized + IC injection boosted, IM immunized + mock IC injection boosted, IM immunized + IM boosted and IM immunized + mock IM boosted treatments at the end of the challenge, respectively. Twenty survivors were maintained at 13°C for 30d, and temperature-stressed by increasing temperature to 18°C during a one-week period. After this period, bacterial persistence and gene expression of immune-related genes were explored in the different treatments.

Both IM and IC vaccines induce an immune response in gills and spleen of immunized rainbow trout. Immune response in vaccinated rainbow trout is stronger than in adjuvant and control treatments. Although both vaccines appear to be safe to trout fingerlings and protective against piscine lactococcosis, IC immunized fish develop a significantly stronger protective response.

REVISITING *Rapana venosa* IN HAMPTON ROADS, CHESAPEAKE BAY AS TBT ABATES

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Rapana venosa (rapa whelk) is a large, long lived, predatory gastropod that is native to the Sea of Japan. It was discovered in Hampton Roads, VA in 1998, arriving via ballast water in coal ships from the Black Sea and eastern Mediterranean. The Molluscan Ecology Program at VIMS enacted a bounty program in collaboration with local watermen to collect rapa whelks. Between 1998 and 2009, over 22,000 whelks were removed from the lower Chesapeake Bay and its sub-estuaries. Rapa whelks have a complex life history that exploits several niches in the Bay, they are voracious predators on local shellfish resources, and there is a lack of a local predator to control them for most of their lives. On prolonged exposure to tributyl tin (TBT), an active component in anti-fouling paints for ships as used in the shipbuilding and repair industry in Hampton Roads for over 40 years, female rapa whelks exhibit development of an accessory male penis, a state called imposex with accompanying impaired sexual function. Strangely, the presence of toxic TBT offered hope for control of an invader. A 2019-2020 survey by the Unger laboratory at VIMS (Figure 1) revisited select sites in Hampton Roads to sample for TBT.

Herein we describe a follow up 2021 survey of imposex in a now endemic *Rapana* population, again implemented as a collaboration with local watermen, in the Elizabeth River region. A total of 174 Rapana were collected (SL range 54-145mm, 53 female + 31 imposex female + 90 male). These data are compared with records from the 2000-2007 period in terms of size, sex ratio, incidence of imposex within the population and TBT concentrations in the foot tissue. The presence of all size ranges in the 2021 collections is indicative of continued reproduction and recruitment into the extant population.

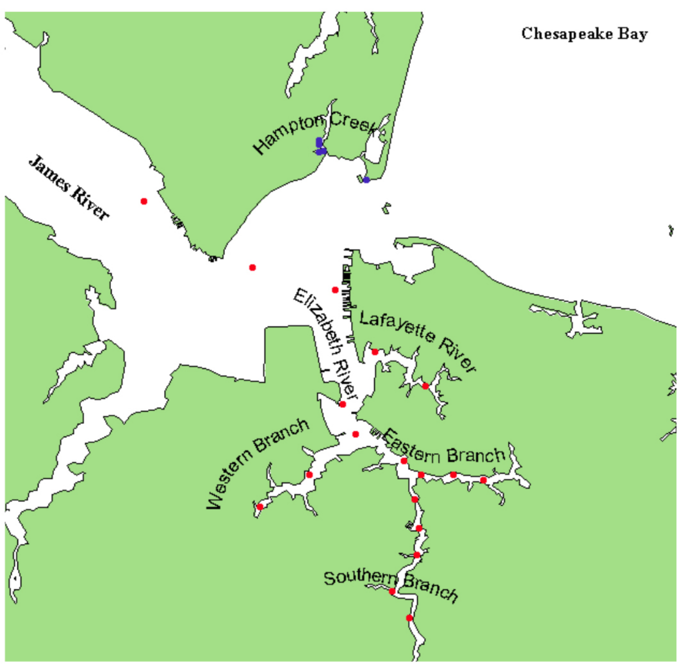

Figure 1. TBT monitoring sites 2019-20.

HEALTH MAINTENANCE FOR THE ENDANGERED WHITE ABALONE (*Haliotis sorenseni*) CAPTIVE BREEDING PROGRAM

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In 2001, white abalone (*Haliotis sorenseni*) was the first marine invertebrate species to be listed under the US Endangered Species Act. The recovery plan identified restoration aquaculture and outplanting as the primary actions to save the species. Early restoration aquaculture efforts, however, were hampered by Withering Syndrome, a disease caused by *Candidatus* Xenohaliotis californiensis (*Ca*.Xc), which caused mass mortality among captive populations.

The California Department of Fish and Wildlife, Shellfish Health Laboratory (SHL), located at the University of California, Davis Bodega Marine Laboratory (BML), has been instrumental in monitoring and improving the health of white abalone in the White Abalone Captive Breeding Program. Careful observation and prevention of infectious disease and pests, as well as treatment of infected captive abalone, have propelled the success of captive production over the past decade, resulting in thousands of outplanted white abalone. The SHL conducts *Ca*.Xc testing via quantitative PCR for all captive white abalone held among a dozen partner facilities. The successful treatment of *Ca*.Xc-infected abalone using an antibiotic bath has created *Ca*.Xc-specific, pathogen-free captive abalone, which is important for maintaining healthy broodstock. SHL also monitors pests such as sabellid polychaetes and *Cliona spp.* (boring sponge), which can compromise shell integrity. Shell abnormalities caused by these pests can make white abalone more vulnerable to infection and/or cause shells to break, sometimes resulting in mortality. SHL is exploring treatment options, including wax and epoxy applications, to asphyxiate pests. The addition of new, wild-origin white abalone starting in 2017 improved the genetic diversity of the captive broodstock, but also resulted in the presentation of pathogens harbored by the wild-collected animals that were not previously present in captive-held white abalone. Managing the treatment and prevention of spread of pathogens new to the White Abalone Captive Breeding Program is a growing focus of the SHL. Routine monitoring and research into new treatments for pests and pathogens are essential for the survival of the species, and also has implications for restoration and commercial aquaculture for other abalone species.

CONVERSION OF SOLID WASTE TO BIOGAS IN A SALTWATER RECIRCULATING AQUACULTURE SYSTEM FOR ATLANTIC SALMON

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Use of Recirculating Aquaculture Systems (RAS) for Atlantic salmon production is rapidly expanding both globally and domestically in the U.S. Domestic production, currently under 5,000 MT a year, is projected to reach 350,000 MT a year within 10 years. However, treating solid wastes from RAS systems is a physical bottleneck in the rapidly expanding U.S. and global RAS Atlantic salmon industry. 1,000 MT of salmon produced in RAS generate 16.7-20 MT of sludge daily, which translates to 6,100-7,300 MT sludge per year.

Performance, biotic/abiotic operational parameters, and efficiency of a pilot-scale Atlantic salmon marine solid waste digester was tested under intensive industry-like conditions in a high density (65kg/m^3 biomass) pilot-scale 0.5 MT salmon saltwater RAS operation. The system attained a stable output of 500-600 L/day biogas (70% methane) and 83% reduction in COD with a HRT of 14 days in a combined hydrolysis/methanogenic 1,000L modified UASB bioreactor. Energy recovery of 1.56 kWh from biogas production achieved 11.7% of the daily energy requirements for the system.

Water quality analyses of the influent and effluent of the anaerobic digester showed that not only are COD and TS reduced in the effluent, but phosphate is also reduced. In addition, alkalinity and pH are increased, which can offset reduction of alkalinity in the nitrification biofilter and prevent phosphate accumulation in the RAS.

Results demonstrate that this technology eliminates a major process bottleneck in the rapidly expanding U.S. and global RAS Atlantic salmon industry by digesting sludge in saline water. It reduces an environmental liability (RAS solid waste) and transforms the majority of the waste into an economic asset, biogas, which can partially offset energy cost of the RAS operation.

POTENTIAL AQUACULTURE MANAGEMENT PROGRAM 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 (PIRO) plans to work with the Western Pacific Fishery Management Council (Council) to establish an aquaculture management program in the Pacific Islands Region (PIR). NMFS prepared a Programmatic Environmental Impact Statement (PEIS) to support early planning for a future management program and evaluate the potential effects of alternatives under consideration. Although the management program is currently conceptual, aquaculture in Federal waters would be managed under revised Fishery Ecosystem Plans (FEPs) and their implementing regulations. The final PEIS supports tiered environmental effects analyses in the future.

Aquaculture in Federal waters in the PIR is not currently subject to extensive management oversight, with limited exceptions. This situation increases the potential for unplanned development and proliferation of unmanaged aquaculture operations in waters of the U.S. Exclusive Economic Zone (EEZ or 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.

Any future management program would be 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 Federal waters, and is intended to help provide operational stability and maintain Council and NMFS commitments to sustainable and environmentally sound fisheries management.

The public can view the PEIS and related comments at https://www.regulations.gov/document/NOAA-NMFS-2021-0044-0003/.

INCORPORATING CLIMATE RESILIENCE INTO NORTH CAROLINA'S SHELLFISH AQUACULTURE INDUSTRY

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Current climate and coastal resilience efforts at the federal, state, and local levels work to address and communicate the impacts from climate change. These impacts include the increase in frequency and intensity of storms, degraded water quality, variable salinity and dissolved oxygen, and increased inundation, which directly and indirectly effect the shellfish aquaculture industry. The impacts to the shellfish aquaculture industry include suitability of shellfish for harvest, mortality, public health risks (i.e., increased occurrences of *Vibrio* outbreaks from shellfish consumption), and disruption to shellfish markets. Climate change also directly impacts management of the shellfish aquaculture industry and long-term planning.

Building resilience is an iterative process that requires planning, responding to an event, recovering, and adapting. When building resilience, planning can include improving forecasts and observation models, connecting decision makers with information, and incorporating green infrastructure. Responding to a disaster can include immediate damage assessments and pollution responses. Recovering and adapting can include assessing damage to communities, economies, and the environment, issuing grants to rebuild and restore habitat, and providing data and tools for analysis. Resilient coastal ecosystems and communities require building back better and stronger in preparation for future conditions, instead of planning for present conditions.

An important component to developing a resilient N.C. shellfish aquaculture industry will be to incorporate these iterative processes and resilience extension best practices for communication and engagement. Sea Grant resilience extension specialists connect climate change science with decision makers and help translate information in ways that are understandable and useful for coastal residents, businesses, and communities. This requires time and the building of trust, while engaging stakeholders and scientists in the framing of critical research questions that are necessary for useful information transfer. These proven efforts in resilience communication and engagement can be adapted and used as a template for the shellfish aquaculture industry.

NUTRIENT MANIPULATION AND MEDIA SELECTION IN DECOUPLED AQUAPONICS SYSTEMS FOR GROWING *Cannabis sativa*

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Aquaponics combines aquaculture with water treatment engineering and hydroponics, a soillessnutrient delivery plant cultivation technique. This method has shown promising results to produce high-quality fish and crops in a controlled environment, where outside risks associatedwith traditional agriculture are negated. Aquaponics' ability to grow robust hemp and cannabis plants provides an encouraging alternative to traditional production of these high value crops.

A commercial, decoupled aquaponics test system was built for growing high-nutrient demand crops. The recirculating aquaculture system (RAS) component consists of two 300-gallon fish tanks, a deep two-walled clarifier, a bead filter, a biofilter, two sump tanks, and two mineralization tanks. Nutrient-rich water from the fish tanks passes through a clarifier and bead filter to remove a majority of solids from the system. After solids removal water enters the biofilter, the filtered water returns to the fish tanks and is added to three aerated, decoupled sumps that each feed Dutch bucket hydroponic rows. Three different media types were tested: light expanded clay aggregate (LECA), coco coir, and a peat-perlite mix. An additional row of hemp was grown hydroponically in coco coir as a control group; nutrient solution was mixed daily to proper concentrations for hemp production. Sludge from the RAS is aggressively aeratedin a two-stage mineralization process in order to enhance breakdown of solid waste into bioavailable forms for plant uptake, and is then added back into the RAS. Temperature, pH, electrical conductivity, dissolved oxygen levels, water macro and micronutrients, and leaf tissue samples were tested to more deeply understand the health of the fish and plants.

Results are still in progress but clearly show the viability of hemp production in an aquaponics system, along with fish production and the ability to translate production for cannabis. While required initial costs, components, and expertise may be greater compared to traditional hydroponics, aquaponics is a zero discharge and fully organic production method. After harvesting, flower biomass, terpene levels, CBD and THC levels will help aid the comparison ofperformance of each media.

TEMPORAL DYNAMICS OF EASTERN OYSTER LARVAL ABUNDANCE IN GREAT BAY ESTUARY, NEW HAMPSHIRE

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Eastern oyster, *Crassostrea virginica*, populations have been declining steadily over the past several decades across the North American East coast. Great Bay Estuary (GBE), located in New Hampshire, is experiencing this loss and restoration efforts have been put into effect. This study characterized abundance in GBE from 2018 to 2020 of two early larval stages of *C. virginica*, D-hinge and veliger, and settled spat. The putative effects of month, year, site, and physicochemical factors were examined. D-hinge larvae and veligers already were present in the water column when sampling began (June), and spat settlement already had occurred, suggesting an earlier than previously thought first spawn of *C. virginica* in GBE. D-hinge larval abundances declined significantly from 2018 to 2020, whereas veliger abundances remained steady and increased slightly in 2020 (Figure 1). Although physicochemical factors are known to play a role in larval abundance, no significant effects other than temperature were noted. Spat settlement was found as early as June (Figure 2), suggesting an earlier than previously thought spawning period of eastern oysters in GBE. The findings are being used to enhance restoration efforts as they suggest that spat brought in to augment current sites of active restoration should be collected and deployed earlier in the season and that recruitment devices should be deployed earlier each year.

Figure 1. *Crassostrea virginica* larval counts in GBE, NH in 2018-2020. (A) D-hinge, (B) veliger.

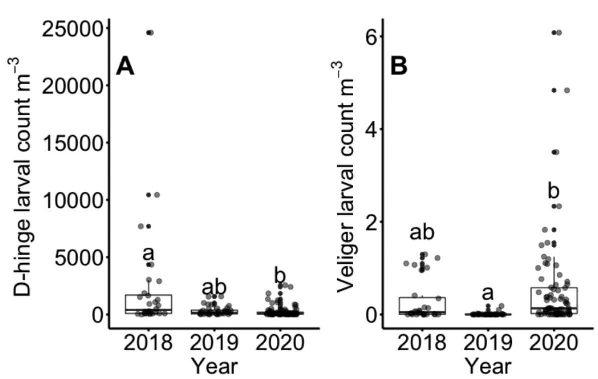
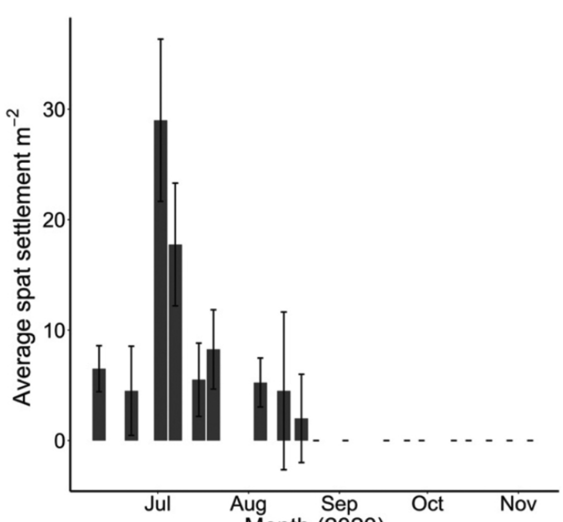


Figure 2. Spat settlement of *Crassostrea virginica* larvae in GBE



CHARACTERIZING FEEDING VARIABILITY IN *Mytilus edulis* USING HIGH TEMPORAL RESOLUTION MEASUREMENTS OF PUMPING RATE AND GUT PASSAGE TIME

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Feeding activity of bivalves is understood to change in response to a suite of environmental conditions including food quantity and quality. Characterizing inter- and intraindividual variability in the feeding activity of bivalves is important to understand the ecosystem interactions of bivalves. The purpose of this study was to explore intra- and interindividual variability in feeding rates of the blue mussel *Mytilus edulis* using natural seston. For five days, physiological rates related to feeding (pumping and ingestion) and digestive processes (gut passage time) were measured every 20 minutes, and 24 hours, respectively using natural seawater in a flow-through system. This high temporal resolution of pumping rate measurements permitted the observation of both intra- and interindividual variability of feeding rates. 10 individual mussels were used for the experiment, and interindividual differences were minimized by collecting mussels of the same length (50mm) and standardizing feeding rates to gill area (mm^2).

Results indicate both intra- and interindividual variability in feeding rates, with some individuals pumping on average at high rates (~5 Lh^{-1}) and some at low (~1 Lh^{-1}), despite being held in similar conditions. Gut passage time also varied both between individuals, and temporally throughout the experiment. To examine drivers of variability in feeding rates, pumping rate was correlated to food availability (Fluorescence (μgL^{-1})) using temporal mismatches informed by gut passage time. Results indicate that there is a higher degree of correlation when pumping rates are matched to food availabilities from 10 hours prior, where pumping rate increased with increased fluorescence. 10 hours was also the average gut passage time of *M. edulis* during the experiment. This finding suggests that digestion may play a role in the variability of feeding activity of bivalves, and that current feeding rates may reflect food availability from some hours prior, although correlation may not imply direct causation. This research contributes to our understanding of intra- and interindividual variability in feeding rates of bivalves and may be informative for *in situ* measurements of feeding, as well as individual growth models.

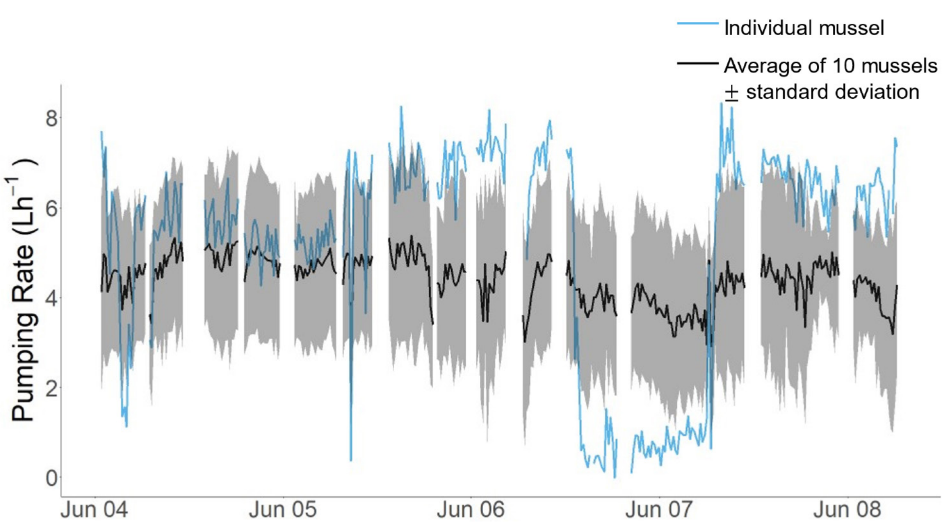

Figure 1. Pumping rates (Lh^{-1}) of 10 (average±SD, black line), and one individual mussel (*Mytilus edulis*) (blue line) measured over 5 days in a flow-through system.

MARICULTURE OF *Saccharina latissima* (SUGAR KELP) AS A SCALABLE MODEL FOR OFF-SHORE CULTIVATION

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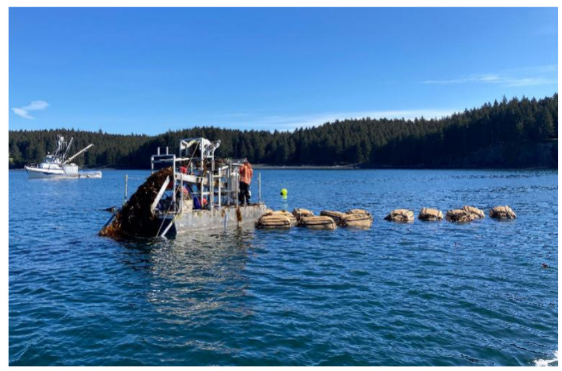
The mariculture of seaweeds is becoming increasingly popular in Alaska. Currently, Alaska has 23 seaweed farm leases with 25 additional leases in the application process. With funds from the ARPA-E MARINER program, we tested different seaweed farm designs, seeding methods and harvest approaches.

Farm designs were based on catenary structures and the use of spreader bars with variable spacing of grow lines. Initial results show that line spacing can be as small as 1 meter with no adverse effects on yield. There was no statistical difference in the growth of *Saccharina* whether in the middle or the outside of the array. Sagging caused by the weight of the mature fronds resulted in lower growth at depth. Seeding lines was done using the current industry standard with meiospores settling on seed string. We experimented with several "direct seeding" methods – spraying or painting both gametophytes and embryonic sporophytes on both strings and on the growlines. Some success was demonstrated with the outplanting of directly seeded growlines.



The *Kelp Buddy* harvester.

Our cooperating farmers tested various approaches for harvesting mature kelps. One innovation that has worked well is the use of large bags for holding the freshly harvested fronds. Although the weight of the fronds on the growlines causes the lines to sink, the bags packed with the harvested fronds float, allowing for easy loading to the transfer vessel. Another advance in harvesting is a specially built harvest vessel, the *Kelp Buddy*, which was in operation for this last season. We also modified a large seiner that allowed for harvesting more than one line at a time. Next season we will continue to test various methods in kelp mariculture in an effort to make kelp farming as efficient and profitable as possible.

Large vessel harvester

COMPARATIVE BIOENERGETICS OF YELLOWTAIL JACKS *Seriola spp.* FOR OPTIMIZING AQUACULTURE PRODUCTION

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As marine finfish aquaculture production continues to expand worldwide there is a burgeoning interest in species of the genus *Seriola*. A number of species within this genus have proven to be prime candidates for culture in both high-energy offshore aquaculture conditions as well as in advanced land-based recirculating aquaculture systems. Globally, there are a number of commercial operations farming *Seriola* species. While aquaculture production of all of the primary *Seriola* species (i.e., *S. lalandi, S. quinqueradiata, S. rivoliana, and S. dorsalis*) has proven to be technically feasible and under certain conditions economically viable, there is still ample room for production efficiencies to be improved in both offshore and land-based production systems. Bioenergetics research offers an opportunity to directly assess production efficiencies, while offering insights into factors which may be altered to help improve the overall aquaculture production of the species. Past and present bioenergetics work on various *Seriola* species will be discussed, with a specific focus on recent comparative bioenergetics research trials of *S. rivoliana* and *S. dorsalis* that have been carried out. As commercial production advances with these species in the U.S., results of this research will help elucidate opportunities for further improvement in profitability and sustainability in the culture of these marine fish species.

PROGRESS IN THE DEVELOPMENT OF LAND BASED FLOUNDER PRODUCTION IN THE U.S.: OLIVE FLOUNDER *Paralichthys olivaceus* AQUACULTURE

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Flatfish aquaculture represents one of the most promising forms of marine finfish production in land-based systems. While currently much of the global production of flatfish, particularly flounder species, is conducted using flow-through systems, there is a growing interest in using recirculating aquaculture systems (RAS) to help overcome many of the issues which have plagued the industry over past decades. In the United States (U.S.), where marine finfish aquaculture is still a rather nascent industry, the search for economically-viable species and production systems that can achieve commercial-scale success in the country continues. The olive flounder, *Paralichthys olivaceus*, also known as the Japanese flounder or hirame, has been identified as one of the most promising species for commercial-scale land-based marine finfish aquaculture in the U.S. The potential for commercial-scale culture of this species is of particularly high interest in coastal areas with ready access to saline groundwater resources, as found in many of the nation's working waterfront communities. Research trials at the University of Miami centered around this species have been aimed at assessing the feasibility of pilot-scale aquaculture production of this species in land-based flow-through and RAS systems to determine the overall viability of domestic commercial-scale culture of this species. Results will be presented detailing production updates at the University of Miami as well as results of market surveys assessing consumer acceptance of farm-raised flounder along the east coast of the U.S. Overall, the production potential for this species in the U.S. appears quite promising, and next steps in process towards the species realizing its full production potential will be discussed. This work has been supported by the Atlantic States Marine Fisheries Commission (ASMFC) and the National Oceanic and Atmospheric Administration (NOAA).

ADVANCING SUSTAINABLE MARINE FISH PRODUCTION FOR THE SOUTHEAST U.S. AND CARIBBEAN: ADVANCEMENTS IN YELLOWTAIL SNAPPER *Ocyurus chrysurus* AQUACULTURE

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Throughout the southeast region of the U.S., including the Gulf of Mexico and Caribbean, the yellowtail snapper, *Ocyurus chrysurus*, is one of the most recognizable and high-value marine fish species available. With all of the market demand for this species being reliant on wild-caught fish, any disruption to the supply from commercial fisheries can have negative downstream consequences for working waterfronts and associated businesses. Aquaculture production of yellowtail snapper has been investigated as a means to stabilize the supply of this high-value species while potentially offering additional benefits related to economic diversification and added resilience for working waterfront communities through the addition of other sources of harvested fish that are immune to fluctuations in supply resulting from fisheries closures and quota limits. Recent research and development efforts have resulted in mass production of yellowtail snapper fingerlings, and grow-out trials under different commercially-relevant conditions are underway. Results will be presented and discussed, including preliminary assessments on key aquaculture performance indices. All indications point to the yellowtail snapper being a prime aquaculture candidate species for further development in the U.S., and directions for upcoming work will be discussed.

PRE- AND POST-CONSTRUCTION ASSESSMENTS OF FISHERIES IN AN OFFSHORE WIND LEASE AREA

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The School for Marine Science and Technology (SMAST) collaborated with the fishing industry, regulatory agencies, and Vineyard Wind to develop a pre- and post-construction assessment of fisheries, associated ecological conditions, and socio-economic aspects of fisheries, in and around the Vineyard Wind offshore wind lease area, as designated by the US Bureau of Ocean Energy Management, on the US Outer Continental Shelf.

First, a series of workshops with fishermen and regulators to present a relatively expansive set of monitoring component options and to identify which elements are most important to local fisheries and which are most important to regulators were organized. Monitoring components included fishery assessments, fishery resources surveys, tagging, oceanographic monitoring and modeling, socio-economic analysis, and geostatistical integration of monitoring components. From these discussions a series of recommendations emerged. Seasonal fishery resource surveys were proposed examining the substrate and benthic macroinvertebrate, groundfish and planktonic communities. Results from these workshops were compiled into "Recommendations for planning pre- and post- construction assessments of fisheries in the Vineyard Wind offshore lese area" dated 24 March 2019 (available at https://www.vineyardwind.com/fisheries-science, and https://www.mafmc.org/northeast-offshore-wind).

The experimental design for the seasonal surveys followed the Before-After-Control-Impact (BACI) design and was set up to coordinate with ongoing large-scale surveys conducted by SMAST and other institutes such as VIMS, NOAA fisheries and state fisheries agencies. This structure would enable the development of large scale Before-After-Gradient (BAG) experimental frames works as well.

Two benthic macro-invertebrate surveys are underway, a drop camera optical survey and a ventless trap lobster/crab/black sea bass survey. A seasonal demersal trawl survey monitors the species abundance, population characteristics and community structure of marine fish and invertebrate communities including commercially important species such as squid, groundfish, summer flounder, whiting and black sea bass. Successful field seasons were completed for all these surveys in 2019 and 2020, despite the difficulted associated with the Covid-19 pandemic. All the documents are available to the public at https://www.vineyardwind.com/fisheries-science, and a data-sharing agreement is in place. These surveys are planned to continue until construction, sample during construction when possible, and ideally then sample for three years post-construction, with the possibility of additions years throughout the life of the windfarm. At the regional scale oceanographic modeling is examining windfarm and fisheries interactions. A 39-year simulation using a refined subdomain grid (up to ~1.0 m) finite-volume community ocean model (FVCOM) suggested that the Vineyard Windfarm field could considerably change the larval distribution in the southeast.

EXPOSED AQUACULTURE OPERATIONS IN NORWAY. KEY CHALLENGES

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Significant parts of the ocean are today unavailable to industrial fish farming due to remoteness and exposure to harsh wind, wave, current and ice conditions. Presently, conventional fish farming is the second most risk exposed occupation in Norway, and operate at the limits for the fish, personnel, and equipment. In exposed conditions, the risks are magnified, making it challenging to avoid injuries, fish escapes, and mortality. Unpredictable weather and sea states lead to short operational windows and delayed or interrupted operations. Regular as well as infrequent operations are challenging. There will be postponed delicing and longer periods of starvation for the fish.

In the Norwegian research centre EXPOSED, we have studied how exposed aquaculture operations can become robust, efficient, and safe. Exposed aquaculture operations are defined as operations on aquaculture localities where work or equipment is strongly influenced by current or weather exposure. Exposed aquaculture operations share a line of challenges, that must be addressed to be able to utilize the tricky, exposed areas along the coast and at sea.

Our studies embrace multiple methods. Some of the existing fish farms in Norway are exposed and have been studied using weather data, accident statistics, technology verification results, digital twins, interviews of personnel, etc. Other methods have been development of technology or experiments with potential materials and constructions for future fish farm concepts.

Through the studies we have identified five key challenges that needs to be solved to achieve robust, efficient, and safe exposed aquaculture operations.

The first challenge is risk in the human working environment. Exposed operations equal demanding working conditions that result in stress and occupational hazards. There is a need for coordinated action to reduce risks in aquaculture, involving industry and the various authorities.

Challenge two is demanding management and daily operations. Some farms have prolonged periods with ocean swell, and we have seen examples of farms that have been abandoned in periods. One needs to develop agreed competence requirements and design methods for robust technology and decision support.

Vessels serving the aquaculture farms also have demanding conditions, and this is the third challenge. The navigation alongside the farm needs to be gentle to fish that is crowded towards the surface and vulnerable to stream. There is a need for specialized vessel design and operational limits.

Challenge number four is fish welfare. Treating fish under rough environmental conditions inflict additional stress, with a potential for higher mortality. Crowding with large relative movements, waves, and cold air/water, represents a risk for the fish. Extreme conditions have caused mass mortality. There is a need for robust fish and adapted stocking strategies, and to understand the limitations of the fish and how to monitor its state.

The last key challenge of exposed aquaculture operations is increased risk of escaped fish. 1 of 3 escaped salmon escapes under bad weather. Extreme conditions also can cause structural failures. One preventive measure can be automated inspections.

To solve these challenges, we have approached them from different sides. We have analysed exposure limits of fish, personnel and equipment, and found techniques to model and measure environmental loads on farms, as well as exposure degree. This has lead us closer to understand how exposure and operational limits can be classified, and how exposed aquaculture operations can be robust, efficient, and safe.

IMPACTS OF THE COVID-19 CRISIS ON TRADE MARGINS IN NORWEGIAN AQUACULTURE EXPORTS

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During 2020 the COVID-19 crisis created a sizeable drop in aggregate trade flows for many industries and countries worldwide. At the same time the total volume of seafood exports from Norway increased by 2 per cent in 2020, while the value was down by only 1 per cent, compared with the record year of 2019. In 2020 the export value of salmon from Norway, the world's leading producer of salmon, was the second highest ever, and in terms of volume 2020 was a record year. As the Norwegian aquaculture industry better tackles capacity problems, controls lice- and disease problems in the production, the industry is anticipated to have a high potential for future growth. A significant share of future production is expected to be exported to foreign markets demanding high quality nutritious products. Understanding the microeconomic mechanisms driving the evolution of aggregate exports is key to the comprehension of the aquaculture industry's reaction to the pandemic as well as to provide knowledge on how to tackle future crisis.

This paper studies how the extensive and intensive margin of exports of aquaculture products was impacted by the pandemic. We utilize highly disaggregated firm-level data for export of aquaculture products from Norway. Export value of different aquaculture products are decomposed into different elements of the extensive and intensive margins of trade such as the number of exporters to a market, the number of importers, average unit value and average volume. Of particular interest is heterogeneity of adjustments of trade margins along the size distribution of exporters.

Our results show that the extensive margin of trade in general was more responsive to the pandemic than the intensive margin of trade. During 2020 the number of exporters dropped compared to 2019. The average export value per exporter was higher during the first months of the pandemic than in the corresponding months of 2019 but fell below the 2019-level by the end of the year. We document that the top exporters tackled the crisis best in terms of maintaining overall sales.

ACOUSTIC FEEDING BEHAVIOR USING ATTRACTANTS IN DIETS FOR *Litopenaeus vannamei*

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Passive acoustics monitoring (PAM) can contribute to improve feed formulations by providing information on shrimp feeding behavior. The inclusion of attractants, especially those derived from marine animals, have been used to increase chemosensory characteristics and feed intake of soybean-based shrimp diets. However, the evaluation of their effect in shrimp feed intake and behavioral responses is challenging on multiple levels. This study used PAM to test the efficiency of attractants included in all-plant diets for *Litopenaeus vannamei*.

The feeding activity and food consumption (FC) of *L. vannamei* (7 g) were evaluated using all- plant diets supplemented with krill meal (2%), squid meal (2%) or fish hydrolysate (4%), as well as a control diet without attractants and a fishmeal diet (12%). Two consecutive trials were performed using "naive" (without previous acclimation to the diets) and "non-naive" shrimp (four days of acclimation) in clear water recirculation system (50-L aquaria). The acoustic feeding activity was monitored by hydrophones connected to a multitrack recorder (16 bit/192 kHz), while FC (g) was measured as the amount of food eaten by the shrimp (n=10) in each aquarium over a 30 min period. The clicks produced by shrimp during feeding activity were automatically detected and quantified using specific acoustic targets. These data were also used to calculate the relationships between the number of clicks and FC.

An overall improvement was observed in acoustic feeding activity and FC for all-plant diets with attractants in comparison with the control diet, while the all-plant diet with krill meal showed a significantly similar feeding activity as the fishmeal diet for non-naive shrimp (Figure 1). A strong positive correlation (~r=0.8) was found between FC and number of clicks during 15 and 30 min. The clicking activity analysis through PAM technology was proved a reliable tool to help us understand feeding behavior and preferences of shrimp in laboratory experiments.

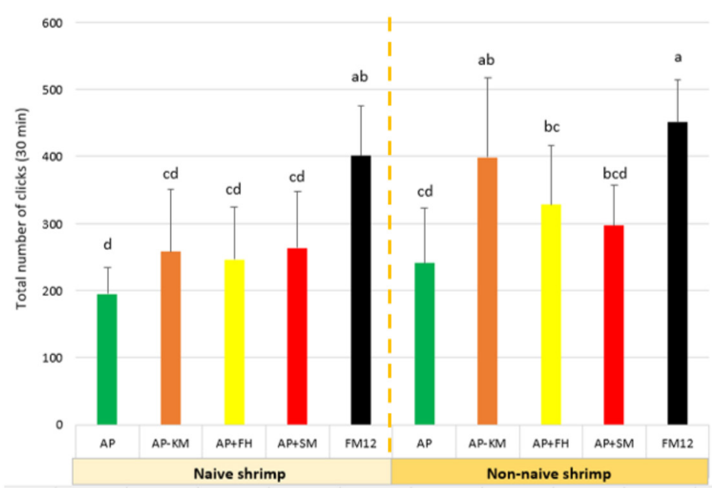

FIGURE 1. Mean (± SD) number of clicks produced by naive and non-naive shrimp fed all-plant diets with inclusion of krill meal (AP+KM), squid meal (AP+SM) or fish hydrolysate (AP+FH), and control diets without attractants (AP) and a fishmeal diet (FM12). Values with different letters are significantly different (P < 0.05).

EVALUATION OF A COMMERICIAL BROODSTOCK DIET FOR ADULT CALIFORNIA YELLOWTAIL *Seriola dorsalis*

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The reliable production of marine finfish is contingent on the quality of the eggs available from the broodstock populations. Inconsistent egg production or poor egg quality will negatively impact the production of juvenile fish available for grow out. Proper broodstock nutrition is critical for maintaining a supply of good quality eggs. Here we have compared a commercial broodstock diet (Vitalis) to fresh sardines and squid diet, when fed to California yellowtail (CYT*; Seriola dorsalis*), to determine the dietary impact on egg production and quality.

The first diet (Diet 1) was a commercial diet (Vitalis Prima, Skretting) and was fed in a 6 mm pellet form. The control diet (Diet 2) was a combination of frozen sardines and squid supplemented with a vitamin premix. Each diet was fed twice over 6 week periods throughout the spawning season (March through September) of a single broodstock population. Diet 1 was fed from weeks 0 to 6 and from 14 to 21; Diet 2 was fed from weeks 7 to 13 and from 22 to 28 to the same fish population. There were nine CYT F1 brood fish (three females; six males) that were 7 years old and averaging 13.2 kg each. The fish were held in a 30 m^3 tank under ambient water temperature and photoperiod conditions.

Diet 1 feeding periods yielded 19 spawn events totaling 10.8 million eggs, with an average viability of 86.2%. When the fish were fed Diet 2, they spawned 28 times for a total of 20.4 million eggs, with an average viability of 84.3%. Egg 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. Proximate composition and fatty acid analysis have yet to be completed. Results from this trial demonstrate that this commercial diet is comparable to the standard fish and squid feeding regime when looking at egg quality. However, the reduced egg output from the fish on the commercial diet needs to be explored.

Table 1. Egg quality results for California yellowtail (*Seriola dorsalis*) fed a commercial diets (Diet 1) and a diet of fish and squid (Diet 2).

	DIET 1 (COM DIET)	DIET 2 (SAR/SQUID)
SPAWN EVENTS	19	28
VIABILITY (% ± SD)	86.2 ± 7.3	84.3 ± 7.8
TOTAL EGGS PRODUCED	10,863,479	21,486,438
EGGS PER SPAWN	571,762	767,373
EGG DIAMETER (MM ± SD)	1.36 ± 0.04	1.34 ± 0.03
OIL DIAMETER (MM ± SD)	0.30 ± 0.01	0.29 ± 0.01
PERCENT OIL VOLUME (% ± SD)	1.05 ± 0.12	1.07 ± 0.12
NOTOCHORD LENGTH (MM ± SD)	4.17 ± 0.22	4.19 ± 0.17
HATCH RATE (% ± SD)	45.3 ± 25.0	41.1 ± 23.4
SURVIVAL TO FIRST FEEDING (% ± SD)	76.4 ± 11.6	71.8 ± 13.7

PROVIDING EDUCATIONAL OPPORTUNITIES DURING THE COVID-19 PANDEMIC THROUGH DEVELOPMENT OF AN ONLINE OYSTER CULTURE COURSE

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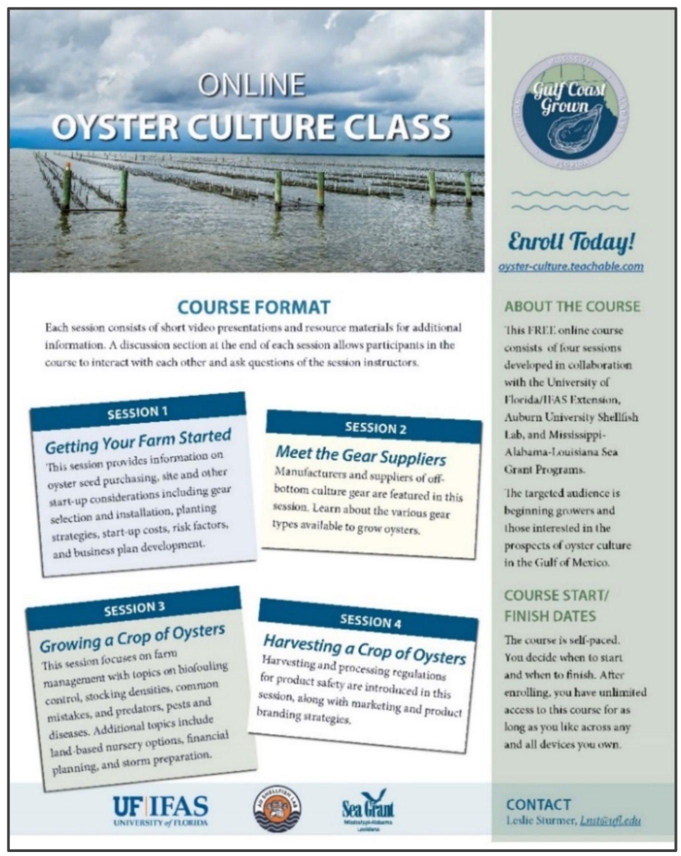
Intensive aquaculture of oysters (*Crassostrea virginica*) in the Gulf of Mexico region represents a new industry sector with development occurring in the past decade. In Florida, over 170 growers operate on 284 leases, a 21% increase in the past two years. Interest in oyster culture continues to increase in other Gulf states. With in-person classes restricted due to the COVID-19 pandemic, a virtual format was considered to provide educational opportunities for beginning growers and those interested in the prospects of oyster culture in the region.



To gauge interest, a survey was sent to 50 new growers in which all (response rate of 36%) indicated they would be interested in participating in an online course, thought 15 to 30-minute presentations would be adequate, and had an internet connected device. The course was created using the Teachable platform, which provides custom domains, creates web pages, hosts content and videos, allows students to auto-matically register, and monitors student activities via a dashboard.

Launched in June 2021, the course format, which consists of four virtual sessions, follows the sequence of starting a farm to growing and harvesting a crop of oysters. Twenty-three instructors from universi-ties, industry associations, gear manufac-turers and suppliers, and federal and state agencies collaborated to provide 39 presentations with videos and resource materials. To increase the learning experi-ence, a discussion section follows each session. Within the first five months, 260 students had enrolled in the course.

Results of a follow-up survey to evaluate the usefulness of the course, determine knowledge gained, and assess other educational needs will be presented. To view or enroll in the course, which is free, go to https://oyster-culture.teachable.com.

ADDRESSING OYSTER *Crassostrea virginica* MORTALITIES IN FLORIDA'S OFF-BOTTOM OYSTER AQUACULTURE INDUSTRY

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Off-bottom aquaculture of oysters *Crassostrea virginica* in Florida represents a new industry sector with more than 170 growers operating on 965 acres of leases located primarily in the state's Panhandle. In 2019, 4.7 million oysters were produced on these leases. Recently, unexplained mortality events during late spring and early summer, amounting to 50-80% loss of triploid oysters reaching market size, have been reported by growers. A variety of factors may account for these mortalities; however, diversity in location, gear, seed, and operational scale, make it challenging to extricate potential causes. In response to industry concerns, "sentinel" farms were established at two lease locations and a monitoring program initiated to examine basic but important relationships between production and health of cultured oysters, and environmental factors.

Juvenile triploid oysters (19-21 mm shell height, SH; n=2000), half produced from traditional Louisiana (LA) tetraploid stocks and half from new Florida (FL) tetraploids were deployed in October 2020 at farm sites in the Alligator Harbor (AH) Aquaculture Use Zone (AUZ) and Oyster Bay (OB) AUZ, where oyster mortalities have occurred. The AH lease site is characterized by high salinity with monthly means ranging from 27.8-32.2 psu in this study, while salinities at OB ranged from 16.2-26.1 psu. Growers provided gear (floating bags) and maintenance during the culture period. Oysters were sampled at bimonthly intervals through harvest in May at the AH site and July at the OB site to determine growth and mortality and assess prevalence and severity of shell parasitism and Dermo disease. From April through June encompassing the period when mortalities have occurred, water samples were analyzed for phytoplankton abundance and composition.

At AH, oysters reached market-size (75.6±11.1 mm SH) within six months (March) but were maintained another two months (May). Oysters at OB averaged 53.7±6.2 mm SH after six months and did not reach market size (76.2±8.9 mm SH) until 3.5 months later (July). Cumulative mortali-ties at harvest were 30.2-32.0% (AH), 25.4% (OB, FL stock), and 40.4% (OB, LA stock) with significant differences between stocks at the OB site (Table 1). Water quality, phytoplankton, and parasitism will be presented that most influenced oyster production both positively and negatively.

Table 1. Average mortalities (interval and cumulative, %) of triploid oysters produced from FL and LA tetraploids at two leases. Means with different letters are significantly different (P<0.05).

Sample/ Harvest Dates	Alligator Harbor (AH) AUZ				Oyster Bay (OB) AUZ			
	FL Stock		LA Stock		FL Stock		LA Stock	
	Interval	Cum	Interval	Cum	Interval	Cum	Interval	Cum
1/26/21	0.9 ± 0.1^{a}	0.9	0.4 ± 0.3^{a}	0.4	0.9 ± 0.1^{a}	0.9	0.2 ± 0.2^{a}	0.2
3/29/21	1.3 ± 0.9^{a}	2.2	0.9 ± 0.5^{a}	1.3	0.6 ± 0.4^{a}	1.5	0.7 ± 0.2^{a}	0.9
5/28/21	28.0 ± 10.9^{a}	30.2	30.7 ± 20.1^{a}	32.0	8.1 ± 2.3^{a}	9.6	12.9 ± 4.3^{b}	13.8
7/14/21	-	-	-	-	15.9 ± 1.8^{a}	25.4	26.6 ± 4.8^{b}	40.4

FRAMEWORK FOR TRANSLATING OCEAN RISK TO INVESTMENT: A SALMON AQUACULTURE CASE STUDY

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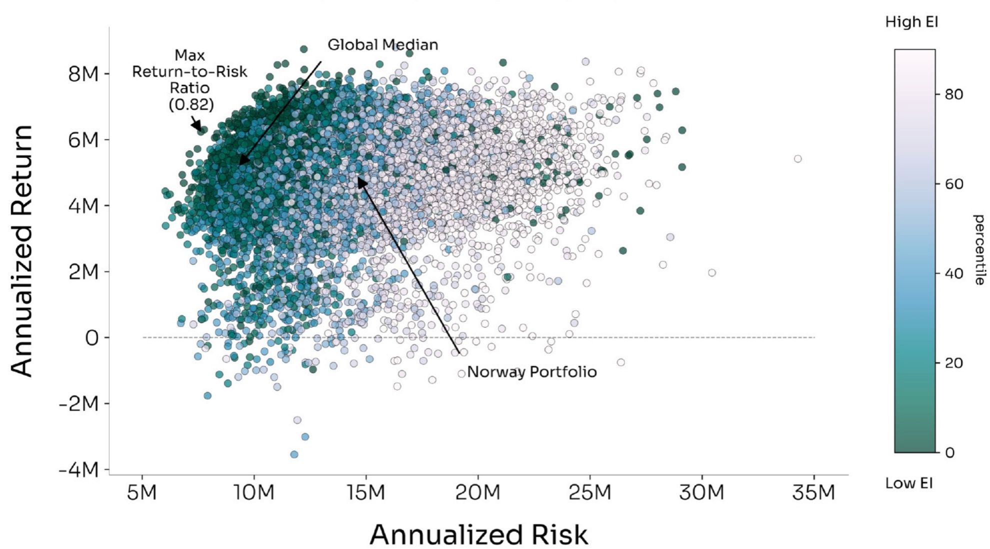
Ocean data is essential to responsible financing in the blue economy. Quantifying the ocean risk that marine operators face can unlock new forms of risk transfer and investments into historically overlooked ocean businesses. To date, there has been minimal private equity footprint in salmon aquaculture, despite its high profitability. This is because the volatility of ocean-driven risk is difficult to navigate without ocean expertise. We developed a global model to simulate the market, biological, and ocean dynamics for over 3,500 Atlantic salmon farm sites. Alongside site-level farm production, our model tracks sustainability metrics, starting with emissions intensity relative to earnings. Translated to an institutional-scale allocation across the wider global industry, we find that salmon aquaculture offered risk-adjusted returns in the top quintile of all US or European stocks across recent decades and a historical impact-and-risk-adjusted return that is 31 times larger than US agriculture and livestock as a whole.

These insights equip institutional investors, such as pension funds and private equity, with the ability to make the three-dimensional tradeoff between risk, return, and impact in a highly specialized industry. The application of well-modeled and constrained ocean conditions to quantify the financial risk of investments is an important step in broadening the blue economy.



EXPANDING GREEN SEA URCHIN AQUACULTURE PRODUCTION BY REMOVING KEY AQUACULTURE CHALLENGES

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In this presentation we will overview a project which addresses the major challenges which inhibit sea urchin aquaculture production in the Northeastern US, funded by the Northeastern Regional Aquaculture Centre. It works towards adapting and improving upon existing technology to improve the success and lower green sea urchin (*Strongylocentrotus droebachiensis*) seed production costs. Hatchery methodologies in the Northeastern US have yielded low settlement success and post-settlement survival for the green sea urchin causing high seed production costs for this emerging aquaculture species. The transformation from planktonic larvae to benthic juveniles, known as settlement, and the survival of these newly settled juveniles are critical aspects for sea urchin aquaculture to be successful. It has been suggested that different biological and chemical cues such as bacteria, benthic diatoms, macroalgae, or even altered temperature can promote settlement. In this study, we test these by examining the settlement success of *S. droebachiensis* larvae exposed to differing biofilms (e.g. *Nitszchia sp., Cylindrotheca closterium,* Adult *S. droebachiensis*) and conditioned seawater treatments (Adult *S. droebachiensis* or macroalgae chemical cues) under two temperature regimes of 12 and 14°C to determine whether settlement can be enhanced. It has been suggested that the low survival rate of newly settled juveniles in hatcheries is associated with the lack of optimal food sources. To address this, an experiment was conducted to determine if differing diatom and macroalgae food sources under two temperature conditions of 12 and 14°C increases survival. This study elaborates on the chemical and biological cues that induce high larval settlement success and post settlement survival, and we discuss the next steps in optimizing cultivation conditions. In tandem we are working towards increasing awareness of the availability of hatchery seed by working through collaboration with regional industry and extension staff towards increasing grower interest in seed uptake.

ADDITIVE EFFECTS OF POLY-B-HYDROXYBUTYRATE ON GROWTH AND IMMUNE RESPONSES OF JUVENILE NILE TILAPIA (*Oreochromis niloticus)* BASED ON *IN VIVO* AND *IN VITRO* APPROACHES

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Aquatic disease outbreaks particularly from bacterial pathogens, represent a major barrier to more efficient fish production. Therefore, novel and innovative disease treatment and prevention strategies without dependence on antibiotics are essential for aquaculture to sustain increased production. Poly-β-hydroxybutyrate (PHB), a biopolymer synthesized by specific gram-negative and gram-positive bacteria, is one such compound with potential immunostimulatory capabilities, as seen in preliminary *in vitro* assays of the current study. For the *in vivo* feeding trial, PHB-synthesizing bacteria, *Zobellella denitrificans*, were produced on-site at the Texas A&M Aquacultural Research and Teaching Facility and then supplemented to a basal diet (36% crude protein and 6% crude lipid) to produce five isonitrogenous and isolipidic experimental diets containing PHB in stepwise increments (0.125, 0.25, 0.5, 1.0, and 2.0% of dry-diet weight). Two experimental control diets of similar proximate composition also were utilized; one practical diet (Basal) contained no supplementation of PHB-producing bacteria while another control contained 0.5% supplementation of a commercial purified PHB product to compare with the PHB produced by the bacteria and included the bacterial cell wall.

Groups of 15 juvenile Nile tilapia (~1.3 g/ fish initial weight) were stocked into 28, 38-L aquaria fashioned as a recirculating aquaculture system with quadruplicate aquaria randomly assigned to each dietary treatment and fed to apparent satiation for 8 weeks. Remarkably, Nile tilapia exhibited significant (P<0.05) dose-dependent linear and quadratic relationships of percentage weight gain (Figure 1), as well as feed efficiency, protein conversion efficiency, and hepatosomatic index. Alternatively, intraperitoneal fat ratio and muscle yield ratio did not exhibit significant (P>0.05) linear and quadratic relationships. Whole-body proximate composition (dry matter, crude protein, lipid, and ash) did not exhibit any significant relationship based on supplementation of graded doses of PHB. Immunological assays, namely intra- and extra-cellular superoxide anion production of head-kidney-derived macrophages exhibited significant (P<0.05) linear and quadratic relationships when graded doses of PHB (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 mM) were added to the cell culture media. However, oxidative radical species production of whole blood was not significant (P>0.05). A disease challenge exposing experimental tilapia to *Streptococcus iniae* is currently being investigated. The current study indicates that supplementation of PHB-producing bacteria in the diets of juvenile Nile tilapia offers significant growth and immunological benefits when compared to a practical reference diet.

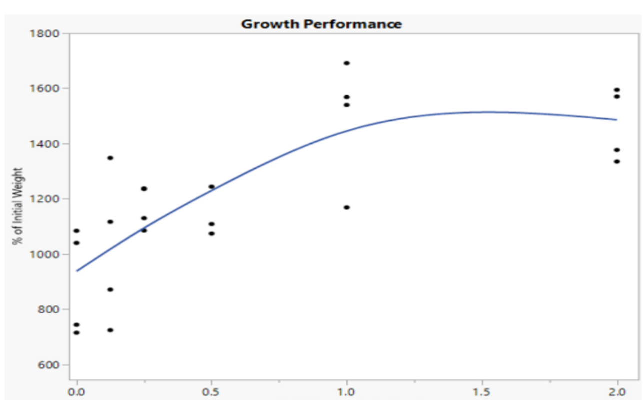

Figure 1. Growth performance of juvenile Nile tilapia fed incremental levels of PHB-synthesizing bacteria.

GROWOUT AND NURSERY CULTURE OF THE GIANT RED SEA CUCUMBER *Apostichopus californicus* IN WASHINGTON AND ALASKA

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Giant red sea cucumbers *Apostichopus californicus* are a known valuable export for the west coast of North America. Aquaculture is still in the pilot stage where growout trials have shown mixed results as research has focused on utilizing existing structures and food sources (figure 1). Water quality, containment systems, fouling and location have been key issues. Wild stock use of other aquaculture systems has been explored and is understood as benefit to the general population. Use includes larval settlement into gear leading to juvenile and adult use of structure and food sources. Nursery phase aquaculture has advanced greatly as food, environment, handling and water quality issues have been addressed. This expertise is being applied in other regions to distribute knowledge to direct stakeholders.

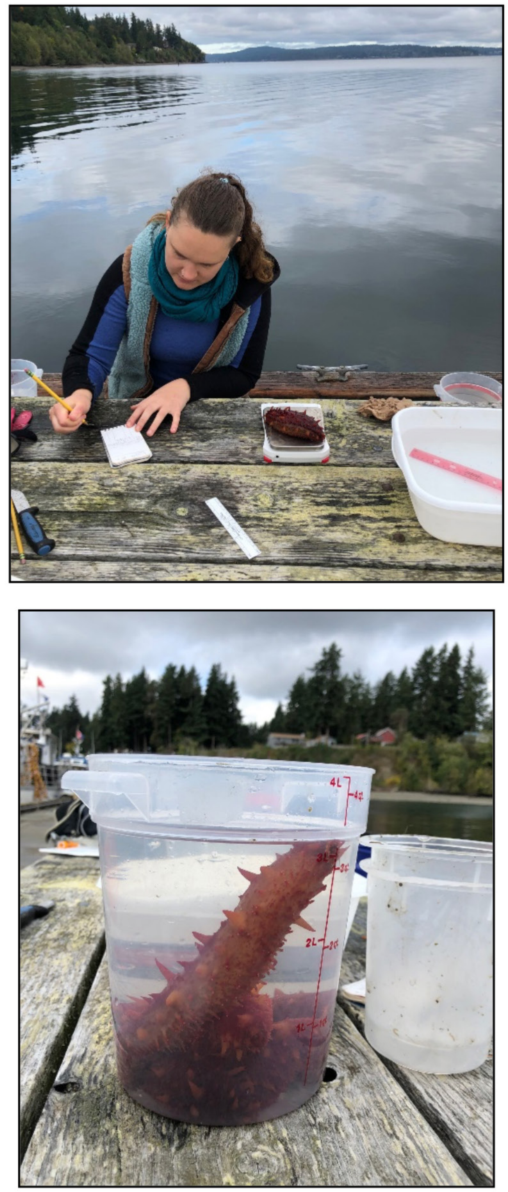

Figure 1. Sea cucumber measurements after deploying in Floating Upweller System (FLUPSY) trough for 1.3 years.

RETAIL SEAFOOD MARKET TRENDS IN THE U.S., 2016 TO 2021

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The U.S. is the largest seafood market globally, with supply dominated by imports. Domestic aquaculture producers seldom have information on the diverse and dynamic trends in retail markets, and the lack of such information became especially apparent throughout the COVID-19 pandemic. To examine retail market trends for seafood in the U.S., seafood scanner data (ScanTrack®) were purchased from A.C. Nielsen. The dataset comprised of weekly sales information from September 2016 to August 2021 for 54 cities, eight regional markets, and the overall U.S. retail market from more than 62,000 retail stores for 53 major seafood categories. The study examined the latest trends and product-specific information in sales values for different seafood products. Overall, retail seafood sales increased by 44% from 2016 to 2021. The largest seafood segment was shrimp with a market share of 29%, followed by salmon (21%), tuna (11%) and crab (10%) in the year 2020-2021 (Figure 1). Those four seafood species also ranked as top species in all regional markets by sales values. The south central region was the largest seafood regional market for many species, including catfish, swai and other major species. Of the top ten seafood products sold in retail markets in 2020-2021, four (shrimp, salmon, tilapia, and catfish) were products that are primarily supplied from aquaculture. Of these the majority of shrimp, salmon, and tilapia sales are of imported products, leaving catfish as the only top-ten product for which sales are primarily from domestic farms. Imported swai/basa ranked 15th in retail market sales. New York was the largest seafood-consuming city in terms of sales revenue for retail seafood sales, and was followed by Los Angeles, Philadelphia, Miami/West Palm Beach, Chicago, and Washington, D.C. Only about 13% sales of seafood products were sold under promotion in retail markets, although promotional sales were greater for species such as lobster, shrimp, salmon, cod & scrod, and tuna.

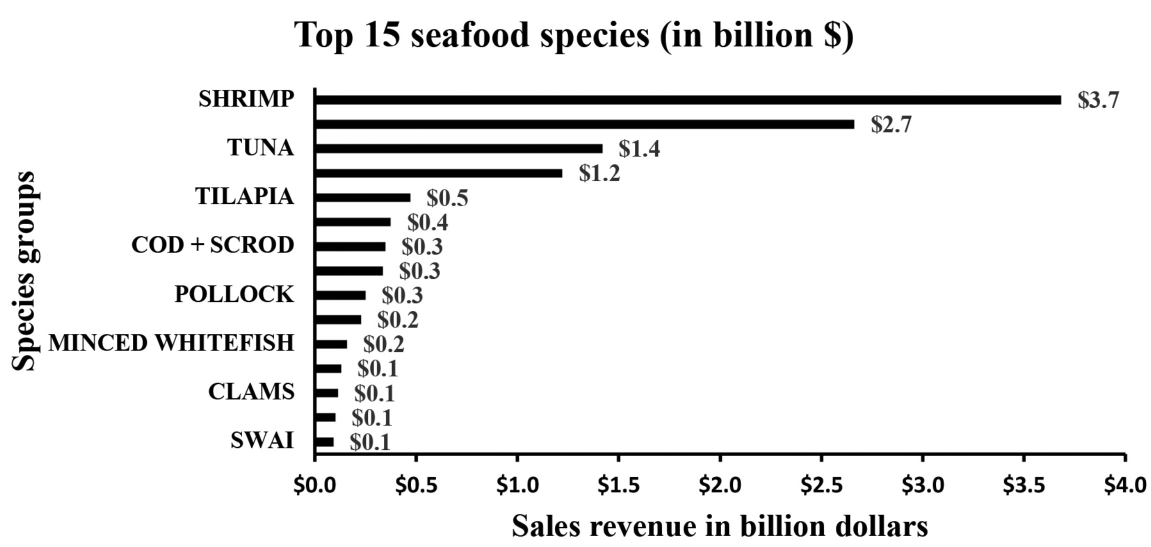

Figure 1: Top 15 seafood species by sales value for 2020-2021 (in $ billion)

EFFECTS OF OCEAN ACIDIFICATION AND WARMING ON DISEASE VIRULENCE IN THE ENDANGERED WHITE ABALONE (*Haliotis sorenseni*) UNDER CAPTIVE CULTURE

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Large-scale changes in ocean temperature and pH driven by carbon emissions are negatively affecting the growth and survival of marine calcifiers. How these stressors influence disease virulence in marine shellfish is poorly understood. To understand this linkage, we conducted experiments investigating the effects of temperature and pH on disease susceptibility in the white abalone (*Haliotis sorenseni*), an ESA protected species that is captively bred as part of restoration efforts. This species is susceptible to withering syndrome (WS), a digestive disease caused by the Rickettsiales-like bacteria *Candidatus* Xenohaliotis californiensis (*Ca*Xc). To evaluate the impact of ocean warming and acidification on *Ca*Xc virulence in *H. sorenseni,* we exposed white abalone to factorial combinations of temperature (12, 15, and 18°C), pCO2 (450 µatm and 1,080 µatm), and *Ca*Xc pathogen (unexposed (U), exposed (E)) over the course of a 9-month experiment, simulating conditions experienced in wild and captive settings.

The combination of elevated temperature and *Ca*Xc exposure reduced white abalone survival by 73% and 84% at 15°C and 18°C, respectively, compared to survival rates observed in animals un-exposed to *Ca*Xc and held at 12°C. In contrast, exposure to high versus low CO_2 did not influence survival. However, mean growth of animals held under high CO_2 decreased by 29.4% when compared to animals held under low CO_2 conditions. These impacts were modulated by the initial size of individuals entering the experiment, with larger animals showing a higher tolerance of climate stress. Interestingly, differences in these responses were observed among our F_2 family groups, indicating that genetic lineage could confer some resilience to temperature, disease, and pH stress. These findings hold relevance for white abalone conservation aquaculture and for understanding how global climate change will impact disease dynamics and challenge the management of California abalone into the future.

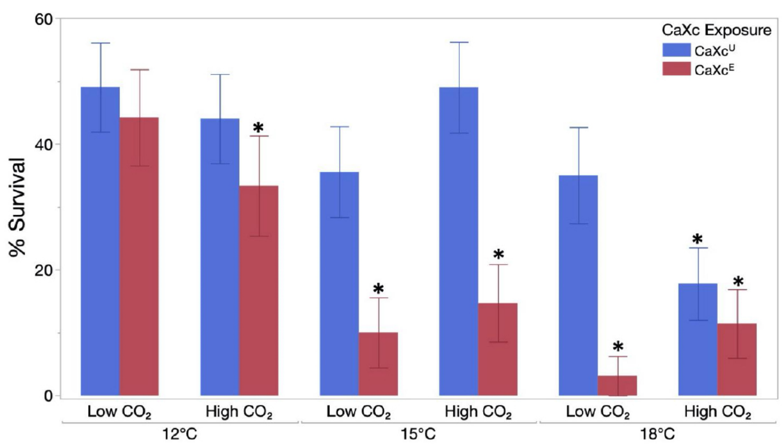

Figure 1: Effects of temperature, *Ca*Xc exposure, and CO_2 level on survival.
Differences in survival across factorial treatment combinations as compared to control conditions. Asterisks (*) indicate significant difference in survival from the control conditions of *Ca*Xc^U, Low CO_2, 12°C

RESTORING KELP FORESTS THROUGH RESPONSIBLE, LAND BASED RAS AQUACULTURE OF OVERGRAZING URCHINS

Brian Tsuyoshi Takeda

CEO and Founder of Urchinomics BV

Problem
Kelp forests are the foundation of our marine ecosystems. However, due to overfishing of predatory species, climate change and pollution, sea urchin populations have exploded around the world, collapsing whole kelp forests and turning them into lifeless, desert-like urchin barrens. The urchins then starve, lose their nutritious and economically valuable roe, and become empty. Fishers do not catch them to produce uni, and predatory species do not eat them as they are not worth the effort. Urchin barren states can persist for decades or even centuries if we do not intervene.

Solution
Urchinomics has developed a novel method to reduce urchin grazing pressure and help kelp forests to recover. Urchinomics specifically targets urchins found in urchin barrens and re-home them into their proprietary land-based urchin aquaculture systems. They are then fed a naturally derived, sustainably produced feed made primarily from the offcuts of kombu kelp production for human consumption to fatten up their roe. And in 6 to 12 weeks, the urchins are full of roe and ready for market. And as a direct consequence of removing the empty urchins from the barrens, urchin grazing pressure is reduced and contributes to kelp restoration.

Presentation Contents
Urchinomics proposes to present:
- how overgrazing urchins are ranched and commercialized from their commercial scale urchin ranch currently operating in Oita, Japan, as well as at their pilot sites in Norway, Canada and the US.
- how kelp forests can rebound when urchin overgrazing pressure is reduced
- how the business model is designed to promote kelp forest restoration
- how the market for sea urchin roe is developing
- how Urchinomics has secured some of the world´s largest investors like Lukas Walton and ENEOS CVC to back its global expansion plans


Before ranching


10 weeks of ranching


urchin aquaculture systems


Feed formulation

EFFECT OF PROBIOTIC TREATMENT ON LARVAL MICROBIOMES OF EASTERN OYSTER *Crassostrea virginica* RAISED IN DIFFERENT HATCHERIES

Evelyn Takyi*, Lauren Gregg, Amanda Chesler-Poole, Jessica Moss Small, Meredith White, Rob Hudson, Cem Giray, David Rowley, David Nelson, and Marta Gomez-Chiarri

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Aquaculture of the eastern oyster (*Crassostrea virginica*) is a rapidly expanding and economically important industry. Probiotics are an increasingly popular approach for prevention of diseases in aquaculture. The marine bacterium *Phaeobacter inhibens* S4 (S4) protects larval eastern oysters against challenge with the bacterial pathogen *Vibrio coralliilyticus* RE22. A liquid formulation of probiont S4 has been developed for commercial use in shellfish hatcheries. The goal of this research is to determine the effect of the S4 formulation on the microbial communities of larvae raised in the hatchery, in order to understand how the larval bacterial community may change due to probiotic treatment and how this microbial community may contribute to larval health, growth, and development. Probiont S4 (10^4 CFU/ml) was delivered daily to *C. virginica* larvae culture tanks from day 1 to day 8 post spawning during eight different trials in four different hatcheries, and structure and diversity of bacterial communities in larval oysters was analyzed as compared to control (no probiont) tanks using 16S rRNA amplicon sequencing. The effect of the probiont S4 formulation on the larval bacterial communities differed by hatchery, season, and type of water treatment (ultraviolet (UV) irradiation/nonUV treatment). The S4 probiont altered the bacterial communities in the larvae and had a targeted effect on the abundance of specific bacteria taxa, including Alteromonadales, Rhodobacterales, Oceanospirillales, Bacillales and Vibrionales. This study contributes to better understanding of the mechanisms of action of the probiont S4 liquid formulation and aids in optimizing its use and benefits for commercial culture of eastern oyster larvae and preventing undesirable side-effects. Further analyses will focus on potential relationships between bacterial community structure and environmental parameters collected during the hatchery trial.

A SYSTEMATIC APPROACH FOR QUANTIFYING BIOSECURITY MEASURES IN AQUACULTURE

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Diseases are the major constraints in aquaculture, and biosecurity is critical for sustainable development of aquaculture. This work emphasizes how biosecurity measures and their relative importance can be quantified and documented in an objective way. The system approaches internal and external biosecurity in a general manner, focusing on transmission routes shared by numerous different types of infectious agents. Norwegian veterinary institute worked with research institutes and farmers from Croatia, Egypt, France, Greece, Italy, Spain, Tunisia, Turkey to estimate biosecurity risk associated with disease introduction and spread into seabass and seabream farms in 8 different countries surrounding the Mediterranean basin (Tavornpanich, S. et al. 2020). The same approach has been tested for Atlantic salmon farms in Norway. This quantitative system helps to identify gaps and weaknesses in the biosecurity plan, assists farmers to allocate resources and tailor the biosecurity programme to fit the risk profile of their farms. If the system is applied in region it also helps to compare a specific farm with an average of the biosecurity scores obtained by neighbouring farms, so that the owners can benchmark their biosecurity and evaluate the risk profile of the region. This benchmarking may give owners impelling reason to improve their farm biosecurity. The system can be modified to fit various farm production characteristics (e.g. RAS), different exposures (e.g. antibiotics), and for different disease agents. This system is developed to be a farmer self-assessment tool with a user friendly automate dashboard containing the functionalities so that the farmers interested in an objective evaluation of farm or regional biosecurity can have a secure access of their own information.

Tavornpanich, S., Leandro, M., Le Breton, A., Chérif, N., Basurco, B., Furones, D., Muniesa, A., Toffan, A., Dalla Pozza, M., Franzago, E., Zrnčić, S., Varvarigos, P., Saleh, H., Cagirgan, H., Dverdal Jansen, M., and Brun, E. (2020). Biosecurity and risk of disease introduction and spread in Mediterranean seabass and seabream farms. Deliverable 4.1 of the Horizon 2020 project MedAID (http://www.medaid-h2020.eu/index.php/deliverables/)

EASTERN OYSTERS SPAWNING AND IMPACT ON RECRUITMENT, WATER QUALITY, AND SPECIES DIVERSITY IN REHOBOTH BAY, DELAWARE

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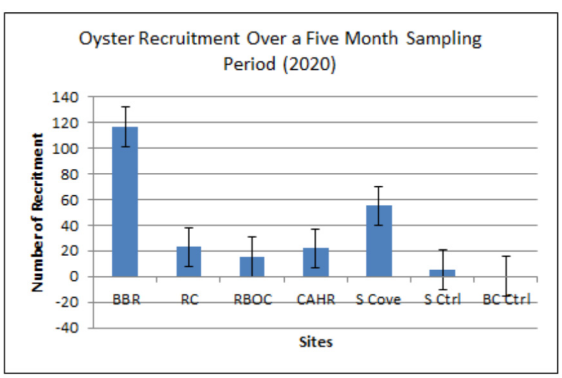
Rehoboth Bay, Indian River Bay, and Little Assawoman Bay are part of Delaware's Inland Bay system. Rehoboth Bay spans thirteen square miles and is connected to the Indian River Bay in the south, the Lewes-Rehoboth Canal to the north, and other freshwater bodies. Eastern oyster (*Crassostrea virginica*) populations in the bays have decreased due to overharvesting and disease. Without eastern oysters, nutrient-rich water increases concentration and frequency of algal blooms that can result in areas in the bays that can cause a reduction in dissolved oxygen and lower the chance for marine wildlife to survive. However, eastern oysters' ability to be natural filter feeders may help control nutrient levels and improve water quality, species diversity, and the productivity of fisheries.

The objectives of this project are to understand how artificial oyster reefs and oyster farms can affect water quality, species diversity, and oyster recruitment as well as understand the spawning timing of oyster larvae entering Rehoboth Bay. Two artificial oyster reefs, two oyster farms, and three control sites were chosen. Four oyster shell bags were deployed at every site. These bags are left in the bay for five weeks before being retrieved. Each site is also monitored by a YSI 556 Multiprobe to collect dissolved oxygen, temperature, pH, conductivity, ORP, and salinity data. Water samples are also collected and processed in the lab for total suspended solids, nitrate, nitrite, and orthophosphate as well as turbidity. Total Chlorophyll-a is monitored at each site using Turner Design Fluorometer. Next, oyster larvae will be retrieved through pump sampling and counted with ZooScan. Recent results from Dr. Ozbay's lab show that artificial reefs have the most and oyster farms have the second highest species diversity, dissolved oxygen, and recruitment, and oyster farms had the highest turbidity due to high occurrences of waves and boat traffic to and around the farms. Literature also shows that eastern oysters begin spawning between mid-June and mid-September and our further efforts will target this timeline to obtain better data outcomes.

Acknowledgments: We acknowledge NOAA LMRCSC Grant #NA16SEC4810007, National Science Foundation EPSCoR Grant No. 1757353 and the State of Delaware, and Dr. Gulnihal Ozbay's Environmental Health Laboratory Team.



LONG-LINE CULTURE OF RED SEAWEED IN THE PACIFIC NORTHWEST

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Seaweed farming is an emerging industry in the U.S. with an annual increase of 132% from 2017 to 2018. While most of this growth has been in the East of the U.S. and Alaska, California's strict regulations have limited the growth of this industry. This changed in 2020, when Humboldt State University, in partnership with Greenwave, installed the first and only commercially licensed seaweed farm to be operational in California state marine waters. The objectives of this project were to: 1) evaluate the growth of Pacific dulse *Devaleraea mollis*, previously known as *Palmaria mollis,* cultivated at a range of depths and during different seasons, 2) estimate nutrients removed by *D. mollis* from the water, and 3) analyze for heavy metals and pesticides to determine if the red seaweed would be considered safe for human consumption.

The study site was located in a pre-permitted lease area of Humboldt Bay in northern California. Two long-lines were used, each consisting of a 107m surface line attached to a mooring buoy at both ends and supported by roughly 25 floats. The mooring buoys were anchored with 100-pound Danforth anchors. Each long-line had 50 vertical weighted grow lines (droppers) separated every two meters. Each dropper was seeded with a 15g starter bundle of *D. mollis* at 0m, 1m, 2m, and 3m deep. Lines were monitored weekly for bundle presence/absence and sampled once a month to measure growth rate over two 4-month farm seasons (September-December 2020, April-July 2021). Water parameters were also tracked and measured. Tissue samples were collected before deployment and at harvest and sent for analysis of nutrient composition, heavy metals and pesticides.

A nonparametric Friedman test was used to test a null hypothesis that seaweed growth for all depths is equal with an alternative hypothesis that at least one depth's growth is different. It rendered a Q value of 6652.800 and p <0.000 for Trial 1, and a Q value of 8209.296 and p < 0.000 for Trial 2 which is significant. Therefore, we reject the null hypothesis at the alpha 0.05 level, and conclude that at least one depth is different and that the best depth to grow Pacific dulse is 0 and 1 m. It is estimated that a total of 453.5g of carbon, 108.1g of nitrogen, and 9.3g of phosphorus were removed from the water. All pesticides were found to be undetectable, and heavy metals were either undetectable or below FDA action levels.

Figure 1. Growth per depth over time. Depths are as follows: blue = 0m, orange = 1m, gray = 2m, yellow = 3m. Trial 1=8/28/2020-12/18/2020. Trial 2 long-line 1=3/23/2021-7/16/2021. Trial 2 long-line 2=4/7/2021-7/30/2021.

SCIENCE TO SUPPORT AQUACULTURE OPPORTUNITY AREAS

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Executive Order 13921, 'Promoting American Seafood Competitiveness and Economic Growth,' calls for the National Oceanic and Atmospheric Administration (NOAA) to identify specific areas suitable for commercial aquaculture termed 'Aquaculture Opportunity Areas' (AOAs). To do so, NOAA is combining scientific analysis and public engagement to identify areas that are environmentally, socially, and economically appropriate for commercial aquaculture. As part of this process, NOAA will develop programmatic Environmental Impact Statements (EIS) for the AOAs under the National Environmental Policy Act, relying on the broader NOAA aquaculture science community to provide important science advice products and material to inform these documents. During this presentation, the NOAA Fisheries Office of Aquaculture will provide an overview of how the NOAA aquaculture science community is supporting the AOAs effort, including both sustained and new projects, capacity, and positions. We will also describe ways in which the broader aquaculture science community can support AOAs, now and into the future.

BACTERIOPHAGE TECHNOLOGY, AN EFFECTIVE SOLUTION TO TACKLE ANTIMICROBIAL RESISTANCE IN AQUACULTURE

Mara Thomas*, Justyna Andrysiak, Ewelina Wójcik, Elżbieta Górecka, Justyna Kowalska, Jolanta Witaszewska, Monika Terenowicz and Jarosław Dastych

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Antimicrobial resistance is one of the biggest threats to public health at this time. The aquaculture industry contributes to this challenge with excessive and often exclusive use of antibiotics on farmed aquatic species. There is an urgent need for safe and effective alternatives that can safeguard animal and human health as well as the environment.

Bacteriophages, also commonly known as phages, are viruses with a high specificity towards their host bacteria. Their function is to eliminate excessively multiplying virulent pathogens without disturbing the microbiome or triggering side effects. They are ubiquitous in the environment and were proved to be safe and non-toxic. Our extensive research has found them to be effective prophylactically as well as therapeutically.

Proteon's technology is particularly unique because it uses a combination of microbiology, biotechnology, bioinformatic and molecular biology research tools to identify, industrialize and deliver bacteriophages to industrial scale farming. Thanks to our advanced methodology, our products pose little to no threat of developing resistance, unlike antibiotics. Proteon Pharmaceuticals has worked with bacteriophage technology for over 15 years, and it develops products for most animal production industries. The product for aquaculture, BAFADOR®, is a 5-component bacteriophage cocktail targeting Aeromonas sp. and Pseudomonas sp. bacteria, in addition to strengthening the fish's immune system. Because of the phage's specificity to bacteria, the species of the treated animal has no influence on the efficacy of the product. For instance, BAFADOR®'s prophylactic efficacy was tested on European carp, by administering it for 5 days prior intraperitoneal infection. The positive control groups experienced an average mortality of 22.5% whereas no mortality was detected in the groups treated prophylactically with BAFADOR®. Bacteriophages are also very resilient, and BAFADOR® was proven to remain stable in various temperatures for long period of times, making it accessible to use in most geographical locations. Proteon Pharmaceuticals also developed patented methods to produce different formats of the product, for instance liquid, on-feed or microcapsules to better suit the need of each species and each industry.

Bacteriophages are a tangible and effective alternative to antibiotics. The aquaculture industry is in need of solutions that can improve the health of fish as well as lower mortality rates. This can consequently contribute to better production quality and important reduction of economic losses for farmers.

INTEGRATING AQUACULTURE IN AND OUTSIDE THE CLASSROOM THAT SUPPORTS STEM EDUCATION: A QUALITATIVE STUDY TO IDENTIFY HIGH SCHOOL STUDENTS' ATTITUDES, INTERESTS, AND EXPERIENCES

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This study explored the impact of an active aquaculture project-based learning program, as perceived by high school students. The purpose of this case study was to discover if participation in the program influenced students' interest, engagement, and future educational and career aspirations in science, technology, engineering, and mathematics (STEM) when integrating aquaculture in, and outside, the classroom. Likewise, the study also wanted to explore students' knowledge about aquaculture and skill development after their participation in the program.

The study employed a qualitative methods approach to explore students' attitudes and experiences. Qualitative data were collected from post student focus groups at three different public high schools in Kentucky. Other qualitative data included teacher journal reflections and public newspaper article. Four emergent themes were found: 1) students show excitement and enthusiasm in the hands-on, aquaculture program; 2) students show attention to detail in the hands-on, aquaculture tasks, it sticks, and are more responsible; 3) students are collaboratively engaged with their peers; and 4) Greater interest and confidence in STEM through practical application.

Results demonstrated that the program engaged learners in real-world problem solving and decision-making situations while working collaboratively in small works. Students also gained an important life skill - responsibility - as well as self-confidence in STEM, after participating in the program.

CUTTING THE BLUE TAPE IN CALIFORNIA: A CASE STUDY ANALYSIS OF BARRIERS AND OPPORTUNITIES TO IMPROVE CALIFORNIA'S PERMITTING PROCESS FOR COMMERCIAL AND CONSERVATION AQUACULTURE

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California is poised to be a leader in contributing to the 10 million new green jobs that President Biden seeks, and an expanding marine aquaculture sector can be part of that green jobs growth. Data show that California is among the U.S. states with the greatest potential to support a robust marine aquaculture sector. The state was also recently granted an unprecedented opportunity by the National Oceanic and Atmospheric Administration (NOAA) to participate in a pilot project to support science-based zoning for responsible marine aquaculture. California has a unique opportunity to leverage these strengths and opportunities to integrate marine aquaculture into its economic recovery and aggressive climate action strategies. Unfortunately, California's permitting system often stifles growth by forcing applicants to navigate an uncertain, laborious, lengthy, duplicative and very costly labyrinth of state and local barriers. These same barriers even hamper conservation aquaculture projects designed to help restore or enhance local ecosystems. While many of these barriers have been identified, discussed among stakeholders, and brought to attention of regulators and policy makers, there has not yet been an effort to methodically collect, synthesize, and aggregate experiences and stories from farmers, researchers, and others seeking permits for marine aquaculture production in California.

The Aquarium of the Pacific's Seafood for the Future (SFF) program, in collaboration with CEA Consulting and a small group of stakeholders, is coordinating a project, titled: *Cutting the Blue Tape for Marine Aquaculture in California*. The project is designed to better understand the permitting barriers and identify opportunities to support a more efficient permitting process for conservation and commercial marine aquaculture in the state. Preliminary results from a series of case studies conducted for the project are providing a clearer picture of specific areas and actors in the permitting process that pose the greatest challenges. More importantly, the results from the case studies are providing greater clarity on potential solutions and how to best achieve them.

GERMPLASM REPOSITORIES AND COMMERCIAL MARKETS FOR GENETIC RESOURCES OF AQUATIC SPECIES: A ROLE FOR OPEN TECHNOLOGIES

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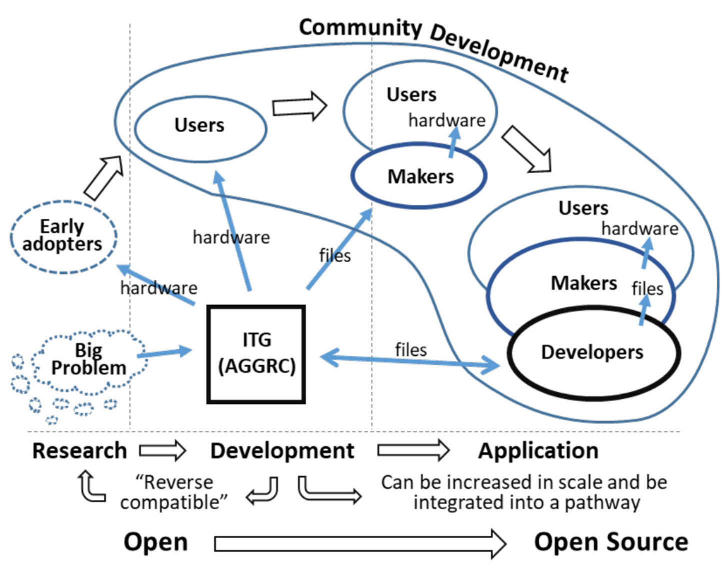
Some challenges are immense and defy resolution despite offering tremendous opportunity. The development of germplasm repositories to protect genetic resources of aquatic species is such a challenge. Despite 70 years of cryopreservation research, fish and shellfish have only minimal frozen collections although there are thousands of publications, primarily addressing creation of freezing protocols. This is in stark contrast to livestock such as dairy for which massive collections exist that drive multi-billion dollar global markets for improved genetics. The lack of repositories suppresses advances across aquaculture, conservation programs, natural fisheries, biomedical models, and efforts to address food security and poverty alleviation. Recognition of this as an immense challenge (not likely addressed by current approaches) is a step towards resolving it. Because large problems such as this are beyond the resources of single entities, other models are required to address them. An emerging model involves use of distributed networks to combine the efforts of large, interconnected communities that share common motivation. This approach was used for example to develop the Linux operating system in the 1990s through open-source software development driven by thousands of volunteer computer programmers. This sharing and community-based approach was in direct response to the limitations of proprietary development. The tremendous success of Linux provided impetus for other open-source projects, and the experience gained opened doors to expand distributed development. This spirit has emerged in renewed form with the advent of new consumer-level design and fabrication technologies that can enable study, distribution, production, modification, improvement, and commercialization (all based on licensing agreements) of computer-aided design (CAD) files shared over the internet. As such, these open technologies provide a powerful alternative to traditional research and proprietary development to combine efforts across multiple communities to establish repositories (Figure 1).

Figure 1. Recognition of a "Big Problem" is a first step in developing solutions such as custom scientific hardware (e.g., tools and devices) to advance aquatic repository development. This hardware can be initially produced by interdisciplinary Technology Groups (ITG), and tested by early adopters. With refinement, it can be adopted by user communities that diversify to produce the hardware themselves ("makers") from shared files (e.g., by 3-D printing), and eventually incorporate their own developers. Sharing of files is an open system with one-way communication; omnidirectional sharing translates into "open-source". Such systems can rapidly increase production and quality.



INTERIM SIZING GUIDELINES FOR SIZING AIRLIFTED POLYGEYSER® RAS FOR THE MARINE SHRIMP *Liptopenaeus vannamei*

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Research into improved RAS techniques for the production of the marine shrimp, *Liptopenaeus vannamei*, under the auspices of a USDA Small Business Innovative Research Grant have led to the propagation of sizing criteria to facilitate commercial evaluations. These conservative guidelines provide sizing ratios for principal components required to produce 18 gram shrimp at a tank density of 10 kg/m^3 without artificial substrate. Total ammonia nitrogen (TAN) and nitrite-N peak concentrations are maintained below 1 mg-N/L under the presumption that elevated nitrite-N concentrations are the principal stressor contributing to mortality, particularly during molt.

Initial testing focused on evaluation of a 4.5 ton tank, a 85 liter PolyGeyser® bioclarifier, and a 7.6 cm airlift driven by a 100 lpm linear air pump with the goal to refine the system configuration and verify sizing assumptions. During the fall 2021 run, one system was lost at 7.2 kg/m^3 as the system was grossly overfed because shrimp numbers deviated from projections. Two systems, also suffering from gross overfeeding, were successfully harvested at 19 grams and an average density of 9.2 kg/m^3 under less-than-ideal greenhouse conditions. The ongoing study (winter 2022) is focusing on the use of feeding trays to monitor the consumption of sinking pellets and the use of pitot tubes as backwash indicators. The winter 2022 run incorporated localized sludge digestors driven by pneumatic exchange to conserve salt as sludge discharge is eliminated. The research team remains confident that the propagated sizing criteria will prove conservative as it is commercially evaluated and minor adjustments can be expected as techniques are refined.

WHY DO WE NEED GENOME EDITING IN AQUACULTURE?

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Aquaculture continues to grow to meet the consumption of an increasing world population. The consumption of the principle terrestrial meat species: beef, lamb, pork and chicken is often compared to aquaculture which consists of at least 20 species, many in the early stages of domestication and breeding. Breeding programmes for farmed aquatic species will gain much from implementation of good practice developed in terrestrial species for pedigree selection, statistical analysis, control of inbreeding, sib selection and selection indexes. But aquaculture breeding has to move quickly to meet the expectation of consumers and retailers in quality, health and welfare. Genome editing offers the opportunity to make targeted changes in the genetic sequence, introducing variation that will result in major improvements in performance health and welfare. Such variation exists at a low level in the wild, but genome editing allows fast and effective introduction of favorable variants into the emerging farmed aquatic species making them better adapted to the farm environment. Where favourable legislation and consumer demand exists, aquaculture breeders should identify genes and variation that improve health, welfare and performance, and employ genome editing to develop strains that are more sustainable. Research is underway to use genome editing to give better control of sex and sterility to prevent escapees breeding in the wild, disease resistance to control disease and improve the production efficiency of high quality, farmed fish.

TOWARDS THE SURVEILLANCE OF HARMFUL ALGAL BLOOMS IN CHESAPEAKE BAY THROUGH THE APPLICATION OF OPTICAL REMOTE SENSING, ECOLOGICAL ASSOCIATIONS, AND CITIZEN MONITORING EFFORTS

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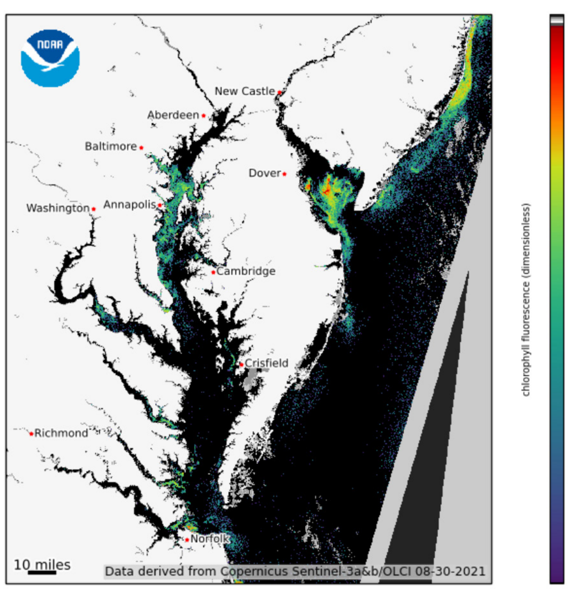
Harmful Algal Blooms (HABs) are a national and international problem associated with risks to animal and human health. Several known human syndromes are related to the consumption of shellfish, which concentrate algal toxins in their tissues and thus are of concern to fishery and aquaculture operations. To identify large scale blooms remotely, satellite-based algorithms have been developed for ocean color sensors. While satellite imagery alone cannot distinguish a HAB from non-harmful algae, it can be a useful tool when combined with other ecological data to identify locations of potential HABs.

High frequency monitoring through satellite remote sensing has been beneficial in protecting public and environmental health in several key US waterways. The success of these tools relies on unique optical signatures of specific high biomass HABs, such as those detected during Gulf of Mexico *Karenia* blooms. Conversely, in Chesapeake Bay a variety of dense algal blooms can appear throughout the year. While these blooms can be detected from satellites, the specific species cannot be determined using only satellite data. The monospecific blooms of *Alexandrium monilatum* and *Margalefidinium polykrikoides* that occur in late summer in the southern bay have similar optical properties. In the northern bay, both monospecific and mixed assemblage blooms of dinoflagellates and diatoms occur and these groups cannot be differentiated with current multispectral technologies. These mixed assemblages may be identifiable by combining satellite algorithms with ecological data (e.g., *Heterocapsa* blooms during the winter). Several algorithms applied to the Sentinel-3 Ocean and Land Colour Imager (OLCI) have improved our ability to detect and characterize algal blooms at higher resolution. Heuristic models constructed with information regarding the ecological niche of individual species (time of year; bloom succession; salinity, temperature, and nutrient regimes) combined with satellite data can potentially lead to HAB identification. A portable microscope/camera (HABscope) provides near real-time cell counts of *Karenia* in the Gulf of Mexico. Expanding HABscope technology to new species and extending it to citizen scientist programs in Chesapeake Bay will further support aquaculture and resource management in the region.



Composited Red Band Difference (RBD) showing relative chlorophyll fluorescence from high (red) to low (violet) for Chesapeake Bay.

A 1,500 FT^2 AQUAPONIC SYSTEM DESIGN CONSIDERATIONS

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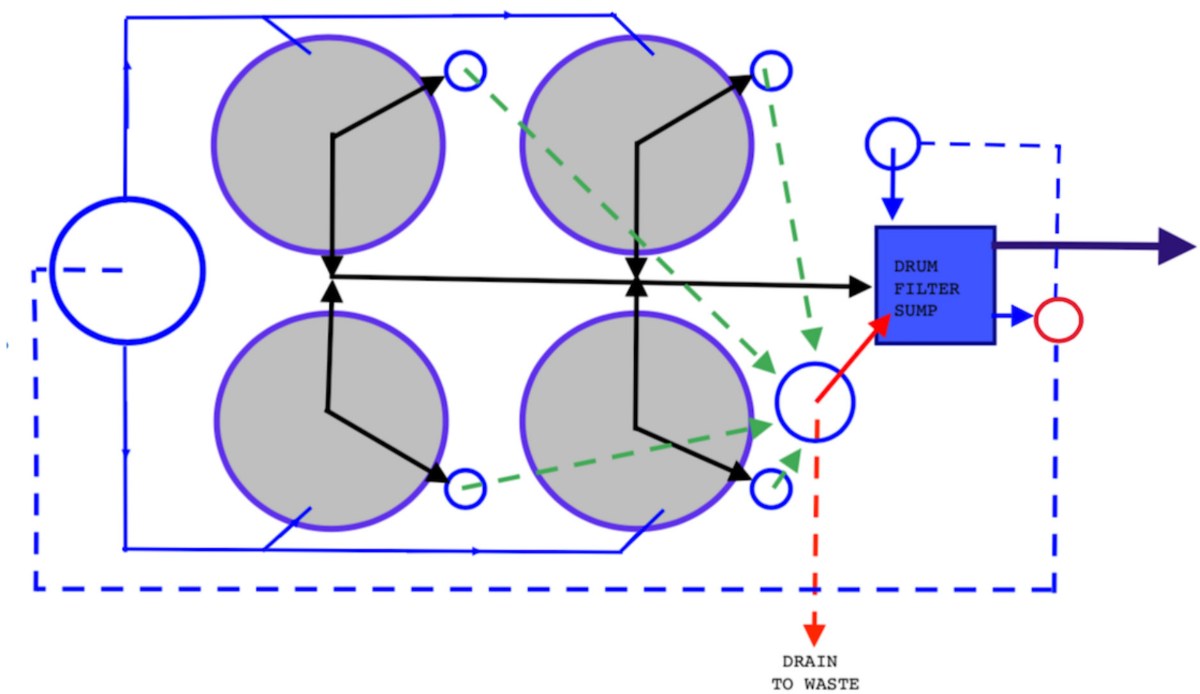
Show design, components, and parts list of a 1,500ft^2- 2,000ft^2 aquaponic system. The goal is to provide a fully functioning and proven system that is large enough for a single person to operate efficiently.

A start up aquaponic system that has been full sized to optimize the efficiency of a single owner/operator. System design includes four fish culture tanks, filtration, mineralization, aeration, DWC, media/wicking beds, water circulation pump and all associated plumbing. All calculations, has been done, including: TDH (total dynamic head), settling velocity, friction loss, pump curve, blower selection (aeration), CFM of air/kg of fish, CFM of air/m^2/in DWC, and fish to plant biomass ratio. Fish waste to plant ratio has been calculated to a wide varieties of plant crops versus greens and herbs.



AQUAPONIC SYSTEM, DYI FOR HOME AND CLASSROOM, 7 yrs. UPDATE

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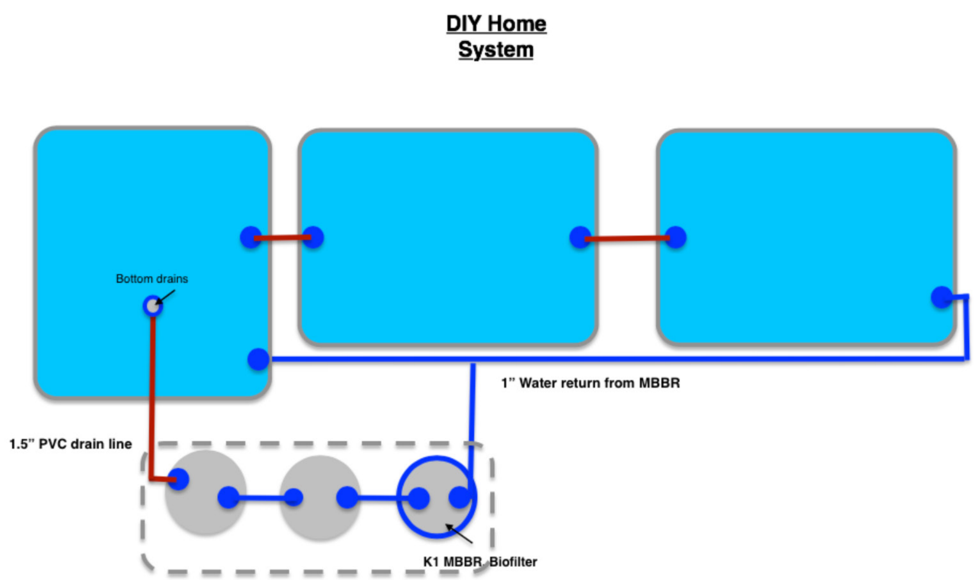
Show design, components, and parts list of a DIY home aquaponic system. The goal is to provide a fully functioning and proven system that is easy to build with local sourced common materials.

A DIY system that has been full sized to optimize the efficiency of a home scale or school system. System design includes fish culture tanks, mechanical filtration, biological filtration, mineralization, aeration, DWC, media/wicking beds, water circulation pump and all associated plumbing. All calculations, has been done, including: TDH (total dynamic head), settling velocity, friction loss, pump curve, blower selection (aeration), CFM of air/kg of fish, CFM of air/m^2/in DWC, and fish to plant biomass ratio.



VIRAL DISEASE DIAGNOSTICS OF PACIFIC WHITE SHRIMP BY POLYMERASE CHAIN REACTION (PCR)

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Global aquatic animal production has been increased, with a minimum impact on the environmental and benefit for human consumption. Aquaculture plays an important role in product of quality nutrition food; however, aquatic animals are susceptible to viral, bacterial, protozoan, parasite and other diseases. It is very important to realize that multiple pathogens are often present in the pond environment, and it is essential to consider the pathogen and the environment in order to prevent or treat disease problems before it becomes widespread. White Spot Disease (WSSV) has been the most problematic infectious agent. If a virus is present in a population and the environmental deteriorates, there will then be a rapid rise in mortality.

Our effort is of interest to shrimp disease diagnostics helping to improve healthy and safe aquaculture farm production. In our shrimp diagnostic work, DNA viruses (WSSV, Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)) and RNA viruses (Taura Syndrome Virus and Yellow Head Virus) can be detected using the PCR detection techniques to screen individual and pool of five samples. Here, we will share our work about the services on screening of a subset of samples sent for Genotyping for two pathogens WSSV and IHHNV with the estimation of their prevalence. It is possible to determine the prevalence with good sensitivity using DNA samples collected for genotyping services for qPCR testing. Table 1 shows a full comparison among lines and estimate minimum prevalence for each pathogen detection. The concentration of the viral load in the pooled samples (in number of copies/mL) can be determined using qPCR with a standard curve. Among other applications, this technique is useful for monitoring pathogen prevalence in all-pathogen exposed breeding populations over time, or confirming groups of broodstock are free of detectable pathogen loads. Importantly, this service can be linked with genotyping work, providing a genetic profile and estimated viral load at reduced cost.

Table 1. Estimated Prevalence for each pathogen detection

Line	Total pools	(+) pools for IHHNV by qPCR	Prevalence IHHNV by qPCR (%)	(+) pools for WSSV by qPCR	Prevalence WSSV by qPCR (%)
Line 1	6	1	3.3	6	20.0
Line 2	6	0	0.0	6	20.0
Line 3	6	6	20.0	1	3.3
Line 4	6	6	20.0	0	0.0
Line 5	6	2	6.7	6	20.0
Line 6	6	4	13.3	6	20.0
Line 7	6	6	20.0	5	16.7
Line 8	6	4	13.3	0	0.0
Line 9	6	2	6.7	0	0.0
Line 10	6	0	0.0	0	0.0

AQUAPONIC SYSTEM BOTTLENECKS IN DWC AERATION/MIXING AND UV/O_3 APPLICATIONS

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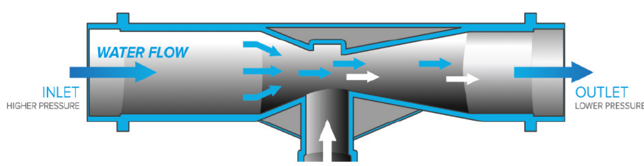
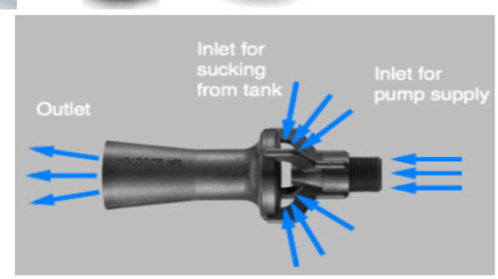
Show system designs, components, efficiency and efficacy of aeration/mixing methods for DWC aquaponic system. Also show when and in what conditions to apply UV/O_3 to aquaponic systems. The goal is to provide a perspective from equipment design to match with application needs.

Equipment sized to optimize the efficiency and cost of operation. System design for aeration of DWC, water circulation. When and how to use UV or O_3 in aquaponic systems.





CRISPR-BASED DIAGNOSTICS FOR DISEASE DETECTION IN SHRIMP

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Infectious diseases cost fisheries and seafood farming operations billions of dollars in lost production per year. For shrimp farming alone, it is estimated that viruses, bacteria, and other microorganisms result in supply and revenue losses amounting to billions of dollars per year globally. Losses of this magnitude make rapid, accurate, and sensitive diagnostics critical for limiting transmission and mitigating outbreaks, thereby protecting farms and their profitability. However, current diagnostic tests require sophisticated equipment, expensive reagents, are time intensive, and cannot be employed in the field. This limits the ability of diagnostic monitoring to translate to immediate and meaningful action to stop disease spread and supply loss.

To address this, we investigated molecular technologies including loop-mediated isothermal amplification (LAMP) and CRISPR collateral cleavage for their potential to be used in a rapid, robust, field-based diagnostic tool for the detection of the common shrimp pathogens White Spot syndrome virus (DNA) and Taura syndrome virus (RNA). We found LAMP to work robustly, but less specifically than CRISPR alone or CRISPR in combination with LAMP. CRISPR in combination with LAMP worked more sensitively but less quantitatively than CRISPR alone. We found targeting multiple genomic regions through multiplexing guide RNAs increased the sensitivity of CRISPR alone. Overall, CRISPR is a promising technology to adopt in field diagnostics for rapid, inexpensive, user-friendly detection of aquaculture diseases that would aid in mitigating disease outbreaks, reducing supply loss, and increasing productivity and sustainability.

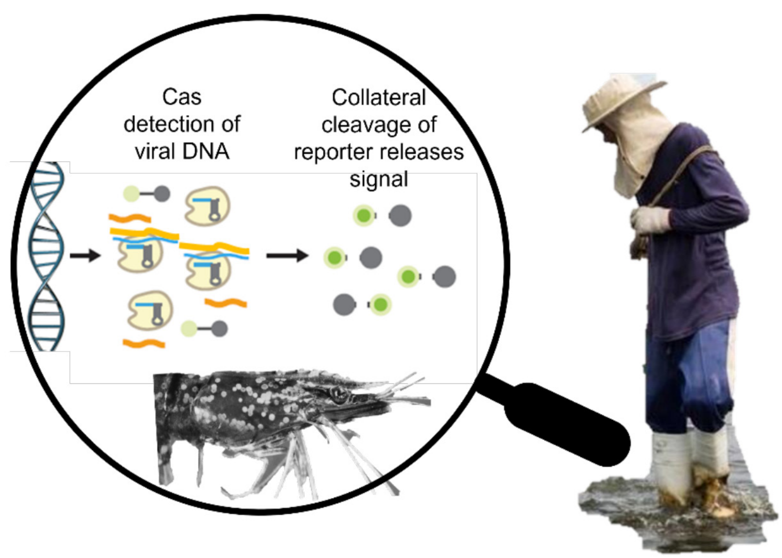

Figure 1. CRISPR diagnostics for field-based detection of White spot syndrome virus in shrimp.

GENETIC GAIN FOR QUANTITATIVE TRAITS BY GENOMIC SELECTION – A SIMULATION STUDY

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Genomic selection has been utilized for the last decade to accelerate genetic improvement in various species. However, field data are insufficient to quantify the long-term effect of genomic selection for a variety of genetic architectures. Simulations based on either genomic selection or traditional genetic selection based on phenotypes and pedigree information could provide a better direction for more efficient improvement in farm animal populations. The objective of this study was to compare genetic progresses by genomic selection and traditional genetic selection for a quantitative trait over the generations, assuming different numbers of QTLs and animals.

Simulations for a population with an effective size of 40 included 21 overlapping generations with phenotypes for a trait with a heritability of 0.5 and an additive genetic variance of 50. Genotypes simulated for the last 10 generation comprised 30K SNPs across 30 autosomes including 1, 10, and 100 QTLs per chromosome for 220, 1100, 11,000, and 33,000 genotyped animals with phenotypes (Table 1). The QTLs explained all the genetic variation for the trait (i.e., no extra polygenic effects). Selection was conducted by mating the top 10% males and 50% females for 20 generations. We estimated and compared genetic trends when selection was based exclusively on either a single-step genomic (ssG)BLUP, a traditional pedigree-based (A)BLUP, or a phenotypic BLUP without considering relationships among animals (IBLUP).

When the number of genotyped animals is smaller, the genetic gain by genomic selection (ssGBLUP) was smaller than that by ABLUP and IBLUP (Table 1). With fewer QTLs, the genetic gain by genomic selection was even smaller than that by ABLUP and IBLUP. However, when the number of QTLs increased, the genetic gain became larger. When weighting SNPs by the percentage of genetic variance explained on the trait, the genetic gain was slightly better for a small genotyped population but not significant. the percentage of additive genetic variance explained by top SNPs was lower (< 15%) for more polygenic traits, indicating that selection based on a few SNPs or QTLs chosen from genome-wide association would lead to less genetic gain. Maximizing the rate of genetic gain under genomic selection would require a large number of genotyped animals that explain the genomic variation significantly, especially for a trait with polygenic effects. The long-term selection study on genetic trend with field data should be conducted with caution.

TABLE 1. Genetic gains estimated for 12 difference scenarios in the simulation

No. genotyped animals	No. QTL	Genetic gain /generation	ssGBLUP /ABLUP in gain
220	30	0.16	1.22
220	300	0.22	1.67
220	3000	0.28	2.15
1100	30	0.07	0.25
1100	300	0.32	1.12
1100	3000	0.38	1.32
11000	30	0.08	0.15
11000	300	0.45	0.85
11000	3000	0.63	1.21
33000	30	0.07	0.11
33000	300	0.57	0.88
33000	3000	0.69	1.07

AQUACULTURE RESEARCH INSTITUTE'S AQUACULTURE WORKFORCE DEVELOPMENT PROGRAMMING

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The Aquaculture Research Institute (ARI) at the University of Maine has developed several new aquaculture workforce development programs with the core objective to promote careers in the aquaculture industry to workers of all types (i.e. professional development and degree seeking students). UMaine is offering a new micro-credentialing program designed to take a learner from foundational knowledge and rigorous training, to application in a real-world work-setting. ARI has collaborated with groups across UMS to develop an aquaculture micro-credentialing pathway. This pathway includes ARI's new hands-on skills development courses in Aquatic Animal Husbandry, Aquatic Animal Health, Recirculating Aquaculture Systems, and more. These hybrid courses allow learners to gain foundational knowledge online that will be implemented with further skills development during the week-long laboratory sessions. Skills gained in these courses will include skills sets identified in Aquaculture Occupational Competencies approved by the Maine Aquaculture Association. Laboratory sessions take full advantage of the aquaculture facilities across UMS giving students experience with industry sized facilities and cutting-edge research. ARI also offers an Industry Partnered Internship Program allowing learners to demonstrate and reinforce their skills within an aquaculture setting. This internship program matches students with industry hosts allowing students to gain experience in the aquaculture industry while conducting projects/research lead by the industry partner. A recently awarded AFRI SAS grant to the Recirculating Aquaculture Salmon – Network, including ARI, will aid in further development of ARI aquaculture education programming and expand workforce development opportunities.

INVESTIGATION OF ENVIRONMENTAL PARAMETERS, TOTAL BACTERIA, *Enterobacteriaceae*, *Vibrio* SPP., AND *E. coli* IN OYSTERS AND SEAWATER FROM SLAUGHTER BEACH, DELAWARE

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Oysters play a significant role in nutrient cycling by filtering large volumes of seawater on a daily basis. This filtration of water results in the removal of sediments, bacteria, and nutrients from the water column and adds nutrients to the bethos. In addition, this leads to the bioaccumulation of different types of microorganisms in oysters such as *Vibrio* spp. *Enterobacteriaceae and E. coli*. *Vibrio* spp. present in marine environments common to human pathogens includes *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vi. cholera,* which may cause wound infections and gastrointestinal illnesses. Other Vibrio spp. such as *Vibrio coralliilyticus* and *Vibrio tubiashii* are known as shellfish pathogens. Enterobacteriaceae is a large, heterogeneous group of gram-negative rods. High level of human sewage disposed into coastal waters resulting in an increase in Enterobacteriaceae pathogens in these waters, hence a higher occurrence of food-borne disease from shellfish. Slaughter Beach, Delaware has been considered as a potential area to establish an oyster hatchery. However, total bacteria, *Vibrio* spp., *E. coli*, and *Enterobacteriaceae* levels remain unknown in seawater and oysters in Slaughter Beach. Additionally, seawater quality may influence oyster larval production, especially *Vibrio* spp. targeting oyster larvae. The aims of this study were to investigate and evaluate *Vibrio* spp., *Enterobacteriaceae*, and *E. coli* in seawater and oysters collected from Slaughter Beach, Delaware and determine relationship between water quality and level of total bacteria, total *Vibrio* spp., and *Enterobacteriaceae* levels in oysters and seawater collected from Slaughter Beach, Delaware.

Water and oyster samples were collected twice a month from the three different sites in Slaughter Beach, Delaware from June to November 2021 at high and low tides for water and low tide only for oysters. A YSI-556 multiprobe system was used to monitor physical water parameters while chemical water quality parameters were monitored using YSI 9500 Photometer. *Vibrio* spp. were monitored by using Thiosulfate Citrate Bile Salts Sucrose (TCBS). Isolated colony were re- inoculated on Luria Broth (LB), then later, was picked to 4-5 ml of LB broth and incubated at the required temperature, at 250 rpm for about 3-4 hours. 30% glycerol stock was prepared by adding 700 µl of the culture to 300 µl glycerol, in a cryotube and then freeze for 16Sr RNA. Trypticase Soy Agar, MacConkey Agar, Luria Broth were used to monitor for total bacteria, *Enterobacteriaceae and Escherichia coli*, respectively. Based on the preliminary data, water quality parameters are found similar at all three sites, while *Vibrio* spp., *E. coli*, and *Enterobacteriaceae* levels are much higher on site two where the creek meets with Delaware Bay with 69 CFU /100ul, 77 CFU /100ul and 55 CFU /100ul, respectively. Vibrio levels have also changed as results of rains. This is an ongoing project and further research results will be presented.

NUTRITIONAL PROGRAMMING OF GILTHEAD SEA BREAM *Sparus aurata*: IMPROVEMENTS TOWARDS BETTER UTILIZATION OF LOW N-3 HUFA DIETS

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There is increasing number of evidence regarding how dietary interventions during the early stages of development can have long-term on the metabolism of different organisms, including fish. In the recent years, in commercially important fish species, it has been demonstrated the possibility of routing the metabolism for better usage of more sustainable formulated diets. In this review, the effects of early dietary interventions, focusing on improving the utilization of the novel feeds on growth, n-3 LC-PUFA utilization and synthesis of Gilthead sea bream will be presented. Our studies showed offspring's utilization of low n-3 LC-PUFA improved if nutritional clues were supplied during the spawning period, not during early or late larval stages in Gilthead sea bream. Obtained progenies from different fed brood fish showed altered gene expression of lipid metabolism gene such as *fads2*, at the larval stage even fed with same commercial diets. This altered metabolism led better utilization of low n-3 LC-PUFA diets at 6 and 18 months of juveniles; we observed lower feed conversion ratios thus higher final weight if fishes were nutritionally challenged with low n-3 LC-PUFA diets in later life stages. Following studies showed the possibility of improving the growth of the offspring by selection of the broodstock by fads2 expression levels at eight-months-old juveniles if fed with low n-3 LC-HUFA diets. On the other hand, our recent studies showed genetic selection of the broodstock can contribute better utilization of the vegetable meal and oil based diets and affects progeny's performance in later life stages during on-growing. Nutritional programming together with a genetic selection of the broodstock shows excellent possibility further improve the utilization of the low n-3 LC-HUFA diets by the offspring during the on-growing. This novel feeding strategy may lead better utilization of low n-3 LC-HUFA, vegetable-based diets this, in turn, can contribute the sustainable growth of the aquaculture sector.

EXAMINATION OF POND BOTTOM SEDIMENTS FOR THE PRESENCE OF COMMON PATHOGENIC BACTERIA IN COMMERCIAL CATFISH PONDS

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Virulent *Aeromonas hydrophila* (vAh), *Flavobacterium columnare*, and *Edwardsiella* spp. (i.e. ESC) are the three most prevalent pathogenic bacteria afflicting catfish aquaculture. It is imperative to evaluate how bottom sediments of commercial catfish ponds impact the prevalence of pathogenic bacteria. This comprehensive study aims to determine the presence of vAh, *F. columnare*, and *Edwardsiella* spp. in renovated and non-renovated commercial catfish ponds in western Alabama and the persistence and antibiotic susceptibility of isolates found iin the pond bottom sediments. Sediments from 36 filled non-renovated ponds (NRPs) and soil cores from 21 drained to-be-renovated ponds (RPs) at six points from each pond were collected and combined to form one composite sample. The cores of RPs were separated into depths of 0.0-5.0 cm, 5.0-10.0 cm, and 10.0-15.0 cm. Samples were vigorously mixed with 30 mL of DI water and allowed to settle. Then, using a sterile inoculation loop, water was plated onto myo-inositol, modified shieh with tobramycin, *E. ictaluri* medium, and blood agar. After 24-48 h of incubation at 30°C, targeted bacterial species were streaked for isolation and frozen for later PCR confirmation. Additionally, antibiotic susceptibility of vAh, *F. columnare*, and *Edwardiella* spp. isolates were tested on Mueller-Hinton agar following the disk-diffusion method, using oxytetracycline, sulfadimethoxine/ormetoprim, and florfenicol. Of the 99 soil samples processed, 77 were biochemically identified as vAh, 28 as *F. columnare*, and 38 as *Edwardiella* spp. Antibiotic sensitivity of vAh isolates was determined by measuring zone of inhibition diameter and assigned a susceptibility designation of susceptible, intermediate and resistance to oxytetracycline (Fig. 1a), sulfadimethoxine/ormetoprim (Fig. 1b), and florfenicol (Fig. 1c). 19.35%, 12.67% and 25.35% of vAh isolates were resistant to oxytetracycline, sulfadi-met-hox-ine/ orm-et-oprim, and florfenicol, respectively. Sediment samples were analyzed for chemical and physical analysis. Two cations of interest, calcium (Ca^{2+}) and iron (Fe^{2+}) had average concentrations of 9346.4 ± 4234.6 ppm and 168.7 ± 44.9 ppm respectively across all sediments. Future research on virulence factors, genetic sequencing, and persistence of these environmental bacterial isolates are necessary in understanding the role of sediments within the pond microbiome.

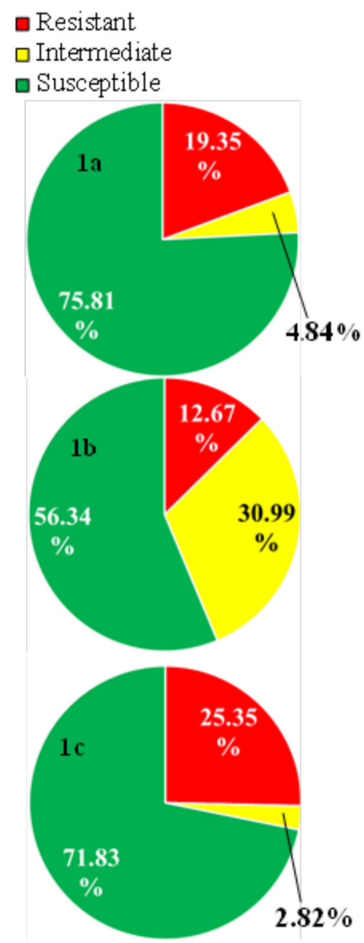

Figure 1. Percentages of field vAh isolate susceptibility to oxytetracycline (a), sulfadimethoxine/ormetoprim (b), and florfenicol (c).

HETEROSIS BETWEEN TWO SELECTED LINES OF THE EASTERN OYSTER *Crassostrea virginica*

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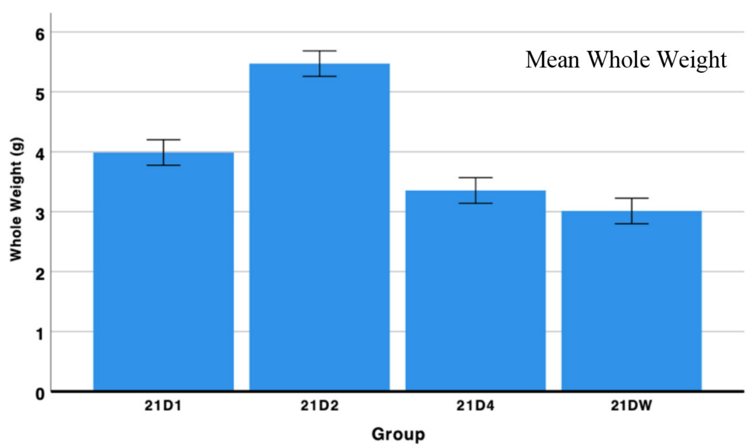
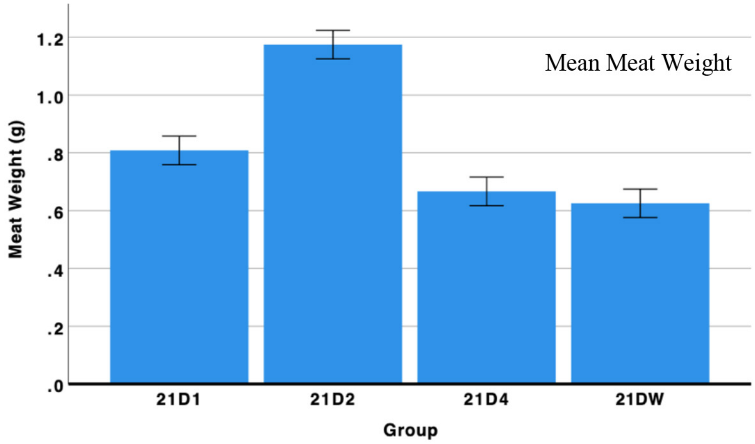
The Eastern oyster *Crassostrea virginica* is a major aquaculture species in the United States. Genetic improvement of cultured stocks is essential for the sustainable development of Eastern oyster aquaculture. Rutgers University has been breeding Eastern oysters for disease resistance since 1960 and produced several lines of selected oysters. In this study, a hybrid cross (21D2) between two selected lines (21D1 and 21D4) was produced and evaluated along with the two pure-line crosses and a wild control (21DW). The four groups were spawned at the Rutgers Cape Shore Facility in June 2021 and were deployed in four replicate bags on Cape Shore flat once the individuals reached 4 millimeters in length. All groups were sampled (25 per replicate, 100 per group) and measured in November 2021 to compare the relative growth of the four groups. No difference in mortality was observed, which was low (1 – 2.4%) in all groups.

The hybrid cross 21D2 showed significantly higher whole weights, with a mean of 5.47g. The wild control 21DW and the selected 21D4 showed significantly lower whole weights, with means of 3.01g and 3.35g, respectively. The same patterns can be seen in the mean meat weights, with the hybrid 21D2 having a mean of 1.17g, the selected line 21D1 having a mean of 0.81g, the wild control 21DW and 21D4 having means of 0.63g and 0.67g, respectively. These results are consistent with the sampled mean heights, lengths, and widths of each group. These results indicate that heterosis exists between the two selected lines and crossing between selected lines is an effective approach for genetic improvement of the Eastern oyster.





BUILDING COMMUNITY CAPACITY IN KELP MARICULTURE SITE SUITABILITY ASSESSMENTS

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Alaska has a nascent kelp mariculture industry currently focused on farming sugar kelp, *Saccharina latissima*, bull kelp, *Nereocystis luetkeana*, and winged kelp *Alaria marginata*. While kelp mariculture has expanded in the last five years, key technical barriers in farm site selection must be resolved to support the industry. Determining the suitability of sites for cultivating kelp is a complex system that involves multiple factors of varying importance, including conflict over area use, geophysical aspects, and biological requirements for effective farming. The deployment of kelp farms in sites that do not provide adequate conditions for kelp growth is of significant concern.

We designed, assembled, and tested a Site Assessment Toolkit (SAT©, Figure 1) to assist farmers and farmers-to-be in evaluating potential leasing sites and determining the suitability of these sites for kelp farming. The successful testing and upgrading of the toolkit resulted from a joint effort between the University Alaska Fairbanks, Alaska Sea Grant, and the Native Conservancy, a non-profit organization integrated by Native Alaskans pioneering kelp farming in Prince William Sound. The SAT© is a final product copyrighted by the University of Alaska Fairbanks.

Figure 1. Site Assessment Toolkit (SAT©) designed, assembled, and tested in Alaska for kelp mariculture.

MAKING PRESCISE GENETIC CHANGES IN THE TILAPIA GENOME

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Efficient tools that dissect gene function and enable introduction of desired genetic modifications at precise locations will radically advance existing genome improvement strategies in animal agriculture, especially in aquaculture. CRISPR/Cas9 has proven very effective to create knock-out alleles in multiple fish species via the non-homologous end-joining (NHEJ) pathway. However, precise insertion of exogenous donor DNA and gene swapping via the homology-directed repair (HDR) pathway has hardly ever been described in aquaculture species. We have achieved high frequency of precise knock-in of donor DNA and further demonstrated the possibility to replace and repair a deficient allele at equally high efficiency in tilapia.

Our strategy co-targeted pigment genes and genes affecting sterility (multiplexing) and used pigment defect as visual selection criteria to identify individuals more likely to carry the desired change.

We successfully generated tilapia lines where b-globin 3'UTR was integrated downstream of dead-end1 (dnd1) coding sequence. We measured ~ 50% of precise homology-directed knock-in amongst depigmented larvae following multiplex gene modification. F2 tilapia homozygous for the b-globin 3'UTR integration developed into sterile adults with un-developed ovaries and testes, revealing the essential role of dnd1-3'UTR in the maintenance of adult germ cells.

In addition to the homology directed knock-in, we successfully repaired a mutant version of the tyrosinase pigment gene for a wild-type version. An albino line of tilapia carrying a 7-nucleotide deletion at the tyrosinase locus (Tyr^{alb7}) was used. We observed the return of pigmented melanophores in 8% of injected embryos and showed germ line transmission of the corrected allele at frequency between 10% and 50%.

Our study indicates that precise genomic modification can be achieved by HDR in tilapia at high efficiency. These results open exciting possibilities in breeding programs allowing, for example, rapid introgression of favorable or de novo alleles into a breeding population opening new possibilities to improve health, welfare and performance of farmed fish by increasing disease resistance, growth rate or enhancing flesh quality and taste.

HOW CAN WE INTEGRATE GERMPLASM REPOSITORIES INTO THE CONSERVATION LANDSCAPE?

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The climate crisis currently at play worldwide has led to a long list of stressors on biodiversity in a wide array of ecosystems. Aquatic environments are often sensitive to seemingly small changes, with many organisms being affected by disruptions to habitat. Germplasm repositories have the potential to advance and complement current conservation efforts, at a relatively low cost, by preserving the genetic diversity of species and allowing correction of genetic effects caused by decreasing population sizes. For example, by reintroducing sperm collected before a disturbance event, captive breeding programs can improve genetic diversity of captive and reintroduced wild populations (Figure 1). While this approach has been used successfully in some species, this approach is often overlooked and has experienced a slow uptake in aquaculture and conservation. National stock centers, such as the *Ambystoma* Genetic Stock Center (University of Kentucky), can provide models for such repositories. *Ambystoma mexicanum* is a valuable research tool for improving treatments for spinal cord and limb injuries, however, is also a relevant example for conservation efforts as this species is considered critically endangered in its natural setting. Our goal is to develop generalizable cryopreservation pathways that can be applied to biomedical model organisms and can be extended to imperiled aquatic species such as amphibians, fishes, gastropods and corals.

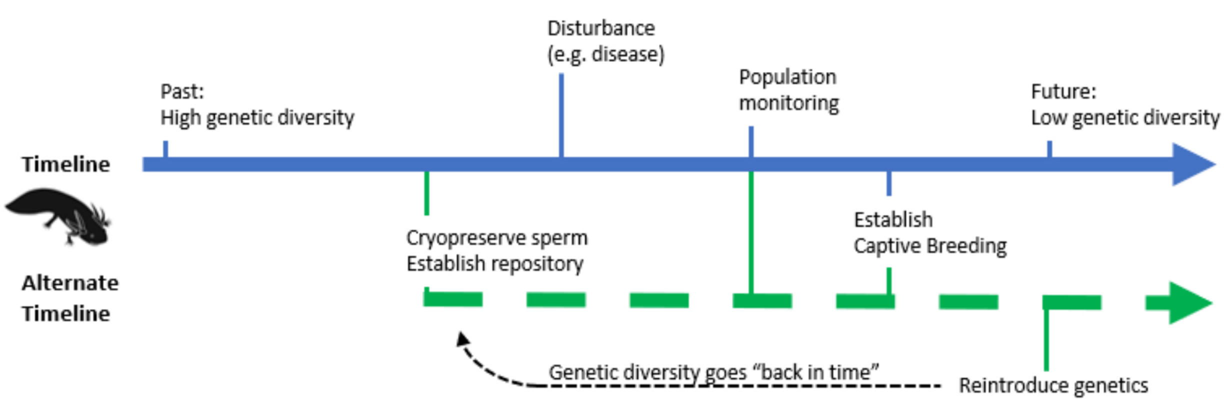

FIGURE 1. Possible timelines with and without sperm cryopreservation to capture genetics in response to potential or actual population disturbance. With decreasing population size, wild and captive populations lose genetic diversity over time, however with addition of cryopreserved sperm from before a disturbance event, the genetic diversity is improved.

A COMPARATIVE STUDY ON METALS AND PARASITES IN SHELLFISH OF FRESHWATER AND MARINE ECOSYSTEMS

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Little has been published about the interactions of metals and parasites in economically important aquaculture species, particularly shellfish. Metal bioaccumulation and parasitic diseases could vary in different species depending on temperature changes and other environmental factors. Most studies conducted on endoparasites, such as acanthocephalans, cestodes, nematodes and trematodes, indicate that only cestodes and acanthocephalans could absorb heavy metals successfully in their hosts, and that only adult worms could be used as indicators of environmental pollution. In *Artemia parthenogenetica*, cestodes increased resistance to arsenic (As) pollution and temperature changes; and infection was associated with improved antioxidant defense system without oxidative damage. The most serious parasite of *Penaeus* spp. is *Enterocytozoon hepatopenaei* (Microsporidia: Enterocytozoonidae). Apostome ciliates are negatively impacting *Pandalus borealis* of the northeastern United States (*Synophrya* sp. that causes "white eggs" or Black Spot Gill Syndrome) and *Penaeus* spp. from the South Atlantic and Gulf of Mexico [*Hyalophysa lynni* that causes shrimp black gill, sBG). In freshwater fish, the larvae of the nematode *Eustrongylides* spp., Jägerskiöld, 1909 (Nematoda: Dioctophymidae), absorbed lead (Pb), mercury (Hg), and cadmium (Cd), when the metal levels were close to zero in water and sediment. The concentration of Pb in the larvae was approximately 17 times higher than in the fish. Like the antioxidant defense reactions in the parasitized *A. parthenogenetica* exposed to As, an improved antioxidant defense system may be available in parasitized fish, limiting oxidative damage caused by metals.

This study presents baseline concentrations of 30 metals in wild *Penaeus vannamei* Boone, 1931 from Ecuador and other species, as well as the current taxonomy for selected shellfish species and their parasites. Research is needed to assess the relationship of metals in parasites and host tissues, and oxidative stress in shellfish. The tools of One Health including molecular ecology, population genomics, proteomics, and epigenetic epidemiology should be used to rapidly and specifically detect parasites and environmental pollution indicators that could threaten aquatic species from freshwater and marine ecosystems, particularly considering climate change and pollution threats.

ACQUIRING DAY-ONE COMPETENCY THROUGH THE FUNDAMENTALS OF AQUATIC VETERINARY MEDICINE LEARNING RESOURCE

Laura Urdes*, Chris Walster, Julius Tepper

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This book is intended for veterinary students looking to acquire day-one competency, as defined by the OIE. This book is organized in such a manner that each chapter builds on the previous one. It is also suitable for veterinary schools wishing to establish a course covering the nine core areas of aquatic veterinary medicine. For veterinarians interested in practicing aquatic veterinary medicine, and aquatic veterinarians looking to become certified through the CertAqV program of the World Aquatic Veterinary Medical Association, it provides a valuable resource to obtain necessary skills and technical understanding.

There is a significant amount of information contributed by dozens of authors from around the world on the nine core areas of the CertAqV certification program, so a companion website has been created with access to supplementary data. Each chapter in the book has links to the companion website, where additional text, figures, and further reading and resources can be found. The diseases presented in this printed version of the text are those that are considered to have worldwide importance. Diseases found primarily in one locality or isolated to only one or a few species can be found in the online text. With respect to exploratory surgery in aquatic birds, mammals, amphibians and reptiles, it should be considered that the procedures and techniques are similar to their terrestrial counterparts except where noted throughout the book.

All proceeds from the sale of this book will go to the World Aquatic Veterinary Medical Association to help support its many member programs.

THE WELFARE CONCEPT – DOES IT APPLY TO SHELLFISH, TOO?

Laura Urdes*, Maria-Antonia Minea and Acacia Alcivar-Warren

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With the rapid expansion of aquaculture and ownership of a wide variety of animal species worldwide, the well-being and welfare of these species has recently gained a great deal of attention.

As most of these species are poikilothermic vertebrates and invertebrates, they present unique issues. Furthermore, commercial aquaculture producers and industries providing farmed seafood for the global market appear to be generally naïve to what is evolving about animal well-being, welfare, and the humane treatment of all aquatic animals.

As the veterinary profession is being looked to for contributions in filling the knowledge gaps in the practice of aquatic animal health, this presentation will outline some of these issues and relate them to approaches commonly used in addressing the well-being and welfare. Comments about pain in invertebrates will also be presented.

THE FUNDAMENTALS OF AQUATIC VETERINARY MEDICINE TEACHING AND LEARNING RESOURCE

Laura Urdes*, Chris Walster and Julie Tepper

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This is a comprehensive clinical reference covering taxonomy, anatomy and physiology of aquatic species, water quality and life support systems, diagnostics, treatment, and prevention of aquatic animal diseases - including parasitic diseases considered to have worldwide importance.

The book is designed as a guide for all relevant professionals involved in practicing and/or teaching aquatic animal health and welfare. It is suitable for veterinary schools wishing to establish a course covering the nine core areas of aquatic veterinary medicine.

For veterinarians interested in practicing aquatic veterinary medicine, and aquatic veterinarians looking to become certified through the CertAqV program of the World Aquatic Veterinary Medical Association, it provides a valuable resource to obtain necessary skills and technical understanding.

There is a significant amount of information contributed by dozens of authors from around the world, so a companion website has been created with access to supplementary data. Each chapter in the book has links to the companion website, where additional text, figures, and further reading and resources can be found.

PARASITES OF FISH AND SHELLFISH SESSION

Laura Urdes[1]*, Nick Saint-Erne[2] and Acacia Alcivar-Warren[1]

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The Parasites of Fish and Shellfish session will address the following topics: The Fundamentals of Aquatic Veterinary Medicine Teaching and Learning Resource; a comparative study on metals and parasites in shellfish of freshwater and marine ecosystems; protozoal parasites of ornamental fish; prevention techniques for selected freshwater parasites; parasitic worms and crustaceans of ornamental fish; interactions of metals, glyphosate, parasites and epigenetics in shellfish and fish: a review; transcriptome modulation of *Salmo Salar* immunized with *Caligus rogercresseyi* vaccine prototype: a host-parasite interaction; novel genomic markers associated with pharmacological resistance in the sea lice identified through whole-genome resequencing; untangling sea lice microbiota: novel insights in the biological role and potential threat associated with the salmon aquaculture; cell-based antigens prospection through transcriptome analysis uncovers vaccine candidates in Atlantic salmon against sea lice: transmission of parasites, larval sea lice (*Lepeophtheirus salmonis*) exhibit behavioral responses to pre-adult and adult conspecific cues, among others. Additional papers will be presented at the poster session including: pain in aquatic invertebrates – the questioning triad; ghosts of oceans past: what can data on historical parasite burdens tell us about the future of marine disease?; and the parasite *Sylon hippolytes* alters the fatty acid compositions of the dock shrimp *Pandalus danae*.

NUTRITIONAL COMPOSITION AND PHYSICAL CHARACTERISTICS OF CAPTIVE REARED PRE-METAMORPHIC BONEFISH (*Albula vulpes*) LARVAE

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Wild bonefish (*Albula vulpes*) were collected from two locations (Biscayne Bay and the Florida Keys) and transported to Harbor Branch Oceanographic Institute where they were acclimated to captive habitats to examine spawning mechanisms. The population underwent monthly hormonal manipulation to induce spawning. Spawning events occurred on October 5, 2020, and October 6, 2020. For both spawns, eggs were collected in an egg collector and transferred to Kreisel tanks to hatch. Samples of eggs were collected prior to hatching to determine biometrics such as size, fertilization success, and hatching success. After hatching, larvae were collected daily for up to 3 days post-hatch and biometric data (total length and oil droplet length) was recorded for a subsample of larvae. Total fatty acids (polar and neutral) were also extracted from egg and larvae samples and analyzed using GC-MS detection methods. Results are expected to show a decrease in essential fatty acids over time as they are utilized by larvae for growth and metabolic processes. This project is part of a multi-year effort to successfully spawn bonefish in captivity and will be utilized to develop suitable diets for captive larvae.

AT WHAT TEMPERATURE DO CULTURED ATLANTIC SALMON *Salmo* salar EXPERIENCE COLD-INDUCED PHYSIOLOGICAL DISTURBANCES?

Émile Vadboncoeur*, Charlotte Nelson, Colin J. Brauner, Rebeaccah Sandrelli, Andrew Swanson and A. Kurt Gamperl

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Salmon reared in sea-cages in Atlantic Canada experience a large range of temperatures during grow out (0-19˚C), and the extremes of this temperature range may be stressful for the fish. The effects of high temperatures on Atlantic salmon are being actively researched given the current, and predicted, effects of climate change. However, climate change is also resulting in more 'cold shocks', and there are sparse data on the effects of cold temperatures (< 2ºC) on salmon physiology, health and welfare.

Commercially reared smolts (~175 g) of St. John River (New Brunswick, Canada) origin were separated into 8 tanks (2 groups x 4 tanks; 35 fish per tank). The first group was the control group, and was kept at 8˚C. The second (experimental) group was exposed to a decline in temperature of 1˚C week^{-1} from 8 to 1˚C, and then held at this temperature for an additional week (see Fig. 1). This temperature regimen mimicked the typical seasonal decrease in temperatures at sea-cage sites in Newfoundland. Photoperiod was 12h light: 12 h dark, and the fish were offered feed twice daily until apparent satiation. Plasma and liver samples were taken from 2 fish per tank after 1 week at 8, 6, 5, 4, 3, 2 and 1°C, and after 2 weeks at 1ºC. Fish morphometrics (incl. HSI; hepatosomatic index) and feed consumption (FC) were measured over the course of the experiment, and blood (plasma) samples were analyzed for enzymes associated with tissue damage, ion and metabolite levels, and for stress indicators.

The salmon fed less starting at 6˚C, and FC was only 10% of that at 8ºC at 1-2ºC. During the 2 weeks at 1˚C, 5% of the fish died, and many of the fish had elevated values of HSI (1.57 ± 0.05 compared to 1.16 ± 0.04). However, there were signs that fish health/welfare was impacted before this point. An ionoregulatory disturbance was apparent at 4˚C, with cold-exposed fish having lower levels of K^+ and higher levels of Na^+ and Cl^-. However, an increase in plasma cortisol levels was only observed 1ºC (18 ng mL^{-1}), there was no increase in plasma lactate (i.e., no indication of the onset of anaerobic metabolism), and none of the plasma levels of enzymes indicative of tissue damage (creatine kinase, aspartate aminotransferase, or lactate dehydrogenase) were elevated. Thus, while Atlantic salmon show changes in appetite and physiology beginning at 6 and 4˚C, respectively, stress, mortality and other signs of compromised health were not apparent until 1ºC. Clearly, even short periods of exposure to temperatures ≤ 1ºC pose a unique challenge to growers of Atlantic salmon in regions that experience episodic low water temperatures.

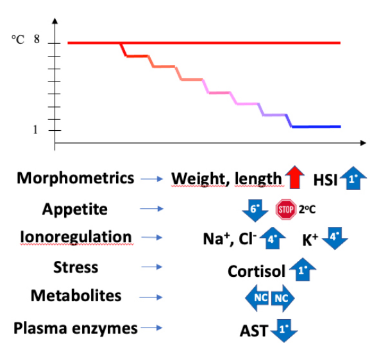

Figure 1. Impact of a seasonal decline in temperature on Atlantic salmon.

UNTANGLING SEA LICE MICROBIOTA: NOVEL INSIGHTS IN THE BIOLOGICAL ROL AND POTENTIAL THREAT ASSOCIATED WITH THE SALMON AQUACULTURE

Diego Valenzuela-Miranda*, Ana Teresa Gonçalves, María F. Morales-Rivera, Valentina Valenzuela-Muñoz, Gustavo Nuñez-Acuña & Cristian Gallardo-Escárate

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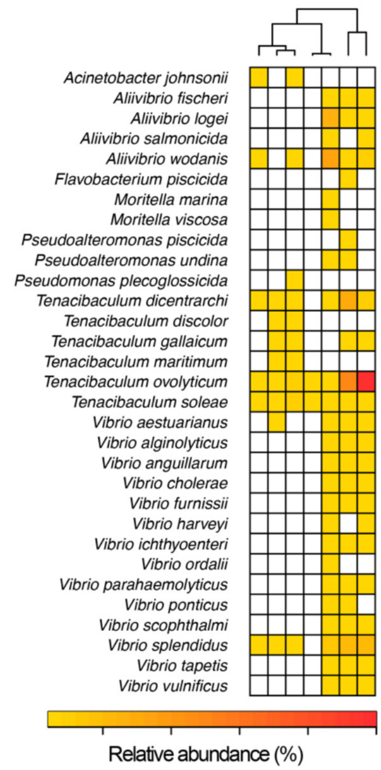
The sea louse (*Caligus rogercresseyi*) is a marine ectoparasite that has become one of the main constrains for the sustainable development of Chilean salmon aquaculture. Besides the well-known deleterious effects of sea lice in salmon farming, novel evidence suggests the presence of a large and diverse microbiota in the parasite. However, the biological roles in the parasite development and the potential threats for salmon farming remains unexplored. In this scenario the present work was aimed to (i) characterize sea lice microbiota from distant populations, (ii) to predict biological roles of the microbial community in the development of sea lice, and to, (iii) to identify bacterial pathogens that could potentially impact salmon aquaculture. To do this, chromosome proximity ligation (Hi-C) coupled with long-read sequencing were used for the genomic reconstruction of the *C. rogercresseyi* microbiota, while nanopore sequencing of the full 16S rRNA gene was used for microbial profiling at specie level. Through Hi-C we were able to assemble and characterize 413 bacterial genome clusters, including six bacterial genomes with more than 80% of completeness. The most represented bacterial genome belonged to the fish pathogen *Tenacibacullum ovolyticum* (97.87% completeness), followed by *Dokdonia sp.* (96.71% completeness). This completeness allowed identifying 21 virulence factors (VF) within the *T. ovolyticum* genome and four antibiotic resistance genes (ARG). Notably, genomic pathway reconstruction analysis suggests putative metabolic complementation mechanisms between *C. rogercresseyi* and its associated microbiota. Regarding possible bacterial pathogens in sea lice microbiota, a total of 30 potential fish bacterial pathogens species were identified. Notably, fourteen Vibrio spp. were predominantly found in the Los Lagos region, while six Tenacibaculum spp. were more equally distributed among the sites. A core of five fish pathogens was observed in all farming zones, including *Aliivibrio wodanis*, *T. dicentrarchi*, *T. ovolyticum*, *T. soleae*, and *V. splendidus* (see figure). Overall, our results evidence that sea lice microbiota might fulfill key metabolic roles in the parasite's development. At the same time, potential threats for salmon farming were found within the microbiota, including fish bacterial pathogens, virulence factors and antibiotic resistance genes.



Funding: ANID-Chile through the Postdoctoral grant FONDECYT (#3200600), and FONDAP (#15110027).

WHOLE-GENOME EXPRESSION APPROACH FOR TRANSCRIPTOME ANALYSIS: ATLANTIC SALMON SEAWATER ADAPTATION AS BIOLOGICAL MODEL

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The available high resolution of genome information and transcriptomes data allows the understanding of complex biological processes. However, the analysis of complex experimental designs involving different tissues, times-points or environment represents the main obstacle. This study proposes a novel approach to analyze complex data sets combining coding and non-coding RNAs at the chromosome-level genome.

Therein, Atlantic salmon smolts were transferred to SW under two strategies. (i) Fish group exposed to gradual salinity changes (GSC), and (ii) exposed to a salinity shock (SS). Gills, intestine, and head kidney samples were used for total RNA extraction, followed by mRNAs and small RNAs Illumina sequencing.

Through a whole-genome transcriptomic approach, different expression patterns among the tissues and treatments were observed. A mRNAs and miRNAs correlations expression were observed at chromosome levels. Chromosome regions highly expressed between experimental conditions included a high abundance of transposable elements. In addition, differential expression analysis showed a higher number of transcripts modulated in response to SS in gills and head kidney. miRNAs expression analysis suggested a low number of miRNAs involved in the smoltification process. However, the target analysis of these miRNAs showed a regulatory role in growth, stress response, and immunity. This study is the first evidencing the interplaying among the mRNAs/ miRNAs and the structural relationship at genome level during Atlantic salmon smoltification.

Funding: ANID-Chile funded this study through the Postdoctoral grant FONDECYT (3190320), grants FONDAP (15110027) and FONDECYT (1210852).

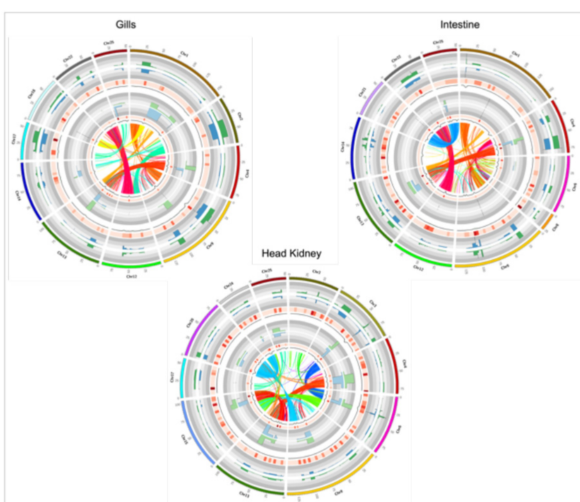

Figure: Chromosomes regions with high CGE index variation between GSC and SS fish groups. Heatmap in red showed the differences expression variation between both groups, CGE index. Black line graph indicates genome coverage of threshold areas. The ribbons, in the Circos plot, represent the homoeologous regions in salmon genome.

DEVELOPMENT OF QUORUM SENSING INHIBITORS AS POTENTIAL TREATMENTS FOR VIBRIOSIS

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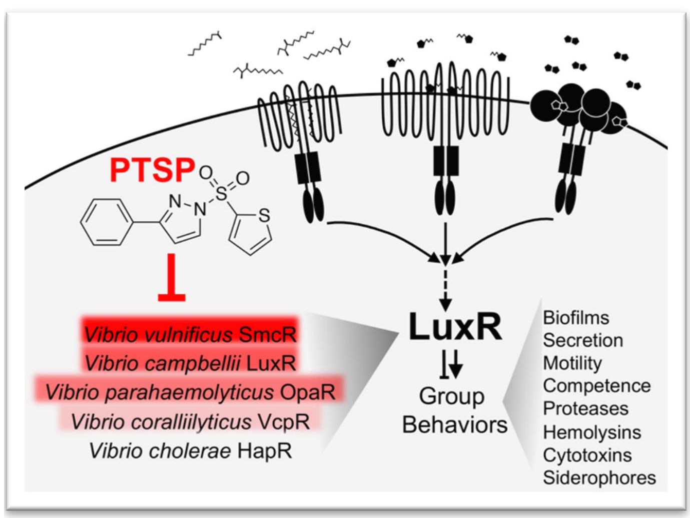
In marine *Vibrio* bacterial species, the cell-to-cell signaling and communication system called quorum sensing directly controls genes required for causing disease in fish, shellfish, coral, and other hosts. Quorum sensing enables bacterial cells to sense and respond to increases in the number of cells around them and engage in activities that are most beneficial when many cells act together (*e.g*., secretion of toxins). *Vibrio* quorum sensing pathways control many pathogenic behaviors that allow these bacteria to cause disease in the host: biofilm formation and expression and secretion of toxins, proteases, hemolysins, and more that target and kill the host. The core regulators of the quorum sensing pathway are conserved in all pathogenic vibrios that have been examined, including *V. parahaemolyticus, V. campbellii*, *V. vulnificus, V. alginolyticus*, *V. anguillarum, V. coralliilyticus*, and *V. cholerae*. Thus, quorum sensing signaling proteins are excellent targets for designing molecules that block disease in marine hosts. We have identified a panel of specific, potent, stable, and soluble inhibitors that block the master regulator LuxR of the quorum sensing pathway in *V. vulnificus*, *V. parahaemolyticus*, *V. campbellii*, and *V. coralliilyticus*. Several of our thiophenesulfonamide compounds, such as PTSP (3-phenyl-1-(thiophen-2-ylsulfonyl)-1*H*-pyrazole) have sub-nanomolar inhibitory concentrations against specific *Vibrio* species; they highly effective in blocking quorum sensing in *V. vulnificus* whereas *V. cholerae* is resistant. Importantly, these molecules do not kill the bacteria but rather block only the quorum sensing signaling system to eliminate expression of disease-causing genes without affecting growth. We aim to develop these molecules as therapeutic treatments for vibriosis for *Vibrio* bacteria.



ECONOMIC STATUS AND CONTRIBUTION OF U.S. AQUACULTURE

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The U.S. is the largest seafood market in the world. However, the U.S. remains a minor player in global aquaculture with either slower or declining growth trends in recent years. This decline has occurred despite seemingly abundant land, water, capital, and research infrastructure in the U.S. Broader macro and microeconomic factors such as stiffer competition from cheaper imports, rising input costs, and complex regulatory environments have contributed to this decline (Engle and Stone 2013; Knapp and Rubino 2016; Abate et al. 2016; van Senten et al. 2018). The situation is exacerbated by a lack of information on aquaculture investments, market uncertainty, and relatively lower levels of profitability (Engle et al. 2020). Promotion of U.S. marine and Great Lakes aquaculture industries warrants information regarding profitability and investment feasibility. Well-established pond-based sectors and marine shellfish sectors struggle to attract capital while emerging species and production technologies such as recirculating aquaculture systems (RAS) have attracted capital but have experienced high rates of failure. This economic paradox is primarily driven by a lack of fundamentally sound information about profitability and investment feasibilities. This project envisions to address the critical lack of economic information related to cost of production, relative profitability, economic contribution, seasonal and regional market trends, and economic risk associated with U.S. marine and Great Lakes aquaculture industries. A new project seeks to promote a greater understanding of economic and market issues of geographically diverse U.S. aquaculture sectors with special emphasis on mariculture and Great Lakes aquaculture industries. Aquaculture sectors such as mollusks (oysters, mussels, and clams), salmonids, crustaceans, recreational and ornamental fish, centrarchids, and other major offshore/inland aquaculture industries will be the focus of the research. The group of 12 Economists and Sea Grant/1862 Land Grant Extension specialists from eight diverse institutions, will work together in the creation of a National Hub envisioning the advancement and sustainable management of U.S. aquaculture sectors alongside cultivating the next generation of experts in aquaculture economics and marketing through training and experiential learning opportunities.

MARKET RESEARCH TO GUIDE U.S. AQUACULTURE RECOVERY FROM THE COVID-19 PANDEMIC

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The shocks from the COVID-19 pandemic resulted in widespread shutdowns and loss of major markets that have threatened the survival of many U.S. aquaculture businesses. The magnitude of business closures and employee layoffs has resulted in an unparalleled shock to the U.S. economy. The combined effect of business closures and reduced discretionary spending of consumers are having strong negative economic effects, the length of which is unknown. This ongoing project focuses on consumer market research to provide science-based information to guide U.S. aquaculture businesses as they attempt to adjust marketing efforts to respond to the abrupt changes in the food supply chain and consumer purchasing of prepared meals (at-home and away-from-home) and groceries for at-home consumption. Through a series of five quarterly surveys, this study is targeting seafood consumers in 20 core-based statistical areas (CBSA) to assess their changes in seafood purchasing and consumption behavior and preferences . These market areas were selected with the input of industry advisors from varying sectors of U.S. aquaculture. The baseline survey of consumer behavior and preferences for seafood and aquaculture products pre-COVID and during COVID have been completed. Results of this first wave of surveys have been disaggregated into responses related to the major U.S. aquaculture species of catfish, trout, and oysters.
Core-based statistical areas targeted:

- Atlanta
- Baltimore/ Washington DC
- Boston
- Charlotte
- Chicago
- Dallas
- Denver
- Houston
- Jackson (MS)
- Las Vegas
- Los Angeles
- Memphis
- Miami
- New Orleans
- New York
- Raleigh/Durham
- Salt Lake City
- San Francisco
- Seattle
- St. Louis

MARKETING AND ECONOMICS OF WARMWATER MARINE FINFISH AQUACULTURE

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Global production of marine finfish has grown both in total volume of production but also in the number of species farmed commercially. With the exception of salmon and redfish, however, there has been little commercial production of marine finfish in the U.S. While regulatory challenges have affected the development of offshore U.S. marine aquaculture, there are other challenges affecting warmwater marine finfish that warrant further investigation. Readily accessible information on the size of markets, consumer preferences, and market opportunities for U.S. aquaculture production of warmwater marine finfish species are currently unavailable. Understanding the existing supply, major geographic markets, and preferences of consumers and supply chain distributors who handle these products are critical elements in successful business planning and business development. The project team has undertaken three analyses to address some of the knowledge gaps for warmwater marine finfish aquaculture; focusing on the finfish species identified in the "Status of Marine Finfish" document developed by USDA ARS (Table 1). The first was an analysis of the current supply of these species of interest; sourced from capture fisheries, domestic aquaculture production, and international trade. Second, the assessment of consumer preferences for selected species that are available in Southern tier states. Third, the preferences of seafood distributors in the Southern tier states for warmwater finfish species and particularly the potential for the selected species of interest.

Table 1. List of warmwater marine finfish species

Common Name	Scientific Name	Common Name	Scientific Name
Almaco Jack	*Seriola rivoliana*	**Red Drum**	*Sciaenops ocellatus*
Atlantic Cod	*Gadus morhua*	**Sablefish**	*Anoplopoma fimbria*
Black Sea Bass	*Centropristis striata*	**Southern Flounder**	*Paralichthys lethostigma*
California Flounder	*Paralichthys californicus*	**Spotted Seatrout**	*Cynoscion nebulosus*
California Yellowtail	*Seriola lalandi (formerly S. dorsalis)*	**Spotted Wolf fish**	*Anarhichas minor*
Cobia	*Rachycentron canadum*	**Striped Bass**	*Morone saxatilis*
Florida Pompano	*Trachinotus carolinus*	**Summer Flounder**	*Paralichthys dentatus*
Greater Amberjack	*Seriola dumerili*	**Tripletail**	*Lobotes surinamensis*
Olive Flounder	*Paralichthys olivaceus*	**White Sea Bass**	*Atractoscion nobilis*

CURRENCY CHOICES OF NORWEGIAN EXPORTERS OF AQUACULTURE PRODUCTS

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The purpose of this paper is to examine the choice of currency for Norwegian exporters of aquaculture products. This choice will affect prices in different markets as well as risk, factors that are increasingly important as the supply chain for aquaculture products are becoming more sophisticated and more transaction mechanisms are introduced. Since Norway is the world´s second largest seafood exporter, and the leading exporter of farmed salmon, Norwegian exporters are of special relevance to study.

Choice of invoicing currency is a topic that has gained attention in the international literature on international trade. If one assumes that an exporter is free to determine the invoicing currency, three different strategies are available. An exporter who is concerned with exchange rate risk would set the prices in the domestic currency. This is known as "producer currency pricing" (PCP). The prices can also be set in the importer's currency, a "local currency pricing" strategy (LCP). This would be the case for an exporter who can exercise market power or conduct so-called "pricing-to-market", but can also be due to other factors that do not imply oligopolistic behavior, such as currency convertibility. Finally, the exporter could set the price in a major "world" currency, a "vehicle currency pricing" strategy (VCP), which is typically the US dollar (USD), euro, or Japanese yen. A well-known insight from the literature of international trade is that trade in homogenous primary goods should be conducted in a single vehicle currency as market efficiency increases if prices are expressed in the same currency.

Looking into fish exporters' currency choices we find some interesting patterns. First, the domestic currency (NOK) is more frequently found compared to studies from other countries. Vehicle currencies, as the dollar, are used, but in far from the same frequencies found for other homogenous products (metals and oil) and what is the case for exporters in other countries. We also compare currency choices from export of aquaculture products with products from traditional fisheries. In the last group vehicle currencies are much more used than what is the case for export of aquaculture products. We find that the most important factors explaining these features are the high degree of liquidity in the market for Norwegian currency as well as a high degree of maturity in the market for aquaculture products.

USE OF QUANTITATIVE GENETICS AND MARKER-ASSISTED SELECTION FOR IMPROVED GROWTH PERFORMANCE IN NORTH CAROLINA BAY SCALLOPS *Argopectin irradians*

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Severe declines in commercial and recreational fisheries of the bay scallop, *Argopecten irradians*, have led to increased interest in US aquaculture. Since its introduction to China in the 1980s from the US, *A. irradians* has become one of the most important bivalves cultured and the Chinese industry has utilized selective breeding for fast growth to increase the productivity of their farms. As part of the growing scallop aquaculture industry in China, researchers have identified molecular markers (size-related quantitative trait loci (QTL) marked by microsatellites and single nucleotide polymorphisms (SNPs) in the myostatin gene) that are predictive of superior performance. The goal of this project was to improve growth performance of bay scallops on commercial shellfish aquaculture farms in North Carolina through the use of quantitative genetics and marker-assisted selection.

Mature scallops were collected from three wild populations in fall 2019 in southeastern NC (Becky's Creek, BC), Core Sound (Big Marsh, BM), and Bogue Sound (Emerald Isle, EI). The wild scallops were brought back to the UNCW Shellfish Research Hatchery (SRH) and each group was spawned separately. Adductor muscle tissue samples were collected, DNA was extracted and analyzed for ten microsatellite markers and four SNPs in three regions of the myostatin gene associated with growth traits in Chinese bay scallops. No significant genetic differences among the three populations of NC scallops and no significant association between the genetic markers and performance in wild scallops were observed.

Offspring produced from the wild scallops in 2019 were used for growth experiments to evaluate the association of the microsatellite and myostatin SNP genetic markers with growth performance. In fall 2020, performance-based selection was utilized with each line (BC, BM, EI) sorted into "Big" and "Little" groups based on shell height and spawned separately. Adductor muscle tissue was collected from all 2020 broodstock scallops, DNA was extracted and genotyped for the ten microsatellite markers and three regions of the myostatin gene for SNP identification. Evaluation of microsatellite and SNP genetic markers revealed significant genetic differences among the six groups; however, no consistent significant association between the genetic markers and performance was observed in broodstock scallops.

COMPARATIVE GENOMICS OF TYPICAL AND ATYPICAL *Aeromonas salmonicida* subsp. *salmonicida* STRAINS AND THEIR RELATIONSHIP TO PATHOGENESIS EVOLUTION

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Aeromonas salmonicida is a globally distributed Gram-negative teleost pathogen, with a wide host range, that live in freshwater and marine environments. *A. salmonicida* is classified as typical or atypical based on host origin of isolation and phenotype. Five-known subspecies of *A. salmonicida* have been described, where *salmonicida* is the only typical subspecies, while the subsp. *achromogenes*, *masoucida*, *smithia*, and *pectinolytica* are considered as atypical. Genomic differences between *A. salmonicida* subsp. *salmonicida* isolates and their relationship with the current classification have not been explored.

Here, we sequenced and compared the genome of four virulent strains to elucidate their molecular diversity and pathogenic evolution. Phenotypes, biochemical and enzymatic profiles were determined. PacBio and MiSeq sequencing platforms were utilized for genome sequencing. Comparative genomics showed that atypical strains belong to the subsp. *salmonicida* with 99.55 ± 0.25% identity with each other and closely related to typical strains. Typical *A. salmonicida* J223 is closely related to typical strains, with 99.17% identity to the European strain A449. Genomic differences between atypical and typical strains are strictly related to very distinctive ISs family distribution and plasmid content. Major differences were driven by virulence factors, transcriptional regulators, and non-coding RNAs. Although, plasmidome plays an important role in *A. salmonicida* virulence and genome plasticity.

In summary, typical strains harbor a larger number of plasmids and virulence related genes, that contribute to their acute virulence. In contrast, atypical strains harbor a single large plasmid and a smaller number of virulence genes, reflected on their less acute and chronic infection. The relationship between the phenotypes and *A. salmonicida* subspecies taxonomy is not evident. Comparative genomic analysis based on completed genomes revealed that the subspecies classification is a description of different *A. salmonicida* strains that are adapted to different environmental niches rather than different subspecies.

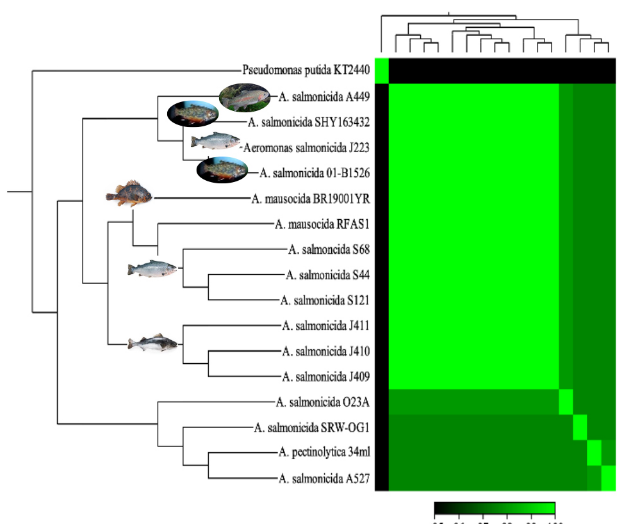

Figure 1. Heat map analysis visualization based on aligned chromosomes of *A. salmonicida* subspecies.

A PILOT SCALE MASS CULTURE PROGRAM FOR *Dunaliella salina* FOR ACHIEVING HIGHEST PRODUCTION LEVELS

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Mass culture of algae in round tank of 500 liter capacity with internal illumination tubes were used for the study. These tanks 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 a 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 in 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 EFFECTS OF LOW TEMPERATURES ON GROWTH AND METABOLIC PROCESSES OF JUVENILE CHANNEL *Ictalurus punctatus*, BLUE *I. furcatus*, AND HYBRID CATFISH *I. furcatus x I. punctatus*

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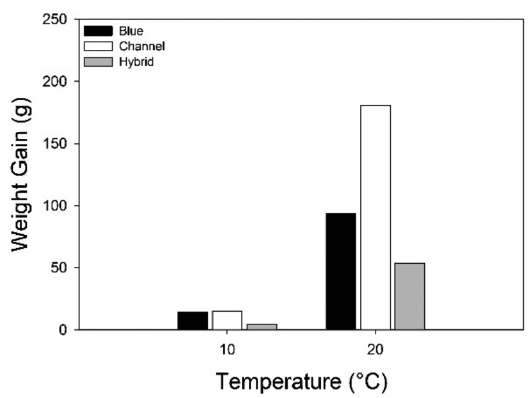
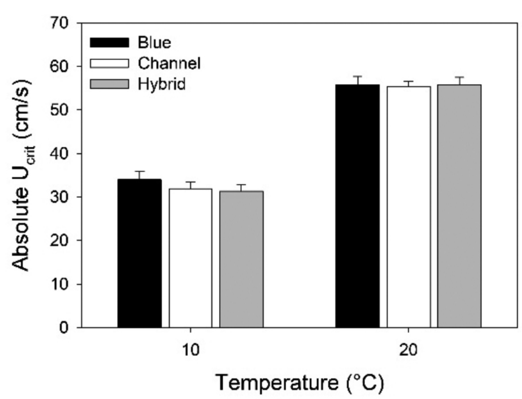
Catfish aquaculture is the largest finfish aquaculture industry in the U.S. and plays a vital role in the U.S. economy. Both channel catfish (*Ictalurus punctatus)* and the hybrid between channel and blue catfish (*I. punctatus x I. furcatus*), are widely cultured, due to their fast growth rates at warm summer temperatures and broad tolerance of environmental conditions. However, because the overall physiology of ectothermic organisms is directly influenced by the environment, less is known about potential limitations of low temperatures on growth and physiological performance. Therefore, the effects of low water temperatures on growth, metabolism, and swimming capacity were evaluated in juvenile channel, blue, and hybrid catfish to guide culture practices.

Fish were acclimated to either 10°C or 20°C in two separate recirculating aquaculture systems and fed to satiation daily for 19 weeks. To measure growth, fish were weighed (nearest 0.01 g) and measured (nearest mm) at the start and end of the 19-week study. Standard metabolic rate ($MO_{2\ min}$) was measured using intermittent respirometry while maximum metabolic rate ($MO_{2\ max}$) and swimming capacity (U_{crit}) were measured using a swim flume.

Weight gain was greater at 20°C than 10°C for all three fish types, with the largest weight increase occurring in channel catfish at 20°C. Hybrid catfish had the highest standard metabolic rate at both 10 and 20°C. At 10°C, channel catfish had the second highest standard metabolic rate followed by blue catfish, yet at 20°C blue catfish had the second highest followed by channel catfish. Swimming performance was affected by temperature, with little swimming capacity evident at 10°C. This study indicates low temperatures can greatly limit energy intake and associated capacity for physiological performance regardless of catfish type.





COLLABORATIVELY DESIGNED GENOMIC TOOLS MAXIMIZE BOTH GENETIC GAIN AND ECONOMIC EFFICIENCY

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Genomics can deliver great benefits to agricultural breeding programs, including efficient management of diversity and inbreeding, accurate parentage assignment, optimal mating designs, improved breeding value prediction, selection decisions, and breeding strategies. Using the appropriate platform for the population of interest is critical. It is often expected that optimal results require a customized tool with a higher level of initial investment and larger ongoing costs. However, it is possible to keep costs reasonable with optimal outcomes through the creation or use of a collaboratively designed universal genotyping platform.

Collaborative genotyping solutions for specific species are created using diverse populations to ensure a design that contains a core set of markers with broad utility among all populations along with markers that capture specific population characteristics. The design also provides the ability to capture published markers associated with key traits. Many industry parties can benefit through using such platforms, creating a sample volume to keep costs reasonable and enabling results and outcomes that are easily compared and evaluated. As the platform is updated and improved, the benefit flows to all users.

A successful example of this approach is in the livestock domain, where the widely used Illumina BovineSNP50 BeadChip array was developed in collaboration with the USDA-ARS, the University of Missouri, and the University of Alberta. The array allows for high-throughput, cost-effective genetic screening using 53,000 markers validated across 18 common beef and dairy breeds. The platform supports many genomic applications, including genomic selection, across both the dairy and beef industries, where its widespread use creates high demand and keeps costs per sample low. More recent extensions of this concept have been deployed as GeneSeek Genomic Profiler (GGPs) arrays. The updated content over time can leverage the continuously developing knowledge base about the genomic structure of a species as new tools and resources become available.

This approach is also proving extremely beneficial for aquaculture species, as exemplified by the creation of a collaborative genotyping platform for *L. vannamei* shrimp designed using samples from eleven populations. This talk will discuss the design of the array, validating the array's results, how to achieve maximal benefit from the array, and the economic impacts of creating and using such genotyping platforms for aquaculture species.

YIELD VERIFICATION TRIALS FOR POND FARMERS IN KENYA TO EXAMINE OPTIONS WHEN EXTRUDED FISH FEEDS ARE EXPENSIVE

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Growth of small and medium scale pond aquaculture in East Africa has been limited by cost of feeds and management strategies related to lack of appropriate technologies. The objective of this on-farm trial was to propose the most profitable and practical pond management options based on our combined experiences and to test them on farms to evaluate their profitability and repeatability. The trials began in December 2020, and were conducted at eight farms in Central, Eastern and Western provinces, Kenya. All ponds, including lined ponds were treated with agricultural lime prior to stocking. Ponds were then stocked with sex reversed male nile tilapia *Oreochromis niloticus* (2.5- 4.5 g) at 3m^{-2} followed by an addition of catfish *Clarias gariepinus* at 0.3 m^{-2} after the second month. All treatments used fertilizer (DAP and urea) to produce natural food for the tilapia. Three supplemental feeding protocols were tested: TRT1, wheat bran (about 16% crude protein (CP), cost \$0.25 kg^{-1}); TRT 2, low protein feed (25%CP, cost \$0.85 kg)$^{-1}$; and TRT 3, high protein feed fed at half rate (30-32%CP, costing over \$1 kg^{-1}). Fish were fed twice daily, according to a feed table and fertilizers applied weekly, until May, then suspended. No aeration was used and water was managed to be static, except for topping off from evaporation or seepage.

The higher protein feed given at half ration (TRT3) resulted in the most reliable net revenues per are (100 m^2), but some farmers found the bran plus fertilizer treatment to be highly profitable. However, leaky ponds managed under the bran plus fertilizer protocol did not perform well. Other reasons for low performance included low survival due to bird depredation (Farm 4); and low fish recovery due to occasional water overflows and incomplete pond draining (Farm3). Previous surveys that showed very low to negative profits from pond fish farming were likely based on farmers who purchased the less expensive floating feeds and fed at high rates, with no fertilization, as evidenced by TRT2 in this trial. Farmers need to be taught to only use feed tables as a guide and to be conservative on feed use. The individualized farm advising provided during the trials helped participating farm managers increase their understanding of pond management, feeding and water quality management. Some owners had never made any profits previously.

Treatment	**Farm1 C/E**	**Farm2 C/E**	**Farm3 C/E**	**Farm4 row1 C/E**	**Farm4 row2 C/E**	**Farm4 row3 C/E**	**Farm5 W**	**Farm6 W**	**Farm7 W**	**Farm8 W**	**Average**
Bran+fert	125	173	11	1	63	0	28	-1	110	29	53.83
LoPro+fert	89	13	-43	21	1	21	108	68	23	50	34.93
HiPro+fert	96	129	30	58	86	70	127	143	132	65	93.66

INTERACTION STUDIES OF THE B SUBUNIT OF PIRAB^{vp} TOXIN FROM *Vibrio Parahaemolyticus* WITH THEIR RECEPTORS: IN SILICO AND EXPERIMENTAL APPROACH

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The main virulence factor of the Acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei* shrimp is the binary toxin PirAB^{vp}, secreted by *Vibrio parahaemolyticus*, which causes massive sloughing of epithelial cells from the tubules of the hepatopancreas. There are reports that the PirB^{vp} subunit possesses lectin activity recognizing amino sugars. Also, has been reported that this damage it's through recognition of a specific sugars sequence of the glycoproteins on epithelial cells hepatopancreas. To explore possible sugar specific linked and effects, we performed in silico analysis and comparative structural studies on the rPirA^{vp} and rPirB^{vp} subunits and the tetrameric complex of PirAB^{vp} purified by affinity chromatography to galactose-sepharose 4B. Analysis of rPirA^{vp} and rPirB^{vp} subunits by circular dichroism, dynamic light scattering, fluorescence-based thermal shift assays and extrinsic fluorescence, in the absence of octylglucoside, showed highly disordered structures that tend to aggregate. However, when octyl glucoside was used as a ligand to both subunits, was observed that only the PirB^{vp} coupled to the ligand and was structurally stable, this was not observed with the PirA^{vp} subunit. When ligand-galactose binding assays were performed for the native tetrameric PirAB^{vp} complex, an increase in the thermostability of the complex was observed. This data suggest that its necessary a sugar, like galactose, for thermostability of pirAB^{vp} complex, and the octylgalactoside for structural stability of the rPirB^{vp} subunit, which suggests that the carbohydrate recognition site is in the B subunit.

FILM AS A MEDIUM TO ADVANCE PUBLIC SUPPORT FOR AQUACULTURE

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Aquaculture has played a small yet increasing role in Maine's marine economy since the 1970s. Today, there are roughly 150 individual aquaculture leases in the state, with an additional 200 operations in the pre-revenue stage. The current value of the Maine aquaculture sector is estimated to be over $100 million and is expected to expand in the coming years.

This growth has helped diversify Maine's marine economy, which has become increasingly reliant on the state's lobster fishery. Recent growth in aquaculture has not been unchallenged, primarily due to lack of aquaculture outreach and education in coastal communities. The Maine Aquaculture Association (MAA) has recognized the need to reach out to Maine communities to help them learn more about aquaculture in Maine – what it is, what we grow, and who grows it. To that end, the association has worked over the past three years to develop a series of educational videos formatted for social media that showcase growers' operations and tell their personal stories. MAA has also spearheaded a 5-part series of short documentary-style films which have been distributed via in-person events, a local TV station and film festival, and social media. Both the educational videos and artistically produced films have generated media attention at the state and national level.

This talk will cover the basics of how these projects were produced, outcomes including metrics and community impact, lessons learned, and current projects in the works for the association. While Maine's case may be unique, the lessons learned and strategies taken by MAA can be applied to other settings across the world where professionals are looking to communicate the benefits of aquaculture to a diverse audience of stakeholders.

Priestia aryabhattai M10 AS GROWTH PROMOTER AND RESISTANCE ENHANCER IN NILE TILAPIA (*Oreochromis niloticus*) CULTURE

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Priestia aryabhattai was isolated from a continuous flow competitive exclusion culture (CFCEC) developed from the intestinal contents of adult Nile tilapia in Colombia and selected based on *in vitro* probiotic potential, with significant antibacterial activity against fish pathogens, acid pH resistance, and adherence capability. The whole genome was sequenced for species identification and functional annotation was carried out.

Also, in vivo effects of *P. aryabhattai* M10 feed supplementation were assessed in Nile tilapia. The probiotic bacteria were lyophilized with the feed (10^6 UFC ml^{-1}), which was vacuum packaged and stored at 4°C until use.

Briefly, 114 tilapia fingerlings (1.05 g ± 0.08) were acclimated for two weeks and randomly assigned to the Control or *P. aryabhattai* treatment groups per triplicate. After four weeks, growth rate, feed conversion, and fish survival after a challenge with *Streptococcus agalactiae* were recorded.

After four weeks of administration to Nile tilapia alevins, *P. aryabhattai* significantly improved weight gain, specific growth rate, and feed conversion ratio in comparison to the control. Also, the survival of Nile tilapia alevins challenged with *Streptococcus agalactiae* was enhanced from 33.33% control to 73.33% with *P. aryabhattai* feed supplementation. To summarize, the research suggests that *P. aryabhattai* M10 is a promising new probiotic option for enhancing the growth performance and survivability of Nile tilapia alevins.

Funding:
Doctoral Fellowship 727-2015 Doctorados Nacionales Colciencias and Project code123080863753, call 808, Evaluation of the probiotic potential of a competitive exclusion culture from tilapia(Oreochromis niloticus) intestinal microbiota. Patrimonio Autonomo Fondo Nacional De Financiamiento Para La Ciencia, La Tecnología y La Innovación Francisco José De Caldas.

The protocol was reviewed and approved by the ethics review board at Universidad de La Sabana N° 57 of 2016, the international ethical guidelines for experiments with animals was also followed according to Directive 2010/63/EU and following Colombian national government regulations. Permission for accessing genetic resources issued by the Colombian Ministry of Environment Number 117, 26 of May, 2015 Otrosí 4.

BROODSTOCK CONDITIONING OF PACIFIC OYSTER IN RAS: EFFECT OF THE WATER QUALITY, CO_2-CARBONATE SYSTEM, AND *Polydora* sp.

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The Pacific oyster, *Magallana gigas* (formerly *Crassostrea gigas*), is one of the most cultured bivalves in Mexico. Nevertheless, the Pacific oyster production has a significant challenge: continuous seed production to meet the current demand. For seed production, the hatcheries frequently use broodstock from the farming zones exposed to natural conditions that cannot be controlled, like those related to the oysters' reproductive cycle or pollutants and parasites as *Polydora* sp. This has resulted in a constant variation of oysters' condition and unpredictable time required to produce mature organisms. For more accurate control of the factors involved in the reproductive cycle of oysters, the use of recirculating aquaculture systems (RAS) may be significant. Little information on the reproductive cycle on bivalves cultured in RAS has been registered, especially the consideration of other interactions as the CO_2-carbonate system (pH, alkalinity, CO_2, carbonates, bicarbonates, calcite, and aragonite) and the water quality (temperature, salinity, nitrogen compounds, and dissolved oxygen). Thus, this work aimed to characterize and evaluate the effect of the water quality and the CO_2-carbonate system and *Polydora* sp. on the reproductive cycle of *M. gigas* cultured in a RAS. The broodstock was conditioning in four different temperatures (18°C, 20°C, 22°C, and 24°C) for ten weeks. After the ten weeks of conditioning, the water quality factors and the CO_2-carbonate system were within the ranges reported as favorable for bivalves. However, no mature organism was observed, and high variability in the condition of the oysters was detected (Fig. 1.). The results suggest a negative effect of the *Polydora* sp. on the reproductive cycle of *M. gigas* cultured in a RAS (Fig. 2).

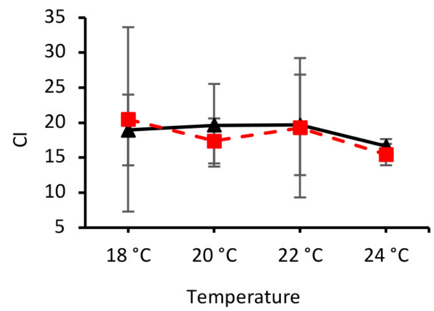

Fig. 1. Condition index of *M. gigas* during the broodstock conditioning in a RAS.

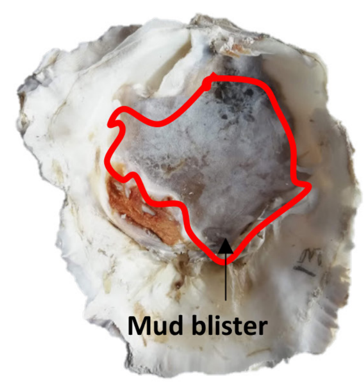

Fig. 2. Mud blister produced by *Polydora* sp. in the left valve of *M. gigas*

ENVIRONMENTALLY FRIENDLY AND SUSTAINABLE ENERGY OPTIONS FOR MARICULTURE WITHIN PROJECT INTEL-MARIC

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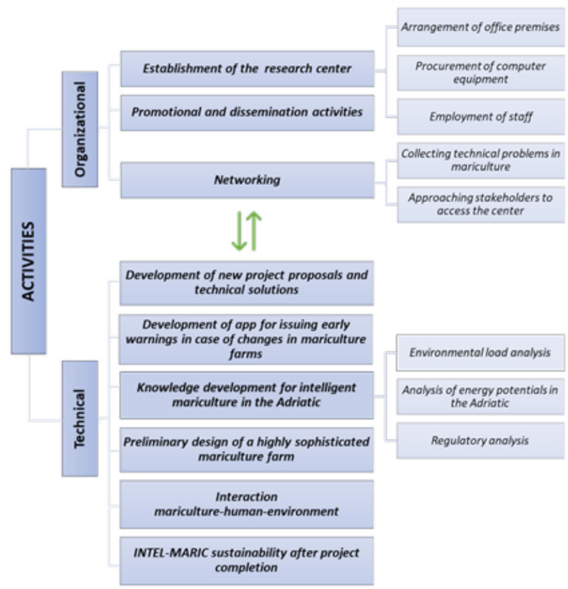
Demands for aquaculture products are ever rising at global level and are resulting in greater investments in the development of efficient and sustainable mariculture farms. However, these developments imply increase in energy needs, which are nowadays mainly ensured from fossil fuels. This work provides an overview of environmentally friendly energy alternatives that are being developed under the Research Center for Intelligent, Inovative, Enviromentally Friendly and Sustainable Mariculture (INTEL-MARIC) that has been founded at UNIZAG FSB. Basic goals, research strategy and activities of INTEL-MARIC are illustrated. Moreover, this work represents extension of investigations presented Korican et al. (2020, 2022) which resulted in alternative configurations of aquaculture systems that enable integration of high share of renewable energy sources. Previous works were limited to the evaluation of carbon footprint, while this one also includes evaluation of other polutants originating mainly from the internal combustion engines onboard aquaculture support vessels as for instance NOx, SOx, PM, etc. Alternative powering options for the complete aquaculture system were evaluated in order to identify those with minimum environmental footprint, simultaneously taking into account costs and environmental regulations. Above developments are illustrated for an aquaculture farm in Croatia, but they are generally applicable if the relevant set of input data is known. Further investigations will include feasibility of the same aquaculture system at different locations along the Adriatic coastline, that have different wind and insolation profiles, and consequently different protential of integaration of high share of RES.



INTEL-MARIC activities

Selected references

Koričan, M., Perčić, M., Vladimir, N., Soldo, V.: Integration of renewable energy sources into the aquaculture systems considering environmental and economic aspects, 12th Int. Conf. on Appl. Energy (ICAE2020), Bangkok, Thailand, 2020.

Koričan, M., Perčić, M., Vladimir, N., Soldo, V., Jovanović, I.: Environmental and economic assessment of mariculture systems using a high share of renewable energy sources, J. of Cleaner Production, Vol. 333, 2022., No. 130072, 13 p.

Acknowledgement

This investigation has been funded by the European Maritime and Fisheries Fund of the European Union within the project “Research Center for Intelligent, Inovative, Enviromentally Friendly and Sustainable Mariculture (INTEL-MARIC)”, granted by the Ministry of Agriculture, Directorate of Fisheries, Republic of Croatia.

CYTOTOXIC RESPONSES TO THE PERFLUORINATED XENOBIOTIC, GENX OF ATP-BINDING CASSETTE PROTEIN AND CELLULAR ACTIVITIES IN *Crassostrea virginica* HEMOCYTES

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GenX is a perfluorinated compound that has been contaminating the Cape Fear River, NC for over 20 years. When organisms are exposed to GenX, it can enter their cells and cause toxicity. ABC proteins are responsible for removing xenobiotics like GenX from cells. However, when these proteins are inhibited, cellular toxicity increases. This project tested the hypotheses that: 1) GenX will inhibit ABC transporters thus increasing mitochondrial membrane potential, cytotoxicity and hypoxia and 2) incubation of hemolymph at a higher temperature to mimic climate change will cause increased inhibition of ABC transporters, and will cause increased mitochondrial membrane potential, cytotoxicity, and hypoxia. Hemolymph from wild adult oysters was exposed to filtered seawater, a protein inhibitor, Reversine, and two concentrations of the xenobiotic, GenX (1.5μM and 15μM). Samples were incubated in 5 replicates at 21°C, and at 30°C to mimic global climate change. The hemolymph was then exposed to various dyes to determine mitochondrial membrane potential, cytotoxicity, hypoxia, and cell mortality. Samples were analyzed using flowcytometry. Though not statistically significant, mitochondrial membrane potential increased in both GenX treatments compared to the control. When compared with the control, cytotoxicity increased in both GenX treatments. Hypoxia also increased in both GenX treatments, indicating that GenX is inhibiting the function of ABC proteins. While no effects of GenX on hemocyte viability were observed, hemocyte mortality after incubation at 30°C was higher than at 21°C. Cytotoxicity and mitochondrial membrane potential both decreased after incubation temperature was raised while hypoxia increased.

DESIGNING AND EVALUATING WIRE-BASED STRUCTURES FOR THEIR ABILITY TO RECRUIT OYSTERS *Crassostrea virginica* AND STABILIZE SHORELINES IN COASTAL SOUTH CAROLINA, USA.

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In coastal South Carolina, the wild population of Eastern oysters (*C. virginica*) consists primarily of extensive (>5,000 acres) intertidal reefs. This population is characterized as substrate-limited, where levels of natural recruitment are high but the presence of suitable substrate for settlement limits population growth. Since 2001, the SCDNR has therefore focused its restoration efforts on substrate supplementation, primarily by deploying natural oyster shell in the intertidal zone to facilitate oyster settlement. In addition to providing habitat to a diversity of other organisms, these oyster-based living shorelines support sediment capture and the subsequent seaward expansion of marsh vegetation. As oyster shell becomes increasingly costly and limited in supply, however, researchers continue to explore alternatives to address the issue of substrate limitations without using natural oyster shell.

In 2011, the SCDNR began utilizing wire-based structures for oyster restoration and shoreline protection. Initially, these structures took the form of repurposed (*e.g.,* cement-coated) crab traps (RCTs), derived from derelict and abandoned traps in the environment, in part as a solution to marine debris issues related to derelict fishing gear in the environment. In 2016, based on the success of RCTs, researchers began developing new approaches to living shoreline installations using purpose-built manufactured wire reefs (MWRs) intended to support high levels of oyster recruitment and facilitate shoreline stabilization and habitat creation.

Over the past five years, these MWR structures have been installed on estuarine intertidal shorelines and monitored for various metrics of performance success to quantify their ability to provide ecosystem services by serving as living shorelines. Monitoring results presented here will support the abilities of these MWR structures to quickly recruit new oysters (see Figure 1), accumulate landward sediment, and facilitate the expansion of natural marsh habitat through vegetative growth. These findings are helping to support new state of South Carolina science-based regulations for living shorelines with a goal being to broaden their adoption as a shoreline protection strategy.

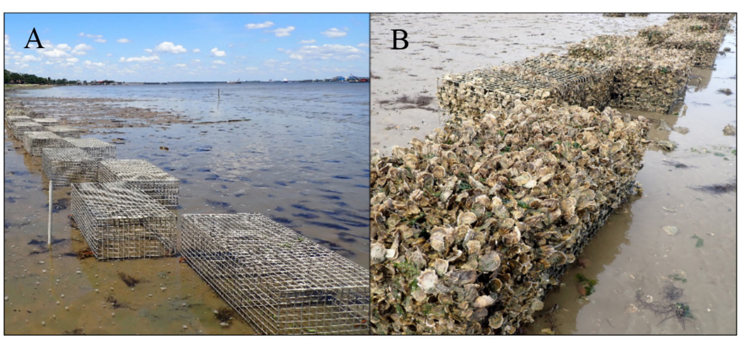

Figure 1. MWRs at Fort Johnson, Charleston Harbor, SC. (**A**) Immediately following installation in June 2020. (**B**) 11 months post-installation, illustrating the rapid development of reef habitat.

USDA PROCESS VERIFED PROGRAM – A MARKETING SOLUTION

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The USDA Process Verified Program (PVP) is a third-party verification service that offers agricultural companies a unique way to market their products to customers using clearly defined, implemented and transparent process points. An applicant's program may include one or more agricultural processes or portions of processes where self-described process points are supported by a documented management system, and independently verified by an Agricultural Marketing Service (AMS) auditor. Process points may include how a company conforms to various internal, industry, international or customer defined requirements. As such, process points must be verifiable, repeatable, feasible and factual and cannot be requirements of regulations or management system criteria.

Examples of claims verified under the USDA PVP include:

- Traceability
- Farm Raised and U.S. Source Verified
- No Antibiotics Ever and Responsible Antibiotic Use
- Animal Handling and Welfare

The backbone of USDA's PVP is the International Organization for Standardization's (ISO) guidelines for quality management systems auditing – an internationally-recognized set of guidelines used for evaluating program documentation and determining how on-site audits should be conducted. The guidelines are designed to ensure that a production system is operating within the parameters that a company has set for itself. AMS auditors undergo extensive training in ISO requirements and audit principles, as well as specific training for the standards they are auditing, which helps ensure the consistency, objectivity, and validity of our auditing services.

Applicants with an approved USDA Process Verified Program may develop promotional materials associated with their process verified points, use the USDA PVP shield in accordance with Program requirements and market themselves as "USDA Process Verified." To learn more about how the USDA PVP, visit the website at http://processverified.usda.gov.

USDA and AMS are committed to the transparency and accountability of our auditing services, so that consumers and buyers can make informed decisions about the products they purchase. We would love to help you find solutions to your marketing challenges through USDA's PVP. You can contact us at any time to discuss your auditing needs.

ASSESSING THE FEASIBILITY OF THE CULTURE OF CALIFORNIA CLAMS IN AN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEM

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The aquaculture industry faces many challenges to expansion, including maximizing yield in limited spaces, addressing environmental impacts, and developing culture protocols for new and emerging products. One solution to these challenges is Integrated Multi-Trophic Aquaculture (IMTA), where species with complementary ecosystem functions from different trophic levels are farmed in proximity to each other. We tested the performance of US west coast venus clams (*Chione* spp.) when suspended and grown out in the presence of seaweed (*Gracilaria* spp.and *Ulva* spp.), and both seaweed and the California sea cucumber (*Parastichopus californicus*). We ran a controlled aquarium experiment (March to August 2021) to understand underlying mechanisms, and a field experiment using a FLUPSY in San Diego Bay (June-Dec 2021; Fig. 1).

Over the 6-month aquarium experiment, clam mortality was highest in the clam-seaweed treatments compared to the others (Fig. 2), while growth rate (weight, length, height) did not differ between treatments (Fig. 3). Preliminary summer data from the bay growout trials reveal that mortality was also highest in the clam-seaweed treatment, lowest in the three species treatment, and the clam only treatment experienced the highest clam growth rates. The findings of this study will inform west coast clam aquaculture efforts which are of rapidly growing interest, and shed light on potential environmental drivers of observed patterns.

Fig. 1. Deploying experiments at the FLUPSY.

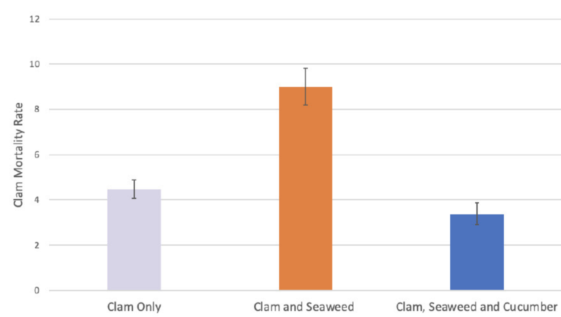

Fig. 2. Clam mortality rates by treatment in the controlled aquarium experiments.

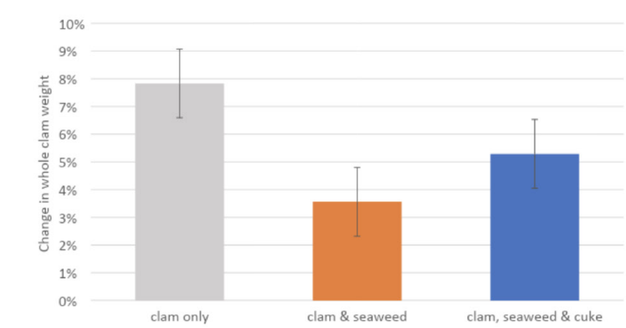

Fig. 3. Clam growth rates by treatment in the controlled aquarium experiments.

GENOMIC STUDY OF HISTAMINE RECEPTOR LIGAND BINDING SITES OF THE BIVALVE MOLLUSC *Crassostrea virginica*

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Histamine is a biogenic amine found in a wide variety of invertebrates. Histamine is particularly well studied in arthropods and gastropods where it is involved in local immune responses as well as regulating physiological functions in the gut. Histamine also functions as a neurotransmitter, especially for sensory systems. Previous physiology work of our lab found that histamine activates the sensory system of *Crassostraea virginica*, eliciting a motor response in the gill. Our earlier cell biology and immunofluorescence work also showed the presence of histamine receptors in ganglia and mantle of *C. virginica*. Recently the genome of *C. virginica* and other bivalves have begun to be mapped. By conducting BLAST searches of the NCBI (National Center for Biotechnology Information) database using DNA and protein sequences of *C. virginica* and other invertebrate and mammalian species we found matches for histamine receptor H1R genes on chromosome 8; H2R on chromosomes 1, 2, 5 and 10; and H3R on chromosome 3. BLASTS of other invertebrates and mammals found matches with very low Expect Values (E Values) and moderately high Percent Identity, signifying similarities of H1R, H2R and H3R of *C. virginica* to those of other bivalves, gastropods, insects, mice, rats and humans. We hypothesize that the ligand binding sites (LBS) for H1R, H2R and H3R receptors in *C. virginica* are evolutionarily conserved and will closely match those of other animals. To study this, we conducted searches of the NCBI database for H1R, H2R and H3R receptors LBS of *C. virginica* and compared them to other animals. We found the LBS for H2R in *C. virginica* was identified and match some other invertebrates well, but did not match humans of other mammals very well. The LBS for H3R matched some other bivalves, invertebrates as well as humans and other mammals well. The LBS for H1R in *C. virginica* and other invertebrates we looked at has not yet been identified. The LBS for H1R in humans and other mammals is very highly conserved. This study complements our earlier physiology and cell biology studies demonstrating the presence and function for histamine in *C. virginica*, and shows that the genome of *C. virginica* contains genes to produce histamine receptor LBS that are similar to those of other animals where it has been identified. This new information is valuable as it shows that the simple nervous system of histamine can be used to expand studies on histamine neurotransmission.

This work was supported in part by grant 2R25GM06003 of the Bridge Program of NIGMS, NIH grant K12GM093854-07A1 IRACDA Program of Rutgers University and PSC-CUNY grants 62344-00 50 and 63434-00 51.

IMPLEMENTING AQUAPONICS PROJECT-BASED INVESTIGATIONS (APBI) IN K-12 EXTENSION PROGRAMS

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Demonstration scale aquaponics systems can be implemented in K-12 aquaculture extension programming to increase student interest in agricultural concepts and promote comprehension of scientific phenomena. The interdisciplinary nature of aquaponics makes it a hands-on extension education tool that can be adapted to engage diverse populations of school-age learners and recruit underserved minority groups into science, technology, engineering and math (STEM) fields. Aquaponics project-based investigations (APBI) expose students from varying age groups and backgrounds to aquaculture, sustainable agriculture and nutritious eating behaviors. The complexity of growing plants and fish together provides tangible representation of aquatic ecosystem requirements and how they respond to biotic and abiotic factors. Aquaponics can also be used to teach students that all living systems have limits to the amount of biodiversity that they can support; this is the carrying capacity concept. The bacterial nitrification process is another scientific concept that can be highlighted in aquaponics extension curriculums. Program participants use STEM skills to disseminate fish and plant production data, perform water quality testing and calculate fish feeding rates. By allowing students to develop scientific inquiry through the use of classroom and extracurricular extension based aquaponics programs, they may also discover interest in STEM careers or agriculture extension focused areas like FFA, 4-H and family consumer sciences. Extension agents can utilize the information in this presentation to develop new programs, or complement existing ones, for youth of all ages.

UTILIZING FEED EFFECTORS AND PASSIVE ACOUSTIC MONITORING FOR SEMI-INTENSIVE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* PRODUCTION

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The pressures placed upon food security by human population growth will be compounded by the effects of continued environmentally unsustainable farming practices. The farming of highly efficient aquatic organisms such as Pacific white shrimp (*L. vannamei*) may provide a solution that accounts for both food security and environmental sustainability. Developments in shrimp farming management and technology such as better pond location and construction, passive acoustic monitoring (PAM), and plant-based protein diets are all contributing to a more efficient and sustainable industry. To further improve the efficiency of shrimp aquaculture, the use of feed effectors (chemoattractants, feeding incitants, and stimulants) has been suggested to increase the attractability and palatability of formulated diets. Our research trial aimed to expand upon previous shrimp research involving plant protein diets, feed effectors, and PAM in a laboratory setting, by conducting a feed trial in outdoor semi-intensive (30 shrimp/m^2) ponds. A 13-week trial was conducted in sixteen 0.1-hectare ponds equipped with PAM integrated, automatic feeders, which allowed for demand-style feeding. Four open formulation soy optimized diets, an "all plant" basal diet and three diets with an attractant (2% krill meal (Krill), 2% squid meal (Squid), 4% fish hydrolysate (Fish Hydro)), were fed to the shrimp for a period of 74-75 days. The growth and general health of shrimp were monitored via weekly sampling and final production values were determined after fully harvesting each pond. Harvest was delayed due to a hurricane, which in combination with multiple blue green algae blooms, led to shrimp mortalities. As a result of low survival, some ponds were excluded from the final data set. No statistically significant differences were found between the treatments for any major production parameter apart from feed input and feed cost (Table 1). Significantly more of the Fish Hydro diet was fed to the respective ponds than the All Plant diet, suggesting that the addition of fish hydrolysate to soy optimized diets increases the intensity of the feed response in Pacific white shrimp in semi-intensive pond culture. However, further research must be conducted to improve our understanding of the relationship between feed effectors and shrimp production aquaculture.

Table 1. Pacific White Shrimp response to four soy-based diets with varying attractants.

Treatment	g/week	Weight (g)	Feed Input (Kg/ha)	Feed Cost ($/ha)	Survival (%)	Yield (kg/ha)	FCR
All Plant[1]	1.41	19.40	6636.3[b]	8,732[b]	69.90	4044.6	1.65
Krill[2]	1.47	20.37	7556.1[ab]	10,845[ab]	77.38	4778.3	1.63
Squid[3]	1.53	20.99	8145[ab]	11,597[a]	76.56	4831.5	1.69
Fish Hydro[4]	1.50	20.42	9147.3[a]	12,333[a]	83.17	5155.5	1.79
P-value	0.7194	0.8128	0.0237	0.0143	0.5155	0.2139	0.9492
PSE[5]	0.0612	0.9099	329.142	451.64	4.9987	286.971	0.1552

[1]n=4
[2]n=3
[3]n=1
[4]n=2
[5]PSE: Pooled Standard Error

SEAFOOD PERCEPTIONS AND PURCHASING: A FOOD SERVICE INDUSTRY PANEL

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***NOTE: This session will be 90 minutes long.**

Culinary professionals, seafood purchasers, and others involved in the seafood service sector play a key decision-making role in the aquaculture industry. With the potential to impact seafood demand through direct purchasing as well as consumer guidance, engaging with these influential industry members to understand perspectives and the factors that shape purchasing in this sector is critical to advancing US aquaculture.

In this panel discussion, members and affiliates of the seafood service industry – including chefs, wholesalers, and seafood writers – are invited to share their perspective in response to the research project used to introduce this session as well as contribute to a broader discussion of the potential to enhance US aquaculture purchasing.

EFFECT OF WAVE ACTION, BIOFOULING CONTROL, AND DENSITY ON THE PERFORMANCE OF EASTERN OYSTERS (*Crassostrea virginica*)

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To better understand the impacts of wave action on oyster performance and its interaction with other farming choices, triploid oysters were deployed utilizing the adjustable longline system at an oyster farm site near Deer Island, Mississippi characterized by frequent wave action. A full factorial experiment consisting of two wave action (natural, dampened) x three biofouling control (desiccated, power washed, submerged) x two stocking density (low, high) treatments was deployed in 96 baskets (n = 8 bags/treatment) at this site in a fully randomized design July 2020 (with oysters with a mean shell height of 28.6 mm) for a total of ~7 months with regular tending and periodic sampling throughout.

Natural wave action generally increased production quality metrics (cup ratio, condition, cleanliness) relative to dampened wave action treatments. Conversely, dampened wave action generally increased production quantity metrics (shell height, whole wet weight) relative to natural wave action treatments. Based on the results of this study, a farmer seeking to maximize product quantity in a lower wave action environment might employ submerged, lower stocking density treatments, while a farmer in a higher wave action environment might employ power washing, higher stocking density treatments. For increased product quality (regardless of wave action), weekly desiccation and lower stocking densities produced the highest quality. In the dampened wave action treatments, the weekly desiccated, higher stocking density treatment seemed to produce the best combination of quantity and quality. In the natural wave action treatments, the submerged, higher stocking density treatment seemed to produce the best combination of quantity and quality. This suggests that wave action may be a feature that farmers may be able to take advantage of in the production cycle.

COMMERCIALIZATION OF AQUADVANTAGE SALMON, A GENETICALLY ENGINEERED LINE OF ATLANTIC SALMON *Salmo salar*

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AquaBounty is now rearing and selling its genetically modified salmon (AquAdvantage Salmon or AAS) in the United States and Canada. Fish produced at the AquaBounty farm in Panama were previously sold in Canada and were accepted by the market with limited anti-GM comments. In preparation for introducing AAS to the U.S. market, AquaBounty conducted consumer research that included focus groups and online surveys. Most respondents expressed a willingness to try the product and the company found the most positive reactions were triggered by information on the sustainability of the product. Concerns about the safety of the product were not a significant factor in forming consumer opinion. Details of the research and the findings will be presented.

Following on the consumer research, AquaBounty has also worked closely with the seafood industry and potential customers to provide information on the product, including evidence of health and safety, product quality, and the benefits of producing Atlantic salmon in recirculating, land-based systems. The company is providing its customers with product information and training on best practices for responding to questions and concerns about AquAdvantage salmon. Examples and feedback from customer interactions will be discussed.

To ensure long-term acceptance of AquAdvantage salmon and the next generation of gene edited products that will emerge, AquaBounty has launched a program with leaders in the field of communication and media relations to counter misinformation and false narratives about our company and our product specifically, and about biotechnology in aquaculture more broadly. The objectives of the project and a description of the process will be provided.

PREDICTING LARVAL DISPERSAL AND POPULATION CONNECTIVITY OF SEA SCALLOPS *Placopecten magellanicus* IN COASTAL MAINE THROUGH COMPUTER MODELING AND POPULATION GENOMICS

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This research combines computer modeling and population genomics approaches to estimate sea scallop (*Placopecten magellanicus*) larval dispersal along the eastern Maine coast and to predict the source and sink populations of larvae. We are modifying an individual-based hydrodynamic computer model of the coastal currents from the Bay of Fundy westward through the Gulf of Maine to include larval development and behaviors, such as growth and mortality rates, swimming speeds, and vertical migration patterns. These additions to the model will ensure that the larvae behave as active rather than passive particles.

Larvae are released from three sites, the Bay of Fundy, the eastern Gulf of Maine, and George's Bank. Within each site, there are six adult beds. A similar model examined dispersal of blue mussel larvae in the same region and found that sites closer to the Bay of Fundy were more likely to house source populations, while sites to the west acted more as sink populations. Although scallop larvae may behave differently from blue mussels, our initial hypothesis is that the same coastal currents would disperse scallop larvae from eastern populations to western populations. In preliminary model runs, larvae from beds in the Bay of Fundy dispersed westward but did not reach the Gulf of Maine within the expected 30-40 day larval duration. Larvae from several of these beds appeared to get caught in eddies and be retained within the Bay of Fundy, preventing further westward transport. Thus, the Bay of Fundy populations may experience greater larval retention.

Model predictions will later be tested using genomic analyses and assignment tests to compare the degree of genetic similarity among predicted source sites and settlement sites. This will increase the number of scallop populations that have been genotyped, provide insight into the population connectivity and genetic diversity of Maine's wild scallops, and allow for the identification of source populations that could be important in sustaining both the wild fishery and seed sources for scallop aquaculture.

ACUTE TOXICITY OF AFLATOXIN B_1 IN THE LARGEMOUTH BASS *Micropterus salmoides*

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Relatively high mortality rates are common in farmed largemouth bass (LMB). These mortalities are associated with chronic anemia and pale necrotic livers. Carbohydrate sensitivity has been the suspected problem as cereal grains are added to feed formulations at >15% to produce floating feed. However alternative explanations should also be considered. Mycotoxins are natural poisons produced by fungi that contaminate cereal grain products prior to harvest and/or during post-harvest storage. Aflatoxin B_1 (AFB_1) is a mycotoxin produced by *Aspergillus sp.* fungi and is one of the most toxic naturally occurring substances. Many of the symptoms of liver disease in LMB are similar to those produced mycotoxicosis in some fish species. To evaluate the susceptibility of the LMB to mycotoxins and determine if some of the health issues in commercial LMB production might be attributable to mycotoxins, researchers at Kentucky State University conducted a trial where increasing concentrations of AFB_1 were fed to LMB.

The diet concentrations were 0 (control), 0.25, 2.5, 5 and 10 mg AFB_1/kg diet. Basal diets contained 40% fish meal, 25% soybean meal, 21% wheat flour and 10% fish oil. Juvenile LMB (3.6g) were randomly stocked at a rate of 15 fish per aquarium into 20 110-L acrylic aquaria. Each of the five diets were randomly assigned to four replicate aquaria. Fish were fed once daily to apparent satiation for 9 weeks. At harvest, all fish from each aquarium were individually weighed and measured (total length). Livers from three fish from each aquarium were weighted and preserved in 10% formalin for histological analysis.

Fish fed the highest concentration of 10 mg AFB_1/kg diet exhibited a poor feed response and higher ($P<0.05$) feed conversion ratios (3.3) than fish fed the other diets, which were similar, averaging 1.2 overall. Survival was reduced in fish fed the 10 mg AFB_1/kg diet (68%) compared to fish fed the other diets, which were similar and averaged 98%. Average weights were similar for fish fed the control diet (26.5g) and the 0.25 mg AFB_1/kg diet (24.5g). Average weights showed a significant incremental reduction as the AFB_1 concentrations increased from 2.5 (15.2g), to 5.0 (9.7g) and then 10 (5.5g) mg AFB_1/kg diet. Hepato-Somatic Index (HSI) was significantly higher in fish fed the control diet (2.2) than for fish fed diets containing AFB_1 which were similar and averaged 1.1 overall. Low HSI values can indicate toxicity, liver disease, or septicemia. Though fish fed the lowest concentration of 0.25 mg AFB_1/kg diet exhibited similar growth to the control diet, HSI values may indicate underlying mycotoxicosis suggesting that LMB may be very sensitive to AFB_1.

MICROPLASTICS AND MOLLUSCS – A BRIEF OVERVIEW OF A FLAWED LITERATURE

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Microplastics are a contaminant of global concern and, as such, there has been a rush to action and publication. Over the past decade, this haste has resulted in a chaotic and cluttered literature that is rife with inappropriate methodologies, unrealistic experimental protocols, misinterpreted results, and overstated significance. A comprehensive critical assessment of the current literature on interactions between particle-feeding molluscs and microplastics and their purported impacts (> 600 publications) is underway. It is not surprising that microplastics have been noted in shellfish guts globally. What is surprising is the extremely low level of particles routinely recorded. The data to date clearly demonstrate low numbers of microplastics in bivalve molluscs globally. There are no unequivocal data demonstrating that their presence in filter-feeding bivalve molluscs is a serious risk to human health and few data to demonstrate negative impacts on the shellfish at environmentally relevant concentrations. Many studies on suspension-feeding bivalve molluscs and other invertebrates are weak. There is a recurring presence in the published literature of misunderstanding of the feeding processes, capabilities for particle selection and rejection, and species-specific differences that all leads to misinformation, misinterpretation, and incorrect assumptions regarding potential impacts. There are major shortcomings to many laboratory studies that examined uptake and accumulation of microplastics by bivalves and their subsequent effects. In most cases, the issues can be traced to poor experimental procedures and animal husbandry, and lack of knowledge of the literature. They are compounded by a misunderstanding of the basic biology and physiology of molluscs. The shortcomings have led to a seriously flawed literature based upon the interactions and impacts of microplastic on these animals. Bivalves and other particle-feeding molluscs are complex living organisms with extraordinary capabilities for the control of selective capture, ingestion, and egestion of particulate material. They should be recognized and treated as such in any attempt to describe impacts of stressors, including different particle types, on their feeding and ability to accumulate materials. Any future experimental studies need to be focused carefully, based upon clear questions, use standardized analytical procedures, and demonstrate a knowledge of the animals being studied and the extant literature. The hype needs to be curtailed and scientists should not imply impacts or potential impacts when there are no data to support the suppositions. Editors of scientific journals must make a stronger effort to engage qualified peer-reviewers and stop the flow of poorly done studies and superficial reviews that do nothing more than confuse the literature and reinforce prior inadequate studies and potential reviews.

EVIDENCE OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS VIRULENCE EVOLUTION AFTER A HOST JUMP INTO RAINBOW TROUT AQUACULTURE, AND POSSIBLE MANAGEMENT STRATEGIES

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Pathogens often make host jumps and subsequently evolve in new systems, as exemplified by the COVID-19 pandemic. A long standing question is will pathogens evolve increased or decreased virulence (host morbidity and mortality) as they adapt to new hosts. Answering this question is an important first step in estimating the threat emergent pathogens pose, and adequately directing resources to manage them. Aquaculture is a particularly unique environment for emergent pathogens, with various aquaculture practices predicted to drive virulence evolution in different directions. However, most of these predictions are based solely on theoretical work, with limited experimental studies. We investigated the virulence evolution trajectory of infectious hematopoietic necrosis virus (IHNV) after it made a host jump into rainbow trout (*Oncorhynchus mykiss*) aquaculture from sockeye salmon (*Oncorhynchus nerka*) in the 1960-70's. We used a collection of 1000's of viral isolates spanning 50 years from the time of the host jump to present day, and *in vivo* common garden experiments in both the novel and ancestral host, to assess the change in viral isolate virulence through time. We found that IHNV dramatically increased virulence in the novel host (rainbow trout) at the time of the host jump and has continued to gradually increase virulence through time (Fig. 1). An initial drop in virulence was also observed in the ancestral host (sockeye salmon) at the time of the host jump, but evidence of subsequent virulence evolution in the ancestral was not observed. Our findings suggest that emergent pathogens may evolve increased virulence in aquaculture. We discuss how various aquaculture practices might drive virulence evolution and offer possible management solutions.

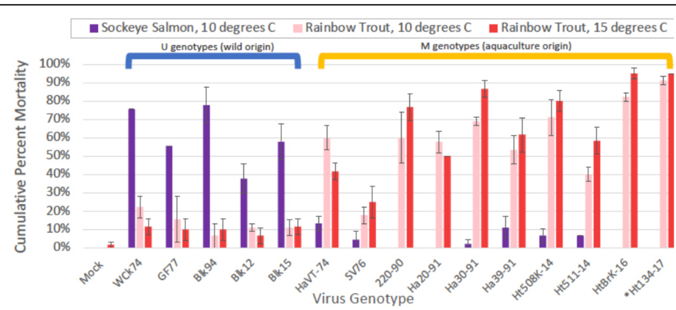

Fig. 1. Virulence of evolved IHNV isolates. Bars show cumulative percent mortality (+/- 1 SE) of triplicate groups of 20 fish exposed to various IHNV field isolates (genotypes). Isolates are arranged from oldest to newest, with year of isolation provided at end of isolate name (1974-2017). U clade isolates represent those evolved in ancestral host sockeye salmon. M clade isolates represent those that made the host jump and evolved in rainbow trout (novel host). Experiments were conducted in sockeye salmon (purple bars) at one temperature and rainbow trout (red bars) at two temperatures. The virus lost virulence in the ancestral host but gained virulence in the novel host at the time of the host jump (U to M clade evolution). There is also evidence that IHNV has continued to increase in virulence as it has adapted to the novel host (M clade through time in rainbow trout).

BUSINESS AND ECONOMIC PLANNING FOR SEAWEED AQUACULTURE SYSTEMS IN THE UNITED STATES

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This presentation will provide an overview of a new project funded under the NOAA Sea Grant funding opportunity titled, "Addressing Economics and Market Needs of the U.S. Aquaculture Industry." Kelp, *Saccharina spp*. are the most common seaweed species cultivated in the United States. Kelp farming, as well as the farming of various other seaweeds, is a significant and growing industry in the US as seaweeds, especially kelp, can be used for food, medicinal products, additives and bioremediation. With any new industry, barriers to its development and expansion always emerge. One of the greatest barriers is the lack of economic/financial information on the cultivation of domestic kelp. There is a need to better understand the realistic economic and financial parameters associated with kelp aquaculture in order for farmers, investors and lenders to make more informed decisions regarding investment in this type of venture.

The goal of this project is to support the development of a vibrant, profitable, and sustainable seaweed aquaculture industry in the United States. 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. Anticipated outcomes include more access to capital, more informed business decisions by farmers, investors and lenders; increased employment; greater success of business; and environmental improvements.

SHRIMP SCAMPI: A CITIZENS SCIENCE PROJECT TO EXAMINE THE LEVELS OF ENDOCRINE DISRUPTING CHEMICALS (METALS, GLYPHOSATE, BISPHENOL A) IN FROZEN SEAFOOD SOLD AT US SUPERMARKETS

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The Shrimp Scampi Project is part of the OHEEI of Environmental Genomics Inc. It was initiated by 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 (G) and other endocrine disrupting chemicals (EDCs) like metals and Bisphenol A (BPA). These EDCs are associated with antimicrobial resistance (AMR), obesity, diabetes, non-Hodgkin's lymphoma, non-alcoholic fatty liver disease, neurodevelopment, congenital malformations, neural tube defects (NTDs) and autism. Exposure to these EDCs cause changes in epigenetic mechanisms regulating gene expression: DNA methylation, histone modifications, and non-coding RNAs.

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 (WSSV, IHHNV) and metals have been detected in frozen shrimp sold at MA supermarkets, no official compulsory testing of contaminants in imported seafood is performed. The goals of Shrimp Scampi project are to (a) perform an in-depth review of the scientific literature about epigenetic mechanisms associated with exposure to EDCs like Glyphosate (G), metals, and BPA, and (b) detect the levels of these EDCs in frozen shrimp sold at US supermarkets.

The effects of Glyphosate (G) alone and 14 of its formulations in plant and human cells were reviewed. Petroleum-based compounds in herbicides were highly more toxic than G. Arsenic, chromium, cobalt, lead and nickel were found in the pesticide formulations.

Preliminary results on the concentrations of metals and G in frozen shrimp sold at MA supermarkets will be presented. All three epigenetic mechanisms have been reported associated with exposure of shellfish, fish and people to metals, G and BPA. Methylation changes are associated with AMR (G); obesity, diabetes and cancer (BPA, G) and exposure of animals to G and BPA during development.

Increased monitoring of EDCs in imported shrimp is recommended. Research is needed to understand transgenerational epigenetic inheritance associated with reproductive toxicity after exposure of shellfish and fish to EDCs.

DISTRIBUTION, ABUNDANCE, AND CONNECTIVITY: A COLLABORATION TO MAP OYSTERS FROM BAJA CALIFORNIA TO BRITISH COLUMBIA

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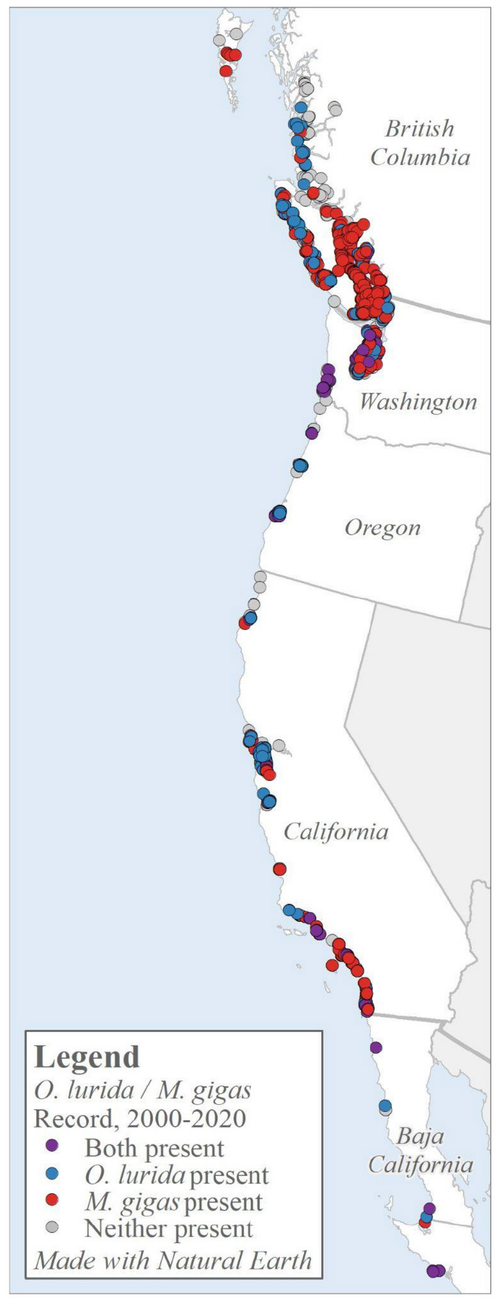
To conserve coastal foundation species, it is essential to understand patterns of distribution and abundance and how they change over time. We synthesized oyster distribution data across the west coast of North America to improve conservation strategies for the native Olympia oyster (*Ostrea lurida*), and also gathered information on feral populations of the non-native Pacific oyster (*Magallana gigas*), the only widespread non-native oyster and a notorious global invader, occurring within this range.

We designed a user-friendly portal for data entry into ArcGIS Online and collected oyster records from unpublished data submitted by oyster experts and entered by interns from the published literature, while self-quarantining during the early phase of the COVID-19 pandemic. The resulting database contained over 2,000 records, which we used to examine spatial and temporal patterns and have made publicly available via an interactive web-based map.

Comparing records from pre-2000 vs. post-2000, we found that *O. lurida* significantly decreased in abundance and distribution, while *M. gigas* increased significantly. Currently the distribution and abundance of the two species are fairly similar, despite one species being endemic to this region since the Pleistocene, and the other a new introduction. We mapped the networks of sites occupied by oysters based on estimates of larval dispersal distance, and found that these networks were larger in Canada, Washington, and southern California than in other regions. We recommend restoration to enhance *O. lurida* network size where it is small, and increase abundance where it declined. We also recommend approaches that restore natural biogenic beds to mudflats and sandflats especially in the southern range, where native oysters are currently found most often on rip rap and other anthropogenic structures.



THE NATIVE OLYMPIA OYSTER COLLABORATIVE: A COMMUNITY OF PRACTICE FOR *Ostrea lurida* CONSERVATION

Kerstin Wasson*, April Ridlon, Edwin D. Grosholz, Kay McGraw, Betsy Peabody, Jennifer Ruesink, Chela Zabin, and Danielle C. Zacherl

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The Native Olympia Oyster Collaborative (NOOC) is a network of oyster scientists, practitioners, educators and aquaculturists who learn from each other and share scientific, management and educational resources.

Our vision is: *Resilient native oyster populations in a network of bays and estuaries from British Columbia to Baja California, valued by people and forming an integral part of healthy coastal ecosystems.*

Over the past years, NOOC has brought together oyster stakeholders and together, we are strengthening and connecting many local efforts to conserve and restore Olympia oysters. We created a NOOC website that provides resources for science, education and management. We also maintain a Zotero database with Olympia oyster and related references.

We compiled information on all past restoration projects of this species, and made this available in a publication and ArcGIS Story Map. We evaluated the risks and rewards of conservation aquaculture in another recent publication. We mapped the distribution of oysters from Baja California to British Columbia. These collaborative efforts, building on local knowledge and expertise, complement place-based conservation for Olympia oysters and allow for range-wide strategic planning.

NOOC accomplishments were made possible due to contributions from many oyster stakeholders along the coast and with partnership and support from the NERR Science Collaborative, Pew Charitable Trusts, and the Science for Nature and People Partnership.

Interested in joining NOOC and receiving quarterly Olympia oyster updates? Email olympiaoysters@gmail.com.

INITIAL EVALUATION OF CRAFT BREWERY SPENT GRAINS AS A POTENTIAL FEED INGREDIENT FOR RED DRUM, *Sciaenops ocellatus*

Dr. Aaron M. Watson*, Dr. Fabio Casu, Justin Yost, David Klett, Molly Milstein, Sara McConnell, Scott McConnell, Chris Brown, Andy Elliot

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As global and domestic aquaculture production continues to increase, identifying and evaluating alternative protein and lipid sources continue to be high priorities for feed manufacturers. Brewery by-products, such as spent grain material, represent a potential source of nutrition. Notably, craft breweries which have increased in popularity and production volumes over the last decade, represent a potentially unique source. While most individual craft breweries may not produce enough by-product material to be of interest to commercial scale utilization, the combination of brewery by-product between breweries or within regions may represent a significant volume of ingredient if their nutritional profiles are similar and acceptable.

This project was undertaken to evaluate the nutritional profile, digestibility, and tolerance of craft brewery spent grains as a potential feed ingredient for red drum. The South Carolina Department of Natural Resources has partnered with Tradesman Brewing Company, Holy City Brewing, and Low Tide Brewing to collect monthly samples from each brewery over the course of a year to evaluate if and how the gross nutritional profile of spent grains changes over time and between breweries. Proximate composition (Figure 1), amino acid profile, fatty acid profile, and Nuclear Magnetic Resonance (NMR) metabolomics (Figure 2) have all been employed in developing the nutritional profiles. Additionally, digestibility diets have been formulated for each individual brewery's material and a digestibility trial has been conducted.

Figure 2. Principle component analysis of monthly material over the course of 1 year from three partner breweries (Avg. ± S.D.).

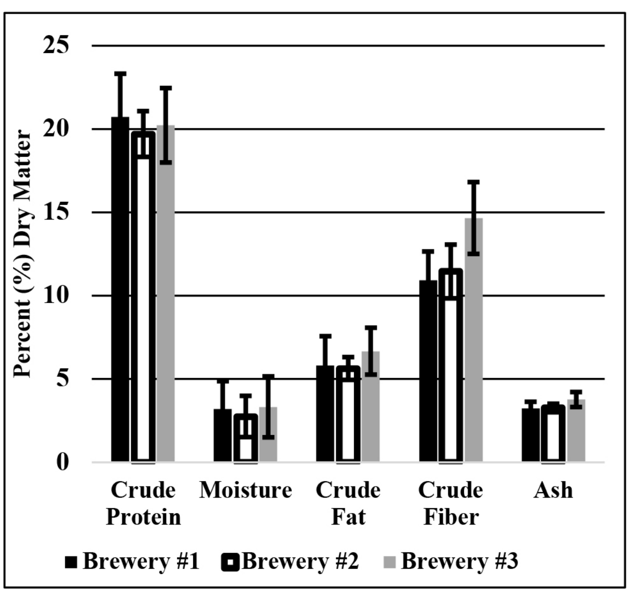
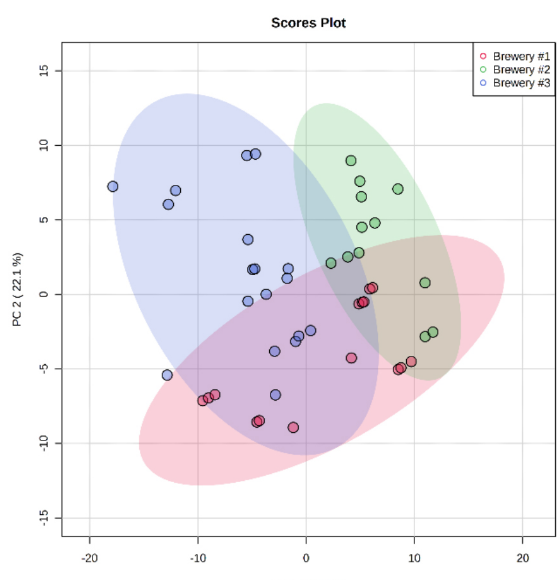


Figure 1. Proximate compositions of brewery by-product Samples from three craft breweries. Dots represent Individual sample metabolite profiles.



MATERNAL TRANSCRIPT PROFILES ASSOCIATED WITH EGG VIABILITY IN RAINBOW TROUT, *Oncorhynchus mykiss,* AND COMPARISONS AMONG POPULATIONS

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Markers that can serve as predictors of egg quality and for diagnosing problems with embryo production would benefit fish hatcheries. Since 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 associate 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, among three populations. Transcript identified as DEGs from unfertilized eggs of different quality based on eyeing rate were compared among two year classes of the same line (A1, A2) and a population from a different hatchery (B) using an assay based on the nCounter analysis data system (Nanostrings Technologies; Seattle, WA). Most of the genes in the assay, 54, are DEGs from a transcriptome analysis of egg viability using RNA-Seq and fish from Group A1.

A total of 32 transcripts were identified as DEGs among the three groups by regression analysis. As could be expected, Group A1 had the most DEGs, 26. Group A2 had 15 DEGs and 14 were shared with A1. Group B which included fish from a different population than the fish from which most of the transcripts were first identified as potential markers of egg quality, had the least DEGs, 12. Seven of the 12 DEGs in Group B overlapped with A1 or A2, and six of these transcripts, *dcaf11*, *impa2*, *mrpl39_like*, *senp7*, *tfip11* and *uchl1*, were found in all three groups.

Our results indicate DEGs for egg quality can overlap among populations of rainbow trout and therefore maternal transcripts found to be differentially expressed between low- and high-quality eggs in one population can be of value for use in other populations, at least when using the same criteria for egg quality. 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 suggests 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.

UNDERSTANDING CONSUMER ACCEPTANCE OF AQUAPONIC FISH: A MIXED-METHOD APPROACH

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Despite the ecological promise of aquaponics, relatively little is known about the impact of this novel production method on fish composition, sensory properties, and consumer acceptance. In this research, we offered a unique, interdisciplinary perspective to examine the market potential of aquaponics by conducting a series of multidisciplinary studies to compare yellow perch (*Perca flavescens*) from a combined Recirculating Integrated Multitrophic Aquaculture System (RIMTAS) with fish from traditional production methods (i.e., wild-caught and farm-raised).

Our quality parameter and macronutrient analyses showed that aquaponic perch were comparable to their wild-caught and farm-raised counterparts in texture, moisture content, total fat, and total protein. We also demonstrated that aquaponic perch were as liked as wild-caught perch in a consumer sensory evaluation (Table 1). Furthermore, in a consumer perception and acceptance study (Table 2), we found that providing information about the environmental benefits of aquaponics significantly increased consumer tastiness perception, healthiness perception, and purchase intention to a level at or exceeding that of wild-caught perch. With proper messaging strategies, aquaponic fish can compete in the market with wild-caught fish.

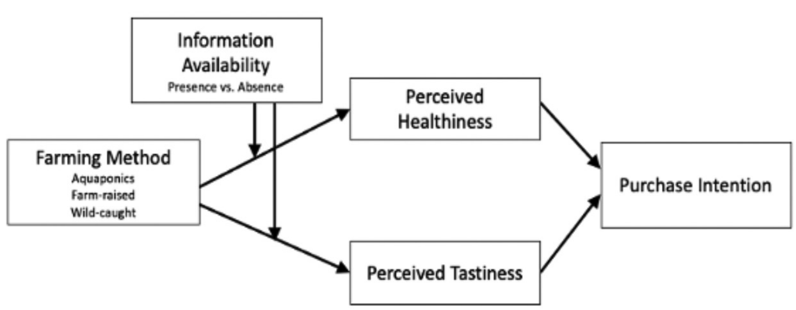
Our findings offer insights to stakeholders in the aquaculture value chain as they explore and establish RIMTAS aquaponics as an environmentally and economically sustainable seafood production source.

Table 1: Liking of Aquaponic vs. Wild-caught Yellow Perch (*Perca flavescens*)

Test	Sample size	Aquaponics	Wild-caught	*p* value
Overall liking	63	6.75 ± 1.65	6.30 ± 1.65	.133
Taste liking	63	6.57 ± 1.60	6.17 ± 1.56	.162
Texture liking	63	6.19 ± 2.09	6.00 ± 1.88	.591
Appearance liking	63	5.76 ± 1.97	5.59 ± 1.80	.605

All outcomes were rated on a 9-point hedonic scale from 1 (dislike extremely) to 9 (like extremely).
Means ± standard deviations are displayed.

Table 2: Consumer perception and acceptance study



IMPROVING PLANT QUALITY AND YIELDS: AQUAPONICS FROM A HORTICULTURAL PERSPECTIVE

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Aquaponics systems are combinations of aquaculture and horticulture sub-systems. Importantly, the aquaculture sub-system is always primary in aquaponics. Aquaculture effluent from the primary sub-system can be diverted to horticulture sub-systems and repurposed to maximize water and nutrient values and to process waste. It is then of utmost importance that the horticulture sub-systems are managed properly to maximize profitability. Among the possible barriers to success in aquaponics are relatively poor plant health and low plant yields, especially when compared to hydroponic systems. Inherent differences between hydroponic nutrient solutions and aquaculture effluent necessitate different approaches to plant production between hydroponics and aquaponics. Horticulture systems should be designed to provide optimizable environments for plant species in any given aquaponics system. In this presentation, we summarize approaches to maximize vegetable yields and quality in controlled environments, recent research on aquaponics, and highlight work from our lab in which we have improved plant quality and yields, hence potential profitability, in aquaponics systems.

STOCK ENHANCEMENT EFFORTS OF SOUTHERN FLOUNDER *Paralichthys lethostigma* IN ALABAMA COASTAL WATERS

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In Coastal Alabama, and throughout the Gulf of Mexico, Southern Flounder (*Paralichthys lethostigma*) is and has been a popular recreational and commercial species. Recent commercial and recreational landings indicate lower abundance of Southern Flounder in Alabama coastal waters – an observation also common in anecdotal reports from fishermen. A stock assessment on of the species was performed in 2018. The results indicated a decline in overall abundance due to an extended period of low recruitment. In addition, the results showed the stock is was not currently overfished, but experiencing overfishing which would result in an overfished condition in the future if management changes were not implemented.

One of the suggested tools for improving management of Southern Flounder was use of a stock enhancement program. The Alabama Department of Conservation and Natural Resources, Marine Resources Division maintains the Claude Peteet Mariculture Center (CPMC) located in Gulf Shores, Alabama. The CPMC facilities include a 23,000-square foot building containing rooms for broodstock maturation and spawning, algae and live food production, egg incubation, larval rearing, and juvenile holding. Complementary infrastructure includes thirty-five - 0.2-acre PVC lined ponds, a greenhouse complex containing re-circulating tank systems, and two seawater pipelines (brackish and full-strength sea water).

Southern Flounder broodstock were acquired locally beginning in 2018 and held in temperature and photoperiod-controlled tanks at CPMC. Utilizing Ovaplant®, an experimental hormone for this species, an estimated 12,236 fingerlings were released to Alabama inshore waters in 2020, and 34,591 fingerlings were released in 2021. In 2021, investigations began at CPMC attempting to identify and refine cryopreservation techniques for Southern Flounder milt to improve fertilization success and gene diversity.

TEMPORAL CHANGES IN *Vibrio parahaemolyticus* POPULATIONS LINKED TO CHANGING CLIMATE AND AQUACULTURE IN NEW HAMPSHIRE

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The Northeast US experienced a rise of *Vibrio parahaemolyticus* infections that contrasts with historical trends. Increasing ocean temperatures and rapid expansion of oyster aquaculture in the region undoubtably played a role in these epidemiological changes. But without the introduction and establishment of a Pacific lineage of *V. parahaemolyticus* sequence type (ST) 36 and expansion of an Atlantic endemic lineage ST631 in the region, disease incidence would not have risen so dramatically. Both strains resided in the Gulf of Maine for decades without causing an increase in infection suggesting ecological changes perhaps linked with aquaculture practices could have shifted population dynamics in favor of these pathogens. Though the Great Bay Estuary (GBE) of New Hampshire lies just to the north of aquaculture areas associated with both strains of regional concern, no local-source infections by these pathogenic strains have yet occurred. A temporal analysis of strains from archived and ongoing samples from the GBE reveals an alarming shift in the prevalence of non-native strains harboring genetic markers (hemolysins *tdh* and *trh*) associated with pathogens. Whereas between 2007-14 hemolysin-harboring strains were rarely detected, they are now routinely detected and cultured from the environment. Though increasing levels of potentially pathogenic strains were first detected in commercial oysters and later increased in wild oysters, disease incidence remains unchanged. The geographic location of the GBE at the margin of pathogen expansion in the Northeast and documentation of these changes highlights that further study of this population could provide valuable insight into the ecology of pathogen invasion.

CREATING MULTI-REGIONAL TRIBALLY LED COMMUNITY PARTNERSHIPS TO MONITOR HARMFUL ALGAL BLOOMS AND SHELLFISH TOXINS

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Native American Tribes and First Nations in the Alaska and British Columbia have a vested interest in protecting traditional natural resources as well as the health of the local community. Regional Tribal networks linked with support from scientist and resource managers have begun detecting harmful algal bloom (HAB) events that pose a human health risk to subsistence and commercial shellfish harvesters. With "eyes on the water" within their communities, Tribes can establish subsistence management plans, integrate aquaculture infrastructure, and continue the cultural importance of shellfish harvesting. Within each regional network, Tribal partners collects weekly samples at key community harvest sites including phytoplankton identification and quantification, salinity, sea and air temperature, whole water for cellular toxin analysis, and shellfish for biotoxins. The data are uploaded to a shared database and are used by resource managers and subsistence harvesters to make informed decisions on harvest timing and risk. Tribal laboratories within each region provide analytical and technical support to each community within the network. By combining weekly phytoplankton observations and shellfish toxin analysis using approved methods developed by NCCOS, Tribal organizations are demonstrating sovereignty through science.

DEVELOPING A SKILLED WORKFORCE FOR ADVANCING RAS THROUGH SUPPORT FROM A NATIONAL COLLABORATIVE EFFORT UTILIZING STRONG INDUSTRY ENGAGEMENT

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The National Sea Grant College Program funded a collaborative project to build capacity of land based Atlantic salmon aquaculture in the U.S. One of the strongest features of this collaborative effort was the immense national partnerships that were involved, including research, education, extension and industry partners from Mid-Atlantic, Great Lakes and Northeast regions of the U.S. Along with the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility (UWSP NADF), other partners included Maryland, Maine and Wisconsin Sea Grant programs, University of Maryland Baltimore County, Institute of Marine and Envoronmenal Technology, The Conservation Fund's Freshwater Institute, USDA National Cold Water Marine Aquaculture Center, Patuxent Environmental and Aquatic Research Lab, and Morgan State University, among others. Industry partners include Superior Fresh, LLC. (WI), Whole Oceans, LLC. (ME), American Salmon (MD), Kennebec River Biosciences (ME), and Riverence, LLC. (WA).One of the main objectives of this project was to gather stakeholder input which included guidance, concerns, ideas and other input regarding industry needs, thoughts on extension, outreach approaches, workforce development, optimal use of available federal/state funding, and other topics.

One of the major bottlenecks identified by industry was access to a skilled workforce, educated in RAS and salmon culture. The UWSP NADF has been successful in its workforce development program, training students in best management practices, rearing techniques and standard operating procedures for various species at all life stages in various systems including incubation, larval and grow-out systems. Specifically this program included technical demonstration tours and workshops, apprenticeships, internships, college level courses and technology transfer initiatives. The success of this program was shown in nearly 100% job placement for students that have undergone this training program.

Due to this success, the UWSP NADF is looking to advance its workforce development program. Over the past several years, the facility has incorporated several new initiatives to help reach a greater audence and increase technology transfer, which includes virtual presentation tours, educational and technical videos, and increased collaboration with industry partners to better the facility's apprenticeship program. This presentation will showcase UWSP NADF's current approaches to increase development of a skilled and educated workforce specific to RAS and salmon, as well as describe future initiatives for advancement and collaboration.

ZOOPLANKTON AS AN ALTERNATIVE METHOD FOR CONTROLLING PHYTOPLANKTON IN CATFISH POND AQUACULTURE

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In pond aquaculture, production of toxins and off-flavor compounds by cyanobacteria can negatively affect fish health and production. Studies have explored chemical or physical methods for controlling algal blooms in aquaculture ponds, which although effective, may be short-lived and can negatively impact non-target organisms, including aquaculture species. Foodweb manipulations have a long history in lake and fisheries management to improve water quality, but have been rarely considered in aquaculture. This study examined zooplankton and phytoplankton communities, cyanobacterial toxins, and nutrients, in nine catfish aquaculture farm-ponds in west Alabama, USA, from whole water samples. The goal of this project was to track phytoplankton and zooplankton abundances with respect to each other, before and after efforts to reduce planktivorous fish in some of the ponds.

During this project, farm managers reduced planktivorous fish abundance in select ponds to create a large-scale field experiment that addressed the role of zooplankton control of phytoplankton in hypereutrophic catfish aquaculture ponds when planktivorous fish were or were not excluded. There was a strong negative effect of zooplankton on phytoplankton, including cyanobacteria, despite high nutrient concentrations. Although high zooplankton ponds sustained elevated zooplankton biomass during much of this study (Figure 1A), including when pond temperatures exceeded 30 ºC, the effect of zooplankton on phytoplankton was most pronounced during the non-growing season (November to April; Figure 1B). In addition, total ammonia nitrogen was significantly higher in high zooplankton ponds, which could lead to ammonia toxicity in fish at elevated temperature and pH. Our findings suggest that zooplankton biomanipulation may be an efficient method to control algal blooms in farm-pond catfish aquaculture.

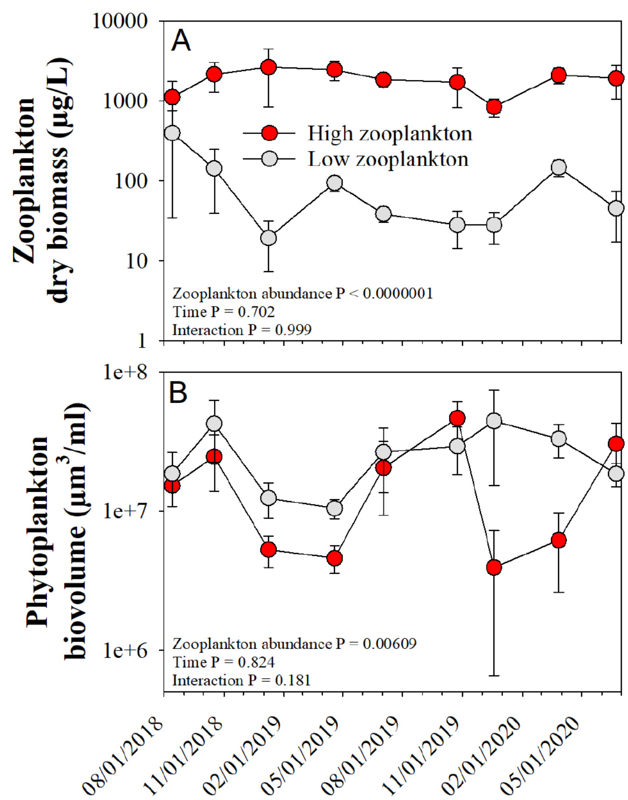

Figure 1. Mean quarterly (A) zooplankton dry biomass (µg/L) and (B) total phytoplankton biovolume (μm^3/ml) for ponds with high (red) or low (grey) zooplankton biomass. Error bars represent one standard error.

ALASKA AQUACULTURE PERMITTING PORTAL – BRINGING IT ALL TOGETHER

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Alaskans' interest in shellfish and seaweed farming is growing. Both the Alaska Mariculture Development Plan in 2018, and the NOAA Fisheries Alaska Mariculture Workshop Summary Report in 2020, identified the need for a central clearing house of aquaculture permitting information and an online permitting portal to aid aquaculture applicants. This nascent industry often found the permitting process confusing and cumbersome. Farmers are required to file multiple permits with four or more state and federal agencies, for a permitting process that can take years.

To address this barrier, NOAA Fisheries Alaska Region and Alaska Sea Grant partnered to create user-friendly tools to guide applicants through the permitting process. An interagency working group of state and federal agencies involved in permitting aquatic farms reviewed each permitting step and drafted tools to help navigate the process. Prospective and existing farmers then reviewed the materials for usability. Experienced farmers provided additional feedback and suggestions for applicants to consider before starting the application process and siting a farm.

This presentation will share information from the Alaska Aquaculture Permitting Guidance document, and walk through highlights of the permitting portal. Both resources contain: "getting started" guidance; an application process step-by-step guide and accompanying flowchart; siting information; permit amendment, renewal, and transfer information; resources for new growers; and the basics of aquaculture governance in Alaska. The portal is scheduled to go live in time for the 2022 application cycle.

DETERMINING LONG-TERM SYNCHRONIZED FEEDING AND RESPIRATION RATES IN EASTERN OYSTERS (*Crassostrea virginica*) USING A COUPLED FLOW THROUGH SYSTEM

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Eastern oysters (*Crassostrea virginica*) are highly valued for the ecosystem services they provide. This is largely due to their filter feeding activity. However, the ability of oyster restoration to improve water quality remains ambiguous because natural variation in biotic and abiotic conditions affects oysters' filter feeding activity. Previous studies on the filtration services of oysters have examined physiological responses under laboratory conditions with monoculture diets and single parameter variation on day to hour-long *in situ* settings with coarse temporal scales. Yet, little is known about how their filtration rates vary over weeks in relation to variations in prey and environmental conditions. Studies that closely track direct effects of environmental changes are labor intensive and time consuming, leading to large data gaps.

This study leverages recent advances in aquatic observing, such as real-time flow-through oyster monitoring coupled with a newly implemented phytoplankton observatory on the Choptank River (Cambridge, MD). This system is able to track long-term feeding and metabolic responses of the Eastern oyster in response to subtle variation in environmental quality. The phytoplankton observing system consists of an imaging flow cytobot, fast repetition rate fluorometer, and CTD to determine algal community composition, health, and water conditions. Feeding and respiration is measured under ex situ, flow-through conditions and logged in real-time using sets of fluorometers and respirometers among replicate oysters over week-long experiments. Oyster feeding and respiration responses to prevailing biotic and abiotic conditions are estimated from signal differences among sensors during post processing. This coupled monitoring system enables a deeper understanding of how algal community and environmental variability directly influence oyster physiology. Additionally, data produced by this system can inform models of the potential variation of oyster filter activity under fluctuations of water quality as well as inform farm placement decisions to provide maximum oyster feeding potential.

PHYSIOLOGICAL AND IMMUNOLOGICAL PROPERTIS OF SELECTED SEA CUCUMBER SPECIES FROM THE WATERS OF UNITED STATES

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Sea cucumbers are known to be utilized as a source of traditional medication and nutrition in Asian countries. Several species have been researched for their potential source of therapeutical properties. It is known that compounds such as carotenoids, triterpene glycosides, bioactive peptides, minerals, vitamins, fatty acids, chondroitin sulfates, collagens, gelatins, and amino acids are present in these invertebrates. Through scientific research, medicinal benefits of some species of sea cucumbers have also been affirmed. We are, therefore, investigating the physiological, immunological, and microbiological effects of several species of sea cucumbers *in-vitro*, found in the waters of the United States of America. Results obtained from these investigations will be presented in the upcoming meeting of the World Aquaculture Society.

EFFECTS OF CANNABIDIOL (CBD) ON THE IMMUNOMODULATION OF FISH (*Oreochromis niloticus*) SPLEEN IMMUNE CELLS COMPARED TO MICE

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This experiment tests how mouse and fish spleen cell proliferation are affected by different concentrations of CBD oil. Spleen cell proliferation assay was conducted in the presence of concanavalin A and lipopolysaccharide mitogens for both mouse and fish spleen cells. Results indicate that for mouse spleen T cells, 11.49 µg/mL of CBD was effective in increasing the T cell proliferation when the cells were simulated by Con A. For mouse B cells, CBD seems to have had an inhibitory effect on the cells by reducing the proliferation caused by the mitogen. Similar results are seen in the case of fish spleen cells stimulated with LPS, where the inhibition of spleen cells is seen at the final CBD concentration of 0.46 µg/mL. From our current research, CBD may have different effects based on the species, whether they need to enhance their immune response or reduce inflammation to be healthy. It also seems to have different effect on different parts of the immune system. By administering the proper dosage of CBD on a case by case basis, health benefits can be achieved.

THE SOUTHERN CALIFORNIA OFFSHORE AQUACULTURE INTERAGENCY WORKING GROUP – A FORUM FOR COLLABORATION AND PROBLEM-SOLVING

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The Southern California Offshore Aquaculture Interagency Working Group was formed by NOAA Fisheries' Regional Aquaculture Coordinator (RAC) in January 2021, to function as a forum for sharing offshore aquaculture permitting information needs and challenges, as well as accessing and understanding best available science. Such information is needed to inform regulatory decisions collectively and proactively, and to engage directly with experts in various related disciplines. Federal and state regulatory agencies in California have seen a marked increase in permit applications submitted to the U.S. Army Corp of Engineers (Corps) for offshore aquaculture project proposals in state and federal waters of the Southern California Bight. With offshore aquaculture still a developing industry in California, this increasing interest has been challenging for the regulatory/permitting agencies, both relative to increasing project workloads as well as varying levels of aquaculture expertise. At the same time, NOAA has maintained sustainable marine aquaculture development as a priority since 1980, through various policies and guidance, thus supporting ongoing and increasing specialized expertise in marine aquaculture science (biological, engineering, socio-economic) within NOAA and with other partners to assist in the regulatory permitting process.

With the increase in aquaculture permit applications, the federal permitting agencies, primarily the Army Corps of Engineers and the Environmental Protection Agency, as well as several state agencies, recognized the need for aquaculture-specific technical expertise and science-based information. The regulatory agencies have found that areas of expertise and data needed include but are not limited to marine engineering, spatial siting, entanglement risk assessment of offshore aquaculture gear, and navigation interactions.

NOAA has under its umbrella several agencies with such specialized expertise, including the National Coastal Centers for Ocean Science and regional science centers, as well as long established partnerships with the US Naval Academy, DOD, and academic institutions engaged in aquaculture-related research. It was for timely for NOAA's California RAC to convene an interagency working group in southern California to focus on offshore/marine aquaculture. The Working Group facilitates exchange of marine aquaculture-related technical information necessary to assist federal and state regulatory agencies effectively navigate the environmental review and permitting processes. This forum is proving to be an effective tool for interagency collaboration and alignment of sustainable marine aquaculture goals across federal, state and local agencies.

ANTIMICROBIAL USE IN CALIFORNIA AQUACULTURE: PRODUCTS, PURPOSE, AND STEWARDSHIP

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California's complex and diverse aquaculture industry confronts many production and husbandry challenges; one of the most prominent and costly being infectious disease caused by microscopic organisms. Antimicrobial products are important tools used by aquaculture professionals to combat various pathogenic bacteria, parasites, fungi, and viruses. Antibiotics are within this group of products and have proven useful against infectious diseases outbreaks in humans and animals. However, antimicrobial resistance (AMR) is a developing concern within both agriculture and aquaculture, with potential human consequences. Bacteria develop resistance mechanisms and are capable of transferring this information to other bacteria, meaning that in many cases, antimicrobial products become less effective. It is important for the growing aquaculture industry to be aware of antimicrobial product usage, issues associated with AMR, and to avoid contributing to further AMR development and spread. Likewise, to support the continued industry growth, it is important for public health organizations and research institutions to provide recommendations and resources to assist aquaculture producers in their decision-making process.

Antimicrobial Use and Stewardship (AUS) in the California Department of Food and Agriculture is a statewide program intended to promote actions that will slow the development of resistant bacteria associated with animal production systems. Aquaculture Cooperative Extension at UC Davis is collaborating with AUS to better understand the types of antimicrobial products used in California aquaculture, the disease challenges experienced, and to provide resources intended to inform industry personnel of antimicrobial stewardship principles and disease management strategies. California aquaculture producers have been asked to participate in a survey designed to inform the AUS of the disease trends and industry needs surrounding animal health and biosecurity. Here we outline the types of antimicrobial products available for use by the aquaculture industry; the purpose of these products and how they are used onsite; and proper stewardship principles intended to help balance the AMR issue with the industry's need for antimicrobial products and disease management strategies.

NATIONAL AQUACULTURE EXTENSION CONFERENCE PLANNING AND DEVELOPMENT

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National Aquaculture Extension Conferences (NAECs) are interregional projects funded by the USDA-supported Regional Aquaculture Centers (USDA RACs). Conferences have been held in Little Rock, AR (1992), Annapolis, MD (1997), Tucson AZ (2003), Cincinnati OH (2007), Memphis, TN (2011) and Boise, ID (2017). Both NOAA Sea Grant and the USDA RACs provided funding for the 2017 NAEC. Joint funding is anticipated for the proposed 2022 NAEC in Portland ME.

Held every four to six years, NAECs provide the only dedicated national aquaculture Extension event that allows participants to present programs and trainings, tour aquaculture operations and exchange information and network. Conference planning occurs at two years in advance in order to secure a venue in the desired timeframe. Efforts are made to locate conferences in various regions of the US to highlight commercial aquaculture industries. Conference organizing efforts reflect suggestions made in previous NAEC participant surveys.

An experienced person(s) in aquaculture education, Extension and outreach acts as the local program host and their employer as the lead institution which processes the grant funds. The planning process involves: finding a volunteer host, securing a conference location, venue and dates, developing a project proposal and venue contract, planning the program and tours, and developing the conference survey and summarizing its results. National Aquaculture Extension Steering Committee and National Conference Planning Committee members assist and advise the conference co-chairs in the various aspects of the planning process.

Dana Morse, University of Maine Sea Grant Program, has agreed to host an in-person 7th NAEC in Portland, Maine in June 2022. Due to the COVID-19 pandemic, a first virtual NAvEC was hosted by the University of Maine Sea Grant Program June 8-10, 2021 when an in-person meeting had originally been scheduled. Approximately 160 aquaculture professionals participated and 18 abstracts were presented. Survey results from the NAvEC were mostly positive and provided useful feedback for future virtual and in-person conference efforts.

HEMOCYTE RESPONSES OF THE OYSTER *Crassostrea hongkongensis* EXPOSED TO DIEL-CYCLING HYPOXIA AND SALINITY CHANGE

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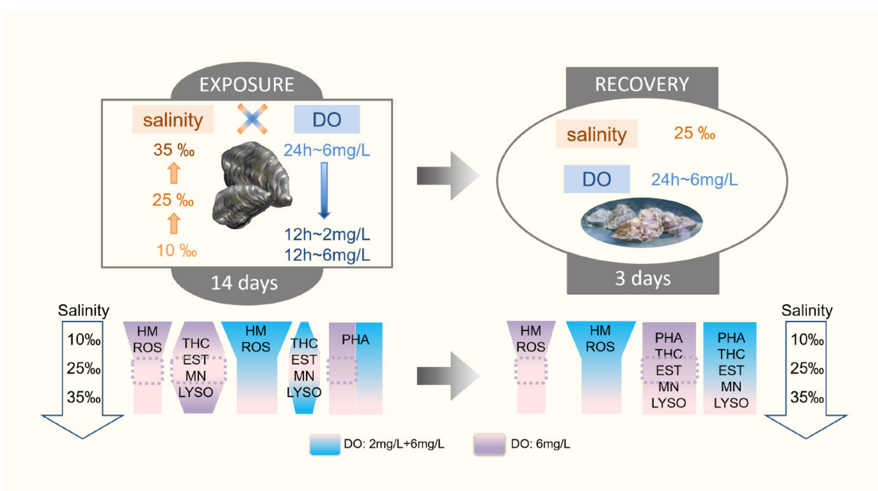
Marine hypoxia caused by nutrient enrichment in coastal waters has become a global problem for decades, especially diel-cycling hypoxia that occurs frequently in the summer season. While sudden rainstorms and freshwater discharge make salinity in coastal ecosystems variable, which often occurs with hypoxia.

To investigate the effects of diel-cycling hypoxia and salinity changes on the hemocyte immune function of *C. hongkongensis*, oysters were exposed to a combined effect of two dissolved oxygen (DO) treatment (24 h normal oxygen 6 mg/L, 12 h normal oxygen 6 mg/L with 12 h hypoxia 2 mg/L) and three salinities (10, 25, and 35‰) for 14 days. Subsequently, all treatments were restored to constant normal oxygen and normal salinity (25‰) for 3 days to study the recovery of *C. hongkongensis*. Hemocyte parameters were analyzed by flow cytometry, including hemocyte mortality (HM), total hemocyte count (THC), phagocytosis (PHA), esterase (EST) activity, reactive oxygen species (ROS), lysosomal content (LYSO), and mitochondrial number (MN). The results showed that diel-cycling hypoxia and salinity changes have obvious interactive effects on immunity of hemocyte. In detail, diel-cycling hypoxia and decreases in salinity led to increased HM and low salinity caused heavier impacts. In addition, low salinity and diel-cycling hypoxia led to decreases in LYSO, EST, and THC, while the decrease of PHA only occurs in the early stage. On the contrary, ROS increased significantly under low salinity and hypoxic conditions. After 3-day recovery, THC, PHA, EST, LYSO, and MN were basically restored to normal, while HM and ROS were still significantly affected by diel-cycling hypoxia and salinity change, indicating that the combined stress of diel-cycling hypoxia and salinity changes had latent effects on the immune function of *C. hongkongensis*.



PRODUCING INFERTILE SALMONIDS FISH FOR AQUACULTURE BY AN IMMERSION-BASED GENE SILENCING TECHNOLOGY

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It is imperative that highly effective containment methods are available to prevent highly selectively bred aquaculture animals escaping from captivity and propagate and/or interbreed with wild stocks. The use of reproductively sterile farmed animals is the most effective strategy for genetic-containment in aquaculture. Sterility carries environmental significance, preventing the escapees from spreading and genetic mixing with natural populations. Sterilization also prevents sexual maturation and minimizes energy input toward gonadal growth while enhancing flesh development and promoting animal health. Furthermore, sterility is a means for producers to protect their valuable strains from unauthorized propagation.

We have developed a technology to efficiently produce infertile fish by disrupting primordial germ cell development in embryos without introducing any genetic modification. This technology administers *Vivo*-conjugated Morpholino oligomer (MO) through bath-immersion targeting at Deadend (Dnd), an essential protein for early germ cell development in fish, which led to the elimination of germ cells and consequently sterile fish.

We have applied this novel sterilization technology to several salmonids, including rainbow trout, Atlantic salmon, and coho salmon. In coho salmon, the treated fish and control fish were reared for 14 months before examining their gonadal development. Infertile coho salmon (Fig.1 C & c) with the absence of gonad were achieved through *dnd* knock-down by immersion. Histology also shows the devoid of any germ cells in treated sterile fish compared with control fish. Optimizations towards better survival and higher sterility rates are ongoing. These sterile animals also provide excellent opportunities to study the involvement of germ cells/gonads in the development and regulation of the reproductive neuroendocrine axis. The contrast of gene expression profiles between 3-year-old sterile and fertile rainbow trout in spawning season is discussed.

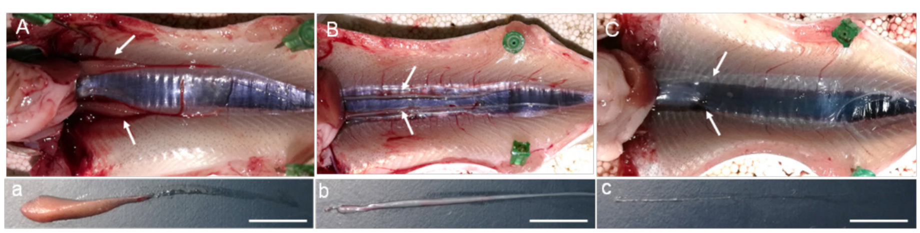

Fig.1 Gross morphology of gonads in 14-month-old treated sterile and control coho salmon.
A & a female, B & b male, C & c sterile. Arrows point to ovary, testis or sterile gonads.

DEVELOPING TECHNOLOGIES TO INDUCE STERILE DIPLOID EASTERN OYSTERS WITHOUT CHROMOSOME SET MANIPULATION

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Triploid oysters are considered sterile and preferred by oyster farmers because of their fast growth and premium meat quality during the summer seasons. However, recent reports revealed the phenomenon of 'triploid mortality' and found that triploid eastern oysters are more susceptible to environmental stressors than diploids even though their gonadal development was delayed and/or diminished. These results highlight the need for alternative sterilization technologies to preserve the market advantages of growing sterile oysters without altering their chromosome sets. We have developed a technology to produce reproductively sterile fish using *dnd*-MO-*Vivo* to transiently silence *dead end*, an essential gene responsible for fish primordial germ cell (PGC) development, by administering Morpholino oligomer through a molecular transporter, *Vivo*.

To apply this bath-immersion technology to produce reproductively sterile diploid eastern oyster (*Crassostrea virginica*), genes that are indispensable for oyster PGC development need to be identified since no *dnd* ortholog was found in oyster. Several genes in the invertebrate model organism Drosophila have been shown to be crucial for PGC development, including germ plasm and germ cell formation, PGC specification and PGC migration. Loss of function mutants of these genes are reproductively sterile. The orthologs of these candidate genes have been identified and cloned, and immersion treatments targeting these genes by *Vivo* conjugated Morpholino have been established in eastern oyster. In addition, to observe the uptake of Morpholino, fluorescent labelling was employed. Immersion of oyster embryos in fluorescent labelling Morpholino compound leads to the delivery of Morpholino shown by the fluorescent signal (Fig. 1). The fluorescent intensity inside oyster embryos increases with the increasing concentration of Morpholino. Unfortunately, none of the treatments produce sterile diploid oyster in these trials. The possible reasons and future perspectives are discussed. Nevertheless, the immersion-based approach provides a new accessible molecular tool to conduct gene function studies in oysters.

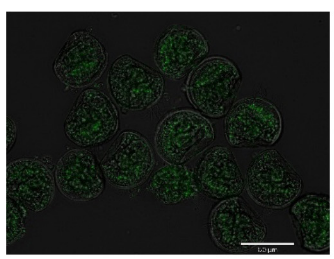

Fig. 1 Fluorescence microscopy of fluorescent labelled Morpholino treated eastern oyster larvae.

THE UNDERLYING PHYSIOLOGICAL MECHANISM FOR MITIGATING THE FEED BORNE IRON TOXICTY IN RAINBOW TROUT VIA DIETAY SUPPLEMENTATION OF VITAMIN C AND BENTONITE

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Iron (Fe) is an essential element; however, higher dose can cause lipid peroxidation in key tissues that could exert toxic effects in fish. Iron toxicity can be mitigated by using functional feed additives such as vitamin-C (VC) and bentonite (BT). Vitamin C is a reducing agent that helps in facilitating iron uptake in gut, and bentonite acts as a chelating agent to remove iron from the body. Bentonite scavenges and binds free iron to prevent reactive oxygen species (ROS) from being produced to prevent peroxidation. Our study investigated the effect of dietary supplementation of BT and VC in mitigating the feed-borne iron toxicity in rainbow trout. Two experiments were conducted to achieve the goal.

Experiment 1: Bentonite study: Six diets [0% BT (Con), 2 and 4% BT (HB), 0.25% iron as $FeSO_4$ (Fe), Fe+2% BT (LBFe), and Fe+4% BT (HBFe) were fed to trout for 8 weeks. Iron exhibited negative impacts on growth and increased the iron load in liver (Figure A), whereas BT supplementation improved the growth and reduce the iron load in liver. Oxidative status was enhanced in BT+Fe fed groups compared to the Fe group. Liver showed infiltration with inflammatory cells and necrosis in Fe and HB groups whereas LBFe group appears to be normal.

Experiment 2: BT and VC Study: Fish fed seven diets: [(i) Control (Con), (ii) low VC and iron (ConFe; 500 ppm; LVC; 0.25% Fe), (iii) BT and iron (BenFe; 2%; BT; 0.25%; Fe), (iv) with medium VC and Fe (MVCFe; 1500 ppm; MVC), (v) MVC with BT and iron (MVCBenFe), (vi) HVC with iron (HVCFe; 3000 ppm VC), and (vii) HVC with BT and iron (HVCBenFe)] for 10 weeks. The result indicated that the supplementation of BT, VC or in combination exposed to a higher dietary iron augmented their growth performance compared to the ConFe group. Also, the relative expression of hepatic antioxidants (CAT, SOD, GPx) were affected with the higher dietary iron; however, with the supplementation of BT and VC helped in restoration their antioxidant activity. Similarly, Hepcidin (HAMP, transferrin (Tf), and Hemeoxygenase (HO1) were upregulated in the ConFe group.

Overall dietary supplementation of vitamin C and bentonite can be an effective approach to mitigate the iron toxicity in trout aquaculture industry.

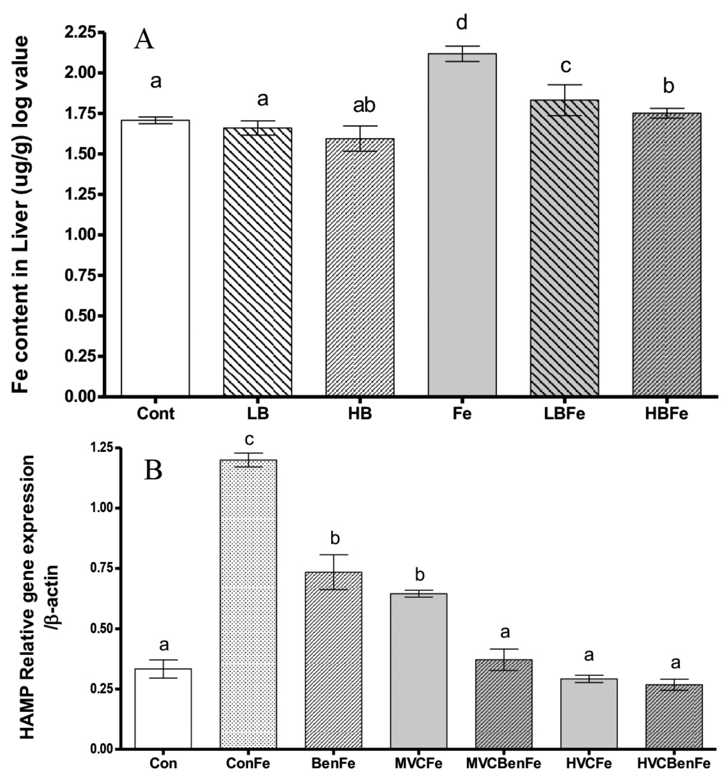

Figure: Iron load in liver (A), Hepcidin (HAMP) gene expression in liver (B)

LIPID SUPPLEMENTATION IMPROVED FEED EFFICIENCY AND PROTEIN UTILIZATION IN CHANNEL CATFISH (Ictalurus *punctatus*)

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A preliminary study was conducted to determine if diets supplemented with 1.5% catfish oil (CFO), or 1.5% menhaden oil (MHO) improved fish condition prior to winter fasting. Fish were maintained in 1,200 L polypropylene tanks supplied with continually exchanged pond water. Fifteen hundred channel catfish juveniles (~20 g) were equally distributed in 15 tanks in a completely randomized block design (n=5), and each tank were assigned to either the control diet (commercial feed), or diets supplemented with CFO or MHO. Fish were fed experimental diets for 8 weeks and feeding was suspended when water temperature fell below 15^{0}C. A subsample of 10 fish from each tank were obtained to compute the conditional indices. The fish remainders from each experimental unit were pooled, ground, and homogenized to measure the whole-body proximate composition. No significant differences were detected for weight gain, survival, hepatosomatic index, and whole-body proximate composition. However, fish fed CFO presented a significantly higher feed efficiency compared to the control diet. Intraperitoneal fat (IPF) was also statistically affected by dietary treatments, with catfish fed CFO presenting the highest IPF, followed by fish fed MHO with intermediate values. Fish fed the control diet had the lowest IPF ratio. While fish fed CFO had the highest IPF ratio, they also presented more efficient protein conversion compared to fish fed control diets. This suggests supplementation of CFO aided protein-sparing effects and prevented amino acids from being used as metabolic substrates. More investigation is warranted to determine how CFO improves protein utilization and whether supplemental lipids can limit protein catabolism during winter fasting.

EFFECTS OF TEMPERATURE AND SALINITY ON CARDIAC FUNCTION AND OSMOREGULATION IN THE THREATENED LONGFIN SMELT (*Spirinchus thaleichthys*)

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Longfin smelt (*Spirinchus thaleichthys*) were once one of the most abundant fish species in the San Francisco Estuary (SFE), California. However, over the last two decades, their abundance has plummeted to <1% of historical values, contributing to their being listed as 'threatened' under the California Endangered Species Act. Developing a captive culture of longfin smelt to be used as a sustainable source of research specimens and for use in supplementing wild populations is one conservation management tool that is currently under development. Unfortunately, high larval mortality has hindered the development of a captive culture and no individuals in captivity have ever survived to reproductive age. To improve culture methods for longfin smelt, we measured the responses of larvae to two environmental conditions that correlate strongly with field abundances of longfin smelt in the SFE: temperature and salinity.

For temperature, we incubated and hatched embryos at three different temperatures (9, 12, 15°) and measured larval heart rate through acute stepwise increases in temperature to identify their Arrhenius breakpoint temperature, a proxy for a fish' thermal optimum, and the temperature at which heart rate peaks, a proxy for a fish's thermal limit. We also measured larval growth and yolk resorption rates at these temperatures to corroborate the findings of our cardiac metrics. For salinity, we reared yolk-sac larvae in five different salinities (0.4, 5, 10, 20, 32 parts per thousand [ppt]) and measured their osmotic and ionic balance, growth, and yolk resorption rates over time to identify their upper and lower salinity tolerances, and potential salinities to use in culture.

Our results indicate that culturing conditions between 9-14° and 5-10 ppt salinity would be appropriate for longfin smelt larvae. We found that 12-14° maximized the proportion of individuals at their thermal optimum, which correlated with the highest growth rates measured at 12°. However, we also found that larvae reared at 9° ultimately grew to the same size as those at 12°. For salinity, we found that larvae experienced disruption to osmotic and ionic balance at 20 and 32 ppt and that larvae in 0.4 ppt had stalled yolk resorption rates. This correlated with the decreased growth at these salinities compared to 5 and 10 ppt. Using these results, larviculture methods were modified and improved survival in culture to the highest rates achieved thus far.

Table 1. Longfin smelt % survival to different ages (dph) in culture between 2010-2020.

Dph	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
40	15	24.1	40.2	10	0	10	2.5	1.6	0	15.3	68
120	0	0	0.1	0	0	6.7	0.7	0	0	0	13
300	0	0	0	0	0	0	0	0.1	0	0	2

USING THE MINION TO RAPIDLY PROFILE THE OYSTER MICROBIOME

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Nanopore sequencing is a rapidly evolving technology useful for profiling communities of bacteria such as the oyster microbiome. By sequencing the rRNA operon (4200 + bp, Fig 1), species- and strain-level resolution can be achieved. Long-read methods provide a much more detailed picture of oyster microbiome dynamics, which can by obscured by short-read approaches that often limit resolution to the genus level or higher. For this study, extraction and sequencing methods were adapted for use on oysters reared in different settings, including semi-sterile lab conditions, an oyster farm, and wild or restored oyster reefs. The Oxford Nanopore MinION was used to profile rRNA operons and assess how the oyster microbiome varies geospatially, as well as the potential connections to oyster disease status (*Perkinsus marinus* infection). Phenomena observed at the species-level, such as the increase in *Mycoplasma pulmonis* in oysters moved to the common environment (Fig 2), would be obscured at higher taxonomic levels (e.g. genus).

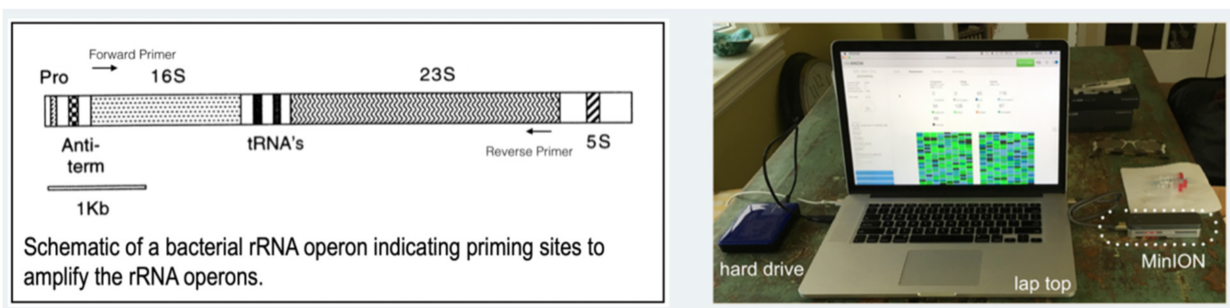

Figure 1: rRNA operon (left) and a picture of a typical MinION sequencing setup (right).

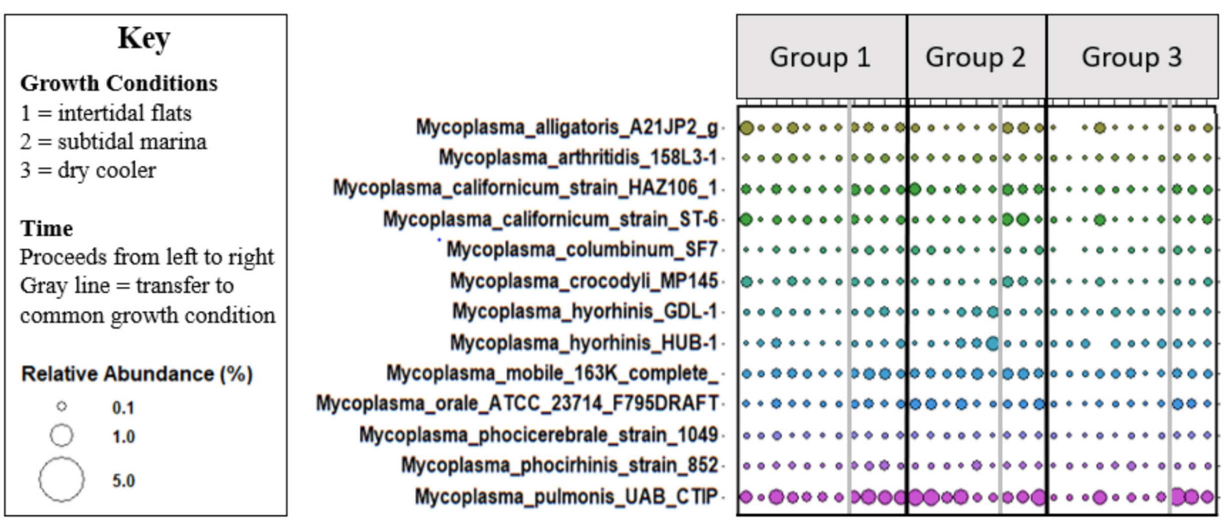

Figure 2: Relative abundance of various *Mycoplasma* species/strains in oysters.

FUNCTIONAL CHARACTERIZATION OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* HEAT SHOCK PROTEIN 90 (L*v*HSP90) IN RESPONSE TO WHITE SPOT SYNDROME VIRUS (WSSV) INFECTION

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Stress proteins (SPs) including heat-shock proteins (HSPs), are molecular chaperones essential for cellular homeostasis. The functions of HSPs include chaperoning misfolded proteins, protecting cells from stress, and participating in the host immune responses. Moreover, HSPs also cooperate with numerous viruses to promote viral infection. In this study, we investigate the role of heat shock protein 90 (*Lv*HSP90) in white spot syndrome virus (WSSV) infection in Pacific white shrimp *Litopenaeus vannamei*. The expression of *LvHSP90* was significantly up-regulated upon white spot syndrome virus (WSSV) infection. Gene silencing of *LvHSP90* followed by WSSV infection resulted in 60% higher cumulative mortality rate in the *LvHSP90*-silenced shrimp. In addition, the transcription of WSSV gene were highly decreased in the *LvHSP90*-silencing group. In WSSV infected hemocyte cells, *Lv*HSP90 protein showed accumulation in the nuclear zone, where viral replication is located and the number of WSSV-infected cells was significantly lower than control groups after *LvHSP90* silencing. Injection of shrimp with WSSV that co-incubated with recombinant *Lv*HSP90 (r*Lv*HSP90) showed an increased mortality rate up to 90% compared to only 40% mortality in the control groups and shrimp also had increased viral copy number. Taken together, these results suggested that *Lv*HSP90 plays a crucial role in promoting WSSV infection.

THE PARASITE *Sylon hippolytes* ALTERS THE FATTY ACID COMPOSITIONS OF THE DOCK SHRIMP *Pandalus danae*

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Rhizocephalan barnacles are infamous crustacean parasites due to their effects on host behavior and morphology. As parasitic castrators, rhizocephalans co-opt the reproductive investments of their hosts, suggesting that they may in turn affect the host nutritive quality. As one aspect of nutrition, fatty acids (FA) play vital physiological roles in organisms and their consumers, and differences in host FA compositions imposed by rhizocephalans could highlight their impact.

Here, we investigated the influence of parasitism on host lipid content and FA composition by the rhizocephalan *Sylon hippolytes* infecting the dock shrimp *Pandalus danae*. We collected visibly infected and uninflected *P. danae* from the docks of Friday Harbor Laboratories, Friday Harbor, WA, USA. We dissected the abdomen tissue, hepatopancreas, and externae from one subset of the shrimp (n = 20) and left the remaining shrimp (n = 24) whole, analyzing the samples for lipid content using gravimetry and for FA composition using gas chromatography-mass spectrometry.

Lipid content did not differ between *Sylon*-infected and uninfected shrimp for whole shrimp, abdomen tissue, nor hepatopancreas. Permutational multivariate analysis of variance (PERMANOVA) showed a significant difference in FA composition by *Sylon*-infection status and by tissues when dissected, but significant differences by infection status were not detected within tissues. Whole shrimp were also significantly different in FA composition by infection status, as shown by PERMANOVA, likely driven by the distinctive FA compositions of externae.

Taken together, these results suggest that while *Sylon* affects the FA composition of whole shrimp, potentially altering nutrition, that the shrimp may compensate for parasitism by having similar fatty acid compositions and lipid contents in the tissues of *Sylon*-infected and uninfected shrimp. As *Sylon* can reach high prevalence in *Pandalus* shrimps, it will be important to determine whether similar patterns are found in other, commercially important pandalids, as well as for other nutritional measures.

IMPACTS FROM TROPICAL SYSTEMS ON HATCHERY CONTRIBUTION, WILD RECRUITMENT, AND DISTRIBUTION OF JUVENILE RED DRUM *Sciaenops ocellatus:* LESSONS FROM A LONG-TERM STOCK ENHANCEMENT PROGRAM

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The South Carolina Department of Natural Resources, SCDNR, first started augmenting wild populations of marine gamefish, including red drum, in the early 1980's through stock enhancement programs. The development of genetic tools, which identify hatchery fish compared to their wild cohort, has allowed SCDNR to use applied fisheries research to answer life history questions and improve stocking techniques to increase survival and contribution of hatchery fish.

The increase in tropical cyclones along the eastern seaboard over the past decade provided an opportunity to examine the effects these systems, primarily increased precipitation, have on hatchery survival and movement. Three tropical events occurred in South Carolina: Hurricane Joaquin in 2015, Hurricane Matthew in 2016, and Hurricane Florence in 2018. All three events were associated with record rainfall and river cresting in localized areas where stocking occurred. Genetically unique families of hatchery fish were released before and after each rain event. SCDNR's inshore fisheries section collected young of the year red drum through their independent fisheries survey using trammel net and electro fishing gears. Each fish was measured, and a small fin clip was preserved for genetic analysis. Hatchery contribution was determined based on the percentage of hatchery fish compared to wild fish caught in the sample.

Results from all three years demonstrate that stocked fish made a larger contribution and had a wider distribution area when stocked after major rain events compared to fish stocked before these events. These findings suggest that major rain events during critically important times such as spawning and larval recruitment may have a negative impact on wild red drum recruitment and potentially subsequent contribution to the fishery. Increases in the frequency of tropical systems, their effects on the red drum population, and decreasing population trends in juvenile red drum abundance indices seen over the past decade in our independent fisheries surveys provide fisheries managers valuable information for potential regulatory efforts to combat population declines.

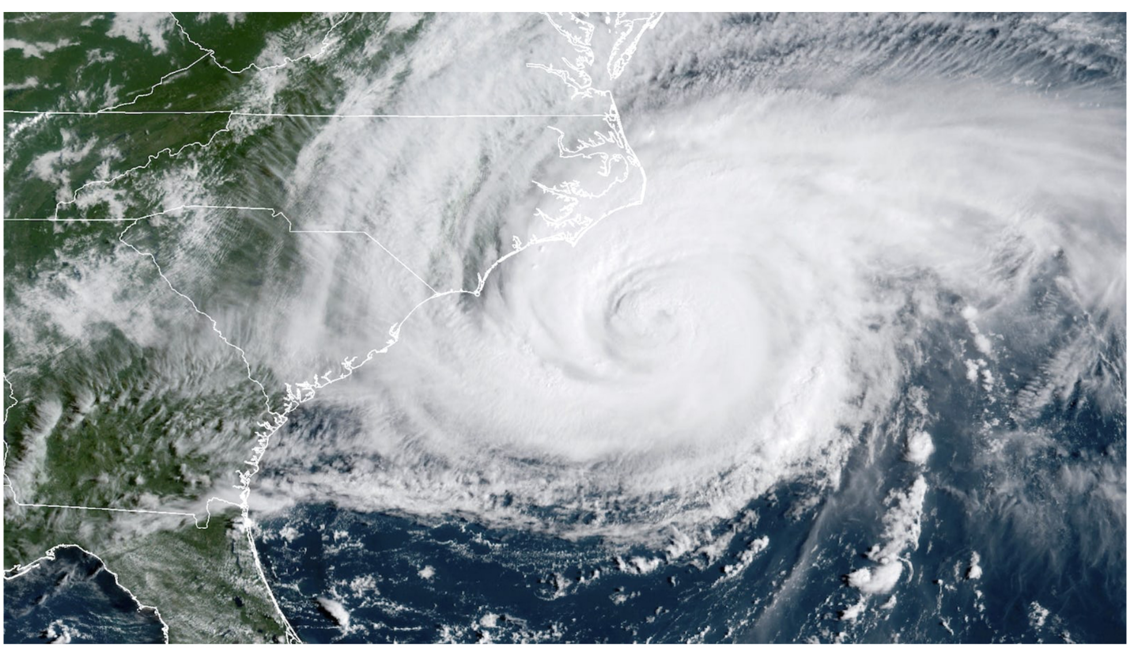

Figure 1: Satellite imagery of Hurricane Florence before making landfall around the South Carolina and North Carolina border.

COMPARING LOW SALINITY TRANSCRIPTOMIC PROFILES AMONG HARD CLAM *Mercenaria mercenaria* LINES

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Assessment of how the hard clam, *Mercenaria mercenaria* (Linnaeus, 1758), responds to environmental changes, like salinity fluctuations, is an existing need. The hard clam is an important ecological and economic resource along the US Eastern Seaboard. In Virginia alone, the farm gate value of the hard clam in 2018 was $38.8 million, making it the largest aquaculture industry in Virginia. This growing industry is primarily limited to higher salinity habitats on the seaside of the Eastern Shore of Virginia or lower Chesapeake Bay. Even in areas of higher salinity, hard clams are vulnerable to extreme precipitation events, which can lead to hyposaline (low salinity) stress and threaten natural and aquacultured hard clam populations. Osmotic stress, like a drop in salinity, can lead to altered gene expression and cell cycle events. Transcriptomic analysis is a powerful tool for exploring the relationship between phenotype and genotype, enabling a better understanding of how hard clams respond to stress.

Genetically distinct hard clam populations originating from varying salinity habitats along the U.S. Eastern Seaboard have been identified and some of these populations were used to establish lines at the VIMS Eastern Shore Laboratory (ESL): Wachapreague Channel, VA (WC); Pocomoke Sound, VA (PS); Mobjack Bay, VA (MB); Great Bay, NJ (NJ); Cape Cod, MA (CC); and Bogue Sound, NC (NC). As small juveniles, clams spawned from these lines were shown to have differences in respiration performance after low salinity exposures as part of the graduate work of VIMS student Anthony Himes. In 2019, F1 generations of WC, PS, MB, NJ, and CC clams were spawned, and in 2021, F1 crosses were spawned and include WC x WC (control), WC x PS, and NC x CC. Three clams from each of the 2019 spawns were exposed to 35 ppt and 15 ppt salinities for 26 hours. After exposures, gill tissue was sampled and placed in RNA preservative. Four replicates of 10 clams spawned in 2021 were also exposed to 35 ppt and 15 ppt salinities for 26 hours. Tissue from two groups of four clams from each replicate was pooled and placed in RNA preservative.

Whole transcriptome shotgun sequencing (WTSS), also known as RNA-Seq, will be used to explore the mRNA expression patterns of the hard clam when faced with ideal (35 ppt) and low (15 ppt) salinity conditions. Comparing mRNA expression patterns among genetically distinct clam lines that are derived from populations that experience different salinity patterns will provide important information about genes involved in response to salinity stress. RNA-seq will also be used to identify genotypic differences in the form of single nucleotide polymorphisms (SNPs). SNPs associated with specific genes that are linked with traits of interest can be powerful molecular markers. A comparative transcriptomic study is the link between the genetic and physiological variation seen among hard clam populations and could lead to SNPs for improved selection of hard clams for better low salinity tolerance by the aquaculture industry.

GENOME SEQUENCING AND ASSEMBLY STRATEGIES AND A COMPARATIVE ANALYSIS OF THE GENOMIC CHARACTERISTICS IN PENAEID SHRIMP SPECIES

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Penaeid shrimp (family Penaeidae) represents one of the most economically and ecologically important groups of crustaceans. However, their genome sequencing and assembly have encountered extreme difficulties during the last twenty years.

In this study, based on our previous genomic data, we investigated the genomic characteristics of four penaeid shrimp species, and identified potential factors resulting in their poor genome assembly, including heterozygosity, polyploidization and repeats.

Genome sequencing and comparison of somatic cells (diploid) of four shrimp species and a single sperm cell (haploid) of *Litopenaeus vannnamei* identified a common bimodal distribution of K-mer depths, suggesting either high heterozygosity or abundant homo-duplicated sequences present in their genomes. However, penaeids have not undergone whole genome duplication as indicated by a series of approaches. Besides, the remarkable expansion of simple sequence repeats was another outstanding character of penaeid genomes, which also made the genome assembly highly fragmented. Due to this situation, we tried to assemble the genome of penaeid shrimp using various genome sequencing and assembly strategies and compared the quality. Therefore, this study provides new insights about the genomic characteristics of penaied shrimps, while also improving their genome assemblies.

REVERSE TRANSCRIPTASE-RELATED GENES AND THEIR POSSIBLE ROLE IN THE HOST CELL RESPONSE TO TRANSITION METAL POLLUTION

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Reverse transcriptase-related (*rvt*) genes represent a remarkable class of reverse transcriptases (RTs) found in bacteria, fungi, protists, plants and invertebrates from aquatic and soil-dwelling environments. These are cellular single-copy genes preserved by natural selection. Their occurrence in prokaryotic and eukaryotic representatives suggests a conserved biological function that is applicable to both prokaryotes and eukaryotes.

RVT proteins from the filamentous gliding bacterium *Herpetosiphon aurantiacus* and the ascomycete fungus *Neurospora crassa* display a peculiar property of initiating polymerization via protein priming. Thus, RVTs represent the first known case of protein-primed RT encoded by a chromosomal non-selfish gene. Moreover, the N-terminal coiled-coil domain allows RVT proteins to form multimers and could potentially interact with metal ions. Since most free-living organisms can be frequently exposed to hazardous pollutants including transition metals, they therefore need potent mechanisms to cope with such stresses.

Three free-living organisms with active *rvt* genes in their genome (*H. aurantiacus*, *N. crassa* and the bdelloid rotifer *Adineta vaga,*) not only display signs of altered growth and behavior after treatment with varying concentrations of several metal ions (Ni^{2+}, Co^{2+}, Fe^{2+} etc.) but also display strongly induced expression of *rvt* genes under metal stress conditions. Interestingly, all examined organisms demonstrate specificity of *rvt* response to a specific metal ion. Moreover, when recombinant bacterial HaRVT is expressed in *E. coli,* it apparently provides an advantage for survival in iron-rich environments. Participation of domesticated RTs in metal stress response could reveal an ancient function of these genes in early evolution and novel pathways to environmental adaptation.

USING AN ELEVATION PORTFOLIO APPROACH FOR SUCCESS IN PASSIVE RESTORATION OF NATIVE OYSTERS IN SAN FRANCISCO BAY

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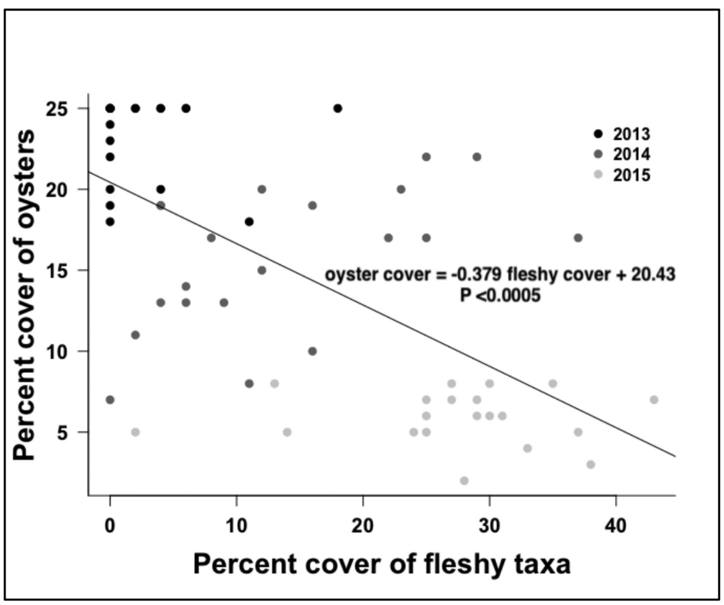
Passive habitat restoration that depends on the natural dispersal of a target species to project sites is reliant on two major sets of processes: those that influence the quantity and timing of the arrival of propagules and those that affect survival and growth post recruitment. Particularly relevant for passive restoration projects is an understanding of the relative importance of rate of propagule arrival vs. post-settlement survival, as the same location might not be ideal for both sets of processes, however these data are often unavailable. Restoration projects are often not monitored more than one year post-construction, with the result that predictors of longer-term success may not be clear. We monitored native oysters *(Ostrea lurida)* that naturally recruited to restoration substrates for five years at two sites in San Francisco Bay. We report on changing rates of recruitment and adult densities over time that show different trajectories at different locations. We document gradients in oyster predation, competition, and physical stress along a short elevation span that correspond with differences in adult densities. These results demonstrate that for restoration to be successful given these gradients as well as interannual and site-specific variability, it is critical to take a portfolio approach that involves using a range of sites and tidal elevations to ensure success at the end of the project. Additionally, our longer-term dataset demonstrates the differences in conclusions we might have drawn from short-term monitoring (<2 years) compared with the understanding gained after 5 years.



At lower tidal elevations, growth of fouling organisms such as sponges and tunicates on restoration substrates reduced oyster densities over time.

REFUTING OFFSHORE MARINE AQUACULTURE MYTHS, CRITICISMS AND ASSUMPTIONS FOISTED ON THE U.S. PUBLIC

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Sustainable domestic aquaculture development is a critical component to achieving greater U.S. seafood security in the future, yet detrimental allegations have corrupted public support. A variety of longstanding and inaccurate myths and assumptions directed at offshore aquaculture farming and its regulation have been foisted on the public. This presentation refutes the most prevalent critiques by reviewing current policies, regulations, research and industry production practices. These criticisms include: inadequate regulatory oversight; portrayal of farms as being high density factories unconcerned by food waste, untreated discharge, use of antibiotic and antifungal treatments; entanglement of marine mammals; impacts on wild stocks and habitats; use of feed additives to pigment fish flesh; unsustainable use of fish meal in feed formulations; potential market disruption by producing cheap, low quality products; and commercial farms and commercial fishers cannot coexist as for-profit businesses. Marine aquaculture is not risk-free in terms of potential environmental, economic, social, and cultural impacts and challenges remain to achieve a sustainable industry. These challenges are well known and addressable by the U.S. and global research community. Current offshore farming realities bode well for the future: 1) there is a clear global imperative to sustainably produce more seafood to meet growing demand and the U.S. has the marine resources to become a major exporter, if U.S. law can be amended to grant offshore farmers a property right or security of tenure for sites in federal waters; 2) U.S. ocean farmers work within a very complex and effective legal, regulatory, science-driven environment to anticipate and mitigate potential impacts; 3) farm level management decisions and federal and state regulatory frameworks have worked together to bring about environmentally friendly siting, operational, and production outcomes, and 4) the farming community and its advocates in government, at universities, and industry recognize it is essential to reach out to decision-makers and the interested public, as well as critics, with the latest research and empirical results to present an accurate picture of risks and rewards to development.

WITHIN-GENERATION POLYGENIC RESPONSES TO SELECTION SHAPE MICROGEOGRAPHIC POPULATION STRUCTURE IN THE EASTERN OYSTER *Crassostrea virginica*

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Cohort adaptation is a within-generation population response to spatial variation in selection below the scale of average dispersal. Cohort adaptation can provide a dynamic response to temporal and spatial environmental heterogeneity and maintain spatially balanced polymorphisms. Using Eastern oysters *Crassostrea virginica* as a model, this study seeks to test for genomic signatures expected from cohort adaptation along the Delaware Bay salinity gradient. A wild transect of adult samples was sampled in 2019 after precipitation generated a protracted period of low salinity leading to substantial oyster mortality on up-bay beds. Based on whole genome sequence comparisons we found very low population differentiation (F_{ST} pairwise average 3e-4), underscoring the dominant role of homogenizing gene flow within the estuary. We hypothesized that hypo-salinity selection was strong enough up-bay to increase the frequency of tolerance traits and genes contributing variation to those traits. A model-based single generation selection (SGS) test was implemented to detect significant allele frequency differences between the transect end samples, beyond that expected from genetic drift in a single population (e.g., sweepstakes reproduction) and sampling error. Thousands of single nucleotide polymorphism (SNP) candidates were identified, distributed across the genome with low to moderate allele frequency change magnitudes. Challenge experiments were used to identify and compare genomic changes resulting from extreme hypo-salinity selection on adults, but parallel responses are only expected for genes of large effect. Therefore, additional metrics were compared between SGS candidate vs. control loci to increase confidence in a cohort adaptation inference.

GENOMES REVEAL GENETIC DIVERSITY OF *Callinectes sapidus* REOVIRUS 1 (CsRV1) IN WILD BLUE CRAB POPULATIONS ACROSS TWO HEMISPHERES OF ITS HABITATION

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Evolution of viruses within a single host can be shaped by host life history and environment. *Callinectes sapidus* reovirus 1 (CsRV1) is a segmented dsRNA virus that infects *C. sapidus* across two hemispheres and a range of habitats. CsRV1 causes systemic infections and paralysis and mortality in the Atlantic blue crab, *C. sapidus*. Previous phylogenetic studies of a portion of the CsRV1 genome in US northern Atlantic coast and Brazil revealed geographically distinct viral genotypes.

To expand our understanding of the genetic diversity of CsRV1 across a broader temporal, geographic, and host connectivity range, we obtained 24 complete or near-complete CsRV1 genomes as well as segment 9 sequences 96 strains from the US Atlantic, Gulf coast, Caribbean Sea, and Brazil/Uruguay. The Maximum likelihood phylogenetic tree of CsRV1 concatenated genomes and segment 9 ORF suggested that CsRV1 genotypes are divided into major lineages of genotypes within the US Atlantic coast, Brazil and Uruguay Atlantic coast, Gulf of Mexico, and Caribbean Sea.

Full and partial genome analyses provide insight into the movement the virus across different scales and through time. For example, one CsRV1 genotype from Texas and several from Louisiana grouped with CsRV1 genotypes from the US Atlantic coast rather than genotypes from the Gulf of Mexico, which suggested long-distance transportation of CsRV1, is consistent with human-mediated movement.

These results of this study can help us understand the origins and evolution of a marine virus and give clues to host population connectivity in the ocean. In addition, identified genetic variations in each genomic segment of CsRV1 can also be employed to gain insights into the evolution dynamics of its viral genome over time.

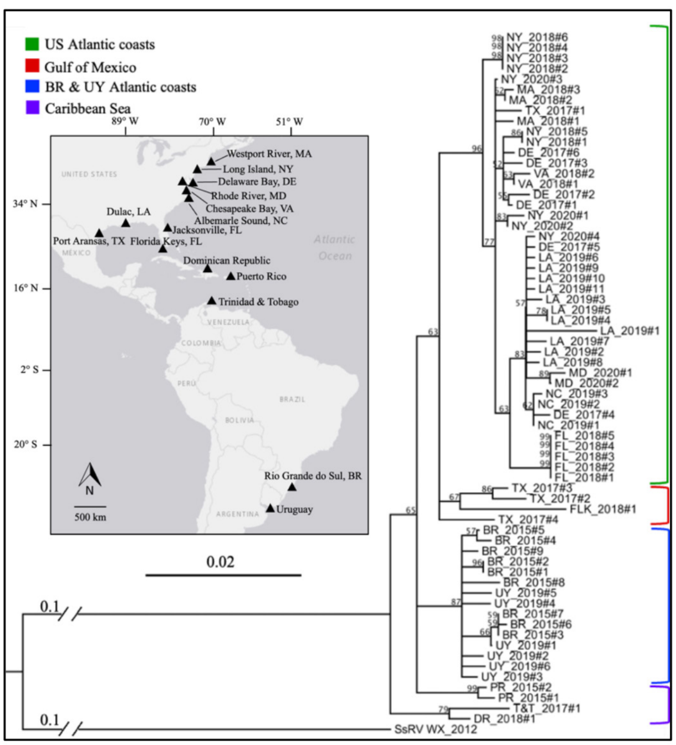

Figure 1. Maximum likelihood tree based on segment 9 nucleotide sequences of CSRV1 collected from geographic locations shown on the map.

MOLECULAR CHARACTERIZATION OF TWO NOVEL TOTI-LIKE VIRUSES GENOMES THAT CO-OCCUR IN BLUE CRAB *Callinectes sapidus* ALONG THE NORTHERN ATLANTIC COAST OF THE USA

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The development of next generation sequencing has greatly facilitated the exploration of viruses that infect marine hosts. For example, there are an increasing number of virus genomes belonging to the *Totiviridae* family described in crustacean hosts. In this study, two novel distinct dsRNA virus genomes were discovered in the Atlantic blue crab *C. sapidus* in the United States. The complete genome sequences of both viruses were determined, which are 6444 nt and 7421 nt in length, containing two overlapping open reading frames (ORFs) that encode capsid and RNA dependent RNA polymerase proteins with similarities to toti-like viruses. The viruses were tentatively named *Callinectes sapidus* toti-like virus 1 (CsTLV1) and *Callinectes sapidus* toti-like virus 2 (CsTLV2). Both genomes have typical elements required for -1 ribosomal frameshifting, which may induce the expression of an encoded ORF1-ORF2 (gag-pol) fusion protein. Phylogenetic analyses of CsTLV1 and CsTLV2 RdRp amino acid sequences suggested that they are members of new genera in the family *Totiviridae*.

The presence of CsTLV1/2 genomes were detected in muscle, gill and hepatopancreases of blue crabs by RT-qPCR. The PCR assay was used investigate the geographic range of these viruses, which may be restricted to the northeast US. The presence of 40 nm totivirus-like viral particles in all these three tissues were validated by transmission electron microscope (TEM). The two virus genomes co-occur most, but not all, of the time. To our knowledge, this is the first report demonstrating the presence of two novel toti-like viruses in the Portunid crab - *C. sapidus*. Transmission and pathogenicity studies are currently underway.

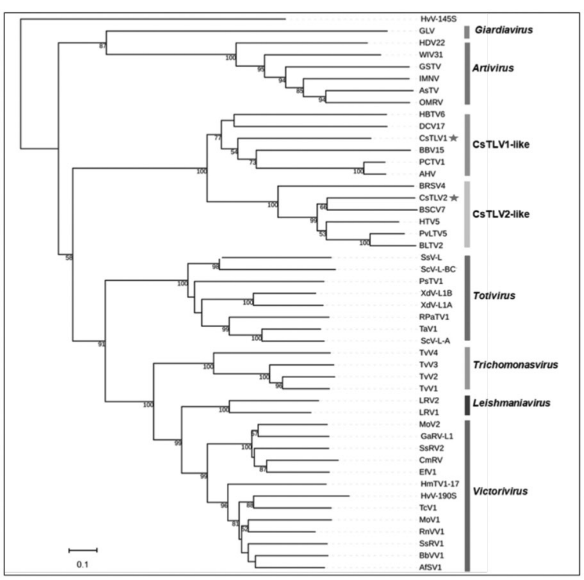

Fig 1. Phylogenetic relationships between putative RdRp amino acid of CsTLV1 and CsTLV2 with other selected *Totiviridae* members.

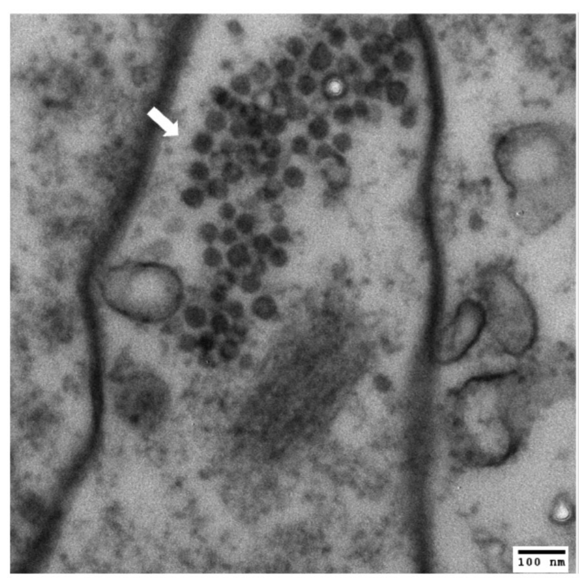

Fig 2. Electronic microscopy image showed putative CsTLV1 and CsTLV2 viral particles in hepatopancreases of *C. sapidus*.

TESTING FOR GENOMIC SIGNATURE OF DOMESTICATION ON EASTERN OYSTER *Crassostrea virginica*

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Selective breeding for commercially valuable aquaculture traits has yielded relatively rapid successes with aquatic, high fecundity species such as salmon and oyster. Discovering the genetic changes associated with trait evolution is an important goal for understanding biological mechanisms, and also can facilitate better predictions about likely fitness of selected strains if they escape the aquaculture farm environment. Here we refer domestication as genetic changes related to increasing ease and efficiency of culture (e.g., higher survivorship at high density or higher settlement efficiencies). Recent investigation of fish domestication revealed that rapid adaptation to captivity could be characterized by numerous, heritable changes in gene expression. In addition, genome-wide comparison between farmed and wild fish populations has identified multiple selection sweeps indicative of adaptation to the captive environment. In comparison with fish domestication, the genetic underpinnings associated with recent domestication process in shellfish are relatively less studied. Using eastern oyster as a model, this study seeks to uncover the genomic consequences of recent domestication between wild populations and selective lines from the newly developed 600K SNP array, as well as whole genome resequencing data. Paired contrasts were made between selected strains (5 - 15 generations of breeding) and the primary natural population it was originally sourced from. Principle component analysis clearly differentiated selected lines from each other and from their natural progenitor populations. Comparisons of within population variation will be presented for these population pairs to quantify the early evolutionary consequences of selective breeding on standing genetic diversity. Genome scans for loci under selection, with tests for parallel patterns across selected strains, will be discussed with respect to selected traits in common (e.g., faster growth) versus unintentional parallel adaptation to culture.

ESSENTIAL OILS IMPROVE THE SURVIVAL OF GNOTOBIOTIC BRINE SHRIMP (*Artemia franciscana*) CHALLENGED WITH *Vibrio campbellii*

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The halophilic aquatic bacterium, *Vibrio campbellii* is an important aquatic pathogen, capable of causing vibriosis in shrimp and fish resulting in significant economic losses. In previous work, essential oils (EOs) extracts from *Melaleuca alternifolia*, *Litsea citrata* and *Eucalyptus citriodora* were found to inhibit the growth of *V. campbellii in vitro*. This study aims to determine *in vivo* EOs' potential protective effect towards gnotobiotic brine shrimp, *Artemia franciscana*, challenged with *V. campbellii*. The study showed that brine shrimp larvae supplemented with EOs of *M. alternifolia* (0.0008%) and *L. citrata* (0.002%) displayed significantly increased survival against *V. campbellii*. The results indicate that supplementation of these EOs increased the expression of immune-related genes (either in the presence or absence of the pathogen), probably contributing to enhanced protection. Furthermore, *in vitro* studies indicated that some EOs modulated the expression of virulence factors including swimming motility, biofilm formation and gelatinase and lipase activity, while flow cytometry data and regrowth assay indicated that these EOs do not exhibit antimicrobial activity as *V. campbellii* grew at the tested concentrations (*M. alternifolia* (0.0008%) and *L. citrata* (0.002%)). Our findings suggest that EOs extracted from *M. alternifolia* and *L. citrata*, can module virulence factor production and immunological responses and might hence become part of an intervention strategy to control vibriosis in a fish or shrimp aquaculture setting, a hypothesis that needs to be validated in the future.

THE SEA URCHIN VALUE CHAIN: CIRCULAR ECONOMY ON APPLICATIONS DERIVING FROM MARINE COLLAGEN

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According to FAO Fisheries & Aquaculture, the annual consumption of fish products is about 17 kg per capita with consumption destined to increase due to the effect of population growth.

Regarding fishing waste, it is estimated to exceed 20 million tonnes each year, about 25% of the annual world harvest. Most wild fish stocks are classified as fully exploited, with several species facing extinction. Moreover, a remarkable share of waste is represented by the discarded fish, which includes many species and by-catches. Therefore, this is an increasingly emerging problem, even because fish industries are suspected of being responsible for significant risk to the environment. In addition, there are also species such as sea urchins, where waste is abundant due to the high content of inedible parts, such as shells, spines, and viscera. Lacking management of fish waste and overfishing have several negative implications on ecosystems and the protection of marine species (e.g., damage to habitats, illegal fishing, endangered fish species).

For the purposes of the research, a relatively niche market is considered in terms of volumes, that is of the sea urchin, which represents a very important food commodity for the culinary tradition of different regions of the world and for its high commercial value. Despite this commodity, belonging to the Echinoid class, is extremely high-profit, the aquaculture activities of the product remain very limited - about 1% of the total - compared to the almost 70,000 total tons of sea urchins sold per year.

Therefore, the aim of this study is to analyse the transition from a linear system to the circular economy, providing an example of reuse of sea urchin waste for the creation of eco-sustainable products deriving from marine collagen obtained by sea urchin waste. Marine collagen is a valid support for production of biomedical devices, dermal implants, cosmetic and pharmaceutical products.

Although the research field and the experimental phases on collagen derived from sea urchin are still quite recent, the future prospect of the use of this product is very promising.

The reuse of sea urchin waste is, therefore, a clear example of how a circular economy model, capable of making profits and recycling of waste, can be adopted, thus enhancing the management of food waste and promoting new products to be destined in increasingly and promising markets.

Acknowledgements
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AUTONOMOUS BEHAVIOURAL FEEDING AND ITS IMPLICATIONS IN LAND-BASED AQUACULTURE

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Land-based RAS systems are complex, fragile, and brimming with potential. There are millions of things that can (and often do) go wrong. At ReelData.AI, we want to ensure that you never have to think about feeding your fish. With our autonomous behavioural feeding system, we can ensure that your fish are perfectly fed, all the time, without the labour intensity of manually checking fish appetite.

In our presentation, our CEO will walk through Autonomous Behavioural Feeding (ABF) and how it drastically changes land-based aquaculture's profitability, consistency, and sustainability. While profitability will be mentioned, it will be spoken about in a more educational tone, as we want to explain how ABF reduces wasted feed and increases the rate at which the fish reach full maturity.

ABF is derived from a type of tech that is not new to aquaculture, pellet detection. Pellet detection tells farmers how hungry their fish are, but it doesn't fix the problem. ABF, on the other hand, works with an AI algorithm to learn how the fish's appetite changes and integrates with the farms feeding system to automatically adjust feed rates significantly more often than even the most diligent farmer could handle in one day.

With Autonomous Behavioural Feeding, you receive a multitude of benefits. From the financial benefits of reducing how much feed you waste, to the water quality improvement that comes from consistent feeding (versus over and underfeeding).

Let us introduce and educate the Aquaculture 2022 audience on the vast range of benefits that ABF can bring to an industry already brimming with potential!

ReelData

BUILDING CAPACITY OF ATLANTIC SALMON RAS PRODUCTION IN THE US - FROM RAS-N TO SAS^2

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Fueled by the growing investment in land-based RAS production of Atlantic salmon in the US, the federal government increased its support towards enabling public-private partnerships and ensuring success of this industry. In 2019, an initial award was made by NOAA/National Sea Grant for a collaborative program, the Recirculating Atlantic Salmon Network (RAS-N), a stakeholder-driven consortium consisting of several academic research institutions and industry partners. The main goal of RAS-N was to identify gaps in knowledge and impediments to the development of the industry, prioritize R&D needs and engage in extension, education, training and workforce development that will enable the success, stability and growth of the industry. RAS-N is generating a Road Map/Strategic Plan, which will help policymakers, federal and state agencies and industry identify and responsibly allocate resources to promote an economically feasible and environmentally sustainable land-based Atlantic salmon industry in the US. Based on progress made in the NOAA-funded program, a broader consortium was established and recently funded by USDA-AFRI to implement the RAS-N findings and recommendations. This program, Sustainable Aquaculture Systems Supporting Atlantic Salmon (SAS^2), is a multidisciplinary, synergistic "hand-on" national partnership in which leading aquaculture scientists, in collaboration with major US producers, carry out research focusing on industry-identified impediments to the expansion of the salmon RAS industry, including developing domestic broodstock and egg production, reducing early maturation, improving ecologically-responsible, RAS-specific feeds, understanding and mitigating off-flavor, increasing water re-use, treating and minimizing waste, and conducting economic analyses. Consortium-funded educators are developing RAS-related STEM curricula and modules at multiple education levels and have focused their efforts on the urgent need to foster a trained and skilled workforce for the rapidly growing industry. Aquaculture extension agents are working with industry to increase public awareness of this new form of farming, engage with local communities, enable efficient technology transfer from academia to industry and ensure fish health and seafood safety. SAS^2 is, therefore, expected to improve sustainable US aquaculture and food systems and enhance life for fish farmers and society.

PRELIMINARY SURVEY OF TRANSPOSABLE ELEMENTS FROM THE FIRST SPECIFIC PATHOGEN FREE (SPF) SHRIMP *Penaeus vannamei* PRODUCED IN THE UNITED STATES: URGENT NEED FOR A NEW WHOLE REFERENCE GENOME FOR SHRIMP

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As part of our efforts to understand the epigenetic mechanisms associated with susceptibility of *Penaeus vannamei* to bacterial and viral diseases, we proposed that horizontal transfer (HT) and methylation of transposable elements (TEs) are potential mechanisms involved in susceptibility to Acute Hepatopancreatic Necrosis Disease (AHPND), white spot syndrome virus (WSSV), and Infectious hypodermal and hematopoietic necrosis virus (IHHNV). To test this hypothesis, we needed access to a fully assembled *P. vannamei* genome sequence (expected size is 2.8 Gb), but none is currently available in the NCBI genome databases. The only genome sequence available for *P. vannamei* is a 1.6 Gb draft prepared with muscle DNA of male shrimp of Kehai isolate from China (GCA_003789085.1; breed Kehai No. 1) originally from the United States.

A pilot genome sequencing effort was initiated through 'The Shrimp Epigenome (ShrimpENCODE) Project' of the FUCOBI Foundation of Ecuador and Environmental Genomics Inc., MA, USA, with help from Amplicon Express and Pacific Biosciences collaborators. High molecular weight DNA from offspring of SPF Kona Line *P. vannamei* domesticated by the breeding program of the U.S. Marine Shrimp Farming Program (USMSFP) maintained at the Oceanic Institute in Kona and Oahu, HI were used for sequencing.

A total of 312 TEs were identified in a partial genome sequence (~470 Mb) belonging either to Class I [Long terminal repeat (LTR) retrotransposons and non-LTR retrotransposons] or Class II (DNA transposons). These included: 105 DNA transposons [EnSpm(1), Harbinger(11), hAT(13), Kolobok(2), Mariner/Tc1(10), Merlin(12), MuDR(1), P(1), piggyBac(8), Polinton(3), Transib(2), unclassified(41)]; 119 LTR retrotransposons [BEL(15), Copia(2), Gypsy(92), unclassified(10)]; 76 non-LTR retrotransposons [CR1(7), Daphne(7), Ingi(4), Jockey(4), Nimb(7), Penelope(18), Proto2(2), RTE(9), R4(2), Vingi(2), SINE2(3), unclassified(11)]; 1 integrated Nimavirus, *Nimav-1_LVa* (279,905 bp), and 11 unclassified. All sequences are deposited in Repbase (https://www.girinst.org/censor/index.php). The four most abundant TE families are: *RTE-3_LVa*, *RTE-2_LVa*, *NonLTR-1_LVa*, and *Nimb-1_LVa*. The complete genome of *Nimav-1_LVa* was found in the genome of *P. vannamei* Kehai isolate from China. This nimavirus seems to insert exclusively into the telomeric pentanucleotide microsatellite (TAACC/GGTTA)n (https://pubmed.ncbi.nlm.nih.gov/31947590/). Future research will focus on (a) sequencing a continuous, fully assembled reference genome from either brood stocks of the original SPF *P. vannamei* produced by the USMSFP or wild shrimp from Ecuador, and (b) understanding the epigenetic mechanisms associated with HT of retrotransposons.