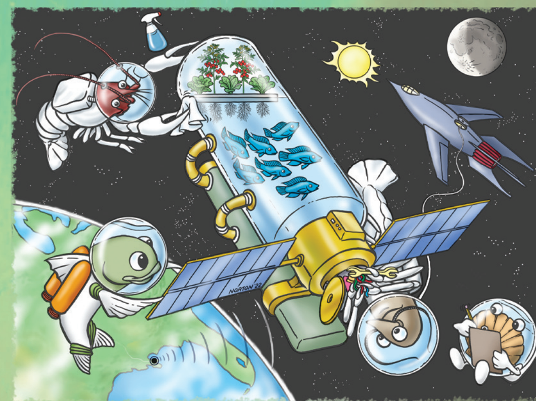
Aquaculture America 2023



Food For The Future

February 23-26, 2023

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WELCOME

"Laissez les bon temps rouler" …… Let the Good Times Roll, New Orleans style. On behalf of the United States Aquaculture Society, the National Aquaculture Association, and the Aquaculture Suppliers Association we welcome you to Aquaculture America 2023. We are so happy to be able to gather "post"-Covid with some sense of normalcy to network with our friends and colleagues, forge new relationships and get the latest aquaculture science and technology updates.

The theme of this year's conference is "Food for the Future". Everything from Aquaponics in space for long interplanetary journeys to the more practical, providing high quality protein and nutrition for the worlds growing population. We know aquaculture will play a major role and each of you will have a part in some way, large or small, in making that happen. Whether you are new to aquaculture or an old hand there is something here for you. With your help we have put together an impressive program of over 750 presentations, posters, panels, and round tables. If you are an expert in a particular area, I encourage you to branch out and see how your knowledge can interact with other disciplines to advance aquaculture in new ways.

Make sure to attend the plenary session to recognize and congratulate your fellow aquaculturists for their contributions to our various organizations and hear from two exciting plenary speakers. Daisey Berg will share her seafood marketing journey and how she and her high-end grocery chain employers learned that "farm-raised' has its place among the previously exclusive "wild caught" seafood items in the seafood case. The second speaker, Dr. Tracy Fanara will share how her creative philosophy and forward-thinking multimedia platforms make science communication relatable, entertaining, and accessible to all, including the next generation of scientists. Speaking of the next generation of aquaculture scientists and practitioners, please engage our student members to make them feel welcome and appreciated.

In addition, please make time to visit the trade show and thank our vendors and sponsors without whom the conference would not be possible. I would also like to take this opportunity to thank the other members of the Steering and Program Committee; Dennis McIntosh, Abigail Bockus, Carla Schubiger and Paul Zajicek for pulling together such an outstanding program. And to John and Noah Cooksey and the whole conference management team for keeping us organized and on track. Wrangling scientists can be a lot like herding cats but these outstanding professionals did just that. So, make the most of our time together and Let the Good Times Role as we step into the Future of Seafood.

Sincerely,
David J. Cline
Aquaculture America 2023 Steering Committee Chair

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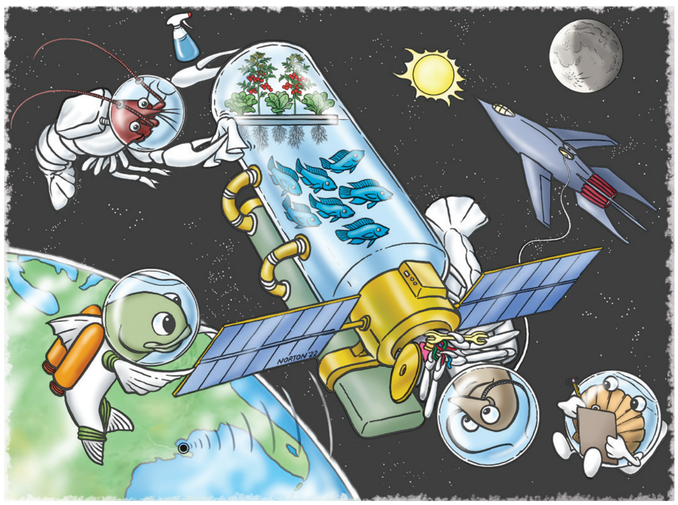

Food For The Future

ABSTRACTS

CATFISH VIRUSES: PROFILING AIDS MANAGEMENT STRATEGIES

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Catfish industry with an economic impact of $1.9 billion per year and 74% contribution to the finfish production is the largest sector of the U.S. aquaculture. Infectious agents can severely impact catfish production. Catfish viruses such as channel catfish virus (CCV) and blue catfish alloherpesvirus (BCAHV) are problematic during the hatchery and nursery phases of catfish culture. Viruses are dynamic entities that constantly evolve and adapt according to the surrounding environment, available resources, and hosts potentially leading to expansion in host range, host switch, and increase in virulence. Profiling catfish viruses will facilitate development of efficient pathogen-targeted management strategies. The external and internal clinical signs of infection, histopathological alterations, and cytopathic effects on cell cultures caused by these viruses are similar. While CCV is more pathogenic to channel catfish fingerlings, BCAHV caused significant mortality in blue catfish. Younger catfish are more vulnerable to viral infections. Crowding of fish and increased viral loads significantly increased viral infectivity and associated mortalities. Development of disinfection protocols, vaccines, and establishment of new catfish cell lines will aid with the management of these viruses. Both *in vitro* and *in vivo* experiments indicated the inactivation of CCV in the presence of a commercial disinfectant (active ingredient potassium peroxymonosulfate). An attenuated CCV vaccine was developed by serial passage of the wildtype virus in catfish cell lines. This attenuated vaccine was administered to channel and hybrid catfish fingerlings via immersion. Thirty days post immunization, the immunized and non-immunized fish were exposed to wildtype CCV. Results indicated that the vaccine significantly improved the survival of immunized channel and hybrid catfish compared to the non-immunized group confirming the protective immunity conferred by the attenuated virus vaccine. Further studies are underway to evaluate the efficacy of the vaccine.

SHOTGUN METAGENOMIC STUDY OF A COMMERCIAL TROUT FARM REVEALS TAXONOMIC AND FUNCTIONAL (DIS)SIMILARITIES ACROSS ENVIRONMENTAL- AND HOST-ASSOCIATED MICROBIOMES

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Microbiomes play a crucial role in the physiology of fish across various environments. Fish microbiomes are shaped by numerous environmental (i.e., water physicochemical parameters) and host (i.e., developmental stage, genetics, etc.) factors. While many studies have begun to explore microbiomes of rainbow trout, the exact metabolic functions of these microbiomes and their interactions with the host and its environment remain unclear. The two common methods used to study microbiomes (i.e., 16S rRNA gene sequencing and shotgun metagenomic sequencing) have their own set of limitations. 16S rRNA gene sequencing, for instance, is limited to identifying bacterial constituents, suffers from phylogenetic biases in PCR amplification, and provides no information of potential microbiome functions. Shotgun metagenomics, on the other hand, is relatively more expensive, computationally intensive, and often requires much deeper sequence to achieve the same level of characterization achieved by 16S rRNA gene studies.

We conducted a large-scale microbiome survey of commercial trout farms in Idaho using 16S rRNA sequencing data (presented here by Overturf). However, because of the limitations posed by 16S rRNA sequencing, a concurrent study, presented here, utilized shotgun metagenomics to evaluate trans-kingdom (bacteria, virus, fungi, etc.) phylogeny and metabolic functional potential of environmental (i.e., water, raceway biofilm, and diets) and host (i.e., fish gills, skin, and gut mucosa) microbiomes.

Raw samples from our large scale on-farm 16S rRNA study were pooled from 4th use rearing units for metagenomic evaluation of six discrete sample-types (i.e., raceway biofilm, diet, water, fish gill, skin, and gut mucosa). Samples were selected for metagenomic analyses according to DNA yield and good 16S rRNA results. Pooled DNA for each sample-type was shotgun-sequenced using Illumina technology. Reads were pre-processed using Trimmomatic and BBTools, taxonomic classification was determined using Kaiju and Kraken2. Metagenomic assemblies, binning, and annotations were conducted using the KBase platform and various command-line tools.

The level of host-contamination varied across sample types. Taxonomy results show a variation in the microbial composition of each sample type, although Protobacteria was the most dominant phylum across sample-types (Fig. 1). Further results presented will include methodological comparisons of bioinformatic steps, a deeper comparison of phylogeny by sample-type and technique (16SrRNA vs. shotgun) and insights on the metabolic functional potential of these on-farm environmental and host-associated microbiomes.

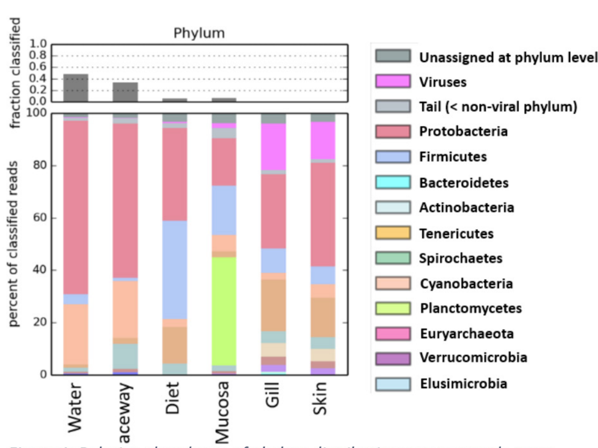

Figure 1: Relative abundance of phylum distribution across sample types

ASSESSMENT OF THE POTENTIAL OF AUTOCHTHONOUS PROBIOTICS FOR ADMISTRATION TO MIRROR CARP *Cyprinus carpio*

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A. hydrophila causes haemorrhagic septicaemia in freshwater fish, with outbreaks leading to up to 80% of losses in some stocks. Previous reports have demonstrated that dietary probiotics can enhance the disease resistance of *C. carpio* against various pathogens, including *A. hydrophila*. This study isolated carp autochthonous candidate probiotic strains, with *in vitro* antagonism against a range of fish pathogens, and used aqueous administration to investigate the potential to inhibit *A. hydrophila* in the rearing medium and on the mucosal surfaces of mirror carp.

Intestinal mucosa and digesta samples from *C. carpio* (n = 5) were homogenized in phosphate-buffered saline (PBS). Homogenates were cultured on tryptic soy agar (TSA) plates and isolated colonies were tested *in vitro* for antagonistic activity against *A. hydrophila, Streptococcus iniae*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Yersinia ruckeri*, and *Pseudomonas anguilliseptica*. Out of 150 isolates, 16 displayed antagonism against *A. hydrophila*. Of these 16, 87.5% were antagonistic against *S. iniae*, 50% were antagonistic against *V. anguillarum*, 6.3% were antagonistic against *V. parahaemolyticus*, 12.5% were antagonistic against *Yersinia ruckeri*, and 87.5% were antagonistic against *Pseudomonas anguilliseptica*. Two promising isolates (C24 and C72) were used in pathogen-mucosal colonisation studies. In order to reduce negative impacts on welfare by challenging live fish, exposure assays were conducted using whole fish bodies immediately post-mortem. *C. carpio* (mean weight, 18.0±3.8 g) were euthanized and distributed randomly into four exposure treatments (n = 5): (T1) control, exposed to sterile PBS, (T2) PBS containing *A. hydrophila*, (T3) PBS containing *A. hydrophila* and probiotic candidate C24, and (T4) PBS containing *A. hydrophila* and probiotic candidate C72. After 90 min exposure, the fish skin, gill, fins, and rearing medium were sampled, and homogenates were spread onto *Aeromonas* agar plates (incubated at 25°C for 24-36 h) to determine *Aeromonas* levels. *A. hydrophila* was not detected in the rearing medium or mucosal tissues in the control group after the exposure. The levels of *A. hydrophila* in treatments T2 and T3 increased by 82% and 89%, respectively, demonstrating the ability of *A. hydrophila* to proliferate in the PBS solutions exposed to carp mucus and the inability of isolate C24 to inhibit *A. hydrophila* proliferation under such conditions. However, candidate C74 significantly ($P < 0.05$) retarded the growth of *A. hydrophila* in treatment T4 (with *A. hydrophila* levels only increasing by 34%, vs 82% in the control). The effects on *A. hydrophila* mucosal colonisation levels were also investigated (data not shown). Further studies are being conducted to observe the effects on mucosal colonization levels on a range of other aquacultured freshwater fish species.

BASIC AQUACULTURE PRODUCTION DESCRIPTORS OF ICTALURID CATFISH RAISED IN COMMERCIAL FRESHWATER EARTHEN PONDS: A SEVEN-YEAR CASE STUDY FROM ALABAMA, USA

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Alabama (AL) ranks second in food-size catfish production, accounting for 26% of the total catfish sales in the U.S. in 2021. Most commercial food-size catfish production in AL is in earthen ponds in seven western counties (Dallas, Greene, Hale, Marengo, Perry, Pickens, and Sumter). Catfish production faces serious challenges such as high feed prices, disease, competition from international markets, and big fish (not accepted by fish processors or accepted at a reduced price). Identifying basic descriptors (such as county, farm area, pond size and number, stocking rate, breed cultured, production, type of feed utilized, protein content of feed, and aeration rate) of the catfish industry provides crucial epidemiological, etiological, and pathological tools. The objectives of this study were to quantify the production area and determine basic descriptors of the AL catfish industry both statewide and at the county level. The Alabama Fish Farming Center conducted annual surveys of all commercial catfish producers in AL during 2015–2021 by mail and/or telephone interviews to achieve these objectives.

Across the study (2015–2021), the annual number of survey respondents ranged from 64–74, with a total of 482 respondents. The yearly survey response rate ranged from 95.6–100% (mean: 98.2%). The annual survey coverage area ranged from 6,410–7,006 ha/year. The majority of reported catfish breed produced was channel only (61.3%), followed by channel and hybrid (30.5%), and hybrid only (8.2%; Fig. 1). Average aeration rates ranged between 2.8 and 18.4 kW/ha (mean ± SE = 7.8 ± 0.25 kW/ha) and was higher in farms producing hybrid only (9.6 ± 0.51 kW/ha) as compared to farms producing channel only (6.7 ± 0.29 kW/ha). Pickens county reported the highest average aeration rate (12.2 ± 1.57 kW/ha) and the highest percentage (84.6%) of farm operations producing hybrid catfish only. The present study provides the first comprehensive assessment of primary descriptors of the farm-raised catfish industry in the freshwater pond environment for a substantial duration.

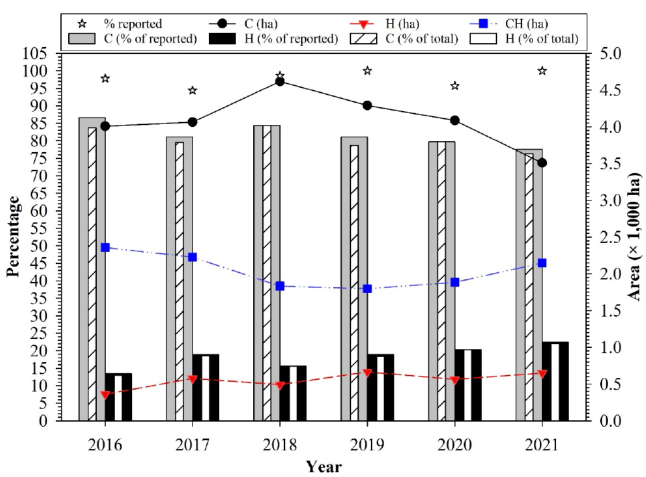

Figure 1. Annual distribution of catfish breeds in commercial catfish farm operations in west Alabama during 2016–2021. Stars indicate the annual area of farm operations reported catfish breeds as a percentage of the total area of respondent operations. Bars indicate areas of farm operations contain channel catfish (C) or hybrid catfish (H) as percentages of the total area of respondent operations (% of total; narrow bars) or percentages of the area of farm operations reported catfish breeds (% of reported; wide bars). Lines with symbols indicate the area (ha; right y-axis) of farm operations that produce channel catfish only (C), hybrid catfish only (H), or both hybrid and channel breeds (CH). The question about breeds was not included in 2015 survey.

EVALUATING THE FEASIBILITY AND SUSTAINABILITY OF AN INTEGRATED MULTI-TROPHIC RECIRCULATING AQUACULTURE SYSTEM USING STRIPED BASS (*Morone saxatilis*), SAND WORMS (*Alitta virens*), AND SEA BEANS (*Salicornia bigelovii*)

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In many ways, striped bass (*Morone saxatilis*) aquaculture production using recirculating aquaculture systems (RAS) is commercially ready in the United States. The broodstock, larval, nursery, and grow-out husbandry techniques are established, wholesale and high-value niche markets exist, and consumer demand is high. However, as with other forms of finfish aquaculture, waste management is a major challenge constraining the expansion of RAS-reared striped bass. The objective of this project is to investigate whether integrated multi-trophic aquaculture (IMTA) is a feasible and environmentally sustainable strategy for mitigating the wastes produced by RAS-reared striped bass. Specifically, we are testing the capacity of the polychaete *Alitta virens* (i.e., *Nereis virens*) and the halophyte *Salicornia bigelovii* to utilize solid and dissolved wastes, respectively. Commonly known as the sand worm, *A. virens* is an important bait species in the Northeast with high commercial value. *S. bigelovii,* also known as sea bean, pickleweed, and sea asparagus, is an edible, salt-tolerant plant with several commercial uses, from animal fodder and biofuel to human consumption. In this ongoing experiment, two nearly identical RAS systems were established. One system is a conventional RAS set-up for striped bass monoculture, while the other system has been altered for IMTA by adding tanks containing sand worms and sea beans. Over the course of the experiment, we are measuring and comparing striped bass growth, survival, and feed conversion ratio, system waste accumulation, and system maintenance metrics (e.g., number of weekly water changes) across the two systems. We are also measuring growth and survival of the sand worms and sea beans in the IMTA RAS. If successful, this novel IMTA combination may have the potential to diversify aquaculture operations, increase RAS sustainability, and give growers new sources of income through the sale of additional crops.

BLACK SOLDIER FLY LARVAE: A NOVEL PROTEIN SOURCE FOR AQUACULTURE

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The aquaculture industry is constantly in search of novel protein sources for many reasons. Insects can potentially provide a high-quality, sustainable source of proteins to salmonid species to help offset the need for animal-based protein in the diet, and to help alleviate soybean meal-induced intestinal Enteritis in salmonids. Crickets, Mealworms, and Black Soldier Fly Larvae (BSFL) are the three common insect species used in animal feeds and pet foods globally. Particularly, the Black soldier fly larvae has received a lot of attention due to its short life cycle and ability to eat ingredients that would normally be destined for the landfill. BSFL can be processed to create at least 4 feed ingredients including whole dried insects which can then be further processed to produce an insect oil and an insect meal. Finally, insect frass is produced from processing the residual material from the insect rearing process including leftover feed and exoskeletons and may also have applications in plant and animal nutrition. The nutrient composition of these ingredients are highly dependent on the insects dietary and rearing practices. Along with being an excellent source of protein in their meal form (50-60% CP), insects also provide an excellent source of fats, vitamins, and minerals. This presentation will describe the nutritional benefits of utilizing BSFL ingredients as a protein source in Aquaculture diets, while also exploring how nutrient composition may change with insect diet and rearing practices.

BENEFICIAL PROPERTIES OF INSECTS: ANTIMICROBIAL PEPTIDES, CHITIN, AND LAURIC ACID

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Crickets, mealworms, and black soldier fly larvae (BSFL) are three common insect species used in animal feeds. BSFL has received a lot of attention due to its short life cycle and ability to consume a wide variety of low-quality ingredients, upcycling them into a high-quality protein and energy source. In addition to the nutritional benefits of BSFL as a protein and energy source, these ingredients also have the potential to optimize animal performance via naturally occurring antimicrobial peptides (AMPs), chitin, and lauric acid. AMPs are small molecules that are a component of the insects' innate immune system and can cause direct killing of bacteria. Chitin provides benefits by acting as a prebiotic, supplying nutrients for beneficial bacteria in the gut microflora. Finally, lauric acid is a major component of BSFL fatty acid composition and has been demonstrated to have many antimicrobial properties. This presentation will examine the beneficial properties of insect ingredients with specific focus on AMPs, chitin, and lauric acid.

ENCOURAGING HEALTHY LIVING THROUGH DEVELOPMENT OF TASTY RECIPES, HEALTHY COOKING, AND EDUCATIONAL VIDEOS BASED ON LEAN PROTEIN FROM LOCALLY GROWN NILE TILAPIA

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The COVID-19 pandemic augmented food insecurity and it compromised health throughout the United States, especially for minority and limited-resource groups that are socioeconomically disadvantaged, highly vulnerable, with high-risk health conditions. This project addresses the need for increased food security by providing support for locally farmed Nile Tilapia for healthier and expanded food choices, healthier cooking, and healthier living. It entails active participation and support by Kentucky State University's (KSU) United States Aquaculture Society Student Subunit, and KSU's faculty, staff, and State Extension Specialist for Dietetics and Human Nutrition. Subunit members participated in training on processing of whole fish and fillets (Figure 1). Still images and videos were recorded for use in development of postcards, brochures, infographics, and other Extension-based publications. Short videos (1 to 3 minutes) were produced on cleaning, processing, and cooking of whole fish and fillets, and healthy recipes and cooking methods were created and disseminated in social media (KSU Aquaculture YouTube Channel, Pinterest, TicTok, Facebook, and Twitter). These activities are in partial fulfillment of the human nutrition objective of KSU's Extension-based Tilapia Capacity Building Project, and they provide tangible products and resources that will be used to impact the daily livelihood and health of Kentuckians and others.

Figure 1. Kentucky State University's United States Aquaculture Society Student Subunit and Aquaculture staff processing Nile Tilapia harvested at KSU's Aquaculture Research Center, Frankfort, KY.

STATUS OF LOCAL FISH FEED MILLS IN NIGERIA: THE CASE OF AKURE METROPOLIS

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Fish requires high quality nutritionally balanced diet for growth and attainment of market size within the shortest possible time. Therefore, local production of fish feed is very essential to the development and sustainability of aquaculture in Nigeria. Sustainable aquaculture can bridge the already existing wide gap between fish demand and supply. Hence, the vital role of locally produced fish feed in reducing production cost, thereby making fish farming sustainable and attractive to both private and commercial investors and eventually boost fish production cannot be overemphasized.

This research looks at the status of local fish feed mills in Akure metropolis in enhancing aquaculture development, and expansion in Akure metropolis and Nigeria as a whole. Both primary and secondary data were used in the study. Socioeconomic and Demographic characteristics of the respondents were analysed descriptively, Snowball method was used to locate the Fish Feeds Mills in Akure Metropolis. Results revealed that 69 percent of the respondents were male while 31percent were females; 61 percent were between the active age of 30 and 49 years; also, 70 percent had formal education. Currently, the status of Fish Feed Mill industry in Akure is experiencing low growth due to high cost of fish feed ingredients which constituting about 60 percent of the total cost of fish production. Other fundamental challenges observed in this study were: lack of funds to acquire the extruding machine to help produce locally floating feeds rather than sinking pellets; technical know-how on the part of the feed millers; poor nutritional value of the locally produced fish feeds; and provision of storage facilities to prevent spoilage of fish feeds.

Plate 1: Pelleting machine
Source: Field survey, 2021
Picture by Omotosho Kehinde Jubril 2021.

The study also revealed that, amid the ten (10) feed mills visited in Akure Metropolis, **none of the mills produces solely fish feed**: only four (4) combined livestock and fish feeds while the remaining six (6) produces only livestock feeds. There was high concentration in the production of livestock feeds compared to Local Fish Feed production in Akure Metropolis. Hence, the need to depend more on imported fish feeds. The study therefore recommends, quick intervention from both the private and public sectors on the observed challenges impeding the development of fish feed mills in the Metropolis and the Nation at large.

A COMPARATIVE GROWTH RESPONSE OF FLORIDA POMPANO *Trachinotus carolinus* AND RED DRUM (*Sciaenops ocellatus)* TO HEMP PROTEIN ALTERNATIVE DIETS WITH/ WITHOUT TAURINE SUPPLEMENTATION

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Fishmeal is the primary dietary protein source for many farmed fishes. The decrease in the supply of fishmeal relative to its demand has heightened research on alternative protein ingredients. Hemp protein meal (HPM), a by-product from the hemp processing plant, has been reported to contain 30 – 40% crude protein and very high polyunsaturated contents, depending on the variety of the hemp plant. Despite the prospect of HPM in the aquafeed industry, information on its nutritive values and utilization by cultured fish is unavailable. Therefore, evaluating HPM productivity in Florida pompano and Red drum becomes necessary in aquaculture.

This study investigated the effect of replacing fishmeal (control) with hemp protein meal on the survival and growth performances of Florida pompano and Red drum. We hypothesized that HPM would produce negative responses on survival, feed intake, feed efficiency, growth, and proximate compositions in both Florida pompano and Red drum. Seven isonitrogenous (40% CP) and iso-lipidic (10%) experimental diets were formulated. In a completely randomized design with the tank as the experimental unit, Florida pompano and Red drum fingerlings were stocked in a recirculating system to triplicate groups of 20 fish/tank and three tanks/diet. Fishes were carefully fed by hand three times daily for 68 and 63 d, respectively, and survival was monitored. Post-feeding trial samples of both fishes (tissues, blood, and gut contents) were collected to determine body indexes, proximate compositions, and growth performance.

Our results showed that HPM had no adverse effect on both fish, with the survival of Florida pompano and Red drum ranging from 85 – 100% and 90 -100%, respectively. However, the growth of both pompano and Red drum (Fig. 1) was significantly affected by replacing fishmeal with HPM ($P<0.05$). Increased levels of HPM without taurine reduced the fish weights, and taurine supplementation offset the negative weight responses ($P<0.05$). Results on proximate compositions, protein efficiency ratio, and amino acid compositions will be presented later.

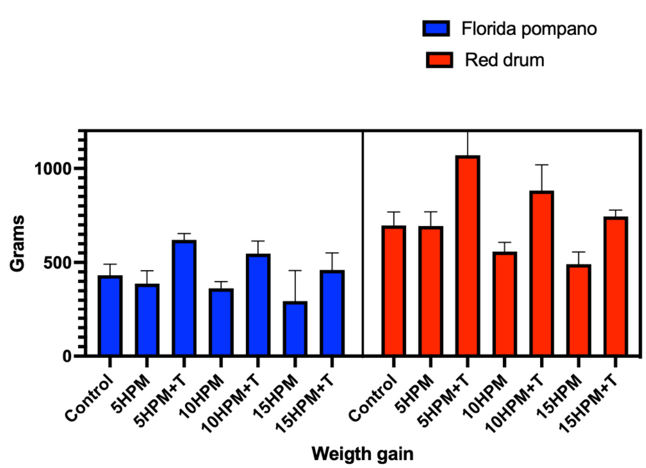

Fig. 1. Growth performance of Florida pompano and Red drum fed HPM diets

ASSESSMENT OF HOUSEHOLD LIVELIHOOD DIVERSIFICATION AND FISHERIES CONSERVATION STRATEGIES AMONG FISHERMEN IN COASTAL AREAS OF OGUN STATE, NIGERIA

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This study assessed the association between household livelihood diversification and fisheries conservation policies among fishermen in coastal areas of Ogun State, Nigeria by adopting a multistage sampling procedure. The sample size was 90 fishermen from six randomly selected fishing communities (Abureji, Agbalegiyo, Ilamo, Imosan, Iseku and Wharf) along the coastline in Ogun State, Nigeria. Data were collected using pre-validated interview schedule and subjected to descriptive and inferential analytical techniques. Results revealed that majority of the fishermen were married (98.9%), in the age bracket of 41-60 years (71.1%) with mean age of 49 years, had household size of 6-15 persons (91.1%) with mean household size being 9 persons, from extended families (90.0%), either either no formal (43.3%) or only primary education (41.1%), were non-members of social groups (62.2%), and had no other occupations (93.3%).

It was also reported that there was generally low level of household livelihood diversification across the fishing communities. Gill nets were the most commonly used fishing gears across the fishing communities (80.0%). This was followed by seine nets (63.3%), traps (56.7%) and trawl nets (53.3%) while fish aggregating devices (35.6%), cast nets (37.8%) and hook and line (24.4%) were the least used fishing gears in the study locations. Results further revealed that coastal fishery was characterised by conflicts among water users (64.4%), absence of protected fishing areas (81.1%), and experience of water pollution (36.7%). Majority (71.1%) of the fishermen across the study locations agreed with closed season policy as a coastal fisheries conservation strategy. This was followed by gear restriction (30.0%). Results of Chi-square analysis revealed that there were significant associations between level of household livelihood diversification and fishermen's agreement with gear restriction (χ^2 = 15.545, df = 5), and closed season (χ^2 = 11.214, df = 4). The study concluded that coastal fisheries is in a poor state and that it could be improved through the introduction of gear restriction and closed season policies. The study recommended that youths in the coastal areas should venture into fishing, and that government and non-governmental agencies should organize sensitization programmes on fisheries conservation policies across the coastal areas in Ogun State.

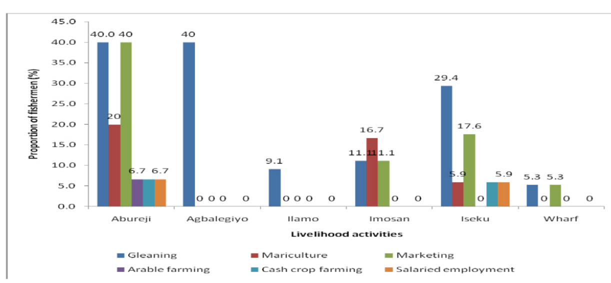

Figure 1: Livelihood activities of fishing households by fishing villages

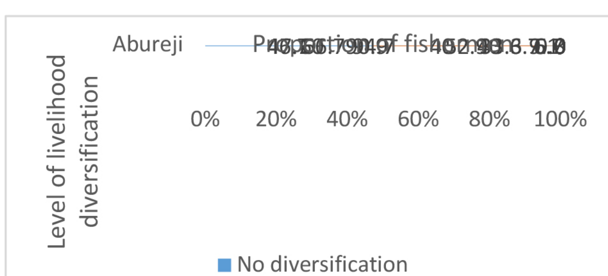

Figure 2: Levels of fishing household livelihood diversification by communities Coastal fisheries conservation

OFFSHORE PRODUCTION OF BLUE MUSSELS *Mytilus edulis*: A SWEDISH PROFITABILITY STUDY

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Blue mussel (*Mytilus edulis)* farming in Europe is an established industry, although production from aquaculture has been declining over the last decade, due to among others, low mussel prices stemming from competition with imported mussels from outside the EU, atomization of the producer sector into small enterprises, lack of sustainable space to enlarge operations and difficulties to obtain permits. Novel technologies that can support diminishing production costs, as well as facilitating expansion of the activity in offshore areas with less marine spatial conflict, can provide improvement for the farming in Europe.

The firm Bohus Havsbruk (BH) has blue mussel farming facilities at seven sites in three different production areas off the west coast of Sweden. The farm uses automated farming technology consisting of floating tubes from which spat collectors (nets) are vertically suspended. The infrastructure used is more robust and flexible than that of traditional mussel farms, thus enabling the farm to operate further offshore. Due to challenges from other stakeholders and governmental restrictions the farm has though not been able to harvest and only maintenance activities are currently ongoing. However, the technology has been tested with good results and has the potential to be up scaled and replicated in other areas.

Using data obtained from BH, we calculated the net present value (NPV) of profits over a period of 10 years. As each production cycle can be up to 36 months long, this allows for three full production periods throughout the whole period, with the company able to harvest 3,000 tonnes per production cycle. Larger mussels are sold directly to fish mongers at a price of 2.06 EUR/kg, while smaller mussels are sold in bulk at a price of 0.84 EUR/kg. The average unit cost of production is 0.68 EUR/kg. Assuming an interest rate of 7%, the NPV of the project is calculated as 0.61 million EUR, indicating that despite very high initial investment costs of 5.4 million EUR, projected revenue for the period can make the operation economically viable. The baseline scenario assumes a predation rate of 10%, mainly from eider ducks, but a higher rate is not sustainable. However, the firm can take on up to 10% higher wages and live with up to 5% lower mussel prices. An interest rate of 9% or higher results in negative NPV.

Sweden is a high-income country, where wages are considerably higher than in many other EU-countries. Setting up mussel farms based on the same or similar technology could therefore be even more profitable in low-income countries in Europe and elsewhere. It is also possible that the technology used will become less expensive through time. Such a development would make this production system even more attractive.

CONSUMER ACCEPTANCE AND WILLINGNESS-TO-PAY FOR LOW TROPHIC AQUACULTURE PRODUCTS

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As part of the Aquavitae project, financed by the EU, a study was undertaken to identify positive and negative motives, perceptions, and attitudes towards consumption of low trophic aquaculture (LTA) products. For this purpose, online surveys were conducted in June 2021 in four regions bordering the Atlantic Ocean: Spain; the west coast of South Africa; coastal Brazil; and the east coast of the USA. A representative sample of 300 participants was collected from each country.

The survey focused on consumer's views on three LTA species; macroalgae, sea urchin, and oysters, as well as LTS freshwater fish. Results indicate that while Brazilian consumers are already open to accepting LTA products into their food choice, North Americans are more hesitant and would have to be specifically targeted with a communication campaign on issues related to sensory appeal, natural content, health, environmental protection, animal welfare, and of course convenience and price.

A discrete choice experiment was conducted as part of the consumer survey to quantitively assess consumer preference. Participants were given a choice set consisting of nine alternatives, each with four attributes, and asked which one they would prefer. The attributes were nutritional value (high or medium), environmentally friendly certification, socially responsible certification, and price (ordinary price, 20% higher, 50% higher). A total of 1,038 individuals took part in the discrete choice experiment. The analysis was based on a mixed logit model, where the parameter associated with price was assumed to be fixed for the sample, but the parameters associated with the other attributes allowed to vary, thus taking into consideration that preferences may differ between consumers. The model was estimated both for each region separately as well as for a pooled sample. The model results were then used to calculate the participants' willingness-to-pay (WTP).

A significant WTP for seafood products with high nutritional values was found in all the cases except the US. The overall mean was 0.5, indicating that consumers would in general be willing to pay 50% more for products with this attribute. Consumers in all regions except South Africa are also willing to pay on average 32% more for seafood products that are certified environmentally friendly. Consumers are however neither willing to pay anything more for seafood products with medium nutritional value nor for products that are certified socially responsible.

ASSESSING THE EFFECTIVENESS OF NCRAC-FUNDED RESEARCH IN AQUACULTURE WITHIN THE NORTH CENTRAL REGION

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The North Central Regional Aquaculture Center (NCRAC) is one of the five Regional Aquaculture Centers mandated by the US government to advance and enhance viable and profitable commercial aquaculture production in the US for the benefit of producers, consumers, and the US economy. Since its establishment in 1988, NCRAC has supported several aquaculture projects that aim to advance and sustain the aquaculture industry in the North Central Region (NCR). However, there has been little analysis of the effectiveness and impacts of NCRAC-funded projects. A review of all NCRAC-funded projects between 1988-2019 was conducted to identify each project's objectives, procedures planned, anticipated benefits and impacts, outcomes accomplished, benefits and impacts reported, and recommended follow-up activities. Information obtained from the funded projects was used to develop a survey instrument to identify which project outcomes and impacts were of benefit to aquaculture producers in the NCR.

Results showed that NCRAC spent approximately $14 million on 125 aquaculture projects between 1988 and 2019. Those projects attracted an additional $12.1 million in leveraged funding support from other federal agencies, universities, and other industry stakeholders. The funded projects focused on advancing the production of nine fish species (yellow perch, walleye, sunfish, salmonids, tilapia, crayfish, baitfish, largemouth bass, and hybrid striped bass) in the NCR and other aquaculture-related topics such as Extension/education-related projects, conferences/workshops/symposia, new animal drug applications, aquaculture drugs, nutrition/diets, aquaponics, waste effluent, white papers, and economics and marketing. NCRAC has contributed to the development of human capital resources in the US by supporting the education of 41 graduate students through its funded projects. High percentages of survey respondents who participated in NCRAC-funded Extension meetings and used NCRAC-funded Extension materials found the information presented to be applicable and had adopted recommended practices on their farms. Futhermore, high percentages of respondents who raised yellow perch, walleye/hybrid walleye, sunfish, trout, tilapia, and baitfish were familiar with information on culture technologies of the species they raised and had adopted the recommended culture technologies. Growth in yellow perch and walleye farming in the NCR in the last two decades can partly be attributed to NCRAC's investment in yellow perch and walleye farming methods. Overall, finding revealed that through NCRAC-funded projects, NCR producers had gained awareness of a number of issues, had adopted NCRAC project recommended practices, and those practices had contributed positively to improve production efficiencies and profitability of their businesses. While no one research effort is ever entirely responsible for adoption of new technologies and their impacts, NCRAC has successfully leveraged other funding, such as Sea Grant, USDA-Animal and Plant Health Inspection Service, and veterinarian services on environmental quality, disease, and other issues.

APPARENT DIGESTIBILITY COEFFICIENTS OF BY-PRODUCTS IN INTEGRATED RICE AND FISH FARMING (RICE BRAN AND FISH OFFAL MEAL) FED TO THE AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822) AND *Oreochromis niloticus* (LINNAEUS, 1758) JUVENILES

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The process of farm diversification through the integration of aquaculture and agriculture (IAA) is a growing innovation in the developing world. By-products generated such as rice bran and fish offal from this intervention (especially in integrated rice and fish farming (IRF)), may be a major environmental concern if not properly disposed of or utilized. Given the important role sustainable feed development plays in aquaculture development, exploring the opportunities of converting these by-products from IAA for the production of good quality and cost-effective feed for aquaculture development is highly imperative. Knowledge-based on the utilization of these IRF by-products in aquaculture nutrition, therefore, needs to be developed. This study, therefore, investigated the nutrient composition and apparent digestibility coefficient (ADC) of nutrients in rice bran and fish offal in diets fed to *Clarias gariepinus* and *Oreochromis niloticus*.

An 8 weeks feeding trial was conducted in the University of Ibadan, Aquaculture Nutrition Laboratory to determine the apparent digestibility of the test ingredients (TI) - rice bran feed (RBF) and fish offal meal feed (FOM) (procured from USAID/MSU IAA intervention project in Nigeria). These TI were analyzed for their Amino Acid Profile and the amino acid contents in each of the samples were further evaluated using Provisional Amino Acid of Egg Scoring Pattern (PAAESP) to determine their Amino Acid Score (AAS) ingredients. In addition, the TIs were included in the diets of *Clarias gariepinus* and *Oreochromis niloticus* to formulate 40 % and 30% crude protein (CP) reference diets (RF), respectively. Test diets contained 70 % of reference diets and 30 % of test ingredients, with chromic oxide as the inert marker. Replicated groups of *C. gariepinus* and *O. niloticus* (10±0.01; 15±0.01 g. respectively) were fed these diets for 8 weeks. Apparent digestibility coefficients for protein, ash and energy were determined following standard methods. Data were analysed using descriptive statistics, and ANOVA at $\alpha_{0.05.}$

In this study, the crude protein (%) of 65.83±0.21 and 10.59±0.11 were recorded for fish offal meal and rice bran, respectively (Table 1). These values compared favourably to fish meal (75.4%) and maize (9.4%). Ash contents were 9.61±0.01 (%) in fish offal meal and 9.13±0.02 (%) in rice bran. The essential amino acid profile (Table 2) revealed that FOM has arginine, lysine, methionine and Tryptophan with RB having methionine and Tryptophan at the concentration required for growth in *C. gariepinus*. The FOM contained histidine, isoleucine, methionine, Phenylalanine, Threonine, and Tryptophan with RB having (Histidine, methionine, Phenylalanine, and Tryptophan) for growth in *O. niloticus*. The limiting amino acid (LAA) for both the fish offal and rice bran was Met+Cys (TSAA). **The** ADC of nutrients (Tables 3 and 4) varied significantly across treatments (p<0.05) in *C. gariepinus* and *O. niloticus*. ADC of Protein was significantly highest in FOM (77.77 ± 0.49 %) and least in RBF (64.17 ± 0.83 %) in *C. gariepinus* and FOM (78.61 ± 0.51 %) and least in RBF (63.11 ± 0.19 %) for O. *niloticus*.

The result of this study revealed that nutrient digestibility by *C. gariepinus* and *O. niloticus* was good for both TI while FOM gave better results for the test fishes.

(Continued on next page)

Table 1: **Proximate Analysis of the Test Ingredients *Rice Bran RBF and Fish Offal Meal FOM**

Samples	Moisture content (%)	Crude protein (%)	Fat content (%)	Crude fiber (%)	Ash content (%)	Carbohydrate (%)
Rice bran	10.21±0.01	10.59±0.11	16.08±0.04	14.06±0.04	9.61±0.01	35.71±0.25
Fish offal meal	7.11±0.01	65.83±0.21	9.41±0.01	1.93±0.01	9.13±0.02	6.60±0.21

Table 2. **Amino Acid Content(g/100g) and a Score of Fish Offal Meal and Rice Bran using Provisional Amino Acid of Egg Scoring Pattern**

Parameters	PAAESP Protein (g/100g)	Fishoffal EAAC	AAS	Ricebran EAAC	AAS
Leucine	7	7.66	1.09	7.24	1.03
Lysine	5.5	6.82	1.24	4.35	0.79
Isoleucine	4	4.03	1.01	2.85	0.71
Tryptophan	1	1	1	1.08	1.08
Valine	5	4.77	0.95	3.22	0.64
Threonine	4	3.81	0.95	3.16	0.79
Phenylalanine + Tyrosine	6	7.96	1.33	6.67	1.11
Methionine + Cystine	3.5	2.74	0.78	1.62	0.46
Total	36	38.79	7.36	30.19	6.63

Table 3: **Apparent Nutrient Digestibility ($) for *Clarias gariepinus***

Parameter (%)	RF	RBF	FOM
ADC Protein	72.51 ± 1.12^{b}	64.17 ± 0.83^{c}	77.77 ± 0.49^{a}
ADC Energy	54.45 ± 6.88^{c}	59.45 ± 4.62^{ab}	65.14 ± 6.23^{a}
ADC Lipid	70.87 ± 0.98^{c}	74.19 ± 3.59^{ab}	77.24 ± 1.88^{a}

Table 4: **Apparent Nutrient Digestibility ($) for *Oreochromis niloticus***

Parameter (%)	RF	RBF	FOM
ADC Protein	66.50 ± 1.98^{b}	63.11 ± 0.19^{c}	78.61 ± 0.51^{a}
ADC Energy	60.77 ± 0.58^{a}	64.30 ± 2.16^{a}	76.46 ± 3.41^{a}
ADC Lipid	71.21 ± 5.67^{a}	73.95 ± 1.07^{a}	75.13 ± 5.61^{a}

MAXIMIZING THE NUTRITIONAL IMPACT OF A FARM DIVERSIFICATION (RICE-FISH) INTERVENTION: A CASE STUDY FROM NIGERIA

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Introduction
A study on farm diversification through rice-fish farming was conducted in two Nigerian states (Ebonyi and Kebbi) between 2020-2023. The farm diversification process provided rice farmers the opportunity to start a new farming enterprise by modifying their fields to produce fish in addition to rice, including developing their capacities on fish stocking, feeding, best rice-fish co-culture management practices, value-addition (fish smoking), among others. The system optimizes the use of available land and water resources for the simultaneous production of two food commodities, rice and fish, in the same production area, resulting in increased total farm yield per unit area, increased income and profitability, and improved levels of household and community nutrition. The ultimate goal of the farm diversification is to increase productivity as well as the farming family's food and nutrition status. Using the food systems approach, this paper described the step-by-step process (impact pathways) required to maximize this goal.

The food system approach
The methodology employed a food systems approach to recognize the role of various actors at all stages of the food chain, from production, processing, to consumption. A theory of change and impact routes were proposed for observation and evaluation in order to identify entry points to be prioritized to perform the necessary transformative measures for better nutrition outcome.

Step 1: Situational analysis
(i) Food security and nutrition profile
The calculated household food insecurity experience scale (FIES) indicator (12-month recall) of the sample studied indicated severe food insecurity. A large proportion of the population reported worrying about running out of food, compromising on quality and variety of food, reducing quantities, skipping meals and/or experiencing hunger within the last 12 months.
(ii) **Overview of the Agriculture sector**
The communities in this study are major rice producers, offering opportunities to include fish production in an integrated agri-aquaculture farming system. The seasonal calendar of farming activities showed the possibility of rice cultivation two times a year depending on annual rainfall patterns, thus presenting the opportunity for fish farming two times a year as well. Two major challenges were identified: (i) Depending on the area, access to land for farming is governed by various customary (community) arrangements or inheritance, however access to land for agricultural practices could be a challenge, especially for women; (ii) The majority of the farmers are familiar with capture fisheries, with little or no experience about fish culture. Therefore, the farm diversification intervention generated entry-level advice for rice farmers with little or no knowledge of aquaculture to modify their rice farms to produce rice and fish simultaneously.

Step 2: Theory of change
The theory of change was designed with the concept that fish production in addition to rice will have a significant impact on food and nutrition security, dietary diversity, and sustainable production for self-sufficiency.

Step 3: Impact pathways and external drivers
The impact pathways followed include: (i) resolving the constraints along the food supply chain impact pathway (fish seed, rice seed, labor, land and water); (ii) strengthening the food environment impact pathway (yield/size of fish at harvest, affordability, value addition (smoking)); and (iii) influencing consumer behavior pathways (more diverse diet (fresh/smoked fish, rice, other collected aquatic foods from the ricefields) available for purchase and home consumption in the community).

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Socio-cultural inclination to a new way of farming, and perceived economic benefits (increased income and profitability) were the major key external drivers.

Conclusion and key findings

In the context of improving food security and nutrition security, the farm diversification trials demonstrated how promoting small-scale, inexpensive integrated agriculture-aquaculture (rice-fish) farming not only provides an immediate boost to the local supply of nutritious food but can also generate income. It has the potential to transform local agri-food systems in rice-producing communities into a more resilient, and economically viable venture.

Acknowledgement

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AQUATIC ANIMAL HEALTH AND TREATMENT

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It is well established that the world population is increasing as is the demand for aquatic food products. Over the years, diseases and epizootics have caused significant production losses to the fast-growing aquaculture sector and to the natural aquatic populations in the wild. As a result, disease control and health management have been considered as key contributors to aquaculture sustainability.

Successful fish health management begins with prevention of disease rather than treatment. Prevention of fish disease is accomplished through good water quality management, nutrition and sanitation. Without this foundation it is impossible to prevent outbreaks of opportunistic diseases. The fish is constantly bathed in potential pathogens, including bacteria, fungi, and parasites. Sub-optimal water quality, poor nutrition or immune system suppression generally associated with stressful conditions allows these potential pathogens to cause disease.

Daily observation of fish behaviour and feeding activity allows early detection of problems when they do occur so that a diagnosis can be made before the majority of the population becomes sick. If treatment is indicated, it will be most successful if it is implemented early in the course of the disease while the fish are still in good shape.

Measures To Reduce Risk Of Aquatic Measures To Treat Sick Fish Animal Disease Transmission

Fish should be anaesthetised during handling to avoid injury to fish and fish handlers.

Gloves should always be worn when handling fish.

Minor wounds should be thoroughly washed with clean water.

Codes of hygienic practice and good aquaculture practices are essential to lower the risk to employees.

Measures to treat sick fish

The best treatment is prevention

Always quarantine new fish

Maintain water quality

Practise good nutrition

ASSESSING POWER PLAY AND GENDER AMONG FISH MARKETERS IN SOUTHWEST STATES OF NIGERIA

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Male and female in most developing countries of the world do not contribute to fish marketing the same way, due to power dynamics. Limited information is available on power structure among fish marketers, pointing to a need to improve the quality of data collected. Female also contribute to marketing activities but may not be the key decision makers regarding fish management strategies, product uses and sales. Awareness on gender studies among fish marketers has increased, but with diverse and limited depth of data analyses. However, assessing factors that influence power play and fish sales among fish marketers are usually not included in the gender roles of fish marketers. Therefore, the study was aimed to analyze the socio-economic characteristics of fish marketers and identify the determinants of power play among fish marketers in Lagos and Oyo States, Nigeria.

Multistage sampling method was used to select fish marketers in Lagos and Oyo States, by proportionate sampling of Agricultural Development Program (ADP) zones of registered fish marketers in each state. A total number of 202 fish marketers were proportionately selected creating equity among male and female respondents. Cultured fish marketers were selected for this study. In depth interviews of key respondents and quantitative data were used for the study. Statistical analyses used include descriptive statistics and Probit regression analysis.

Socio-economic characteristics of fish marketers showed that women are more involved in fish marketing than males. The study revealed that age, marital status, and gender had significant influence on power play among fish marketers with Probit index -0.129, -0.241, and -0.129 respectively. Therefore, it is important for male and female to be represented equally in decision making and political leadership among fish marketers to achieve Sustainable Development Goal 5 (SDG 5).

ISOLATION AND CHARACTERIZATION OF *Klebsiella* AND *PseudomonAS* SPECIES FROM FARMED AFRICAN CATFISH IN NIGERIA AND THEIR IMPLICATIONS

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Bacteria, one of the smallest and first life forms, are present in almost every environment, including aquatic systems, where they are abundant. They can be good indicators of environmental contaminants with potential implications for fish and human health. There is a need to differentiate between environmental and fish-pathogenic bacteria.

Liver, kidney, and spleen collected aseptically from apparently sick African catfish (*Clarias gariepinus*) farmed in Delta and Ogun states, were transported on ice to the University of Ibadan for bacterial culture, isolation, identification, and DNA extraction. Bacterial isolates suspected to be *Klebsiella* and *Pseudomonas* species were sent for whole-genome sequencing, biochemical and antibiotic susceptibility tests. Ribosomal RNA-containing contigs were identified and their corresponding 16S rRNA gene was extracted and used to BLAST against the NCBI microbial 16S database. Mass screening of contigs for antimicrobial resistance and virulence genes was performed.

A BlastN result from suspected *Klebsiella spp.* had a 99.738% similarity with *Klebsiella pneumonia*, while that of the suspected *Pseudomonas* species had a 99.804% similarity with *Pseudomonas aeruginosa*. Analyses of the assembled *K. pneumonia* genome revealed eight antimicrobial-resistance (AMR) genes; *fosA_gen* and *fosA_5* (Fosfomycin), *oqxB20*, *oqxA11* (Phenicol and Quinolones), *oqxB_1*, and *oqxA_1* (Nalidixic acid and Ciprofloxacin), and *blaSHV-145* and blaSHV-145_1 (Beta-lactamase). *P. aeruginosa* possessed fifteen AMR genes; *catA4*, *catB7*, and *cat_1* (Chloramphenicol), *tet(J)* and *tet(J)_1* (Tetracycline), *blaOXA-50*, *blaPDC-374*, *blaOXA-50_1*, and *blaPAO_2* (Beta-lactamase), *aph(3')-IIb* and *aph(3')-IIb_2* (Kanamycin), *fosA-354827590* and *fosA_4* (Fosfomycin), and *crpP* and *crpP_1* (Fluoroquinolone). The antibiotic sensitivity tests carried out on *K. pneumonia* showed susceptibility to Florfenicol, Oxytetracycline, Sulfamethoxazole/Trimethoprim, and Tetracycline but resistance to Novobiocin, while *P. aeruginosa* showed multi-drug resistance to all the antibiotics. Multiple genes coding for putative virulence factors were identified in the genome assemblies of *K. pneumonia* and *P. aeruginosa*.

The presence of both bacteria in farmed catfish is an indication of polluted aquatic environments. Identification of AMR genes and virulence factors in these two organisms has implications on environmental, animal and public health.

PREVALENCE, ANTIBIOGRAM, AND ANTIBIOTIC RESISTANT GENES (ARGS) OF *Vibrio cholerae* ISOLATED FROM FARMED AFRICAN CATFISH (*Clarias gariepinus*, BURCHELL 1822)

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The exponential increase and impact of aquaculture production systems on the global supply of fish have been confronted with an increase in diseases and antimicrobial use, hence presence of antimicrobial resistance and antibiotic residues in aquaculture. The prevalence, antibiogram, and antibiotic-resistant genes of *Vibrio cholerae* isolated from apparently healthy farmed *Clarias gariepinus* were assessed in one hundred and twenty-seven (127) adult fish and thirty-nine (39) fingerlings. A total of four hundred twenty (420) samples comprising 381 organs and 39 fingerlings were analysed. Tissue samples were enriched in peptone water and later cultured using Thiosuifate Citrate-Bilesalts Sucrose (TCBS) media (Millipore®, Germany) and incubated at 30℃ for 24 hours after which biochemical characterisation was carried out for detailed identification. Fourteen (14) representative isolates were selected for Antibiotic Sensitivity Test (AST), and Antibiotic Resistant Genes (ARGs) determination using polymerase chain reaction.

A total of one hundred and eighty-two *Vibrio cholerae* organisms were isolated which translated to 11.6% prevalence. All the *Vibrio cholerae* isolates (100%) were susceptible to Chloramphenicol (C), Tetracycline (TE), and Florfenicol (FFC). 88.9% susceptibility to Kanamycin (K), and 77.8% to Ampicillin (AP), Streptomycin (S), and Trimethoprim/Sulfamethoxazole while they were 100% resistant to Nitrofurantoin (Ni), and Oxytetracycline, 88.9% resistant to Colistin sulphate (CO), Nalidixic acid (NA), and Novobiocin (NB). Out of the fourteen (14) ARGs considered, 88.9% of the isolates possessed Sul-1 resistant gene, 55.6% had Sul-2 and TetB, 33.3% possessed TEM, 22.2% possessed APH-3 and TetH, 11.1% possessed CTX resistant gene while CMY-1, CMY-2, MCR-1, MCR-2, Sul-3, TetA, and ermF resistant genes were absent.

The presence of *Vibrio cholerae*, a zoonotic pathogen in a fish culture environment has public and environmental health implications for the AMU/AMR menace. The presence of Antimicrobial Resistance characteristics and acquisition of antibiotic-resistant genes in the *Vibrio cholerae* isolates suggests a significant abuse of antimicrobial agents by the farmers. This also renders the fish and fish products unwholesome and portends grave public health risk. A one-health strategy to sustainable aquaculture development in Nigeria, including biosecurity measures and adequate oversight by competent authorities is hereby recommended.

RNA-BASED TARGETED THERAPEUTICS REACH UNDERWATER THROUGH AN ORAL DELIVERY PLATFORM USING A SHRIMP VIRAL VECTOR

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Aquatic invertebrates farmed at an industrial scale continue to face the risk of viral outbreaks due to a lack of antiviral therapies. While RNA interference (RNAi) by sequence-specific dsRNA has been identified as a promising antiviral tool, its application to farm-scale bottlenecks with intramuscular (IM) injection being the only delivery method. Only through an oral delivery platform with a vector capable of delivering therapeutic RNA molecules into shrimp cells shall RNAi reach its potential against viral diseases of aquatic organisms. We present here an oral delivery platform using a shrimp virus capsid protein as the viral vector. The reverse-engineered *Macrobrachium rosenbergii* nodavirus (rMrNV) was made replication-deficient by replacing its RNA-dependent RNA-polymerase (RdRp) with a cargo RNA represented by green fluorescent protein (GFP). Efficient production of the viral vector was achieved using baculovirus dual-expression system capable of simultaneous production of both capsid protein and GFP RNA cargo facilitating assembly. To ensure its applicability to farm-scale, we demonstrate here the oral delivery and detection of GFP RNA into shrimp cells as well as proving MrNV^{cp} is indeed replication-deficient by testing its viability and screening for any pathological effects. We went on to explore its applicability against white spot syndrome virus (WSSV) by replacing GFP with WSSV VP28 hairpin RNA.

Here we demonstrate that our viral vector is non-replicating and can deliver the cargo GFP RNA through the diet. For this, detection of the cargo RNAs (MrNV^{cp} and GFP) were done in shrimp fed with commercial diets incorporated with MrNV^{cp}-GFP. Results show that RNA copy number of MrNV^{cp} gradually declined to 10 copies after 8 days post-feeding (Fig.1A) as also seen for GFP RNA detection (Fig. 2B). These results are proof that MrNV^{cp} is effective in delivering RNA cargo via oral route comparable to injection method. Histological analyses ruled out any pathological damages post-feeding that may be indicative of a viral infection. Combined with the detection results (Fig. 1A), the generated MrNV^{cp} viral vector is non-replicating and non-infectious. These results show a promising shrimp viral vector-based oral delivery platform for RNAi therapeutics.

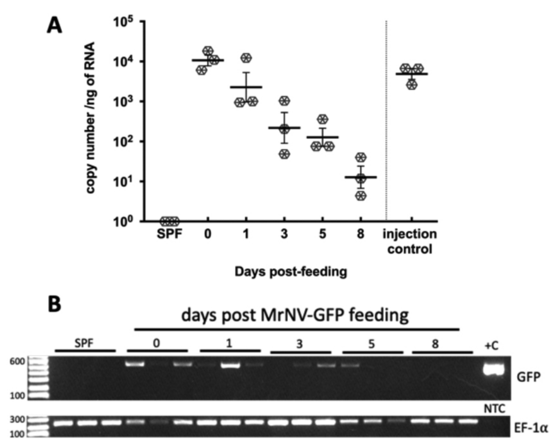

Figure 1. Detection of delivered cargo RNA. Detection MrNV^{cp} using RT-qPCR (A) and GFP by RT-PCR (B) in hepatopancreas of *Litopenaeus vannamei* shrimp fed commercial feeds with rMrNV-GFP. All detections were run in triplicates. Naïve (SPF) was used as negative control and shrimp injected with rMrNV-GFP, as positive control.

EFFECTS OF LOW TEMPERATURE ON PHYSIOLOGICAL PERFORMANCE AND TISSUE ENERGY STORAGE IN CATFISH

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Although U.S. commercial catfish aquaculture ponds are in temperate regions and experience cool to cold winter temperatures, the effect of low temperatures on catfish physiological performance is not well understood. In particular, the accumulation of stored energy during cold conditions can be important for facilitating physiological performance and ultimately survival. Therefore, growth, metabolism, and liver and muscle energy storage were compared between channel (*Ictalurus punctatus*), blue (*I. furcatus*), and hybrid (*I. furcatus* × *I. punctatus*) catfish acclimated to 10 and 20°C over a 17-week period. The experimental design included 18 treatment tanks, with 3 tanks per treatment combination. Feed consumption, growth, standard and active metabolic rates, and corresponding metabolic scopes were greatly reduced at 10°C compared to 20°C in all catfish types. Channel catfish were noticeably different than blue and hybrid catfish at 10°C in liver energy storage, whereas blue and hybrid catfish were generally similar. Relative liver size was much greater (measured as hepatosomatic index) in channel catfish at 10°C than blue and hybrid catfish. Total fatty acids, fatty acid profiles, and proximate composition also were markedly different in channel catfish at 10°C. These results indicate cold temperatures reduce feeding which is compensated by reduced metabolic rate, and increased energy storage in liver tissue, particularly in channel catfish. However, metabolic scope is also reduced suggesting prolonged cold conditions reduce performance capabilities of catfish to respond to additional culture or environmental challenges.

QUALITY AND FISH WELFARE – LINKING CAUSES FOR QUALITY DOWNGRADING AND PRODUCTION RELATED DRIVERS IN NORWEGIAN ATLANTIC SALMON FARMING

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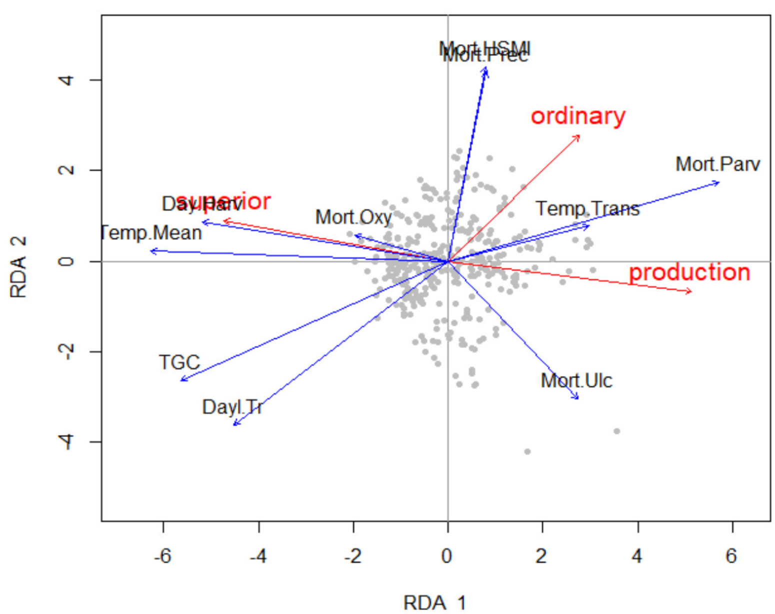
Quality downgrading of fish with quality defects is a considerable cause for economic losses to farmers due to reduced marketability and sales prices, as well as increased processing costs. Several causes for quality downgrading can be linked to adverse welfare states during fish rearing and should therefore be considered as welfare indicators. In this study, we linked the production records of 24 sea cage sites to slaughter records from a processing plant in northern Norway. We quantified the prevalence of proximate causes for downgrading and used ordination and regression-based approaches to model the variation in quality traits due to selected production related drivers. The most important causes for downgrading were ulcers (39 % of downgraded fish), dark spots (17 %), deformities (12 %), and early maturation (10 %). The presence of ulcers was by far the most severe cause for downgrading. Important drivers for overall quality reduction were growth during the seawater stage, seawater temperature (mean and standard deviation), certain cause specific mortality counts, and day of harvest. Ulcers were linked to low seawater temperatures during production.

Our study demonstrates that proximate causes for quality downgrading at slaughter are relevant retrospective welfare performance indicators and provides an overview of how production related drivers can affect slaughter quality throughout the year. It highlights the link between improved welfare and improved production outcomes, as well as the utility of systematic data collection and analysis in aquaculture production.



ASSESSMENT OF FOODBORNE PATHOGENS AND HYGIENE PRACTICES ALONG THE FISH SUPPLY CHAINS IN BANGLADESH

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Although fish in the retail markets in Bangladesh is generally conceived to be fresh, they might carry human pathogenic bacteria. Microbial contamination of fish can occur at different stages of the supply chain and therefore a thorough assessment of the dynamics of changes in the level of contamination is important for understanding ways to control such risks in Bangladesh. Data from samples can serve as input into a microbial risk assessment that will aid in future evaluations of potential mitigation strategies focused on reducing human pathogenic bacteria in aquaculture. This study assesses contamination of tilapia and pangas with fecal pathogens and practices of stakeholders along the supply chain that might be associated with the contamination.

A total of 368 whole fish samples (tilapia, n=188 and pangas, n=180) were collected from 17 retail markets (n=188), 5 wholesale markets (n=100), and 8 grower ponds (n=80). Fish samples were tested for *E. coli,* extended-spectrum β-lactamase producing *E. coli* (ESBL-Ec), *Salmonella* spp. and *Vibrio cholerae* following standard methods. Surveys were used to collect and analyze data on handling practices of 410 selected actors in the fish supply chain comprising 144 retailers, 144 cut-up table workers, 72 wholesalers, and 50 producers.

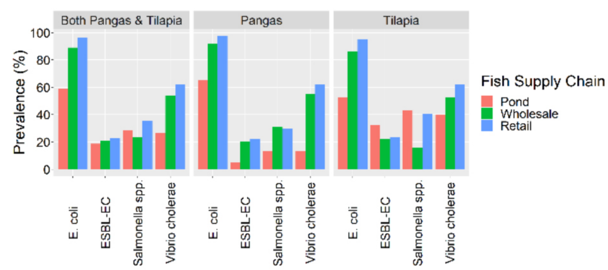

Fig 1: *E. coli*, ESBL-Ec and pathogenic bacteria in tilapia & pangas supply chain

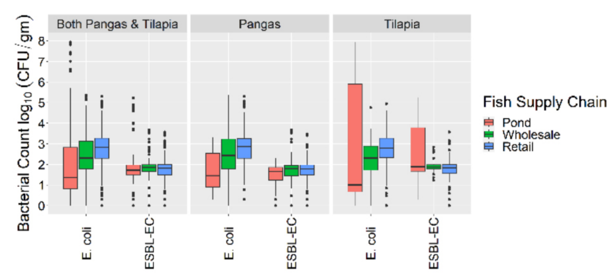

Fig 2: Abundance of total *E. coli* and ESBL-Ec in tilapia and pangas

The prevalence of *E. coli* ($p<0.001$), and *V. cholerae* ($p<0.001$) but not ESBL-Ec ($p= 0.747$) and *Salmonella* spp. was significantly higher in both tilapia and pangas in the retail markets compared to wholesale markets and grower's ponds. ESBL-Ec and *Salmonella* spp. were more prevalent only in tilapia from ponds (Fig. 1). The median count of *E. coli* in both fishes increased significantly from grower's ponds (1.4 $\log_{10}$ CFU/g of fish, IQR= 2.0) to wholesale (2.3 $\log_{10}$ CFU/g, IQR= 1.3) and retail sale (2.8 $\log_{10}$ CFU/g, IQR= 1.0) ($p=0.015$) whereas ESBL-Ec was consistent (Fig. 2).

Majority of fish producers (92%) used antibiotics in ponds without prescription. Around 97% of the wholesalers transported fish in trucks lacking a cold chain. Fish were often processed at unhygienic landing sites using a single knife with improper washing due to the lack of running water. The high prevalence of fecal bacteria and unhygienic practices by supply chain actors implied a poor knowledge and infrastructure for post-harvest management in aquaculture.

MOST TREATMENTS TO CONTROL ALGAL BLOOMS ARE NOT EFFECTIVE: META-ANALYSIS OF FIELD EXPERIMENTS

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Harmful algal blooms negatively impact freshwater, estuarine, and marine systems worldwide, including those used for drinking water, recreation, and aquaculture, through the production of toxic and non-toxic secondary metabolites. Consequently, water resource managers often utilize chemical, bacterial, physical, and plant-based treatments to control algal blooms. In aquaculture, copper sulfate, one of the only two approved algaecides, is commonly used because of its relative inexpensiveness and ability to quickly reduce algae. However, awareness or availability of alternative treatments may be limited due to disagreement among algal bloom treatment effects across studies, especially when comparing lab and field experiment results. Such variation within the literature and lack of knowledge of other tested treatments leave uncertainty for water resource managers when deciding what treatments to use to control harmful algal blooms. The objective of this research was to synthesize published and unpublished data from 39 studies that used one of 28 chemical, bacterial, physical, and/or plant-based algal bloom treatments used in field experiments on various algal measures (i.e., phytoplankton pigments and cell density, microcystin, and off-flavors). Overall, only a handful of chemicals, including copper sulfate, hydrogen peroxide, peracetic acid, and simazine, mediated algal blooms either measured at the day of most significant algal decline following treatment or at the end of the experiment. None of the bacterial, physical, or plant-based treatments were shown to significantly control algal blooms, toxins, or off-flavors by themselves. Results from this synthesis quantitatively showed that most treatments for algal blooms do not significantly improve water quality and highlight the need for more research on alternative treatments.

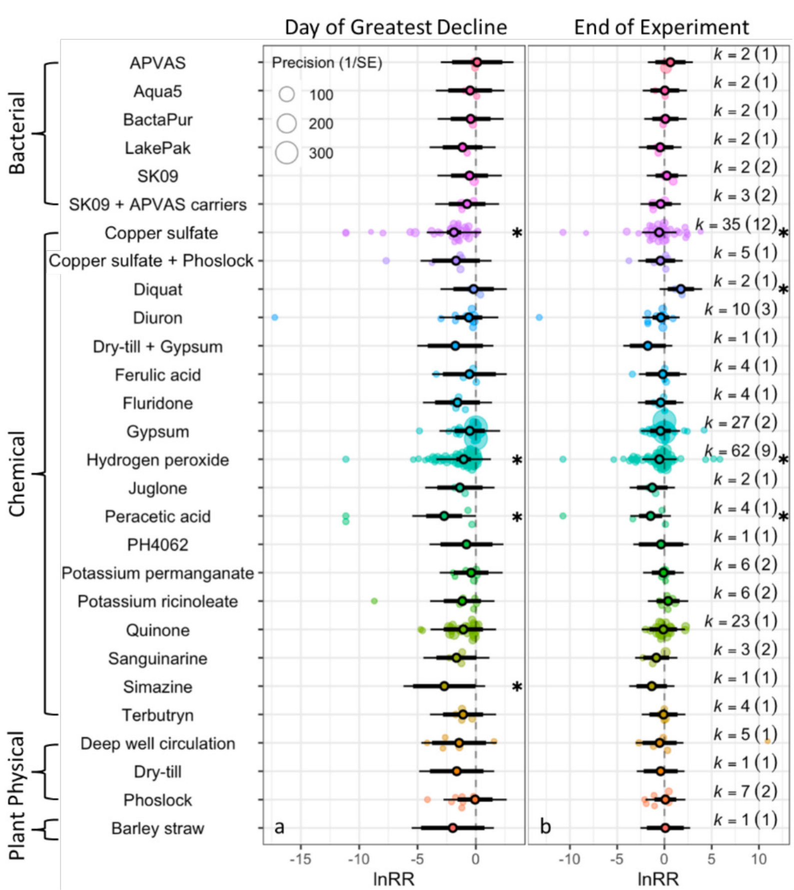

Figure 1. The effects for different treatments on algae are shown of both the (a) day of greatest decline and (b) end of experiment data. Asterisks indicate statistical significance (p≤0.05).

MACHINE LEARNING ANALYSIS OF INTEGRATED TRANSCRIPTOMIC AND METABOLOMIC PATHWAYS OF STRIPED BASS *Morone saxatilis* GROWTH

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Striped bass (SB, *Morone saxatilis*) are an important aquaculture fish as a parental species of hybrid striped bass, the fourth largest US finfish aquaculture industry, and an emerging standalone industry. SB have been bred in captivity for superior production traits (i.e., domesticated) for decades in the National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry with noteworthy success in areas such as spawning without exogenous hormone compounds and reducing time to market size. Despite gains in SB production traits made through breeding, subgroups of SB exhibiting inferior growth and overall failure to reach the desired market size are consistently present in each cohort. An integrated machine learning analysis of fifth generation domestic SB (N=72 fish) transcriptomes and metabolomes was conducted in order to better understand this discrepancy in growth at the cellular level and to identify targets for future breeding efforts and/or biotechnological interventions. The SB sampled were from half-sibling families produced by crossing two female SB ('dam') with six male SB ('sire') each. Dams were crossed with three sires categorized as "Large" (by weight and length) and three as "Small", to enable a comparison of omics profiles of SB produced from sires of distinct growth phenotypes. Transcriptomes were generated from fast-twitch, white muscle tissue of eighteen month old SB reared in a recirculating aquaculture system (RAS) and metabolomes were generated from the liver of the same SB. The machine learning analysis identified between 35–300 muscle gene transcripts as determinant of growth performance, dam, sire, or sire size and a pathway analysis of these gene expression patterns revealed distinct differences in critical metabolic pathways. Specifically, genes up-regulated in SB exhibiting inferior growth suggest that protein ubiquitination and skeletal muscle degeneration is active in these fish, rather than critical signaling pathways that regulate genes involved in growth (e.g., HIF-1α Signaling, JAK/STAT) and muscle homeostasis, which were up-regulated in fish of superior growth. The machine learning analysis identified between 25–122 liver metabolites as determinant of SB growth performance. The determinant metabolites identified were predominantly sphingolipids, which are involved in multiple functions such as tissue development, cellular proliferation and differentiation. These metabolites were generally present in higher concentration in the fish of inferior growth, suggestive of differences in synthesis and degradation processes related to liver dysfunction between SB growth performance groups. This metabolite analysis when integrated with the transcriptomic analysis is consistent with observed gene expression differences in the white muscle, suggesting muscle wasting in inferior growth fish. The causes of this dysfunction, whether genetic, dietary, or husbandry factors, remain unclear and therefore are a recommended topic of future research and breeding efforts.

INVESTIGATING THE RELATIONSHIPS BETWEEN PHYSICAL WATER QUALITY PARAMETERS AND CHLOROPHYLL-A IN REHOBOTH BAY, DELAWARE

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Oyster aquaculture returned to the Delaware Inland Bays with the issuing of shellfish leasing areas in 2018, bolstering local economies, improving water quality, and providing structured habitat for fish and invertebrates. Additionally, the Delaware Center for Inland Bays established three pilot artificial oyster reefs beginning in 2019 to restore native oyster populations and further improve the Inland Bay environment. Successful management of oyster farming and restoration efforts depends on continuous monitoring of growing conditions. Our goal was to monitor and identify relationships between physical water quality parameters, and chlorophyll-a in Rehoboth Bay, DE.

A pilot water quality monitoring program was established in Rehoboth Bay, Delaware, which included multiparameter continuous water quality instruments (sondes) deployed at sites of oyster aquaculture and artificial oyster reefs from summer through late fall in the 2020 and 2021 field seasons. Trends in water temperature, salinity, dissolved oxygen, pH, turbidity, total suspended solids, calcium hardness, and chlorophyll-a were examined with respect to oyster growing conditions. Overall, not much variability present throughout the seven bay sites, though there were some differences present between 2020 and 2021. Generalized additive models revealed chlorophyll-a had significant relationships with both dissolved oxygen and temperature. These preliminary findings are expected to inform the management of Delaware oyster aquaculture and restoration efforts and shape the direction of future monitoring efforts.

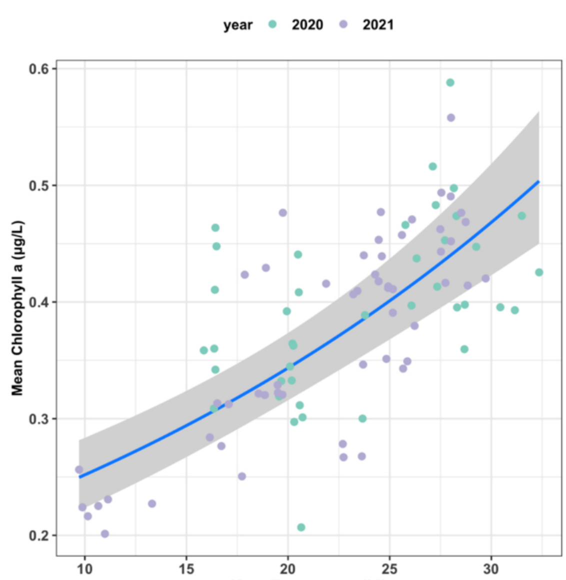

Fig. 1. GAM smooth for Chlorophyll –*a* (µg/L) and Temperature (°C) in Rehoboth Bay in 2020 and 2021. TSS and pH (both non-significant) held at mean values. Confidence intervals (95%) shown in shaded areas.

THE MAINE SEAWEED EXCHANGE

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The Maine Seaweed Exchange is a 501(c)(3) non-profit corporation with a mission to support the development of an organic, sustainable, and restorative seaweed aquaculture industry. The organization works to support the seaweed aquaculture industry through seaweed farming education and training, research on seaweed farming, aquaculture diversification, and products, developing markets and distribution channels, assistance with branding and marketing opportunities, including organic certification, by creating and supporting innovation, and by facilitating key networks for the industry. The MSE hosts events and workshops, which have included the Maine Seaweed Fair, a day long free family event that showcases Maine wild and farmed seaweeds, and the Practical Seaweed Farmer's Conference, which provided educational, networking, and exchange of ideas among existing and potential seaweed farmers, potential buyers and investors, regulators and researchers, and others involved or interested in the seaweed aquaculture industry. We offer a wide range of seaweed farming courses and training opportunities that cover all aspects of seaweed farming in order to promote responsible, informed farmers for a more professional industry. Recently, we have launched the Organic Kelp Collaborative, where we work to train, support, and connect new organically certified seaweed farmers and processors in order to develop a network of organic producers that will be able to aggregate products and sell into larger markets.

USE OF MEALWORMS AS AN ALTERNATIVE INGREDIENT IN FISH FEEDS

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Fishmeal and fish oil, used in production of aquaculture feeds are widely recognized as limited resources, due to strategic efforts in conservation and management of the species that are the source of these ingredients. In order to significantly and sustainably advance the growth of domestic aquaculture, viable alternatives for fishmeal and fish oil are needed. Aquaculture is the largest growing food production sector globally, providing a key role in meeting the protein demand of a rising global population. Insects are an underexplored alternative for sustainable aquaculture and aquafeeds in the US, that could help grow the domestic aquaculture industry, and increase the environmental sustainability of aquaculture. Our study looked at the use of insects, specifically mealworms *Tenebrio molitor*, as a potential alternative for fishmeal and fish oil. Insects such as mealworms are sustainably cultured; they can be grown from organic by-products and have good nutritional profiles making them a well-suited protein alternative ingredient. Three experimental diets were prepared with varying degree of fishmeal and fish oil replacement by whole dried mealworms. In addition, a reference group of fish were fed a commercial salmon feed that was high in fishmeal and fish oil. Diets were fed to apparent satiation to juvenile sablefish *Anoplopoma fimbria*, a cold-water marine fish, for 10 weeks to evaluate any differences in feed and growth performance.

Comparable growth across treatments was observed, demonstrating the ability of mealworms to fully replace fishmeal and fish oil in sablefish feeds (Figure 1). Proximate analyses conducted on whole fish collected at the end of the study showed no significant differences in total protein and crude fat in between each treatment.These results show that mealworms show promise as an alternative ingredient in marine fish feeds, however more detailed examination is needed to determine how fish produced from mealworm diets nutritionally compare to wild fish and fish produced from conventional fishmeal diets.

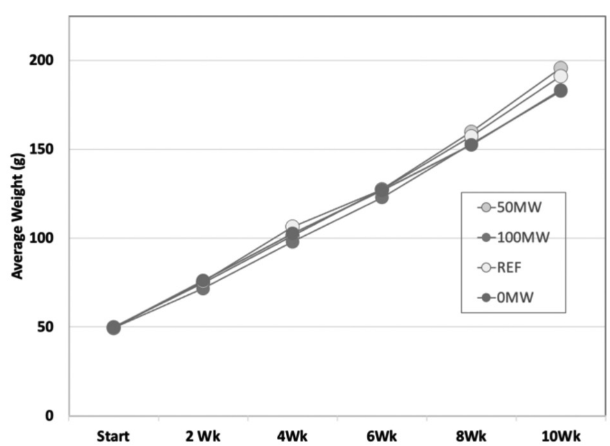

Figure 1.

PRODUCTION EFFICIENCY OF SOLAR AND PETROL-POWERED GENERATOR RE-CIRCULATORY AQUACULTURE SYSTEMS FOR AFRICAN CATFISH *Clarias gariepinus* FINGERLINGS PRODUCTION

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Accessibility of quality fingerlings is dependable on a hatchery system with the capacity to produce all year round. There are two hatchery systems operational in Nigeria namely, the flow-through and re-circulating systems. These two systems require electricity to power periodic or constant water supply and aeration. When electricity supply fails or non-existent, this will result in anxiety and hardship to hatchery operators with the subsequent low output.

This paper, therefore, assess the production efficiency of solar and petrol-powered generator re-circulating aquaculture systems for African catfish *(clarias gariepinus)* fingerlings production. The results obtained from the growth performance and survival percentage of *Clarias gariepnus* reared in the two recirculating systems (solar and generator powered recirculating systems) showed no significant difference (P> 0.05) in the initial weight, specific growth rate (SGR) and feed conversion efficiency (FCE) of *C. gariepnus* from the two-recirculating systems. However, the result showed significant differences (P<0.05) in the final mean weight, weight gained, feed intake, feed conversion ratio, protein intake, protein efficiency ratio and survival rate in the two-recirculating systems. The growth of *C. gariepinus* reared in solar powered recirculating system had highest final weight gain, specific growth rate, protein efficiency ratio; (29.02±0.68, 10.3± 0.13 and 2.16±0.05), respectively. The solar powered recirculating system had the higher survival percentage of 5091.5± 8.17 than the generator powered recirculating system (4994±6.16).

Correspondingly, the mean value of percentage hatchability from the fertilized eggs incubated shows that hatchings had relatively high mean value of 82% and 82.1% in generator and solar powered recirculating system. Survival of hatchlings were observed to be high (5091.5) in solar powered recirculating system compared to (4994) number recovered from the generator powered recirculating system. Thus, this study revealed that solar energy might become the most promising energy source that can replace the usual source of energy for hatchery operations in Nigeria.

TILAPIA (*O. niloticus)* PRODUCTION EVALUATION BETWEEN HIGH, LOW STOCKING DENSITIES IN TRADITIONAL PONDS AND IPRS

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Tilapia producers in Latin America are adopting the In-Pond Raceways System -IPRS- but need a good comparison between traditional earth pond stocking and IPRS. This study evaluated two traditional ponds with high stocking densities 5.5 fish/m² (treatment TA), fish initial weight of 11 grams, final harvest weight of 488.5 grams; two ponds with low stocking densities of 3.9 fish/m² (treatment TB), fish initial weight 20 grams, harvest weight 1143 grams and one pond with four IPRS units, initial weight 49.2 grams, harvest weight 589 grams. ANOVA analysis show a variability among treatments, in harvest weight, culture days, weight gain (P-value <0.05) and FCR (P-value >0.05) see tables below.

TA average tilapia production was 14,657 kg/Ha/Harvest in 230 days, TB was 26,576 kg/Ha/harvest in 247 days and IPRS was 36,971 kg/Ha/harvest in 146 days with (t=0.045) for TA, TB and IPRS respectively. Weight gain in grams/day TA = 2.12, TB 4.74 and IPRS 3.69. FCR TA = 1.07, TB = 1.31 and IPRS = 1.29, showing better results for TA, but data indicate that feed regime was decrease because endemic low DO, due to high stocking densities.

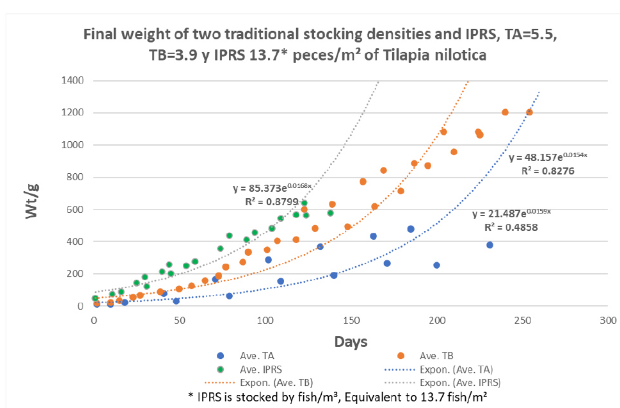
Dissolved oxygen in treatments TA and TB were kept in concentration above 4.0 mg/L on the first 5 months, descending at values between 1 – 2 mg/L on the rest of the culture period, meanwhile in IPRS DO levels were kept above 3.0 mg/L dropping to 1 -2 mg/L on few occasions.

This research was originally planned to have to have vaccinated and non-vaccinated fish as treatment, unfortunately vaccinated fish show high mortalities due to handling and extra un-vaccinated fish was needed to be stock on top of the already vaccinated.

Feeding rates for TA = 70.98, TB = 147.9 and IPRS 247 kg/Ha/day. Survival percent of stocking as follow: TA = 73.5, TB = 76.6 and IPRS = 61.8 The latest treatment, survival was low due to uncounted initial mortalities. Overall high productivity of the IPRS system is due to it capacity to extract fish waste out of the aquatic environment, maintain a better water quality, higher concentrations of dissolved oxygen. Results show that in traditional earth ponds, stocked at more than 4 fish/m² negatively affect fish production, weight gain and culture days, however IPRS technology is an option for water conservation, bird predation, better yields.

	TA	TB	IPRS*
Stocking density (Fish/m²)	5.5	3.9	13.7 *
Final Weight Wt/g	488.5	1143.0	589.2
Kg/Ha/Harvest	14,657 a	26,576 b	36,971 c
Culture days	230.5 a	237 a	146.5 b
Weight Gain g/D	2.12 a	4.74 b	3.69 c
FCR	1.07 a	1.31 b	1.29 a,b
Survival %	73.5	76.6	61.8

* IPRS stocking base on m³ Equivalent to 13.7 fish/m² to traditional ponds.
a = Tratamientos con la misma vocal incan no diferencia estadistica.
ab = Treatment with stadistic differences.
TA= Treatment with high density
TB= Treatment with low density



EFFECT OF DIFFERENT LEVELS OF CRUDE PROTEIN AT DIFFERENT FEEDING RATES ON PACIFIC WHITE SHRIMP (*Litopenaus vannamei*)

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Feed management is one of the most important factors in shrimp culture, as feed makes up around 60% of the total costs. The aim of this study was to evaluate the effect of different protein intake levels on growth performance, feed utilization efficiency, and whole-body composition of Pacific white shrimp using different levels of crude protein (CP). This was done by feeding four practical diets with 40%, 35%, 30%, and 25% of CP, which were fed at two different rates, one of them being the standard feed rate (100%) and a second adjusted rate to match protein intake, resulting in a total of 8 treatments with four replicates each. Juveniles (0.41g ± 0.01 [mean ± SE]) were stocked into a green water system with 32 culture tanks at a density of 30 inds/tank and reared for 77 days. At the end of the trial, growth performance parameters such as final weight, weight gain, biomass, and feed conversion ratio were found to be significantly different among treatments ($p<0.05$). All final body composition values (dry matter, CP, and minerals) did not show significant differences between the treatments except for fat ($p<0.05$). However, feed utilization measurements including apparent net protein retention (ANPR), and phosphorus retention did show to have significant differences ($p<0.01$). This study demonstrated that higher intake levels of CP in shrimp enact better with respect to growth performance and feed utilization.

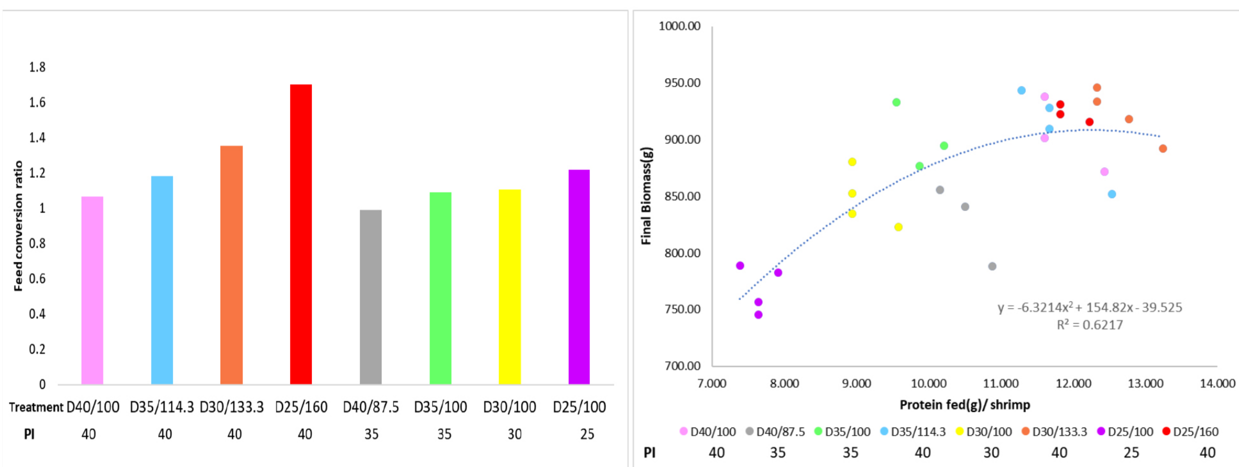

Figure 1. Biomass and FCR of pacific white shrimp reared in a green water recirculating system over an 11-wk culture period fed with four diets (40%, 35%, 30%, and 25% CP) at two different feeding rates. Juvenile shrimp (0.41g) were stocked at a density of 30 ind/tank. (PI = Protein intake).

INVESTIGATING SENSORY THRESHOLDS FOR OFF-FLAVORS IN FARMED FISH

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Off-flavors imparted by the environmental chemical geosmin (GSM) can render aquacultural food products objectionable to consumers. Therefore, sensory evaluation (or "flavor checking") is often used for quality control. A sensory threshold is a stimulus level (e.g., a flavor intensity) sufficient to produce a change in response, such that: a flavor is just noticeable (detection or difference threshold), the flavor can be identified (recognition threshold), or an off-flavor renders the product unacceptable (rejection threshold).

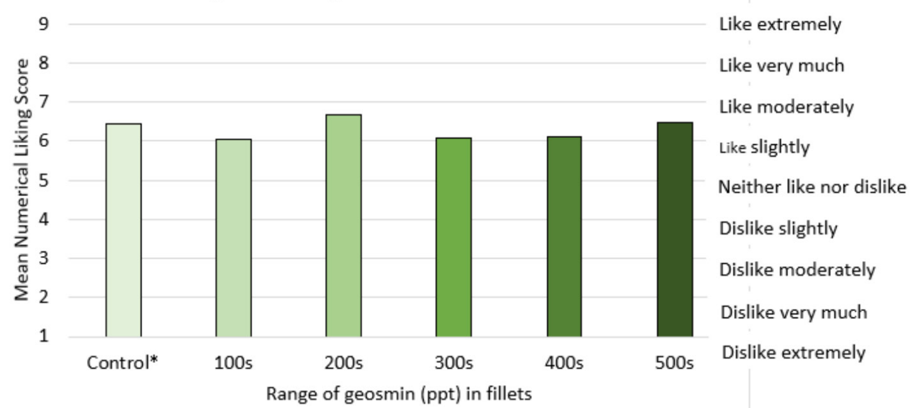
The present research investigated: 1. The terms consumers use to best describe aroma and flavor of GSM in water, and 2. Sensory thresholds for GSM in Rainbow Trout fillets.

To determine a recognition threshold for GSM, appropriate flavor descriptors must be used. Therefore, a sensory test was conducted with 188 consumers to select the most relevant terms. Across low (20 ppt), medium (200 ppt), and high (1000 ppt) GSM solutions, "earthy" was the most commonly used word to describe GSM flavor and aroma.

GSM was imparted into trout fillets through their diets using a newly proposed model system involving spiking feed to known concentrations. Preliminary detection, recognition, and rejection thresholds were investigated for GSM in air fried trout fillets. However, based on 132 consumer responses, concentrations of 500-600 ppt racemic (+/–)-GSM in fillets did not present significant differences in overall aroma/flavor, liking, nor acceptability, and thresholds were not identified.

Data from these two studies highlight the differences in perception between GSM in water versus a more complex food matrix such as fish muscle. Whereas no sensory thresholds were found in fillets containing up to 588 ppt GSM, GSM taste intensity was considered moderate by 31% and strong by 24% of consumers when present at 200 ppt in water. Therefore, higher concentrations are needed in fillets to find reliable thresholds of interest.

Figure 1. Liking of Trout Fillet Flavor



* Control fillets contained less than 33 ppt geosmin.

SUSPENDED FLOATING RACEWAY DESIGN, CONSTRUCTION, AND PRELIMINARY EVALUATION OF A 9.5 CUBIC METER SYSTEM

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Floating in pond raceways have 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. This work is oriented to the small farm with existing impoundments that are not drained.

Following an initial assessment of a floating raceway for production of fingerling largemouth bass, a suspended design with integrated floatation was scaled up for greater production potential (photo). These raceways feature a uniform square cross section and hard flat bottom such that workers in waders can crowd, grade, and harvest the fish. Though the entire unit is 9.75m long, rearing space occupied by fish is 9.5m^3 (21'x4'x4'). The device moving water is a grid airlift with air from a 0.5 hp regenerative blower at the head of the raceway. Sustained measured flow exceeds 4000 Lpm for a corresponding exchange rate in excess of 20/hr. Though carrying capacity has not been empirically determined, a ton of fish would correspond to 66 kg/m^3 (6 lb/ft^3) and a loading rate of 0.24 kg/L/min. It is estimated a ton of catfish @ 454g/fish would remove about 2 mg/L dissolved oxygen at 30°C (Table).

This design may be constructed on-farm with material costs for the raceway, floatation, air blower, screens, and grid airlift estimated to be about $2,500. The system can be configured for both fingerling production and grow out.

	Number of 1 lb catfish to consume 2 mg oxygen					
Flow	Flow		Water Temperature (°C)			
gpm	L/min	10	15	20	25	30
500	1893	4700	3200	2200	1500	1050
1000	3785	9400	6400	4400	3000	2100
1500	5678	14100	9600	6600	4500	3150

SPERM CRYOPRESERVATION IN *Xenopus laevis:* FROM PROTOCOLS TO PATHWAYS

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The African clawed frog (*Xenopus laevis*) is used in laboratories worldwide as a tool to investigate biochemistry, genetics, cell biology and development biology in normal and disease states. The number of transgenic and mutant lines is rapidly increasing and maintenance as live organisms is costly and risky. Storage of cryopreserved germplasm in repositories can provide a way to protect lines and reduce the number of live animals. In collaboration with the National *Xenopus* Resource (NXR, Marine Biological Laboratory, MA), we aim to develop a cryopreservation pathway for this species. Currently, the NXR cryopreserves sperm, but routine quality evaluation of samples is not performed, and a high variability among males in fertilizing capacity with frozen sperm has been found, with 30% failing to produce enough embryos to recover a line. We developed methods for evaluation of sperm viability and motility in this species (Figure 1). We tested the effect of cryoprotectants (DMFA, DMSO, methanol), concentration (5%, 10% and 20%), addition of sugar (sucrose), and cooling rate (5, 10 and 20 ºC/min) on sperm quality. Also, the fertilizing capacity of testicular sperm frozen at different concentrations (30, 50 and 100 × 10^6 cells/mL) was assessed. This protocol will help in reducing post-thaw variability among *X. laevis* males and in setting standards for samples to be accepted into the repository at the NXR. In addition, we are using a center-based approach for application of this protocol to develop a high-throughput cryopreservation pathway that is scaleable and generalizable, integrating processing and quality management to establish repository capabilities to serve the needs of research communities. This repository would be integrated into a larger aquatic species repository network developed in cooperation with the USDA National Animal Germplasm Program located in Ft. Collins, CO and the AGGRC. A major design consideration is that the practices and operations at this center will be in accordance with those of other centers to facilitate network activities. Thus, this collaborative initiative will consider repository development in a multi-level approach that addresses more than protocol development.

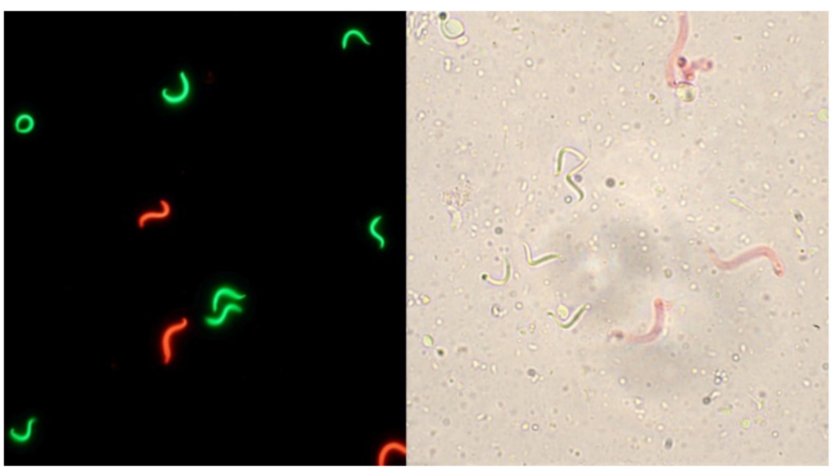

FIGURE 1. Two different approaches for evaluation of sperm membrane integrity according to available microscope resources. Left panel: fluorescent staining with SYBR14 and Propidium Iodide which presents green (intact membranes) and red sperm (compromised membranes). Right panel: brightfield staining using Eosin Y for the visualization of unstained (intact membranes) and stained sperm (compromised membranes).

BACTERIAL PROFILE AT A GLANCE IN A RAS SYSTEM – A TOOL FOR MONITORING AND ACTIONABLE PLANS

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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 utilization 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 Recirculation Systems, emphasizing which parameters are essential for a fast turnaround of actionable information. In addition to core interpretations (such as alpha and beta diversity indexes, heatmaps, composition bar-plots at different taxa and other common indicators of microbial ecosystems) customized lists including cyanobacteria profiles; “watch lists” and “pathogen lists” and presence/absence of off-flavor compound producing species are included. Other in-depth analyses of the bacteria identified by full length 16s sequencing may include the description of general functional categories such as environmental, probiotic, nitrifying, iron-reducing, or predatory and parasitic species; and these analyses may be carried out as part of a routine monitoring program.

Considering that the list of detected bacterial species across several samples can reach the order of thousands, our intention in the report is to highlight the concerning points and the healthy balance, as well as a general overview of the system. In that way, decision makers can focus their attention on what matters and take the appropriate decisions on time for the wellness of the RAS system and the animals. Common applications of these studies include 1.- monitoring for correlation with water quality and animal performance and productivity; 2.- evaluation of disinfection treatments in RAS systems; 3.- forecasting of possible outbreaks or major issues; 4.- evaluation of probiotics, diets, or drug/vaccine treatments.

PRODUCTION GROWTH, COMPANY SIZE AND CONCENTRATION: THE CASE OF SALMON

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Global aquaculture production has been rapidly growing in recent decades, and while there has been significant attention given to the species being produced in which country, less attention has been given to the companies actually conducting the production. We know that in some sectors they are all small, while in some sectors like salmon and shrimp at least some companies are becoming very large. In this paper we have access to firm data on production for all major salmon producing countries from 2010, as well as data on the wild salmon fisheries.

We report production development by species, showing how farmed Atlantic salmon is becoming increasingly dominant, and how the relatively constant landings of wild fish make this sector an increasingly marginalized part of the salmon market. We use Herfindahl-Hirchman Indexes (HHI) to measure concentration in a number of settings. We start by computing HHIs for the five largest farmed salmon producers. Not surprisingly, these are lowest for the largest producers – Norway and Chile, and higher for the smaller ones. We continue by computing global HHIs first for Atlantic salmon, then all farmed salmon and then all salmon including the wild salmon. The concentration declines with the wider selection of species, indicating that these sectors consist of smaller companies than the farmed Atlantic salmon. This is particularly the case for the wild fisheries.

EFFECTS OF NIGER CAKE INCLUSION IN FISH FEED AS A MAJOR PROTEIN SOURCE ON CATION DYNAMICS IN NILE TILAPIA-LETTUCE AQUAPONICS SYSTEM

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In aquaponics system fishmeal is mainly used as a major protein sources as a fish feed which is not affordable to developing countries despite its high cation compositions. Therefore, this study was conducted to investigate the cation dynamics in tilapia-lettuce aquaponics system by providing Niger cake in different proportions as replacement of fishmeal in fish feed to compare its cation loading capacity with that of fishmeal & a standard hydroponics.

The experiment was done in five treatments with respect to the proportion of Niger cake; 0% (Control), 12.5% (TA), 25% (TB), 37.5% (TC), and 43% (TD) and each treatment in duplicates. One hydroponic treatment (H) was included in the experiment as a standard to check cation concentration in lettuce and water samples. Water, fish and lettuce samples were collected from each system every week for cation analysis.

The result of the experiment showed that the higher Niger cake inclusion in fish feed (43%), TD) resulted higher cation loading to the system than the control diet and less cation loading than the hydroponic solution. However, iron concentration in the aquaponics water showed significantly higher value than hydroponic solution ($P<0.05$). The lettuce cation content also showed that higher percentage of the alternative feed could provide higher concentration of calcium, iron and potassium than the control fish feed.

Therefore, the higher percentage of alternative feed from plant sources such as Niger cake could provide higher cations for the cultured plants in an aquaponics system.

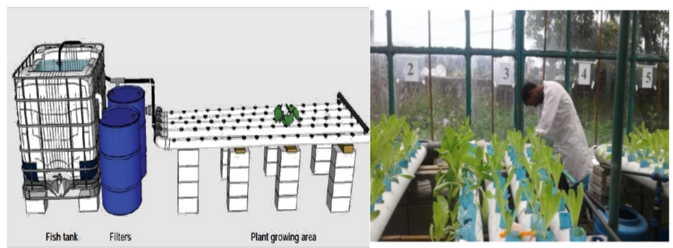

Figure 1. Aquaponic system and sampling

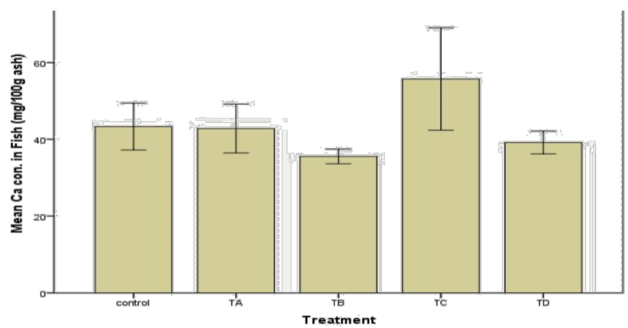

Figure 2. Calcium concentration in fish (mg/100g ash weight)

Table 1. Calcium concentration of the water samples in mg/l

Control	9.27±2.22[a]
TA	15.31±4.28[b]
TB	7.38±1.52[a]
TC	7.28±3.63[a]
TD	11.31±5.02[ab]
H	301.49±28.5 [c]

ASSESSING EASTERN OYSTER *Crassostrea virginica* PREDATION UTILIZING REAL TIME MONITORING AND EDNA ANALYSIS IN DELAWARE INLAND BAYS

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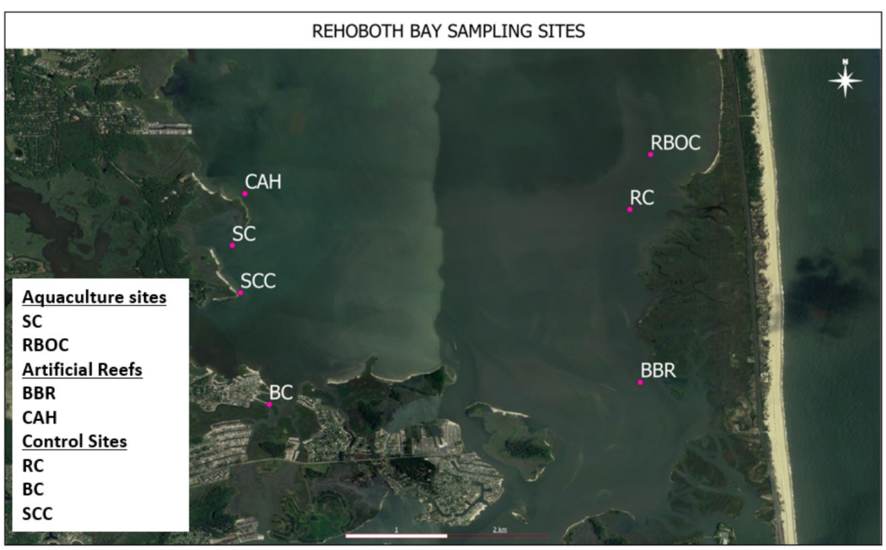
Eastern Oyster (*Crassostrea virginica*) aquaculture is an important industry in Delaware that generates tens of thousands of dollars in sales every year. In addition to the economic value of oysters, they are known as keystone species that offer vital ecosystem services including water filtration and providing habitation and food for many aquatic species. Eastern oysters are predated on by different species of fish, crustaceans, and gastropods.

In this study, we deployed real time monitoring (camera) and molecular biology approaches to monitor the Oyster predation and species diversity at five different oyster sites around Rehoboth Bay. The sites include artificial reefs, aquaculture farms, and control sites (Figure 1). Cameras are secured into recreational crab traps equipped with battery extenders. They are deployed for approximately two to three hours before retrieval. All documented aquatic species are identified and recorded for comparisons between sampling sites. Isolation of Environmental DNA (eDNA) is also performed as a compilatory tool for species identification. This study will provide us an up-to-date information on the oyster restoration efforts in Rehoboth Bay, Delaware.

Figure 1.



LAND-BASED CULTIVATION OF TROPICAL RED MACROALGAE WITH A FOCUS ON PRODUCTION

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Tropical macroalgae culture potential is constrained by the oligotrophic nature of most tropical oceanic waters. Our trials tested the addition of Deep Sea Water (DSW) and an IMTA model using fish tank effluent to provide nutrients for culture of macroalgae. This presentation reports on the cultivation of three red macroalgae (*Halymenia hawaiiana, Gracilaria parvispora* and *Agardhiella subulata*) in our land-based system at NELHA (Natural Energy Laboratory of Hawaii Authority) in Kailua-Kona, HI (Figure 1). For the marine finfish effluent, we co-cultured with the following two marine finfish; *Seriola rivoliana*, aka Kampachi or amberjack, and the herbivorous reef-fish *Kyphosis vaigiensis*, aka chubs or nenue.

Research focused on optimizing production and yield based on DSW and finfish effluent concentrations, stocking density and irradiation rates. Nutrient levels (total nitrogen, total phosphorus, ammonia/ammonium nitrate/nitrite, and ortho-phosphate) at the inflow and outflow of the macroalgae tanks gave measures of nutrient uptake rates by macroalgae. Macroalgal tissue composition, including % tissue ash, carbohydrate, and nitrogen will be presented.

Red macroalgae cultivation in conjunction with fish culture could mitigate nutrient loading of effluent waters, as well as provide increased productivity of commercially valuable macroalgae. The addition of DSW has the potential to drive macroalgal yields and Specific Growth Rates (SGR). This research is setting a baseline foundation for growing tropical macroalgae offshore for food, feed, and biofuels.

Figure 1. Three red macroalgae species cultivated at Ocean Era; (from left to right) (*Halymenia hawaiiana, Gracilaria parvispora* and *Agardhiella subulata*).

UNBUNDLING SUSTAINABLE COMMUNITY-BASED DAM AQUACULTURE FOR BLUE GROWTH

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Theme: Conservation/Management/Stock Enhancement

Dam restocking has emerged as one of the enhancement techniques to riparian social-ecological systems for livelihood and nutritional security advancement. Dams can thus promote community-aquaculture initiatives to increase fish production and availability in rural areas with proper management and husbandry. This paper highlights the development of the Framework for Community-based Dam Aquaculture (FCODA) which lays a solid foundation for blue growth and which are based on poverty alleviation and food security. In the implementation of FCODA, countries are encouraged to focus on environmental, economic, and social considerations that are critical for the Blue Economy's long-term development. The FCODA herein was developed using expert opinions and various literature sources such as national and international policy documents such as the African Agenda 2063, and the United Nations Sustainable Development Goals (SDGs. The framework's three KRAs are: (i) Enhanced economic benefits to the communities; (ii) infrastructural and human capacity development; and (iii) Enabling environment for sustainable dam aquaculture. During the framework's implementation, the resources required to operationalize a given business enterprise over a given period could be mobilized through ploughing back mechanisms, lobbying development partners and the government for additional funding, and other stakeholders. To ensure that the framework is implemented effectively, monitoring and evaluation should be done on a regular basis.

STATUS OF THE U.S. FARM-RAISED CATFISH INDUSTRY - 2022

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U.S. farm-raised catfish acreage decreased by 2% from 24,000 hectares in 2021 to 23,525 hectares in 2022. U.S. acreage has decreased by 70% since its high of 79,600 hectares in 2002. The primary factors associated with this decrease are high feed costs, low fish prices, market disruptions, and increased competition from cheaper imports.

American catfish producers continue to improve production efficiencies (Figure 1). As recently as 2009, gross yield was only 4,332 kg/ha. Producers were yielding 7,271 kg/ha in 2021. The primary technologies leading to these increases are hybrid catfish (channel catfish *Ictalurus punctatus* females X blue catfish *Ictalurus furcatus* males), improved production systems like intensively-aerated small ponds and split-ponds, and improved fish health treatments such as vaccines and rapid diagnostic tools.

Feed prices continue to increase. Prices for 32% floating catfish feed in September 2022 were up 17% from the 2021 average price and 47% from the 2020 average price.

During the first 10 months of 2022, the U.S. imported 132,588 mt of Siluriformes products. If this trend holds for the remaining of 2022, 73% of the Siluriformes products consumed in the U.S. are imported.

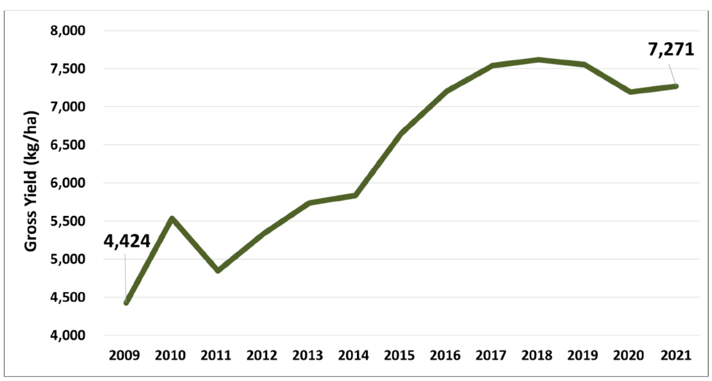

Figure 1. Gross yield in U.S. farm-raised catfish ponds.

MISSISSIPPI STATE UNIVERSITY - THAD COCHRAN NATIONAL WARMWATER AQUACULTURE CENTER

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The Mississippi State University - Thad Cochran National Warmwater Aquaculture Center (NWAC) is a multi-disciplinary, multi-institutional research and Extension program located in Stoneville, Mississippi. The aquaculture program in Stoneville began in 1980 with the NWAC formally organized in 1997. Its mission is to provide solutions to the most pressing problems of the aquaculture industry through basic and applied research, Extension, and diagnostic services. Mississippi State University is represented by the Mississippi Agriculture and Forestry Experiment Station, Extension Service, and College of Veterinary Medicine. USDA is represented by the ARS Warmwater Aquaculture Research Unit and the NIFA Southern Regional Aquaculture Center. The NWAC has over 6,300 m^2 of lab and office space and 119 hectares of ponds. Current staffing includes 15 scientists/faculty and 40 support staff.

MSU and USDA scientists conduct research to solve problems that can be solved in the short-term as well as those that threaten the long-term viability of the industry. Research program areas include water quality, pond ecology, production system development, economics, nutrition, fish health management, genetics, diagnostics, and clinical research. NWAC Extension activities focus on the dissemination of research-based information to the aquaculture industry and to provide technical expertise to those commodity organizations that support aquaculture.

Research milestones include facilitating the registration of the antibiotics Romet® 30 and Aquafor®, identification of the causative organisms and management strategies for off-flavors in channel catfish, developed treatment strategies to combat the *Bolbophorus* digenetic trematode, alternative feed ingredients, developed a live attenuated vaccine for ESC disease, development of the split-pond and intensively aerated pond systems, hybrid catfish production, improved broodstock and spawning technologies, genome sequencing of channel catfish, blue catfish and microbial pathogens, and much of the research used by EPA to develop the final rule on aquaculture effluents. Extension milestones include development of programs pertaining to disaster assistance, foreign competition, and transition to the USDA FSIS Inspection program.

Current water quality and fish heath research emphasis includes chemical control, epidemiology, surveillance, and rapid diagnostics for various pathogens and environmental stressors. The nutrition group is focused on intestinal health and microbiota, feed additives/alternatives, and feed management. MSU Economists are conducting research on production costs and adoption of various production practices. USDA scientists are improving catfish genetics through selective breeding of channel and blue catfish lines, improved hybridization techniques, and improved production environments through pond ecology and water quality research. Current Extension emphasis includes adjusting to an increasingly stringent regulatory environment and producers' adoption of new production techniques.

UNITED STATES FISH AND WILDLIFE SERVICE WILD FISH HEALTH SURVEY AND NEW GIS-BASED MAPPER - PROTECTING WILD FISHERIES

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The U.S. Fish and Wildlife Service's (FWS) fish health centers have launched a new tool to help in the fight to protect wild fisheries and to provide useful information to the aquaculture industry. The Wild Fish Health Survey Mapper provides near real-time surveillance of pathogens in wild aquatic animal populations.

For most of the 20th century, very little was known about the presence or distribution of naturally occurring viruses and pathogens that cause diseases in wild fish. That lack of knowledge put conservation efforts at risk because natural resource managers often have to move fish and other aquatic animals between hatcheries and ecosystems in order to support Tribal and recreational fisheries and conserve some of the most imperiled organisms in the world.

Since 1997 the FWS fish health centers, through opportunistic sampling in partnership with States and Tribes, have conducted a National Wild Fish Health Survey (NWFHS) to improve our ability to conserve fish and waterways. The survey provides information on the presence or absence of aquatic animal pathogens in wild fish populations to Tribes, State and Federal fisheries managers, the aquaculture industry, conservation groups, researchers, and the public.

In 2022, the NWFHS database was upgraded and fully integrated within a FWS-supported GIS-based system. The newly developed, searchable GIS mapper provides novel access to NWFHS data, which is the most robust data set of this type, containing over 200,000 tabular records from 1500 cases and covering nearly 1400 watersheds throughout the United States from 1997 to the present.

If used properly and within the limitations of the dataset, this data can help:

- Establish safe zones and areas where movement of aquatic animals can take place without endangering the health of wild fish populations
- Serve a source of information for modeling and risk assessment.
- Understand the distribution of pathogens in the environment.
- Identify what fish are susceptible to pathogens and where they can be found.

TheMapper and the NWFHS dataset can be found at: https://fws.maps.arcgis.com/apps/webappviewer/index.html?id=db2d6619d8a14c1dab4ec9fd00dc9cfa

PARTNERING TO EXPEDITE THE IMPORT OF HEALTHY SALMON AND TROUT INTO THE UNITED STATES: A USFWS TITLE 50 PROGRAM PRIMER

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One of the primary missions of the Service is to protect U.S. citizens, and our nation's natural resources. The United States Fish and Wildlife Service (Service) partners with the aquaculture industry to safeguard the natural fisheries resources of the United States through it's Title 50 Salmonid Fish Import Program. The Service implements regulations to ensure that only healthy trout and salmon are imported into the United States.

The Lacey Act of 1900 is the United Sates law that regulates the trafficking in illegal wildlife. One part of the law covers the importation of wildlife deemed a threat to the wildlife of the United States. Per 50 C.F.R. § 16.3, "Any importation or transportation of live wildlife or eggs thereof, or dead fish or eggs or salmonids of the fish family Salmonidae into the United States or its territories or possessions is deemed to be injurious or potentially injurious to the health and welfare of human beings, to the interest of forestry, agriculture, and horticulture, and to the welfare and survival of the wildlife or wildlife resources of the United States…"

To expedite the import of healthy salmon and trout into the United States, the Service has established a step-wise process to efficiently certify Salmonid importations. The steps can be found at the following website: https://www.fws.gov/service/steps-importing-salmonids-united-states-america. The new process, along with its new forms, database and webpage, was created after consultation with partners. As a result of these developments, the T50 Program has quadrupled Salmonid fish imports without increasing health risk to natural resources. In addition, processes to streamline approval of Certified Signing Officials has made endorsing them easier and more effective. As a result, many more domestic and international Officials have been approved than in the recent past.

In 2022, the T50 Program has authorized 20 shipments for approximately 4.3 million fish and eggs (4 species, 3 countries). During the same period, six new individuals have applied to be a Signing Official from 3 new counties. The program now endorses 25 officials from 8 counties.

Support and input from the aquaculture industry are essential for the success of the T50 program and for the protection of our nation's natural resources and to the aquaculture industry. The Service continues to strive to improve the T50 program in the future in concert with our partners and adapt to emerging importation needs.

LESSONS LEARNED ON A SCALABLE COASTAL MARCROALGAL FARM

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As part of the Department of Energy ARPA-E MARINER program, an experimental test farm has been deployed over the past four years in Kodiak, Alaska with the goal of increasing yield per unit area and increasing operational efficiencies. During that time, we have worked to improve all aspects of the farming process, with our fair share of successes and learning experiences. The farm utilizes a TendOcean™ catenary system that provides the structure required for dense farming while optimizing production and enabling operational efficiencies unachievable with traditional farming practices. The system has gone through several iterations since its inception to increase size, yield, and operational efficiency with the addition of longer grow-lines, buoyancy techniques, connections, and tensioning mechanisms. The process and techniques used to fabricate and deploy these complex farm systems has been iterated and refined; now entire catenary systems can be shipped to the farm site and deployed as a single unit. The operational efficiencies provided by the catenary system required rethinking traditional methods of seeding, monitoring, and harvesting. We describe some of the advancements we have made in component design and testing to mechanize seeding and harvest and improve material handling and product quality. We are confident that the efficiencies developed from this project can reduce the cost of production, which will allow the industry to supply new markets.

THE EFFECTS OF DIFFERENT LEVELS OF DIETARY PROTEIN ON THE GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED IN INTENSIVE BIOFLOC CULTURE SYSTEM

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Dietary protein is the most expensive component of the feed and an important determinant in *L. vannamei* growth. Therefore, understanding the interaction between dietary protein levels and daily protein intake which drives the shrimp's growth is crucial. A 70-day feeding trial was conducted to illustrate the effect of dietary protein levels on the growth response of *Litopenaeus vannamei* reared in individual biofloc system each containing 800L of culture water stocked at 150 shrimp/m^3. Four diets with crude protein levels of 25%, 30%, 35%, and 40% fed at the standard feeding rate were used for this experiment as four treatments. 30% CP and 35% CP diets were also fed at 133.3% and 114.3% of the standard feeding rate (equivalent to 40% CP), respectively as two other treatments. This resulted in a total of six treatments with four replicates each per treatment. At the end of trial, significant differences (P<0.05) in growth and FCR were observed. Results showed that the increase in crude protein content of the feed resulted in the increased final mean weight and weight gain. The shrimps fed with 35% CP diet at 114.3% had the highest final mean weight (14.1 ± 0.93g), weight gain (13.87 ± 0.94), and weight gain percentage (4490.73%). This trial demonstrated that the shrimps raised in intensive biofloc system perform better in terms of growth and feed utilization when provided with higher levels of crude protein diet.

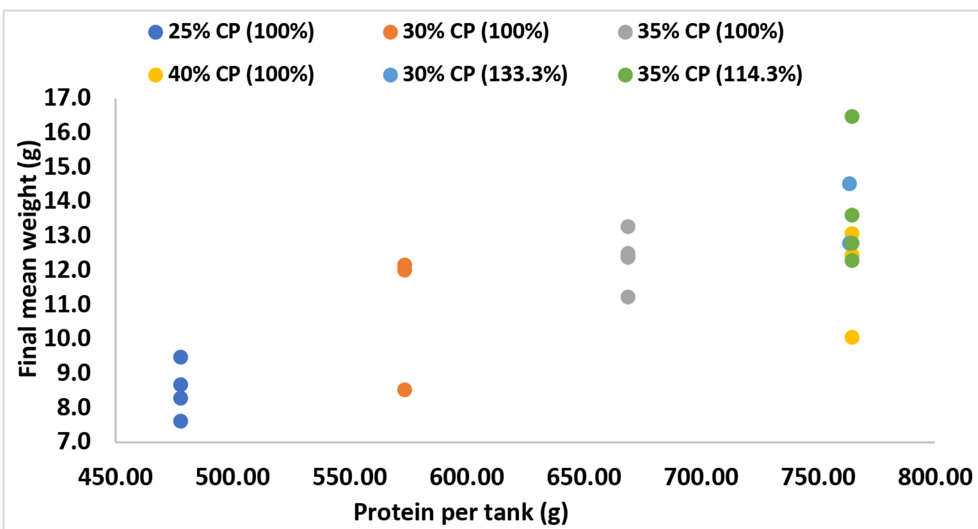

Figure 1: Growth in response to different level of protein diets in 70 days trial.

THE EFFECTS OF DIFFERENT FEEDING RATES ON PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) IN A RECIRCULATING BIOFLOC CULTURE SYSTEM

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In shrimp culture, managing feed and nutrient inputs are important factors to optimize. It is well known that the amount of nutrients consumed affects an animal's ability to grow, survive, and the amount of metabolic and excreted waste products entering the system. The aim of the study was to observe the response of *L. vannamei* to different levels of feed inputs of the commercial shrimp diet (Zeigler Shrimp Grower SI-35, CP 35%) in an intensive biofloc production system. The experiment was conducted in an indoor biofloc recirculating aquaculture system consisting of 24, 800L culture tanks stocked at 150 shrimp/m^3 and grown for 42 days. The treatments included eight different feeding rates (64%, 80%, 96%, 112%, 128%, 136%, 144%, and 152% of the standard feeding rate) which were offered via a belt feeder. At the end of the trial, significant differences ($P<0.05$) in growth and FCR were observed. The shrimps from the treatment fed at 144% of the standard feeding rate had the highest final mean weight (10.4 ± 0.06g), weight gain (9.4 ± 0.10g), and weight gain percentage (927.4%). There is an inflection point above 144% of the standard feeding rate where growth and feed utilization decrease rapidly, causing an increase in FCR and lesser growth increases as maximum gain is approached. The findings of this feeding trial indicate that the current standard feeding protocol could be improved to increase shrimp's final weight, but it could be at a cost of increased FCR and with the risk of feed wastage (which can cause water quality problems).

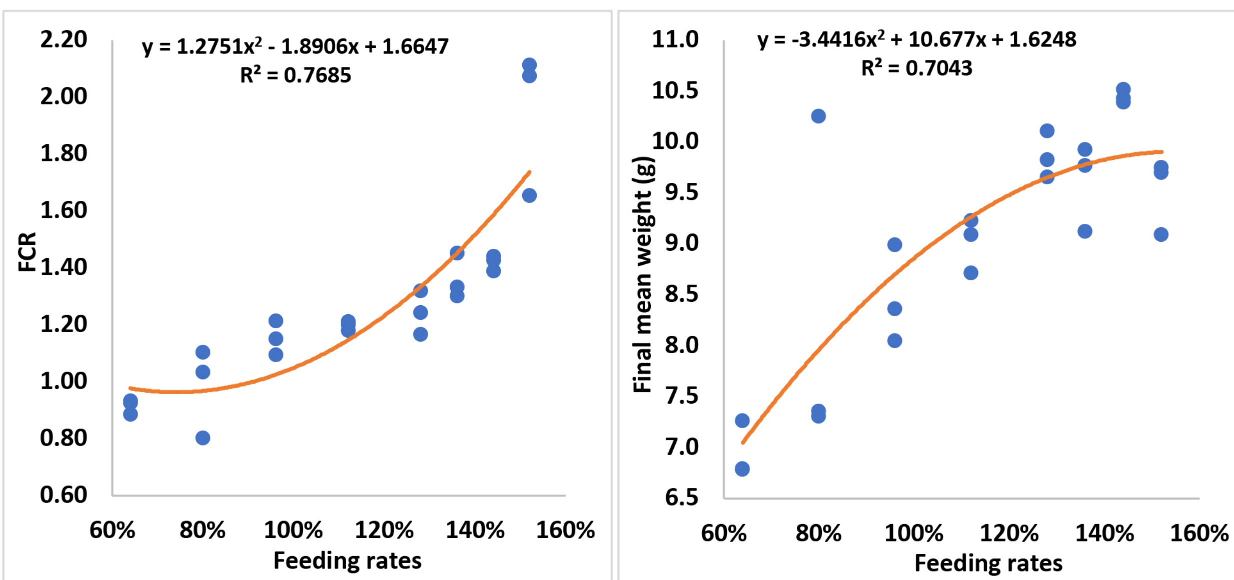

Figure 1: FCR and final biomass at different feeding rates in 42 days trial.

LINKING *vgll3* GENOTYPE AND AGGRESSIVE BEHAVIOUR IN JUVENILE ATLANTIC SALMON (*Salmo salar*)

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Aggressiveness can affect social hierarchies and result in the unequal distribution of resources, with aggressive individuals monopolizing access to food influencing growth. In Atlantic salmon, aggression has been found to have a genetic component, and growth also influences maturation timing. Maturation timing associates with a large-effect locus around *vgll3*, which is also linked to growth and condition, with *vgll3**EE (early maturation) individuals having higher condition factor than *vgll3**LL (late maturation). Here, we examine the possibility that aggressiveness may play a role in juvenile *vgll3**EE individuals having higher condition factor by having increased food intake due to higher aggression promoting increased food access. This prediction was tested under a social context: aggressiveness and feeding activity in four size-matched individual salmon, two from each genotype, were observed over 95 trials. Contrary to our prediction, *vgll3**LL individuals, not *vgll3**EE, were more aggressive. Increased aggression of *vgll3**LL individuals was independent of their sex and size, and genotypes did not differ in their condition factor nor feeding activity. These results imply that aggressiveness may have an energetic cost impairing growth and condition, especially when food cannot be monopolized. This may have critical implications for individual fitness and aquaculture practices.

EFFECTS OF MORPHOMETRICS AND ULTRASTRUCTURE ON SWIMMING KINEMATICS OF BLUE CATFISH (*Ictalurus furcatus*) SPERM

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The United States catfish industry appears sustainable after the adoption of superior channel catfish, *Ictalurus punctatus* female × blue catfish, *I. furcatus* male hybrids. However, there are still challenges in reproductive management due to the nature of the breeding process. For instance, blue catfish males reach maturity after 4-7 years. Unlike other fish in which sperm can be extracted by stripping, sperm collection from blue catfish is accomplished by removal and maceration of testes. As the males must be sacrificed to obtain sperm, a substantial investment in sperm production is required. Typically, a mechanistic relationship exists between sperm form and function, where it is predicted that longer sperm have enhanced swimming and higher fertility. As such, a complete assessment of sperm morphology will provide important information to support captive breeding and broodstock management for hybrid catfish production. The aim of this study was to link sperm ultrastructure and morphometrics to sperm swimming kinematics of blue catfish.

Blue catfish males (n = 43) were seined from aquaculture ponds. Males were euthanized and testes dissected, washed with Hank's balanced salt solution, and sperm cells extracted for assessment of form and function. Computer Assisted Sperm Analysis (CASA) software was used to quantify sperm velocity, which is the primary determinant of fertilization success. Males were ranked based on mean velocity and separated into two groups: high (n = 8; 200.5 ± 8.9 μm/s) and low velocity (n = 8; 158.0 ± 5.4 μm/s) groups. Sperm from these high- and low-quality males were then fixed in glutaraldehyde and imaged with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for morphometric and ultrastructure analyses.

Preliminary results show that blue catfish sperm are biflagellar (Fig 1a), lack an acrosome (Fig 1bc), and are differentiated into a head, midpiece, and flagellum (Fig 1bc). Mean (± SD) head length was 1.74 ± 0.32 μm and width was 2.21 ± 0.25 μm. The midpiece was 1.42 ± 0.30 μm in length and 2.75 ± 0.30 μm in width, while the length of flagella was 81.53 ± 6.87 μm. SEM shows several sperm with unidentified projections on the tip of their heads.

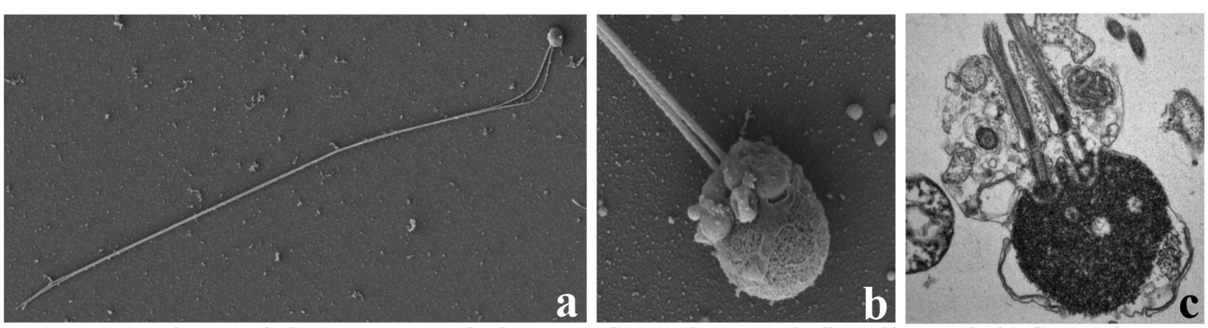

Figure 1. Blue catfish sperm morphology: a) SEM showing biflagellar tail; b) SEM showing midpiece and lack of acrosome; c) TEM of head showing midpiece and centrioles.

AQUACULTURE OPPORTUNITY AREAS: A NEW APPROACH TO U.S. AQUACULTURE DEVELOPMENT

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With a new vision and strategic plan for aquaculture, NOAA is leading a shift-change in U.S. aquaculture development through the identification of Aquaculture Opportunity Areas (AOAs). NOAA is working with its federal partners to develop a process 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.

The AOA process will result in the identification of geographic areas that, through scientific analysis and public engagement, are determined to be environmentally, socially, and economically suitable for multiple aquaculture farm sites of varying types. NOAA will combine input received through consultation and coordination with Federal and non-Federal stakeholders, public comments, and spatial modeling by NOAA's National Centers for Coastal Ocean Science (NCCOS) that is based on the best available science.

NOAA began the process to identify AOAs in Federal waters within the Gulf of Mexico and off Southern California, south of Point Conception. NOAA will provide updates on our progress in these first two regions, including plans for the PEIS for each and the continued opportunities for stakeholder input into the process. NOAA will also discuss the status of the process to identify AOAs in other regions of the country.

CANADA'S AQUACULTURE MONITORING PROGRAM

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Aquaculture activities can result in environmental and ecological impacts. Impacts associated with aquaculture inputs (e.g., organic matter, trace metals, drugs, pesticides) and depletions (e.g., phytoplankton and zooplankton) are better characterized near and within aquaculture lease areas but less is known about the impacts outside of the lease area. Fisheries and Oceans Canada initiated the Aquaculture Monitoring Program (AMP) in 2017 to conduct long-term monitoring outside of aquaculture lease areas. AMP includes data collection, sampling, and analysis with the objective to detect, monitor, and model aquaculture-related changes to the benthic and pelagic environment near select coastal aquaculture locations. Parameters measured include sediment grain size, organic matter, trace metals, sulfides, infauna communities, drugs, seston, phytoplankton, and zooplankton. A national database is being developed to store data from sampling activities. Information from this program is being used to support research initiatives and will be used to inform decision making. AMP has focused on the development of nationally consistent methods for sample collection, sample analysis, and statistical design and is now progressing toward the establishment of consistent long-term data collection and monitoring.

PRODUCTIVITY OF MICRONUTRIENTS FROM INTEGRATED AQUACULTURE-AGRICULTURE SYSTEMS: EVIDENCE FROM BANGLADESH

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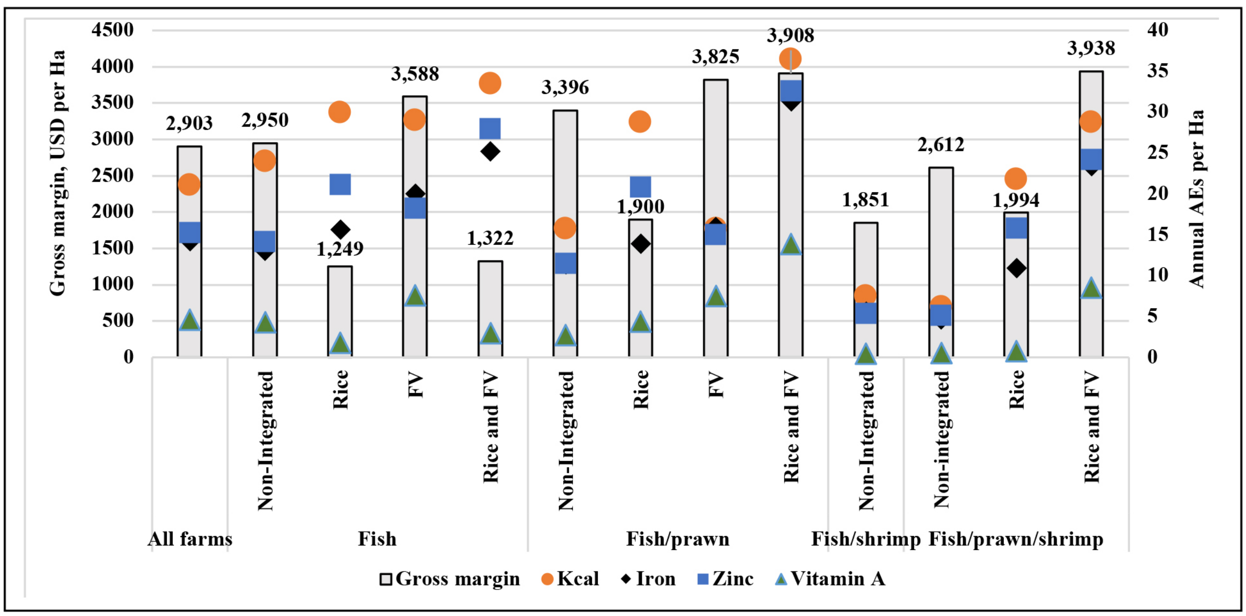
Integrated aquaculture-agriculture (IAA) has been widely promoted as a form of farm diversification that can promote more efficient resource use, improve productivity, and lead to higher household incomes and more diverse diets. Farm productivity is usually measured in terms of biomass or income produced per area of land. Here, we extend the concept of productivity to measure production of energy (kcals) and micronutrients, and explore the relationship between the economic and nutritional productivity for 12 distinct types of IAA system, identified from a survey of 721 farms in Southwest Bangladesh.

Nutrient productivity is expressed as the number of adults able to meet their total recommended annual intakes of selected nutrients from the food produced on one hectare of land (AEs/ha). We present productivity of energy (kcal), iron, zinc, and Vitamin A under different IAA systems. Farms integrated with fruits and vegetables, and farms producing fish with freshwater prawn tend to have higher economic productivity than non-integrated farms, and those producing fish only. (Fig.1). Farms integrated with rice have higher energy productivity. Farms integrated with fruit and vegetables produce slightly more vitamin A. OLS regressions confirm that, in general, integrated farms produce more nutrients per hectare than non-integrated farms. Vegetable production is a key driver of both economic and nutrient productivity. These findings have important implications for the design of Nutrition Sensitive Agriculture programs that can enhance the contributions aquaculture makes to nutrition security in Bangladesh and other countries.

Figure 1: Economic and nutrient productivity by IAA system (USD/ha & AE/ha)



THE AQUACULTURE PERFORMANCE INDEX (APIX) APPLED TO AQUACULTURE SPECIES SELECTION FOR THE STATION PADREOIL RIG OFFSHORE AQUACULTURE PROJECT

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Biological, technological and market criteria have been evaluated to rank several pelagic, demersal, reef and coastal fish species for the Station Padre Aquaculture project development. Feasibility levels (*experimental, technological* and *commercial/economical*) were also considered according to species prospects for commercial aquaculture development using offshore net cages. Red Drum (*Sciaenops ocellatus*), Almaco jack, also known as Kampachi (*Seriola rivoliana*), Pompano (*Trachinotus carolinus*), Red Snapper *(Lutjanus campechanus),* and Cobia (*Rachycentrum canadum*) were considered top candidate species for commercial aquaculture development in offshore systems in the area selected in the Gulf of Mexico and were ranked taking into consideration the characteristics of the site. We applied a methodology termed Aquaculture Performance Index (APIX) In short, the APIX equation considers the following basic criteria, each assigned different weights to calculate a respective final value: feasibility level, feed conversion rate, growth rate to market size, survival rate, stocking density, feed costs, fish processing yield and level, marketability (market demand and price). A full APIX equation was constructed to assign each species a final APIX score. Red Drum (*Sciaenops ocellatus*) was the top selected using this methodology. Even though Red Drum has a lower market value when compared to other fish species considered, it was found to be the most feasible for this operation since it thrives at the temperature range of the site (22 – 26 ºC), allows for a much higher stocking density >30 kg/m^3, and has a lower feed cost since good growth and survival results can be obtained using a high percentage of soy protein as a substitute for fish meal. In addition to that, there is a long history of Red Drum production in Texas, which makes fingerlings and feed readily available as well as an established market. As a competitive advantage, the Red Drum produced in the offshore cages will have a different taste (no *off-flavor*) when compared to pond-reared fish and for this reason, it has a possibility of being marketed as premium. However, we suggest a thorough market research to establish the price and run the economic analysis model for the species in offshore cages. The team is considering an innovative approach of conducting trials combining the proposed marine fish operation with seaweeds and/or bivalves culture in a Multi-Trophic Integrated Aquaculture (IMTA) system. The species of native bivalves and seaweeds were suggested on a commercial feasibility level basis and according to the temperature ranges of the site. The bivalve molluscs suggested were the Lion's Paw scallop (*Nodipecten nodosus*), the Atlantic oyster (*Crassostrea virginica*) and the hard clam (*Mercenaria mercenaria*). *Euchema* spp., *Gracilaria* spp., and *Hypnea* spp were the seaweed species suggested based upon their application for agar and carrageenan production among other potential uses (biostimulants, feed, cosmetics, etc).

VALORIZATION OF BY-PRODUCTS THROUGH FEED FORMULATION FOR *Tilapia sp*: ZOOTECHNICAL PERFORMANCE STUDY

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In recent years valorization of biowaste attracts lot of attention worldwide owing to its high nutritional value and low price. In this work biowaste of animal (sardines) and plant (tomato) biowaste was used to formulate a new feed for red tilapia, that showed to be competitive in its price and zootechnical performance in comparison to commercially available tilapia feeds. Mathematical modelling was used to formulate optimal feed composition with favorable chemical composition and the lowest price. Formulated feed had high protein content (40.76%) and energy value of 279.6 Kcal/100 g. Optimised feed was manufactured and compared to commercially available reference feed in respect to feed intake, feed efficiency, specific growth rate of fingerlings of Tilapia sp and, most important, zootechnical parameters. With fish survival rate of 100% calculated feed conversion index for the formulated feed was 2.7.

The Feed Conversion Index (CI) is an indicator commonly used in aquaculture farming, it gives an idea of the feed efficiency of a feed or feeding strategy. Slightly higher CI was observed for feed T_1 (2.7). In T_0 calculated CI was 2.5. For both tested feeds their CIs were similar to those obtained by Abdel-Warith et al (2001) which ranged from 1.25 to 2.80 for fingerlings fed with feeds based on avian by-products. Feed conversion rates obtained by Fagbenro et al (1999) ranged from 1.52 to 2.27 depending on whether the diet was based on soybean meal or arugula meal with percentage of incorporation of 19.5, 39 or 58.5%. The feed conversion rates obtained by Giri et al (2000) in their experiment of substituting fishmeal with chicken viscera meal or vegetable meals (soybean, peanut and mustard) were 3.0 and 2.9, respectively. Sandamali et al (2016)

Table . Zootechnical performance of formulated and reference feeds in Red Tilapia

Zootechnical parameter	T_1	T_0
Initial average weight (g)	5.50±1.33**	7.36±2.90**
Final average weight (g)	18.95±7.90***	28.07±10.64***
Initial average length (cm)	7.56±1.94*	6.66±0.73*
Weight gain (g/d/ind)	0.27*	0.46*
Food conversion index	2.7*	2.5*
Cost per feed unit (USD)	1.01	3.97

INSIGHTS ON BUILDING AN AQUAPONICS BASED URBAN AQUACULTURE FOOD SYSTEM IN PHOENIX ARIZONA

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With some media suggesting that there is hunger in America, the advent of the COVID-19 pandemic revealed a number of weaknesses within the US urban food system. Across the United States, municipalities stepped forward, some with the help of American rescue funds, to address this issue. Food banks, food, distribution methodologies, and other channels were rapidly employed. In addition, food production from urban agriculture has become increasingly of interest. The question is, within urban ag, what is the role that aquaculture, specifically, Aquaponics including its potential to produce markable fish and crustaceans, can play?

This study with a focus on the Phoenix Arizona metropolitan area, examines the question of urban aquaculture through aquaponics. It summarizes the characteristics of urban aquaponics, and explores the social, environmental and economic opportunities and constraints to its implementation in a desert city.

EVALUATION OF BREWERS' GRAINS AND EXOGENOUS ENZYMES IN PRACTICAL DIETS FOR NILE TILAPIA (*Oreochromis niloticus*)

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Increasing demand for conventional fish feed ingredients has resulted in rising fish feed costs, which are considered a hindrance to the aquaculture industry's growth. Brewer's spent grains (BSG) are byproducts that are not suitable for human consumption and whose safe disposal raises environmental concerns. Brewer's waste is one of the promising protein source by-products for fish diets.

An 8-week feeding trial was conducted at Kentucky State University Aquaculture Research Center to assess the nutritional value of practical diets containing different amounts and combinations of BSG and Allzyme® SSF in Nile tilapia (*Oreochromis niloticus*). A factorial design was used, with three levels of BSG (0, 270, and 550 g/kg) and three levels of ENZ (0, 0.2, and 0.4 g/kg) being used to examine the fish's production performance and whole-body proximate composition. At the conclusion of the study, linear reductions in growth of the fish as dietary BSG increased were observed, while no significant effects of ENZ or interactions between ENZ and BSG were found for all response parameters evaluated. Further studies are recommended to define optimal dietary levels of BSG and enzymes that degrade non-starch polysaccharides in these species' feeds.

NOAA FISHERIES ALASKA FY22 AQUACULTURE ACCOMPLISHMENTS

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NOAA Fisheries Alaska (Alaska Regional Office and Alaska Fisheries Science Center) has continued coordinated efforts to prioritize projects and actions to support the growing Alaska aquaculture industry.

We achieved a number of notable accomplishments this year in our Aquaculture Program despite the continued challenging circumstances of the COVID-19 pandemic. This presentation provides a summary of the accomplishments for each of our identified priority areas.

Marine aquaculture—also referred to in Alaska as mariculture—helps support Alaska's blue economy, contributing to sustainable seafood, and local economies. Seaweed and shellfish aquaculture have been shown to provide ecosystem benefits including creating habitat for mobile fish and invertebrates species. Aquaculture also establishes economic opportunities by creating jobs, supporting eco-tourism, and other pathways.

We developed the Joint Aquaculture Action Plan to strategically align the work being done by both the regional office and science center, help accomplish the goals of NOAA Fisheries Marine Aquaculture Strategic Plan, and meet industry and management needs identified in the Alaska Mariculture Development Plan.

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 in 2022 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 nascent marine aquaculture industry continues to grow, NOAA Fisheries Alaska Regional Office and Alaska Fisheries Science Center will play an increasing role in the management, policy, and research that helps build this sustainable food source. Implementing our Aquaculture Action Plan and Aquaculture Research Strategic Plan helps our agency prioritize efforts based on our agency's strengths and management and industry needs, focusing our efforts to best serve Alaska.

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

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

FOLLOWING THE SNAIL TRAIL: INTERACTIONS OF THE MARINE SNAIL *Lacuna vincta* WITH KELP FARMS IN THE GULF OF MAINE

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The removal of kelp farms before the summer and rigorous surveying prior to lease placement may help deter fouling by other invertebrates, but the small marine gastropod *Lacuna vincta* (family Littorinidae) has proved to be an unpredictable and continuous obstacle for kelp farmers on both U.S. coasts. Though the snails themselves are easy to brush or wash off kelp blades, their eggs become embedded in the blade's surface, making removal post-harvest labor-intensive, time-consuming, and ultimately, impractical. Current management strategies, such as harvesting early, are costly for farmers who often get paid in dollars per pound.

Not much is known about the snails' interactions with kelp farms as most research has been done on *L. vincta* grazing and settlement in wild kelp communities. We aim to pinpoint the timing of *L. vincta* settlement on kelp farms in Maine through frequent visual inspections of grow lines throughout the winter growing season. Additionally, using a novel *L. vincta* PCR primer, we are testing marine environmental DNA methodology as a possible tool for early detection of this species.

Snail distribution is patchy even within kelp farms that have large infestations. Work with industry partners has focused our research to include snail preference experiments to determine if the biology of the kelp itself is a factor in snail preference. In the first experiment, farmed sugar kelp (*Saccharina latissima*) was removed and kept in two treatments: one treatment where it was fed f/2 nutrient solution, and another treatment where kelp was deprived of nitrates. *L. vincta* snails were then exposed to identical squares of each. The second experiment exposed the snails to identical squares of sugar kelp and skinny kelp (*Saccharina angustissima*), two commonly farmed species in Maine. Snail herbivory and settlement preference over time were recorded for both experiments. Results suggest that there may be a sweet spot between peak health and stress in kelp at which snail preference changes. Preliminary results for the second experiment suggest that snails prefer skinny kelp, however more trials are needed.

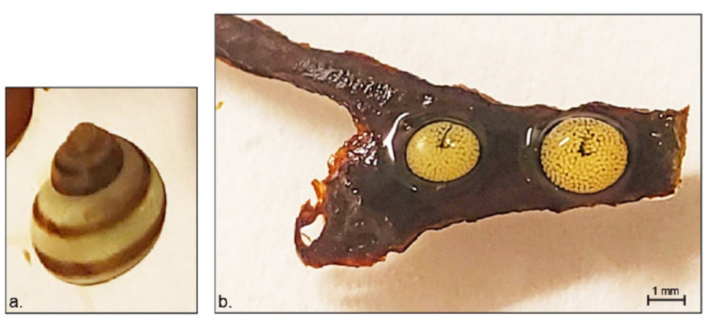

Figure 1. *Lacuna vincta* snail (a) and eggs (b).

TEXTURE QUALITY DIFFERENCES BETWEEN CHANNEL AND HYBRID CATFISH FILLETS

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The texture of cooked and raw fillets of both channel (*Ictalurus punctatus*) and hybrid (female channel x male blue, *I. furcatus*) catfish were measured by instrumental texture profile analysis (TPA) methods. Catfish from commercial and experimental ponds were used, that included differences in geographical region, harvest season, fish gender, and the type of fillet cold-storage (fresh, frozen, of IQF), with or without polyphosphate treatment.

Comparing baked fillets across all three cold-storage types combined, channel catfish were found to have significantly ($p<0.05$) higher values for six of the seven texture attributes, firmness, toughness, cohesiveness, chewiness, resilience, and springiness, with only adhesiveness not showing any difference. Channel-hybrid differences, based on each cold-storage type (Table 1), showed fresh fillets to have more than a 60% difference, while frozen had the largest number of significant attributes and IQF having the fewest significant differences. Significant differences between cold-storage types, not based on catfish type, were only seen for cohesiveness, adhesiveness, resilience, and springiness. This indicated the channel-hybrid differences were predominantly based on the firmness, toughness, and chewiness attributes.

Fillet thickness also showed a covariance with most texture attributes, but based on statistical analysis calculations, firmness was significantly different for the full range of thicknesses used in the study, while toughness became significantly different above 11.9 mm fillet thickness (cooked), with 96% of fillets having larger average thicknesses.

TABLE 1. Instrumental textural differences [1] between cooked channel and hybrid catfish fillets according to cold-storage type. Values expressed as means ± standard deviations

	Fresh (n = 30)		Frozen (n = 40)		IQF (n = 49)	
	Channel	Hybrid	Channel	Hybrid	Channel	Hybrid
Firmness	**274 ± 36**	**167 ± 19**	**248 ± 30**	**183 ± 24**	**235 ± 31**	**174 ± 20**
Toughness	**216 ± 37**	**118 ± 20**	**179 ± 35**	**131 ± 32**	**177 ± 39**	**123 ± 17**
Cohesiveness	0.47 ± 0.03	0.46 ± 0.03	**0.47 ± 0.03**	**0.44 ± 0.02**	0.49 ± 0.02	0.48 ± 0.02
Adhesiveness	−1.2 ± 0.3	−1.4 ± 0.3	−1.1 ± 0.3	−1.1 ± 0.3	−1.0 ± 0.2	−0.9 ± 0.3
Chewiness	**93 ± 18**	**52 ± 8.4**	**85 ± 14**	**57 ± 10**	**82 ± 13**	**59 ± 10**
Resilience	**22.5 ± 1.9**	**20 ± 1.8**	**21 ± 1.7**	**19 ± 1.1**	21 ± 1.7	22 ± 1.9
Springiness	**71 ± 2.6**	**66 ± 2.3**	**72 ± 3.1**	**69 ± 4.2**	71 ± 3.1	69 ± 3.8

[1] Differences in TPA attributes were based on t-tests, at significance level of $\alpha = 0.05$. Values that differed between channel and hybrid, within each cold-storage type, are in bold font.

MULTI-TISSUE DIFFERENCES IN GENE EXPRESSION FOLLOWING EXPERIMENTAL *Flavobacterium columnare* CHALLENGE IN THREE SELECTED LINES OF RAINBOW TROUT WITH VARIED GENETIC RESISTANCE

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Genetic selection has been shown to be an effective strategy to increase specific-pathogen resistance in cultured populations of fish and multiple lines of rainbow trout have been selected for resistance to particular pathogens. Interestingly, in disease challenges conducted by our group, our strain selected for growth performance and resistance to intestinal enteritis caused by high plant-protein diets (PSel) has shown evidence of elevated non-specific pathogen resistance. Here, we used multi-tissue RNAseq to compare the acute transcriptional response to experimental infection of *Flavobacterium columnaris* (*Fc*) in three lines of rainbow trout with disparate selection backgrounds (Commercial Selection w/ *Fp* resistance; PSel: Plant-based diet Selection; VSel: Viral-resistance [IHN] Selection). At 0, 4, 12, 24, and 48 hours post-challenge, six fish from each strain were sacrificed and kidney, liver, intestine, and spleen samples were collected for RNAseq. Illumina sequencing of mRNA-selected libraries yielded 8.6 ± 1.4 M filtered reads per sample. Differential gene expression and gene ontology annotations were conducted within each tissue to detect differences in acute responses to *Fc* infection. Among the selected lines, the CSel strain, which is under selection pressure for *Flavobacterium* resistance, showed the highest surivial rates throughout the 21-day disease challenge (Fig. 1) and also exhibited the most drastic change in gene expression profiles immediately (≤ 24 h) following *Fc* challenge (Fig. 2), including the most diverse upregulation of immune genes. Further genetic and temporal differences in transcriptional responses to *Fc* will be discussed. Results provide insights on molecular mechansims behind varying levels of genetic resistance to specific pathogens, in lines under different selective pressures.

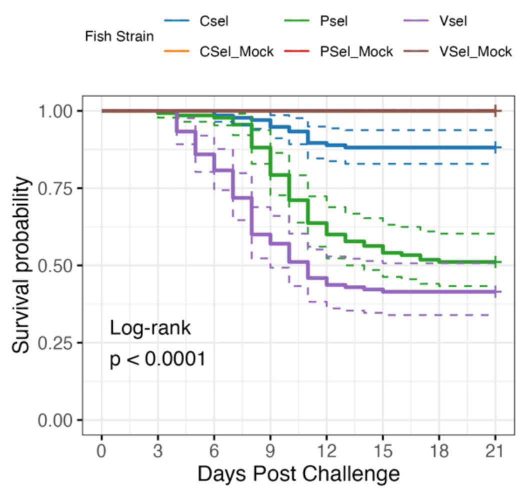

Figure 1. Survival curve from experimental *F. columnare* infection in three selected lines of trout (CSel: Commercial Selection w/ *Fp* resistance; PSel: Plant-based diet Selection; VSel: Viral-resistance [IHN] Selection).

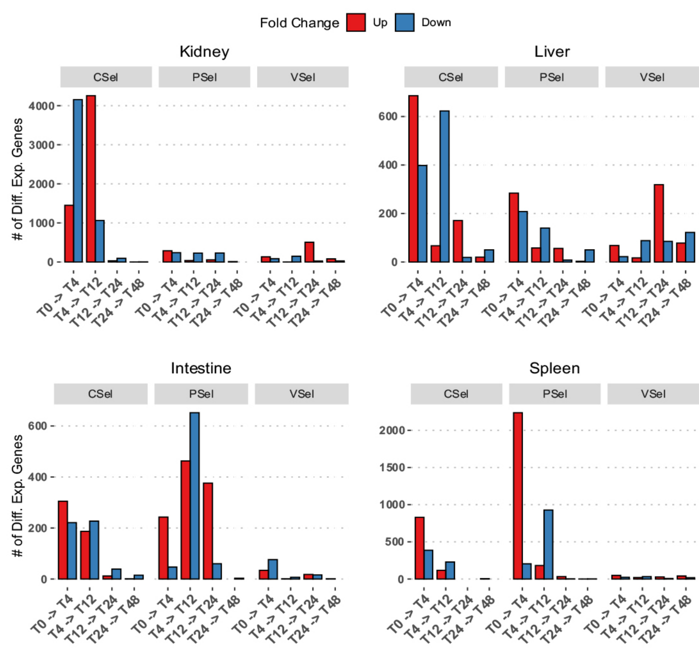

Figure 2. Tissue comparison of the numbers of differentially expressed genes (x-axis) observed between acute timepoints post-challenge (y-axis) with *F. columnare* in three selected lines strains of rainbow trout (facets).

GENE CO-EXPRESSION NETWORKS GIVE INSIGHT INTO REGULATION OF INTESTINAL MICROBIOTA COLONIZATION DYNAMICS IN RAINBOW TROUT LARVAE

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In rainbow trout, intestinal microbiota are known to influence a range of physiological functions, from proper epithelial development, to nutrient digestion, and immune activation. Microbiota colonize the trout gut at early life stages, even before exogenous feeding, and multiple studies have elucidated the role of environmental factors in regulating these early colonization dynamics. However, few studies have explored host-microbiota interaction during these early developmental stages, despite evidence that early colonization dynamics have a strong influence on the structure and function of microbiomes later in life. To study host-microbiota interactions in the gut of developing trout larvae, we characterized homeostatic intestinal transcription (RNAseq), and microbiota composition (16S rRNA gene sequencing) in two distinct lines of rainbow trout at early life-stages known to be critical in the development of host-microbe interactions (20- and 65-days post hatch). All female fish of both strains were reared in the same environment starting from eggs. Intestinal samples from 5 fish per group (2 trout strains x 2 developmental stages; 20 samples total) were used for mRNA-seq, while fifteen fish per group were sampled for gut microbiota analysis. RNAseq data was quantified at the transcript and gene level prior to testing for differential transcript usage and differential gene expression between the strains and developmental stages. Overall, 74 genes were shown to be differential transcribed (alternative splicing) (36.5% and 43.2% by strain and timepoint alone, respectively). At the gene level, 118 genes were differentially expressed between the two strains, while 2,413 genes were differentially expressed across timepoint (Fig. 1A). Gut microbiota followed a similar pattern to gene expression, with more differences observed by timepoint than by fish strain. Significant correlations in intestinal gene expression profiles and gut microbiota composition were also detected (Procrustes $m^2 = 0.19$, Cor. = 0.90, $p < 0.001$), suggesting host and microbiota undergo ontogenetic development in concert. To further evaluate host regulation of gut microbes, gene co-expression networks were constructed, and gene modules were annotated (GO) and tested for correlation with the abundance of bacterial genera in the gut (Fig. 1B). These findings provide insights on host-microbe interactions at early stages by linking specific host gene co-expression modules to the abundance of specific bacterial genera.

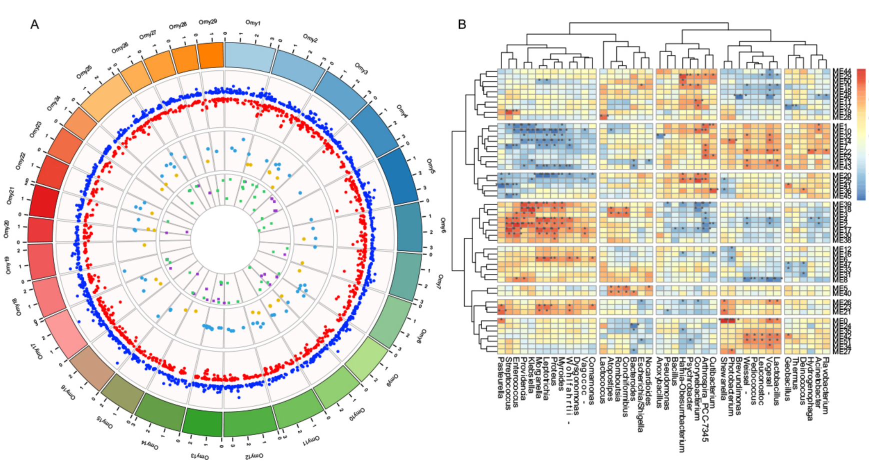

Figure 1. (A) Genomic distribution of differential expressed genes (two outer panels) and differential transcript usage (inner panel) in the intestine. (B) Heatmap showing correlation between gene co-expression modules (x-axis) and bacterial genera abundance (y-axis).

SIMULATION ANALYSIS OF HIGH-THROUGHPUT OYSTER CRYOPRESERVATION AT THREE SCALES OF PRODUCTION

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Cryopreservation and germplasm repositories offer a variety of potential benefits to aquaculture industries. With repositories, farmers and hatchery owners can safeguard important genetic lines and easily transport breeding material to facilitate rapid genetic improvement. Despite these benefits, comprehensive cryopreservation and repository systems do not exist for any aquaculture species. One such species that could greatly benefit from the use of germplasm repositories is the eastern oyster, *Crassostrea virginica*. Many growers in the northern Gulf of Mexico are experiencing oyster mortality events, the exact causes of which remain unclear. Cryopreservation, repository storage, and selective breeding programs can offer solutions to the problem of high oyster mortality. Furthermore, high-throughput cryopreservation protocols already exist for this species, although protocol research has not yet resulted in commercial application. To address the gap between cryopreservation protocols and repository development in the oyster industry, this study created simulation models to evaluate oyster cryopreservation needs at three different scales of production. We analyzed the effects of high-throughput device options and key parameters such as Straws Frozen per Oyster and Number of Operators on production capacity, time, and costs. Recommendations were developed for building cryopreservation and repository systems at each scale of production.

In general, systems that used high-throughput device options were more flexible and less affected by greater production demands, such as the number of straws frozen per oyster (Figure 1). Furthermore, repositories operating at higher production levels benefitted from the economy of scale, and could purchase automated, high-throughput equipment and employ more operators without drastically increasing production costs. By harnessing the power of simulation modeling, repositories can be planned and integrated into oyster aquaculture, and be operated in an economical, non-disruptive, and sustainable manner.

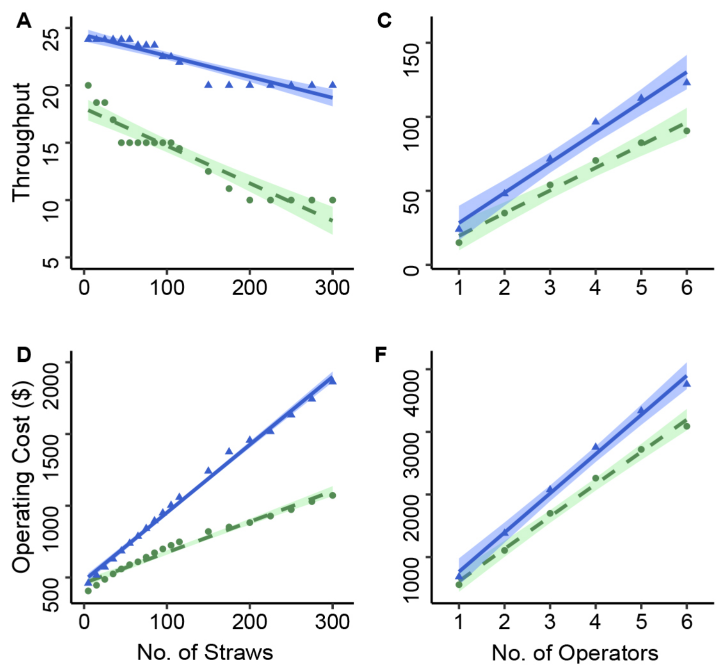

Figure 1. Linear regressions analyzing the effects of two parameters (Number of Straws and Number of Operators) on the Throughput and Operating Cost in the basic-equipment model (green, dashed lines) and the high-throughput equipment model (blue, solid lines). Shapes (circles and triangles) represent data points and shaded areas indicated confidence limits.

ASIAN CARP HYDROLYSATE AS A DIETARY PROTEIN SOURCE FOR LARVAL YELLOW PERCH

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Larval Yellow Perch (YP) are characterized by underdeveloped digestive tracts unable to utilize dietary protein efficiently for growth. Hence, development of diets based on proteins of proper molecular weight and optimal amino acid composition are critical to enhancing tissue protein synthesis. Protein hydrolysates have been obtained using *in vitro* methods and it is evident that enzymatic specificity is crucial to the final protein profile, and therefore, the uptake efficiency of the dietary protein. The objectives of this study were to: 1) Obtain hydrolysate derived from Silver Carp (SC) muscle using adult YP digestive tracts to generate an optimal protein source for YP larvae; and 2) Evaluate the effect of dietary inclusion of the SC hydrolysate on growth, survival, and morphological and molecular responses of the gut.

Briefly, freshly harvested YP stomachs and intestines were ground and used as a direct source of digestive enzymes. Each digestive tract homogenate was added with ground dorsal SC muscle and processed through conditions that mimic stomach digestion (3-4 pH; 2 hours) followed by intestinal digestion (7-9 pH; 4 hours). The final SC hydrolysate was then heated to 90°C to halt any further enzyme activity. Omitting the acidic and basic digestion time, the intact SC muscle (control) was obtained using the same procedure.

At 6 dph, ~4,150 larvae were volumetrically stocked into twelve 280L black tanks with a starting water inflow rate of 2 L/min. Clay (10-16 NTU), overhead light (at feeding only), and water surface sprinklers (90^o and 45^o) were used to deter cannibalism, improve feed intake and swim bladder inflation. The one-month study included 4 dietary treatments in three replicates: SC hydrolysate-based diet (SCH), Intact SC-based diet (ISC, control), and two reference groups: live food (LF; Rotifers/*Artemia*) and commercial dry diet (CD). The feeding regime included three feeding phases: live food only (7 to 15 dph), dry food transition (16 to 20 dph), and dry food only (20 to 32dph). The experiment finished at 32 dph.

The study found no significant differences in growth performance (final weight/total length); however strong trends were detected. Specifically, the SCH group tended to have higher total length (17.92mm ± 1.02) compared to the ISC (15.57mm ± 1.29) but similar compared to LF (17.68mm ± 1.02) and CD (17.73mm ± 0.68). At 32 dph the cumulative mortality was lowest in SCH (2178 ± 569) and highest in ISC (3668 ± 2026); followed by LF (2888 ± 523), and CD (2345 ± 1168). During the dry feed transition phase, the total mortality of the SCH (454 ± 153) vs. ISC (740 ± 469) likely implies that dietary inclusion of SC hydrolysate improved dry feed intake and mitigated the weaning stress during that phase. Additionally, total mortality of LF (964 ± 63) was over twice that of SCH (450 ± 100) once larvae were weaned. This possibly suggests that larval YP can efficiently utilize dry diets much earlier than previously believed, given the protein fractions are cleaved to species specific profiles. Further results will be shared during the oral presentation.

LEAVING ROTIFERS BEHIND: ARTEMIA DECAPSULATION SUPPORTS BRIGHT NEW FUTURE FOR LARVAL YELLOW PERCH PRODUCTION

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Yellow Perch (YP) production is notoriously challenging during the larval stage. Intensive YP larviculture has traditionally used expensive and unpredictable methods in supplying rotifers at first feeding. The rotifer cultivation process requires immensely more labor than most other diet regimens and it is not easily replicable. However, the YP industry has consistently struggled to solve and simplify the inefficient live feed stage at first-feeding. The objective of this study was to determine if decapsulating *Artemia* could sufficiently replace and remove traditional rotifer feeding without inhibiting larval YP performance.

At 6 days post-hatch (dph), ~4,150 YP larvae were volumetrically stocked into six 280L black tanks with a starting water inflow rate of 2 L/min. Clay (10-16 NTU), overhead light (during feeding only), and water surface sprinklers (90^o and 45^o) were used to deter cannibalism and improve feed intake and swim bladder inflation. Two treatment groups with 3 replicates, Rotifer (RG) and Decapsulated *Artemia* (DA), were fed *ad libitum* from first-feeding (7 dph) until 21 dph. The DA group was fed two separate strains of *Artemia*. The DA group was given San Francisco DA until 9 dph, then switched to the larger Salt Lake DA until 17 dph. The RG group was fed *Brachionus plicatilis* until 9 dph, then fed traditional (non-DA) Salt Lake *Artemia* only until 17 dph. From 17 dph both groups were slowly transitioned to formulated diet, until fully weaned at 21 dph when the feeding trial ended. Weight and body length measurements were taken at 11 dph just after the RG group had fully transitioned to feeding on *Artemia*, and at 21 dph just after the groups fully weaned to the formulated diet.

At 11 dph, the DA group had significantly ($P < 0.05$) longer body length and greater body weight than the RG (Table 1). At 21 dph the DA group remained significantly ($P < 0.05$) longer in total body length and greater in total body weight than the RG. No significant difference ($P > 0.05$) in survival was found between the RG (53.64 ± 9.18%) and DA (55.70 ± 20.17%) groups. Using a simple *Artemia* decapsulation protocol the study creates an alternative route for the cost-intensive larval YP stage, eliminating the need for rotifer culture completely.

Table 1. Growth performance of YP throughout the study.

	Body Length (mm)	Body Weight (mg)
11 dph		
Artemia	7.49 ± 0.77[a]	2.59 ± 0.62[a]
Rotifers	6.44 ± 0.97[b]	1.87 ± 0.57[b]
21 dph		
Artemia	13.46 ± 1.59[a]	18.23 ± 5.50[a]
Rotifers	12.75 ± 1.23[b]	14.94 ± 4.58[b]

DETERMINATION OF GONAD REPRODUCTIVE STATE USING NON-LETHAL ULTRASONOGRAPHY IN ENDANGERED BLACK *Haliotis cracherodii* AND WHITE ABALONE *Haliotis sorenseni*

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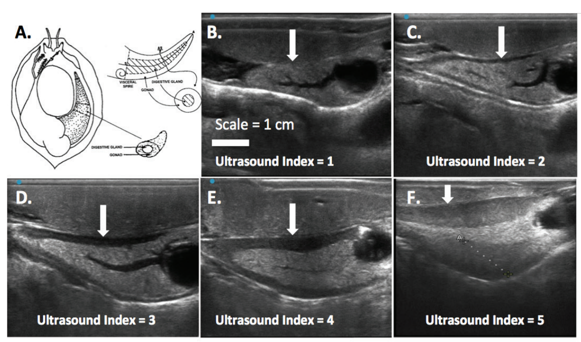
Populations of California abalone (*Haliotis* spps.) were once abundant; however, due to historic overharvesting, disease, kelp forest decline, and habitat degradation all of these species have dramatically declined and today the black (*H. cracherodii*) and the white abalone (*H. sorenseni*) are listed as federally endangered species. Research efforts to conserve and protect endangered black and white abalone have been enacted by the United States Navy Pacific Commander Fleet, the National Oceanic and Atmospheric Administration, and the California Department of Fish and Wildlife, as well as industry and non-profit stakeholders. Conservation efforts rely on captive breeding programs; however, determination of the reproductive state of individual abalone can be difficult using traditional visual assessment methods. Ultrasonography is a well-recognized technology used to accurately and non-lethally assess gonad reproductive condition in cultured and wild fish, and more recently cultured red abalone (*H. rufescens*). Ultrasound image technology allows researchers and conservation managers to quantitatively assess cyclical changes in gonad maturation state. Here, we apply the use of ultrasound imaging technology to monitor the gonad condition of endangered black and white abalone. Repeated ultrasound assessments of the gonad were used to assess seasonal changes in reproductive development in wild black (n=20), and captive white abalone (n=25). An adjusted ultrasound gonad index score was developed to incorporate multiple species of abalone. The ultrasound index scores ranged from one to five, with a score of one being the lowest (gonad margin is wrapped slightly around the digestive gland or absent) and an index score of five being the greatest (gonad margin is thick and significantly compressing the digestive gland). We show that non-lethal ultrasound imaging technology is useful for tracking cyclical changes in the gonad reproductive condition. Utilization of ultrasound technology can provide a more accurate assessment of abalone reproductive condition compared to visual gonad score and without using lethal histological methods.



(A) Diagram of abalone (*Haliotis* spps.) gonad and digestive gland (Rogers-Bennett et al., 2004). Ultrasound visual index classification of multiple species (*H. cracherodii, H. rufescens,* and *H. sorenseni*) of California abalone (modified from Boles et al., 2022): (B) index = 1, (C) index = 2, (D) index = 3, (E) index = 4, (F) index = 5. Scale bar = 1 cm. White arrows indicate progressive thickening of abalone gonad (black band) enveloped around the gray cone shaped digestive gland. Notice absence of coelom in the digestive gland in image (F).

REDUCED FREQUENCY FEEDING IN GROWOUT OF DOMESTIC STRIPED BASS (*Morone saxatilis)* IN RECIRCULATING AQUACULTURE SYSTEMS

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The striped bass, *Morone saxatilis,* is an emerging new aquaculture species in the U.S. due in large part to its rapid growth rate and ability to be grown to 1.3 – 2.3 kg (3-5 pounds) within a 24-month production cycle in salinities ranging from freshwater to full-strength seawater. The successful culture of this species has been advanced by successive generations of selective breeding and domestication that has improved growth characteristics by around 8% per generation. Our previous work in the omnivorous tilapia demonstrates that feeding on alternate days improves feed efficiency by 50% relative to fish fed daily with little impact on growth or yields in ponds supplemented with fertilizers to enhance natural foods. It is unclear however whether reducing the frequency of feeding might provide benefits to feed efficiency or labor costs in the growout of the carnivorous striped bass. To this end we evaluated the production characteristics of fifth generation selectively bred striped bass fed either 3x/week or every other day on Mon., Wed., and Fri., versus those fed 5x/week or everyday (Mon – Fri). Fish (60 g) were stocked in 1080-L tanks at 30 fish/tank with 5 replicates per group and grown for 5 months and then transferred to larger 2125-L tanks (4 replicates per group) and grown out for an additional 7 months. Both groups of fish were fed to satiation on their respective days of feeding and weights and lengths of fish was assessed periodically (every 2-3 months) throughout the study.

Within 2-months of initiation of the growth trial, mean weight of fish fed 5x/week was significantly higher (121 g) than fish fed 3x/week (109 g). This growth difference persisted throughout the course of the study. By12 months at the termination of the study, fish on a 5x/week feeding schedule attained an average final body weight of 1298 g while those fed 3x/week had an average body weight of 1094 g ($P < 0.05$). Fish fed 5x/week had a marginally better, 2.7% improved overall feed conversion ratio (FCR), relative to fish fed 3x per week (FCR: 1.485 versus 1.525). However, the FCR changed with fish age. Fish fed 5x/week initially had lower mean FCR (1.397 versus 1.487) over the first 6- months, a similar FCR (1.563) at around 9 months, and a higher FCR (1.667 versus 1.586) between 9-12 months of the growth trial relative to fish fed 3x/week. A plot of the FCR indicated the shift in FCR with age occurs at around 475 g body weight.

Results indicate that shifting from daily to alternate-day feeding after fish reach 475 g may reduce feed costs by 13% and labor costs by 19%, respectively, while maximizing growth and feed efficiency of domesticated striped bass grown in recirculating aquaculture systems.

This work was supported by the USDA and NOAA-National Sea Grant (StriperHub).

UPDATE ON THE AGRICULTURE RESEARCH SERVICE, WARMWATER AQUACULTURE RESEARCH UNIT'S CHANNEL CATFISH GERMPLASM DEVELOPMENT AND RELEASE PROGRAM

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The USDA-ARS initiated a breeding program in 2006 to develop improved channel catfish germplasm for release to U.S. catfish producers. The Delta Select line was selected based on genomic breeding values for growth and carcass yield. In Spring 2020, 2-year-old channel catfish were released to qualified U.S. catfish producers. Twelve catfish producers, representing over 90% of the channel and hybrid catfish fingerling production, received Delta Select channel catfish. Producers report Delta Selects have superior growth compared to their existing catfish lines, with no reports of negative performance. At present, three major producers currently have transitioned to 100% Delta Select broodfish.

Researchers evaluated other production traits in the Delta Selects to determine indirect effects of selection and possible inclusion of additional traits in the selection index. The Delta Control line, a randomly selected population from the same base population as the Delta Selects line, has been maintained for comparison.

Delta Select fingerlings and Delta Control fingerlings were compared for feed conversion efficiency and whole-body proximate composition during an eight-week feeding trial, weight-loss during a two- week feed deprivation trial, and survival following bacterial challenges with virulent *Edwardsiella ictaluri* (immersion challenge) and *Edwardsiella piscicida* (injection challenge), bacteria that cause significant mortality in farm-raised catfish.

The feed conversion trial revealed that, compared to the Delta Controls, the Delta Selects had better feed conversion ratio (1.50 vs. 1.82, SE 0.06, p = 0.001), higher percent weight gain (121.3% vs. 95.8%, SE 3.4, p = < 0.0001), and lower percent visceral fat (1.01% vs 1.38%, SE 0.20, p = 0.0008). The feed deprivation trial, the Delta Selects had lower percent weight loss than the Delta Controls (4.71% vs. 5.15%, SE 0.15, p = 0.004). In both bacterial challenges the high dose resulted in greater mortalities than the low dose. There was no difference in cumulative percent mortality between the Delta Selects and Delta Controls following the *E. ictaluri* challenge (High Dose - Delta Select 54.8 % vs. Delta Control 48.1 %, SE 4.52, p = 0.15; Low Dose - Delta Select 29.8% vs. Delta Control 32.7%, SE 4.52, p = 0.53). Following *E. piscicida* challenge the Delta Selects had lower mortality than the Delta Controls at both challenge doses (High Dose - Delta Select 63.0% vs. Delta Control 79.8%, SE 5.20, p = 0.003; Low Dose - Delta Select 31.8% vs. Delta Control 48.6%, SE 5.20, p = 0.003).

Feeding trial results suggest selection for growth in the Delta Select line has resulted in improved growth, better feed conversion and reduced fat accumulation. The feed deprivation study suggests selection has impacted physiological processes related to weight-loss in the Delta Selects. Selection does not appear to have negatively impacted susceptibility to *E. ictaluri,* while potentially improving resistance to *E. piscicidia*.

ECONOMIC CONTRIBUTIONS OF FLORIDA'S AQUACULTURE INDUSTRIES: FOOD FISH COMMODITY GROUP

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Florida's aquaculture industry is extremely diverse, producing approximately 1,500 species or varieties of fish, plants, mollusks, crustaceans, corals, and reptiles for food and non-food markets. This industry is expected to grow due to a suite of factors, including demand for seafood products and recently established recommendations by the state government to promote improved aquaculture development. Continual production growth within the aquaculture industry will generate additional economic activity throughout Florida's economy via purchases of input goods and services and the re-spending of employee income. However, the estimation of the total economic contributions for aquaculture is quite difficult as data is extremely limited, specifically information consisting of detailed industry expenditure patterns. This research addresses these data limitations by using the best publicly available economic data in conjunction with previously published expenditure pattern data for the food fish aquaculture commodity group. We use an Input-Output analysis approach and IMPLAN© regional economic modeling software to estimate the economic contributions of this commodity group. We find that this industry generated approximately \$7.5 million in industry output while supporting approximately 57 jobs in 2020. This study provides a better understanding of the food fish aquaculture commodity group's role in the state economy while detailing future requirements to consider when quantifying the economic role of various aquaculture industries.

THINKING ABOUT SMALL-SCALE FISH PROCESSING IN THE MIDWEST? THNGS TO KNOW

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Fish farmers in the Midwest are interested in expanding their businesses by processing their fish. By processing their fish, fish farmers would diversify their product line and increase available products supplied to the market. The farmers would also potentially increase their profit through producing and selling processed items. However, processing fish for the market requires planning: What will be the finished product? How much finished product should be produced? Will it involve packaging, storage, and transportation of products? Who are the customers/buyers? Where would the product be sold (i.e., in-state or out of state)?

The process of starting fish processing can be lengthy with many regulations that must be followed. This is because of food safety concerns when producing a food product for consumption. It is important that fish handling, processing, storage, and distribution activities be carefully executed at all points along the food chain; from the time of harvest to the point of sale to customers.

This research aims to collect all information needed before a fish farmer begins to process fish. The information gathered will provide a better understanding of the entire process. Our research will help fish farmers who intend to process fish make informed decisions on what it takes to process their fish. We will focus on the use of local commercial kitchens and/or on-farm processing facility as potential places for fish processing. We will research federal regulations, state regulations, county regulations, insurance, liability, equipment needed, inspections, taxes, food traceability, labeling, sanitization, environmental regulations, waste management, food safety, health guidelines, and more.

Some training requirements we explore for fish farmers include Hazard Analysis and Critical Control Points (HACCP), ServSafe Food Handler, Sanitation Control Procedures (SCP), and Good Manufacturing Practices (GMP). The training programs will involve both virtual and in-person instructions.

APPLICATIONS AND RELEVANCE OF NEXT GENERATION DNA AND RNA SEQUENCING TO WARM WATER MARINE FINFISH AQUACULTURE

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Application of next generation sequencing (NGS) technologies to explore problems with or improve aquaculture have been increasing in recent years due to the continued lowering of costs associated with these methods. NGS is used to quickly and efficiently determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA. Using these technologies allows for the exploration of normal biological processes as well as how those processes change in a diseased or otherwise altered states (stressed by pollutants, environmental changes, etc..).

DNA-based NGS technologies can provide information regarding an organism's potential suite of genetic functions and a look into its potential molecular mechanisms. Having an organism's annotated genome through whole genome sequencing provides a database of genes with known or predicted functions to compare to. This could include using genotyping by sequencing to find single nucleotide polymorphisms or biomarkers associated with production relevant traits such as growth or disease resistance. Individual fish can then be tested for these positive traits and used for in selective breeding.

Finfish microbiomes (internal and external) are important to the normal functioning of an organism's metabolism, growth, development, and immunity. When an organism's normal microbial community is disturbed, this leads to disruption of these normal physiological processes. Amplicon metagenomic sequencing focuses on a specific region of DNA associated with particular types of organisms such as bacteria/archaea (16S) or eukaryotes (18S) to gain taxonomic information. Shotgun metagenomic sequencing is untargeted allowing for sequencing of any of the DNA within a sample providing information regarding both taxonomy and function for any type of organism present in the community. The tradeoff for more information is that it is more expensive and computationally intensive than amplicon sequencing. Understanding how the whole or partial microbiome changes in an altered state allows for discovery of differentially abundant organisms. These could become targets for disease diagnosis or biomarker detection on the organism or in its surrounding environment to respond to potential issues before they lead to physiologically changes in the fish. Potential probiotics can also be identified with these methods by comparing organism with low and high positive traits such as growth.

Transcriptomics aka RNA-seq allows for exploration of gene expression at a specific point in time. Whereas an organism's genome is static, the transcriptome is constantly changing to respond to environmental and biological cues. Similar to metagenomics, comparing the transcriptome of normal and altered states can help discover differentially expressed genes between states. This allows for detection of molecular markers of growth, immunity, stress, and toxicology. NGS technologies can help to make significant advances in our ability to improve warm water finfish aquaculture by unlocking the potential of DNA and RNA.

HOW TO FINANCE THE FARM – MAINE AQUACULTURE FINANCING RESOURCES MANUAL

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The *Maine Aquaculture Roadmap (2022-2032)* laid out a set of action items to be taken to develop Maine's aquaculture sector. Among them was to increase financing opportunities, loans, and insurance specific to aquaculture. In addition, many farmers in Maine have reached out asking for more information about financing – what is it? What kind is best for me? What is available? In response to both of these factors, the Maine Aquaculture Association took the first step in highlighting financing opportunities in Maine by creating the Maine Aquaculture Financing Resources Manual, with support from FocusMaine.

The manual includes an overview of financing options – loans, grants, economic development funds, tax incentives, investment/equity, and risk management. It highlights the advantages and disadvantages of each option, with an emphasis on speaking with your own counsel and finding what is right for you and your farm. It also includes extensive resources and links of options within each category. A financial advisory committee, made up of financiers across Maine, reviewed the manual. A meeting was held in the winter of 2022 of financing institutions across Maine, including state agencies, to discuss the manual, identify financing gaps within Maine, and assess whether or not the creation of additional financing options should be considered. Webinars, social media, email, and distribution through business planning meetings were utilized in the winter of 2022 to share the manual statewide, and beyond.

FARMING SEAWEED - IS IT WORTH IT? USING FINANCIAL BENCHMARKING TO ASSESS SEAWEED FARMING PROFITABILITY IN MAINE

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With all of the hype around seaweed farming some have begun to ask – is it worth it? This report explores the finances and profitability of seaweed through benchmarking, using Maine as a case study. An initial study was conducted in Maine based on 2017 data, when roughly 15,000 wet lbs. of seaweed were harvested. Two of the six participants had total losses. The numbers did not look promising.

This study presents an update and assesses changes in the sector since 2017. 16 farmers were interviewed and provided data based on the 2022 growing season, when over 1 million wet lbs. of seaweed were harvested. Benchmarks were calculated for production, expenses, breakeven prices and yields, profitability, financial, loan repayment, and efficiency (labor, capital, financial). Preliminary analysis shows strides have been made – farms have grown, yields have improved, and total losses were avoided. The sector has matured, with three different farm size groupings emerging and increased processing capacity. The presentation will highlight profitability metrics for farms across each size grouping in Maine, including how production practices have changed since 2017 and what practices provided the best yields.

LAUNCHING THE COUNTRY'S FIRST AQUACULTURE APPRENTICESHIP PROGRAM IN MAINE

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The country's first aquaculture apprenticeship program is launching in Maine in the spring of 2023. The Gulf of Maine Research Institute, Maine Aquaculture Association, EducateMaine, Southern Maine Community College, and the Maine Department of Labor worked together to create a 2,000-hour shellfish and seaweed apprenticeship program that provides on-the-job training and experience. Apprentices will serve as employees on host farms, where they will follow a schedule of work, recording specified numbers of hours in specific categories based on the Maine Aquaculture Occupational Standards. Apprentices will then take 144 credit hours of coursework through Southern Maine Community College.

The presentation will overview the creation, rollout, and future of the Maine aquaculture apprenticeship program, including lessons learned.

EVALUATION OF THE POTENTIAL TO REDUCE *Artemia* LEVELS DURING THE LARVAL PHASE OF MARINE BATIFISH

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The larval phase of marine fish is one the most challenging stages of production. Small mouth gapes and historical poor performance on exclusively artificial diets have resulted in feeding regimes that rely heavily on live zooplankton, primarily rotifers and *Artemia*. Effective culture protocols have facilitated the incorporation of *Artemia* into larval feeding regimes, but recent issues with supply, demand, cost, and other concerns have warranted investigation into reducing or eliminating the amount of *Artemia* in feeding regimes. As *Artemia* are usually the live prey item fed before or during weaning onto artificial micro diets, partial or complete reductions in either the period of time *Artemia* are fed or overall daily amounts fed during weaning could result in cost savings and also simplify larval culture protocols. Thus, the objective of this experiment was to evaluate the potential to reduce *Artemia* levels during the larval phase of different marine batifish- spot (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboides*), and pigfish (*Orthopristis chrysoptera*).

Eggs for each of the three species were obtained from conditioned, captive broodfish at the Waddell Mariculture Center in Bluffton, South Carolina. Larvae were batch-cultured under standard conditions in 140-L tanks in-line with a recirculating aquaculture system utilizing best culture practices available for each species until the day at which *Artemia* feedings were to begin (approximately 17 days post hatch). The morning *Artemia* were to be fed, larvae were redistributed into new 140-L tanks at 250 larvae per tank. Two trials were conducted for each species and 17 tanks were stocked each trial to generate one starvation tank (negative control) and 16 experimental tanks (4 tanks per treatment). The first trial examined feeding *Artemia* at reduced rates (66%, 33%, and 0%) compared to a standard satiation ration (100%) and supplementing the reduced rations with a 1:1:1:1 mix of micro diets (Otohime A and B1, and ArteMac No.2 and EconMac No.2) fed five times per day at 0.5 g per feeding. The second trial examined feeding a standard satiation ration (100%) for different durations (2, 4, 8, and 14 days) with acute switches to the 1:1:1:1 mix of micro diets fed five times per days at 0.5 g per feeding the day after *Artemia*

feedings ended. Experiments were conducted for 14 days and larval survival of each tank along with larval growth ($n \leq 25$ larvae per tank) were recorded.

For spot, reductions of *Artemia* in both a daily ration and total duration fed resulted in significant decreases in survival and growth (e.g. 47.1±1.6% compared to 33.2±.5% for a 100% and 0% ration, respectively). However, larvae were still able to be produced without using any *Artemia* at all or only for a duration of two days. Thus, future refinement of micro diets and the regime used could facilitate protocols that rely on minimal or no *Artemia* at all for the species. Additional results for the other baitfish species will also be discussed.

SHRIMP AQUAPONICS RESEARCH AT PURDUE UNIVERSITY – OPTIMAL SALINITY, SHRIMP/PLANT RATIO, AND OPTIMAL DIETARTY CRUDE PROTEIN

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A series of studies was conducted focused on establishing the basic production parameters of a marine shrimp and halophytic plant aquaponic scenario. Shrimp species was Pacific whiteleg, *Litopenaeus vannamei* and three halophytic plant crops familiar in Asian cuisines (red orache, okahijiki, and minutina) were evaluated. In the first study, optimal environmental salinity was evaluated. Salinities of 10, 15 and 20 ppt were established in replicated systems using a flocponic approach. Results indicated 15 ppt was the best compromise salinity for maximum growth of the shrimp and plants. In the second study, three ratios of shrimp to plants (2:1, 3:1 and 5:1) and two C/N ratios were evaluated. A ratio of 3:1 with a C/N ratio of 15 was recommended for maximum production. In the third study, dietary crude protein concentrations of 30, 35 and 40% were evaluated. Shrimp growth was not significantly affected by dietary crude protein concentrations. Results indicated higher dietary crude protein concentrations should be offered in the early stages of production, but the lower dietary crude protein concentration could be used after initial harvest of plants. Longer term studies are underway as well as evaluation of three additional halophytic plant crops. These data provide the framework for establishing a marine/brackish water aquaponic system using shrimp and halophytic plants.

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IMPACT OF FLOATING RACEWAYS ON WATER QUALITY IN A KENTUCKY WATERHSED POND

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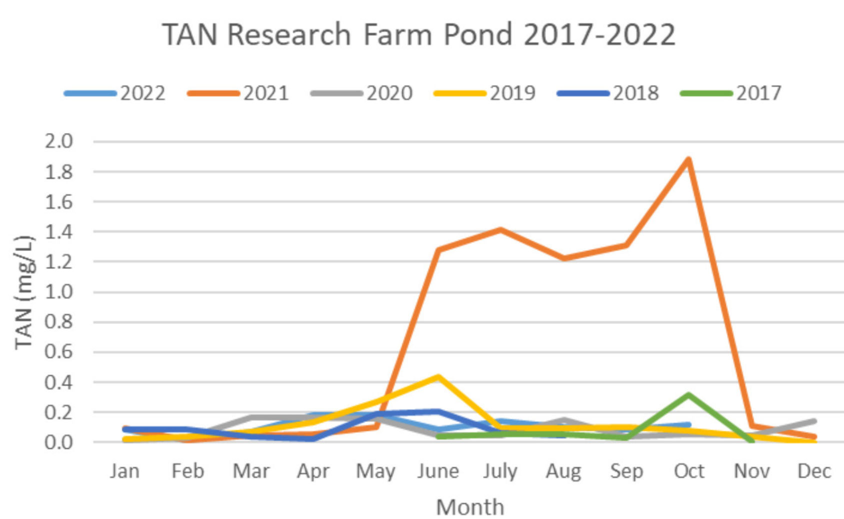
Water quality in an existing 0.65 ha watershed pond with a maximum depth of 4.6 m was monitored before and after installation of floating raceways. Grass and koi carp were stocked in 2016 to control rooted aquatic vegetation. Large catfish and paddlefish were stocked on top of largemouth bass and bluegill populations. Vertical circulation to destratify water began in 2018 and horizontal circulation began when floating raceways were installed in 2019. Intensive feeding of channel catfish occurred in 2021 with maximum daily feed input of 102 kg/day. Largemouth bass production in 2022 resulted in much lower feed input.

Sampling of water quality began in 2017 and continued throughout the project. Dissolved oxygen concentration (DO) and temperature were measured at 30 cm intervals to generate a profile over a depth of 3 m. Weekly grab samples taken at the water surface was analyzed for total ammonia nitrogen (TAN), nitrite, nitrate, alkalinity and pH.

Without circulation, the pond stratification was evident during summer months when dissolved oxygen concentration and water temperature decreased with increasing water depth. As expected, circulation eliminated stratification and created relatively uniform DO throughout the water column. The first year of full production occurred in 2021 when each of three raceways were stocked with 4,400 stocker catfish (158 g/fish). During the year approximately 10,400 kg feed pellets were fed to fish in the raceways and pond. TAN rose to a mean of 1.28 in June and sustained or rose above this level through October. Nitrite rose to 0.186 mg/L in July and did not fall below this level until November. Increased nutrient input significantly impacted water quality, disease pressure, and feeding opportunity. Our protocol has changed to limit the amount of feed applied to 9000 kg/ha annually if there is no removal of solid waste.



ZERO-DISCHARGE AQUACULTURE AS AN ECONOMIC OPPORTUNITY FOR MIDWESTERN FARMERS

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As the costs of conventional agriculture continue to rise many Midwestern farmers are asking, "Can aquaculture provide an additional or alternative source of farm income?" However, Midwestern farms, in particular Missouri farms, lack the warm climate (degree-days) needed to compete with pond aquaculture production 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 or 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 7,200 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

OXYGEN AND NITROGEN DYNAMICS IN ENHANCED CATFISH PRODUCTION SYSTEMS

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Catfish production practice/technology has changed substantially within the last four decades, as fish farmers are driven to intensify production. Average pond electrical aeration in southern catfish ponds increased (1982 to 2020) from 1.87 kW/ha (1 hp/acre) to 2.25 kW/ha (4,2 hp/acre), with a significant percentage (in 2010) using in excess of 7.9 kW/ha (3.8 hp/acre). Efforts at Clemson University, from 1996-2001, focused on intensification of fish culture and reduction in water use though application of a "high-rate algal pond' entitled the Partitioned Aquaculture System (PAS) The original 0.33-2.0-acre PAS prototypes use slowly rotating paddlewheels to circulate water through shallow (2-ft) channelized ponds (95% of area), increasing algal photosynthesis and enhancing treatment rate of nitrogenous waste (NH_3). Catfish are confined to 5% of area in high density raceways (6.0 lb/ft^3) at mixing/aeration energy of 4.5 hp/acre. At the time, conventional catfish ponds (CP) were utilizing 100% of pond volume/area for fish culture at aeration energy of 2.0 hp/acre. The Split-Pond (SP), developed at the National Warm-water Aquaculture Center (NWAC) beginning in 2002, represents a lower cost adaptation of Clemson's PAS. Prototype SPs at NWAC range in size from 5.0-7.0 acres utilizing 5.7-7.0 hp/acre mixing/aeration, with fish culture confined to 20-25% of surface area with 75-80% devoted to waste treatment. NWAC also reported on evaluation of prototype 4.0 acre intensively aerated catfish ponds (IP), utilizing 100% of pond volume for fish culture at elevated aeration energy levels of 8.0 hp/acre.

A performance analysis provided on prototype CP, SP and IP and PAS units suggested fish carrying-capacity in CP of 5,000-7,500 lb/acre, PAS at 15,000-18,000 lb/acre, SP at 12,330-19,000 lb/acre and IP at 9,200-18,245 lb/acre. Average/maximum sustained feed loading was 100/150 lb/acre-day for CP, 160/250 for PAS, 110/280 for SP, and 84/270 for IP. The PAS, SP and IP are highly photosynthetic (3.3-4.3xCP) however, the PAS provides the highest degree of net photosynthesis and ammonia removal. The PAS aggressively re-mineralizes settled algal biomass which is recaptured as additional algal biomass. The SP aggressively removes algal biomass (via sedimentation in the waste treatment zone, WTZ) and promotes nitrification in a WTZ anoxic layer. The IP provides a higher degree of settled algal storage in the sediment. The IP is more subject to sporadic release of sediment ammonia driven by climatic changes, whereas the PAS and SP provide more consistent operator control of oxygen and nitrogen flux.

Currently 7% of U.S. catfish production is from SP with 33% in IP. The PAS was designed to provide maximum nitrogen treatment via optimized algal photosynthesis. Capital costs for PAS are similar to in-pond raceways at $22,630/acre with a breakeven catfish production cost of $1.32/lb. In contrast, SP capital cost is projected at $6,904/acre with BEC of $0.92/lb and IP at $8,380/acre with BEC of $0.93/lb. The SP provides 78% of the net photosynthetic capability of the PAS at 70% of the production cost/lb. The IP provides 87% of the net photosynthesis at 69% of the production cost/lb. The IP requires minimal modification of existing ponds, the major cost being addition/maintenance of aerators. The SP requires substantial modification of existing ponds but provides more consistent/reliable treatment of ammonia nitrogen. SP provide advantages in fish feeding/harvesting and predator control. Fish farmers have reported excessive accumulation of ammonia (3-6 ppm) in IP, particularly during late summer to winter months.

Max Oxygen Flux (lb/acre-day)

	Gross-Phot	Resp	Net-Phot	%Res	% Fish
PAS	+240	-60	+180	25%	120%
SP	+270	-130	+140	52%	78%
IP	+314	-157	+157	50%	66%
CP	+72	-40	+32	56%	64%

Max Nitrogen Flux (lb/acre-day)

	Feed	Recycle	Phot	Nitrify
PAS	+6.1	+5.8	-11.9	0
SP	+5.0	+3.7	-8.7	20%
IP	+6.9	+3.6	-10.5	0
CP	+2.2	+0.5	-2.2	0

MARKET PERCEPTIONS OF LIVE TILAPIA IN KENTUCKY

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Based on the limited production of small-scale aquaculture producers in Kentucky, it is key for farmers to be connected to niche markets where customers are willing to buy live aquaculture products in order to limit the burden of processing costs. Kentucky State University researchers have investigated ethnic markets in the past and have found that there were market opportunities that were not being utilized by current producers. This project looked at other markets in the state in order to determine if there were any other niche markets interested in purchasing live fish.

During August and September of 2022, Kentucky State University extension personnel conducted a market research study at a local butcher shop with a diverse customer base. Live tilapia from a local producer were live hauled to the butcher shop and sold to customers. Questionnaires were given to customers who purchased fish in order to determine willingness-to-pay and purchasing preferences. Through the continued exploration into niche markets in the state of Kentucky, small scale aquaculture producers can continue to be connected to niche markets willing to support the sale of live, locally produced aquaculture products.

IMPACTS OF BLACK SOLDIER FLY LARVAE INCLUSION DIETS ON THE DEVELOPMENT OF JUVENILE RED DRUM *Sciaenops ocellatus*

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Fish meal has a great nutritional value and meets the high protein requirement for culturing fish. However, its production is not sustainable for global economies and marine food webs. Insects, a valuable alternative, are high in protein, amino acids, and lipids. They are easy to rear and carnivorous species can digest them more efficiently than plant-based alternatives. Black soldier fly larvae (BSFL) substitute diets have been proven effective in the culturing of many fish species (i.e. salmon, catfish, tilapia, etc.) but have not been intensely studied on warm water marine species such as Red drum (*Sciaenops ocellatus*). Red drum are regarded for their desirable taste and have a very high market value. They are a carnivorous species that feed on crustaceans and small fish. We believe BSFL has the potential to serve as an effective protein substitute in pelleted diets for this species. The objective of this study is to calculate the appropriate BSFL substitution for optimum growth, while also investigating any potential nutritional limitations or negative health effects of BSFL diets. In this study, juvenile red drum were fed five different pelleted diets. The diets were formulated with fish meal and BSFL substitutions of 0%, 25%, 50%, 75% and 100%. The fly larvae that the diets were derived from, were grown on a substrate composed of apple by-product. The fish initially weighed SD 40g each and were fed daily at 5% body weight over two separate feedings. Fish were weighed every two weeks so adjustments could be made to feeding quantities. After 8 weeks, the fish will be accessed for growth, survival, physiology, and nutritional content. After 4 weeks, fish fed the 0% and 25% substitutions both experienced a standardized growth rate of 78%. Fish fed the 100% substitution experienced the lowest growth rate at 53%. Although 4 weeks are remaining in the study, we expect these trends to continue. The outcome of this study will help further the development of BSFL derived feeds for commercial use in US finfish aquaculture for food production.

GENOME EDITING FOR GENETIC IMPROVEMENT IN FINFISH AQUACULTURE

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Increasing ability to harness the power of genomics is forcing a rethinking of aquaculture genetic improvement strategies. Successful breeding programs will always be built on the careful selection of the next generation of broodstock, detailed record keeping, and accurate collection of phenotypic data. Genomics allows this base of phenotypic selection to be enhanced, and ultimately accelerated to increase genetic gain per generation. This is currently done in finfish at the most sophisticated level through the use of Genomic Selection. However, another exciting technology is on the horizon that will fundamentally change how we deliver genetic improvement. This technology is Genome Editing.

Genome Editing is a technology that can thought of as “precision breeding”. It will be an important tool in the future toolbox for genetic improvement in aquaculture. The current state of the art in Genome Editing in aquaculture is impressive and on the cusp of significant commercial application. The basic concept is that enzymatic tools (such as CRISPR technologies) can be used to create variants in specific DNA sequences that create a desired phenotype (such as sterility, monosex, rapid growth, or disease resistance). The technique does not involve adding new DNA, so is not transgenic and does not create a GMO. It simply involves understanding the genomics and underlying genetic variant that is needed for a trait to be expressed, and harnessing natural processes to create that variant rather than sorting through many thousands of broodstock and many generations to achieve the same effect.

The power of genomic research is that we are beginning to understand the exact genes involved in performance traits, and how variation in those genes leads to improved performance. Harnessing the power of Genome Editing allows us to transfer this knowledge to application in commercial breeding programs for heritable, quantum advances in genetic improvement. Importantly, sterility will be a requirement in most applications of GE in aquaculture as a method of biocontainment to prevent escape to the environment, or the inadvertent application of genetically improved animals.

This presentation will provide background on how genome editing works, an update on regulation, and how this tool may be used to improve aquaculture genetics in the very near future.

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.

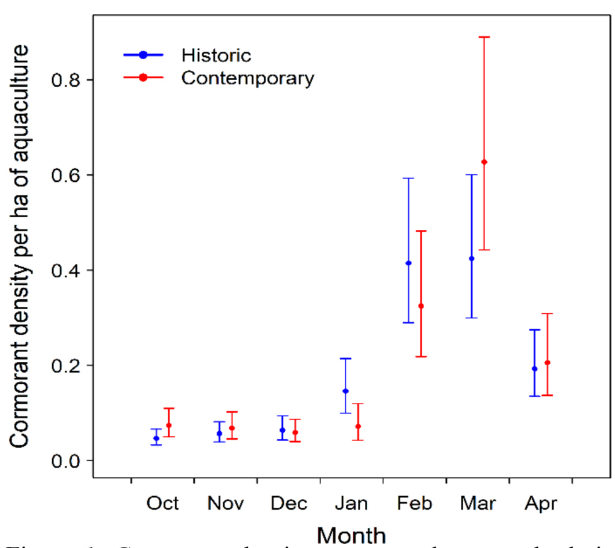

Figure 1. Cormorant density on aquaculture ponds during historic (2001–2004) and contemporary (2015–2018) periods.

USE OF MICROALGAE AS AN ASTAXANTHIN SOURCE IN ATLANTIC SALMON (*Salmo salar*) FEED

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Cultured salmon (*Salmo salar*) is an important carnivorous fish with an estimated2.5 million metric tons in 2018. The pink to red color of the fillet is one of the most important quality criteria for salmon. This pigmentation is due to deposition of carotenoids (mainly astaxanthin) in the muscle of salmon, and to a lesser degree canthaxanthin, in farmed salmon. Since salmon is unable to synthesize astaxanthin, the carotenoids originate from micro-algae in the wild, or are synthetically produced for inclusion in the diets of cultured fish.

Two 30-day stability tests were conducted with microalgae (*Haematococcus pluviatilis*) containing high levels of astaxanthin. Microalgae was dried or encapsulated in 1, 2, or 3% sodium alginate at a concentration of 1, 2 or 3%. The samples were then either kept in the dark in a cool room where feed is normally stored or heated for 5 min at 150ºC and then stored as described. The encapsulated samples that contained the highest levels of microalgae and lowest levels of alginate had the greatest astaxanthin degradation (samples 6, 8, and 9) compared to the lowest levels of microalgae and highest levels of alginate (samples 1, 2, and 4) and the dried algae alone (sample 10). An *in vivo* and *in vitro* study will be conducted to determine if Atlantic salmon can access the astaxanthin from the encapsulated algae.

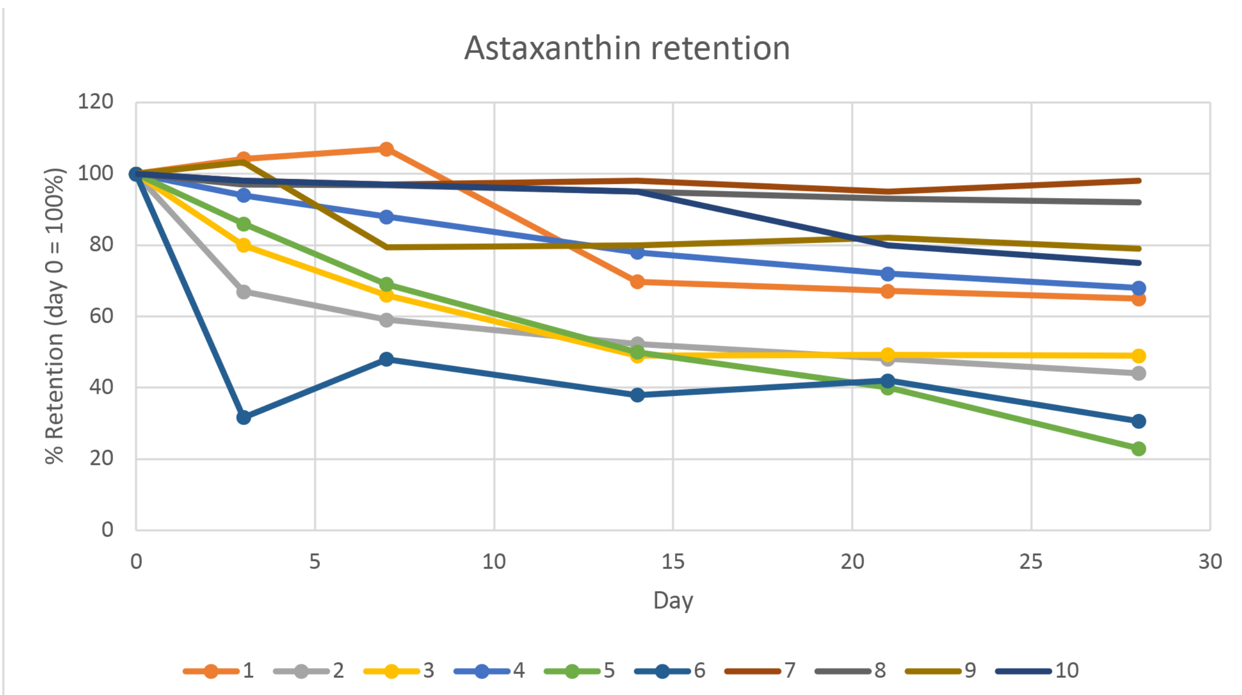

Figure 1. Astaxanthin retention, compared to the original amount of astaxanthin, in samples after heating.

EVALUATION OF OPEN-HARDWARE CRYOPRESERVATION DEVICES WITH AMPHIBIAN SPERM, AN EXAMPLE WITH *Xenopus laevis*

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Sperm cryopreservation is a valuable tool to support preservation and management of valuable genetic resources. Cryopreservation of sperm requires the balance of multiple steps such as the cooling rate and method. The cooling step is commonly performed using a programmable freezer that provides a precisely controlled temperature profiles for cryopreservation. However, controlled-rate freezers can cost $15,000-$50,000, and most breeding and research programs cannot afford this equipment. Three-dimensional (3-D) printed open-hardware devices offer a low-cost alternative to programmable freezers while providing easy fabrication and standardizable reproducibility. In this study, cooling rates and post-thaw sperm quality processed by two open-hardware devices developed at the AGGRC were compared with a commercial programmable freezer (IceCube, Minitube). Both devices, designed for 0.25-ml and 0.5-ml French straws, provided various predetermined cooling rates. The Cajun Ejector (Figure 1A) utilized vertical freezing inside a standard nitrogen vapor shipping dewar. Different cooling rates can be set by adjusting the height of the straw holder into the shipping dewar. The CryoKit (Figure 1B), a horizontal rack was designed to float on liquid nitrogen inside of a Styrofoam box, was set for different cooling rates by adjusting the height of samples above liquid nitrogen, and with different 3-D printed configurations. Testes from five male *Xenopus laevis* were collected and fresh quality was assessed by estimating sperm concentration, percentage of viable sperm and percentage of motile sperm. Sperm concentration was adjusted to 1.6×10^8 cells/mL, samples were diluted 1:1 with a cryoprotectant (10% dimethylformamide and 10% sucrose), and 100μL loaded into straws and cooled at -10C°/min using the three different devices. Then, samples were thawed by immersion in a water bath at 40ºC for 5s and sperm quality was assessed and compared among the devices. Preliminary results (n=2) showed a similar cooling rate between the programmable freezer and the Cryokit (-10±1ºC/min) and a faster rate when using the Cajun Ejector(>-40°C/min). Consequently, there were no differences between the sperm viability using the programmable freezer (35±2.5%) and the Cryokit (45±7.1%) but lower sperm survival was obtained when using the Cajun Ejector (17±4.2%). Communities performing cryopreservation of amphibian sperm with biomedical models such as *Xenopus* or endangered species could benefit from use of these portable, inexpensive, and reproducible devices.

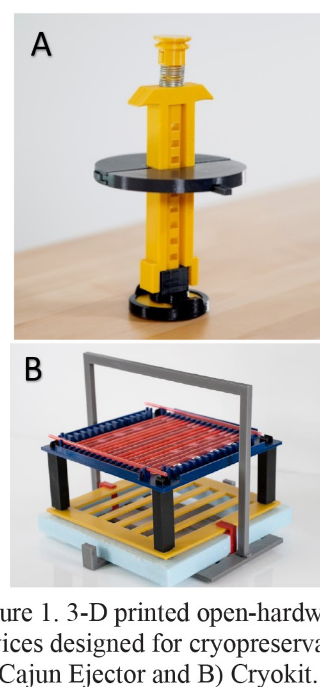

Figure 1. 3-D printed open-hardware devices designed for cryopreservation. A) Cajun Ejector and B) Cryokit.

NUMERICAL MODELING OF CONTINUOUS MUSSEL LONGLINES IN NEW ENGLAND OFFSHORE WATERS

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The feasibility of sustainable aquaculture in nearshore waters can be challenging due to the rise in stakeholder conflicts, coastal environmental pollution, and spatial constraints. While offshore aquaculture farms have evolved as a potential solution, currently, these systems require comprehensive engineering analysis-based design to withstand extreme weather conditions such as storms and strong currents. In this study, historical wave and current data from New England offshore waters were collected, analyzed, and sta- tistically modeled. The information was used as input for the numerical simulations of a continuous mussel longline system design at different mussel growth stages using Hydro-FE to predict the dynamic response of the longline at several wave/current/water depth configurations. Hydro-FE [1] is an advanced version of the finite element software, Aqua-FE [2,3,4] developed at the University of New Hampshire to analyze submerged flexible structures. The components of the mussel longline are modeled using truss and beam elements and the input parameters, such as the normal and tangential drag coeffi- cients. The coefficients are estimated separately via computational fluid dynamics (CFD) simulations in OpenFOAM. The Hydro-FE simulation results are analyzed based on the mooring line stress, anchor forces, and displacement time series to find the possible anchor failure, rope breakage, line entanglement, and mussel falloff in different configurations. General guidelines for a typical offshore mussel longline system are provided based on the quantitative analysis.

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PROBIOTIC EFFECTS ON THE GROWTH AND DEVELOPMENT OF RAINBOW TROUT *Oncorhynchus mykiss* DURING THE EARLY REARING PERIOD

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Rainbow trout production is one of the most significant aquaculture segments of U.S. fish farming, with most of the industry located within Idaho and North Carolina. Bacterial pathogens, such as *Flavobacterium psychrophilum*, are largely responsible for mortalities within this sector. The industry has access to only three approved antibiotic treatments, and antimicrobial resistance has become a concern for fish producers. U.S. aquaculture has shown a great interest in antibiotic alternatives, such as probiotics or immunostimulatory compounds. A probiotic strain of *Bacillus velezensis* has been previously shown to confer protection against warm water pathogens, such as virulent *Aeromonas hydrophila* (channel catfish) or *Streptococcus iniae* (Nile tilapia). Given these health-related benefits, a study was designed to evaluate the application of this probiotic in rainbow trout fry throughout the early rearing period.

Rainbow trout eyed eggs were obtained from a commercial source (Troutlodge) and were incubated within Heath trays (12°C) before transfer into aquaria following hatch and swim-up. Newly hatched fry were fed a probiotic-amended diet (1×10^7 CFU g^{-1}) or a reference diet (without probiotics) for 30 days (Fig. 1). Each dietary treatment was assigned to a separate recirculating aquaculture system with five 50L replicated aquaria stocked with 100 yolk-sac fry per aquarium. Throughout the rearing period, water quality was maintained as follows: temperature (13.4±0.2°C), dissolved oxygen (10.9±0.1 mg/L), pH (7.72 ± 0.5), total ammonia nitrogen (0.18 ± 0.3 mg/L), nitrite (0.07 ± 0.02 mg/L), nitrate (20.3 ± 17.1 mg/L), hardness (96 ± 6 mg/L), alkalinity (95 ± 3 mg/L). Every week, whole fish were sampled for biometric measurement, and samples were flash-frozen for immune gene expression. Fish are currently being measured using ImageJ for seven morphometric characteristics, including standard length, notochord length, yolk sac area, eye diameter, jaw length, myotome height, and body area. Proinflammatory cytokines (i.e., IL-1β, IL-8, and TNF-α) are now being assessed for gene expression using qPCR. Together, these data will shed light on probiotics and molecular ontogeny of their immune system during early larval development during early rearing stages for cold-water species.

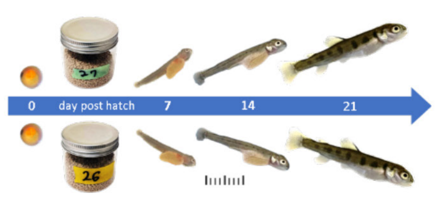

Figure 1: Rainbow trout larvae at 0, 7, 14, and 21 days (Scale = 1cm).

PERFORMANCE ANALYSIS OF EMBEDDED AI FOR PHYTOPLANKTON CLASSIFICATION[1]

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Phytoplankton species play a critical role in marine ecosystems. Various environmental parameters may lead certain species to proliferate, resulting in harmful algal blooms (HAB). HAB can potentially cause adverse effects on aquatic life, human health and associated economic activities. The diversity in species makes communities highly heterogeneous in size, shape, and morphology. Early detection of phytoplankton species has significant importance to continuous HAB monitoring. However, it is a time-consuming and challenging activity even for experts. Embedded system solutions coupled with advanced Artificial Intelligence (AI) models are an early yet effective solution to support a HAB monitoring strategy. Based on the MobileNetV2 and NASNet architectures, these AI models take phytoplankton images as input and process them to provide helpful classification feedback to end-users.

Benchmark deep learning models [1-2] tailored for HAB monitoring within multitrophic aquaculture applications [3] have been embedded into an NVIDIA Jetson Nano platform. Overall system performance assessment aimed to check system resource usage and the impacts on FPS (image frames per second), which supports HAB early detection. The models were built using the TensorFlow library and Tensor-RT framework. Results indicate that the chosen hardware and software platform (Jetson Nano with Tensor-RT) can run the Deep Learning Phytoplankton classification at an acceptable image frame rate. Additionally, the results suggest additional bottlenecks to the already resource-constrained embedded boards. The use of CPU and GPU is acceptable, but memory usage becomes a crucial problem (Fig. 1). Provided all the benefits of the Tensor-RT framework, it still introduces an issue causing the system memory limit to be exceeded.

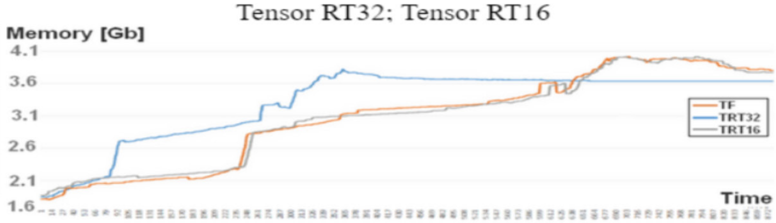
[1] Sandler, M., Howard, A., Zhu, M., Zhmoginov, A., & Chen, L. C. (2018). Mobilenetv2: Inverted residuals and linear bottlenecks. In Proceedings of the IEEE conference on computer vision and pattern recognition (pp. 4510-4520).
[2] Zoph, B., Vasudevan, V., Shlens, J., & Le, Q. V. (2018). Learning transferable architectures for scalable image recognition. In *Proceedings of the IEEE conference on computer vision and pattern recognition* (pp. 8697-8710).
[3] Guterres, B., Khalid, S., Pias, M., & Botelho, S. (2022, April). A data integration pipeline towards reliable monitoring of phytoplankton and early detection of harmful algal blooms. In *NeurIPS 2021 Workshop Tackling Climate Change with Machine Learning* (Vol. 2021). NeurIPS.

This work is part of the ASTRAL (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) project. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement Nº 863034.

Table 1 - FPS results for the two target models.

Model	Tensor Flow	Tensor RT16	Tensor RT32
MobileNet V2	41	82	84
NASNet	34	73	80

Figure 1 - RAM use for MobileNetV2: Only with TensorFlow; Tensor RT32; Tensor RT16



DEVELOPMENT OF A PILOT SCALE SHELLFISH HATCHERY IN DELAWARE

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Seafood plays a critical role in Delaware's economy and culture like most of the other coastal states in the US. Aquaculture has shown consistent growth over the last decade, accounting for more than half of the seafood processed for human consumption globally. Since 2018 when leases first became available, shellfish aquaculture in the state has grown to 10-15 farms. These growers are leasing only 23 of the 343 acres that are available in the Inland Bays. Prior to 2022, Delaware was one of the few states on the East Coast lacking an operational shellfish hatchery. Importing seed from out of state that requires expensive disease testing poses an additional hurdle for growers on top of high startup costs, regulatory restrictions, and COVID setbacks. The University of Delaware, in collaboration with Delaware Sea Grant and Delaware State University, built a pilot-scale shellfish hatchery in an effort to expand our seafood industry and take advantage of the economic, cultural, and environmental benefits that shellfish aquaculture could bring to Delaware. Following more than 5 months of construction, we are now fully operational, equipped with two saltwater intake pumps, multistage filtration, a 3,000-gallon filtered holding tank, two raceways, and five 160-gallon cone tanks. Currently, we have the capacity to produce approximately 50 million eyed larvae per season, with the goal to grow to an industrial scale. As the pilot facility develops, we are working with local schools including the Indian River school district and the Delaware Technical Community College to develop and implement aquaculture-focused activities and curriculum, utilizing our facility as a demonstration and extension space, fostering parallel educational efforts and workforce development.

SURVIVAL AND GROWTH OF TETRAPLOID EASTERN OYSTERS *Crassostrea virginica* IN RESPONSE TO DIFFERENT SALINITY ENVIRONMENTS

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Triploid oysters, the predominant choice in commercial off-bottom oyster farming, are relatively reproductively sterile and often exhibit faster growth and higher meat yield compared to diploid oysters, especially during spawning season. The mating of tetraploid male broodstock with diploid female broodstock results in the production of 100% triploid oysters. Understanding how different environmental variables may influence the survival and growth of tetraploids is crucial in producing a more resilient triploid oyster.

Two tetraploid broodstock lines maintained at the Auburn University Shellfish Laboratory (AUSL), 4MGNL20 (Louisiana origin) and 4MAPCK20 (Florida origin), were spawned to produce four experimental cohorts: two pure lines (4MGNL22 and 4M3APCK22) and two reciprocal crosses between the two lines (4MAPCKGNL22, and 4MGNLAPCK22). The tetraploid lines were produced in conjunction with 4Cs Breeding Technologies, Inc with the GNL tetraploid line originally produced at the Louisiana Sea Grant Program's Oyster Research Laboratory at Grand Isle, LA and the APCK tetraploid line originally produced at AUSL. Four replicates (400 oysters/replicate) of each experimental cohort were deployed in Oyster-Gro floating cages at three differing salinity sites in Alabama: Mobile Bay (low salinity), Grand Bay (moderate to high salinity), and Dauphin Island (high salinity). Mortality and size metrics were assessed monthly. Temperature and salinity measurements were taken at all three experimental sites.

Preliminary data suggest a significant effect of site and tetraploid line on survival and shell height ($p < 0.01$). At the low salinity site, 4MGNL experienced a higher mortality ($p < 0.05$) (Figure 1).

This is an ongoing experiment and data will continue to be collected over 12 months. Trends will be reassessed to determine the next steps in selecting a more resilient tetraploid broodstock for triploid production in the northern Gulf of Mexico.

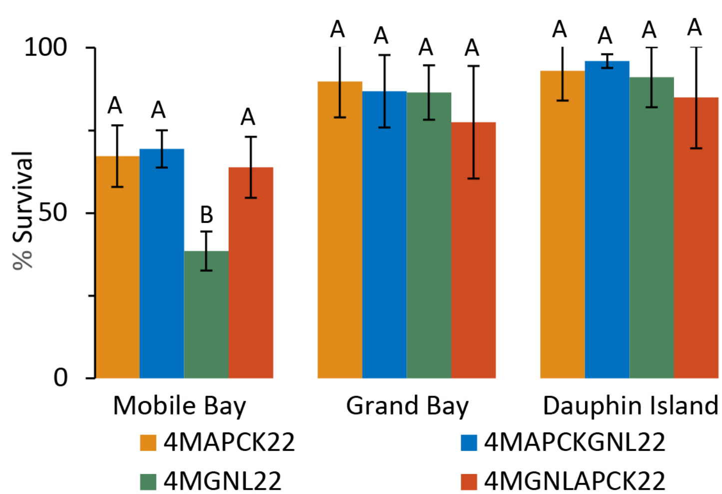

Figure 1. Average percent survival of each tetraploid line at three sites in Alabama (n = 4). Differing letters signify significant difference in survival within sites ($p < 0.05$).

THE SOCIOECONOMIC IMPACT OF SALMON AQUACULTURE IN CHILE

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Global aquaculture has been presented important growth rates over the last decades, has contributed to the food security agenda and SDG's objectives. However, has been considered a controversial activity raising concerns about environmental impacts, sociocultural and economic issues, and health. Chilean salmon aquaculture has shown impressive development in terms of production growth, but controversies are not exempt in this industry. Despite having created employment at the local level, and is 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, this may condition the development of an important industrial activity such as salmon aquaculture. The development of this industry raised expectations for its potential positive impact on the communities' livelihood. 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, the main socioeconomic dimensions analyzed are employment, salaries, poverty, unionism, income distribution, migration, and local economies. The socioeconomic effects that the industry initially triggered are widely criticized in the literature, however, recent performances indicates that these conditions have improved steadily over time.

EVALUATING TUMBLING EFFICIENCY OF DIFFERENT AIR DIFFUSERS ON THE MEAN GROWTH OF *Ulva* AND TURKISH TOWEL SEAWEEDS

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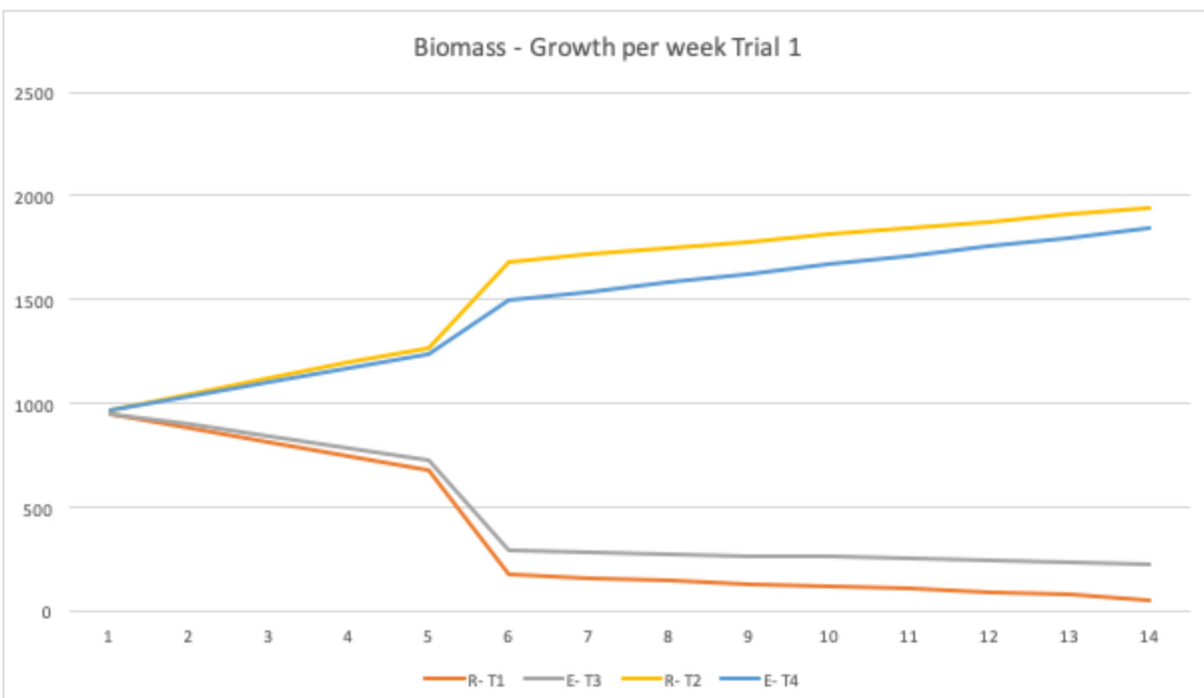
Tumble culture of seaweed and kelp is an emerging form of mariculture that can lead to high growth rates and efficient nutrient absorption. A commercial industry with demand for various types of seaweed for cosmetic, medicinal, or human consumption could be met by tumble culture in land-based systems. This project aims to understand the difference in growth between a commonly used air diffuser and designs created by our group that could lead to more growth and greater nutrient absorption in *Ulva lactuca* (Sea Lettuce) and *Chondracanthus exasperatus* (Turkish Bath Towel). Comparison between the diffuser types will be conducted via analysis of water quality parameters as well as starting and end weight of the seaweeds. The first set of data is conducted with both *Ulva* and Turkish Towel while the second set of data consists of only *Ulva*. The first set compares two types of diffusers across four tanks while the second set compares three diffusers across the same four tanks. Results indicate diffuser type does lead to a difference in growth. Small variations can lead to vastly different results and maintaining consistency is key to understanding any observed effects. This has implications for commercial mariculture with respect to efficiency, as diffusers vary greatly in cost of materials and complexity. Understanding the potential benefits of diffuser type could allow those employing tumble culture to make educated decisions when creating new systems.



MODELING RISK PERCEPTIONS TOWARD GENETICALLY MODIFIED FARMED SALMON

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Studies of attitudes toward and risk perceptions of genetically modified (GM) foods have largely focused on GM plants and have generally found that people have negative attitudes toward and perceive risk from GM food. With the recent FDA approval of a GM salmon in the US, there is an increased need to study attitudes and risk perceptions toward GM animal protein. Here, we report on a survey of midwestern US agriculture and natural resources extension personnel's perceptions of GM salmon. Overall, we found that the respondents perceive relatively low risk from GM salmon (averaging 2.20 on a 5-point risk perception scale). We constructed a linear mixed model of risk perceptions and found that higher levels of food consciousness (i.e., preferences for locally grown or organically grown food) and, to a lesser extent, egalitarian communitarian cultural values were associated with higher levels of risk perceptions. We found that higher levels of objective knowledge about GM foods were associated with lower risk perceptions. The remaining variables in the model (age, being a hierarchical individualist, and aquaculture knowledge) had little or no effect. The model results indicate that the formation of GM salmon risk perceptions is complicated and multi-factorial, spanning both cognitive and affective factors. However our findings suggest that outreach and communication about those issues may help to dampen risk perceptions among this population and, potentially, the general public. More research is needed to better understand the interplay of knowledge, food consciousness, and values, especially among the broader population.

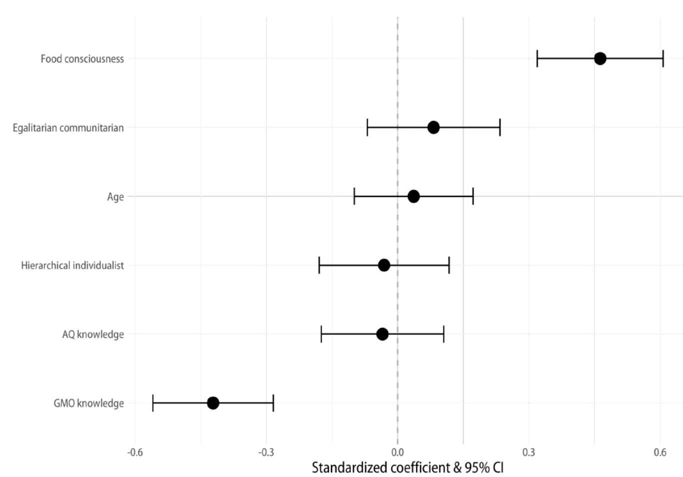

Figure 3: Standardized estimates and 95% confidence intervals for the final model.

KEEPING MY HANDS WET: 30 YEARS OF WORKING IN AQUACULTURE

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Little did I know that raising fish in my cramped, shared bedroom, as a kid that it would shape my entire life. When I was 10 years old, I knew I wanted to be a marine biologist. Every book report and job shadow assignment through high school lead me to enroll for B.S. in marine biology at the Florida Institute of Technology. Confident, in my goal to become the next Jacques Cousteau, I quickly learned of another degree option that was right up my alley… Aquaculture! I had been doing it since I was a child and never knew what it was called. A double major allowed me to follow all my dreams. For the last 28 years, I have combined marine biology and aquaculture in all my varied jobs including teaching them. Among my many jobs, I have been an aquarist in a public aquarium, propagated corals on a coral farm, and spent the last 20 years growing and restoring species along the California coast. I have grown everything from freshwater prawns and American alligators to giant kelp and pismo clams. Keeping my hands wet and watching species grow is my passion and I've been lucky enough to make a career out of it.

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A NEW APPROACH TO ASSESS THE IMPACT OF ANTHROPOGENIC ACTIVITIES ON MARINE ECOSYSTEMS: SMALL-SCALE FISHERIES AND AQUACULTURE

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Globally, oceans and coastal areas represent a critical resource to the livelihoods of millions of people that routinely depend on the sea with fisheries and aquaculture being key sectors for the economy and food security. According to the FAO, the total production of fisheries and aquaculture reached a historic record of 214 million tonnes in 2020 and the production of aquatic food is expected to increase by further 15% by 2030. Therefore, the need to balance the environmental, social, and economic sustainability of the sea-related human activities is greater than ever.

Understanding the potential impact of anthropogenic activities on marine habitats, including Vulnerable Marine Ecosystems (VME), is crucial to protect them, maintain or restore biodiversity, and identify the eventual mitigation measures that should be implemented. Nevertheless, the impacts on habitats are still poorly known and therefore new approaches need to be developed and applied to better identify areas where small-scale fisheries (SSF) and aquaculture may be having a detrimental environmental impact.

In the framework of the research project CABFISHMAN (INTERREG Atlantic Area Programme, European Regional Development Fund), a methodology was proposed to assess perceived impacts of all the most commonly use SSF fishing gears on habitats along the European Atlantic Area. For this purpose, a new standardised questionnaire-based approach and multi-criteria analysis was adopted to collect experts' knowledge and stakeholders' perception (fishermen, managers, researchers, and NGOs) and generate a fishing gear impact score. The developed evaluation matrix comprises physical-chemical, biological-ecological and fishery components. The diverse impacts and interactions (e.g., sea bottom damage and degradation, nutrient enrichment, threatened or protected habitats and species, litter, and conflicts with other fishing gears) were scored as a function of their frequency, severity, and duration. This work presents and highlights how this evaluation matrix can be applied to assess aquaculture impacts, to rank potential areas for future implementation of aquaculture production, and how this new ecosystem-based approach could be a useful tool for planning and managing human activities in the maritime space.

THE ONTOGENY OF ORGANIZED NASOPHARYNX ASSOCIATED LYMPHOID TISSUE IN RAINBOW TROUT: IMPLICATIONS FOR VACCINATION

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Vaccination on young fingerlings requires understanding the development of adaptive immunity. We recently described that the nasal cavity of rainbow trout is lined with an extensive lymphoreticular epithelium that forms lymphoid aggregates. This structure, named organized nasopharynx-associated lymphoid tissue (O-NALT), was described in 30g fish and its composition consisted of 56% $CD4^{+}$, 24% IgM^{+}, 16% $CD8\alpha^{+}$, and 4% IgT^{+} lymphocytes. O-NALT has molecular markers of mammalian germinal centers and likely the home for the maturation of the adaptive immune response in response to mucosal vaccines. Thus, the goal of this study is to determine when O-NALT first appears during rainbow trout development and whether its cellular composition changes over time. Using routine histology as well as immunofluorescence (IF) staining with specific antibodies against CD4, CD8, IgM and IgT, we found that, histologically, O-NALT was first visible at 1490-degree days (DD) or 100 days post-hatch (mean weight 12g). IF staining showed that there is a developmental progression in lymphocyte composition in the O-NALT. Specifically, in 1490 DD young rainbow trout, $CD8^{+}$ T cell numbers dominated O-NALT followed by IgM^{+} B cells. As fish developed, $CD4^{+}$ T cells and IgM^{+} B cells become the dominant lymphocyte types in O-NALT. IgT^{+} B cell numbers did not change during development. These results indicate the early vaccination of rainbow trout may stimulate very different types of mucosal immunity depending on the age of the fish. Further studies are necessary to ascertain whether O-NALT of young fish also expresses molecular markers of mammalian germinal centers and whether it can support B cell selection in response to antigenic stimulation.

UNLOCKING AQUARIUM REEF FISH EGG AND LARVAL IDENTIFICATION WITH DNA BARCODING

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The global marine aquarium trade relies on wild fisheries for 98% of ornamental reef species for display. Overexploitation and harmful collection techniques targeting ornamental species threaten fish populations and reef ecosystems. Aquaculture can reduce the demand for wild supplies while increasing our limited knowledge on the early life history of aquarium species. Standard protocols for egg collection, identification and larval rearing in public aquaria can facilitate research and development towards large scale aquaculture by providing an untapped source of fishes from frequent voluntary spawning events.

The aim of this study was to identify pelagic eggs and larvae collected from the mixed species Living Coral Reef exhibit at the Texas State Aquarium. Each sample was photographed and measured under a stereomicroscope to document unique characteristics for each species. Sample identities were confirmed with DNA barcoding. Mitochondrial DNA was then extracted using Chelex methodology. The mitochondrial cytochrome oxidase 1 (CO1) gene region was amplified by polymerase chain reaction (PCR) and purified with MagBind beads before sequencing. Sample sequences were compared to DNA of adult tissue and genetic information in the GenBank database.

Smallmouth (*Haemulon chrysargyreum*) and cottonwick (*Haemulon melanurum)* grunts had egg diameters of 860 ± 45 μm (n = 20) and 972 ± 30 μm (n = 11), respectively, and were genetically identified. Bicolor damselfish (*Stegastes partitus*) larvae, 1 day post hatch, were recognized by characteristic brain, stomach, and ventral pigmentation. Morphological characteristics, such as average egg diameter and pigmentation pattern, were distinct for each species.

Utilizing photos, measurements, and DNA sequences of adult fishes, eggs, and larvae in a public aquarium setting will allow for rapid sorting of eggs, aiding in assessing egg viability and implementing species-specific larviculture techniques of target aquaculture species. This study discusses new applications in ornamental larval culture by strengthening current research, calling for further support of sustainable fisheries and commercial aquaculture, and presenting opportunities for outreach to the public sector.

FEEDS AND FEEDING IN SUPER-INTENSIVE SHRIMP FARMING SYSTEMS

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Aquaculture production of fish and shellfish represents almost 50% total annual world seafood production. Nevertheless, with increasing global demand for high quality protein, continued growth will be needed to meet demand. Expansion of the area devoted to aquaculture production is constrained by land cost, availability and use conflicts. Therefore, much attention has been given to technologies which enable increasing biomass harvested per unit area by raising the stocking density. Biofloc technology represents a viable strategy for intensification of shrimp farming. In these systems, microbial bioflocs provide control of nitrogenous wastes while also supplementing the food supply for the shrimp.

Maintaining optimal ratios of carbon and nitrogen in the water is critical for supporting the development of bacterial community. Although a wide range of C:N ratios have been reported in the literature for biofloc systems, ranging from 5-6:1 to 20-25:1, most agree that the ideal C:N is somewhere between 9:1 and 12:1. Bioflocs are dominated by heterotrophic bacteria, which are capable of converting organic carbon from the feces and excess feed into bacterial biomass. They also are able to convert inorganic nitrogen in the form of ammonia into organic nitrogen in the form of bacterial protein. It has been demonstrated that shrimp can consume microbial bioflocs, extracting additional nutrients in the process. Some authors have suggested that biofloc can reduce dependency on formulated diets as a feed source in super-intensive environment. Following this rationale, it would be possible to use low-quality diets in shrimp biofloc systems without damaging performance numbers. However, studies have shown that the use of nutrient-deficient diets negatively affects microbial dynamics, biofloc formation, biofloc nutritional profiles, water quality, and animal performance. An unbalanced biofloc system results in stress on the target crop, which requires increasing management inputs further cutting into profit margins. Shrimp grown in biofloc systems, like shrimp grown in other types of production systems, require a complete, highly digestible diet fed at appropriate feeding rates. Proper feeds and feeding methods are essential for production and profitability under super-intensive conditions.

The future of aquafeeds continues to evolve towards cost-effective, high-quality products that are manufactured efficiently and formulated with sustainable certified ingredients designed to meet all the nutrient requirements for each life stage of the target species. Regardless of the production system adopted, advanced aquafeeds and effective feeding programs are crucial to minimize environmental impacts while maximizing profits.

INVASION EXTERNALITIES AND FIRM HETEROGENEITY: THE CASE OF SEA LICE

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Aquaculture is a rapidly growing food production technology, primarily in developing countries, and now accounts for over 50% of global seafood production. While aquaculture provides nutritious food and can alleviate poverty, diminish income inequality, and increase food security, there are significant environmental impacts and disease concerns. Environmental externalities are generally addressed with government regulations, although the extent to which this is possible depends on a country's governance capacity. However, many environmental challenges in aquaculture are not pure externalities but also have private incentives associated with them as firms benefit from, e.g., lower disease pressure and faster growth with improved water quality. Hence, industry structure may also be important as these private benefits incentivize firms to collaborate. However, collaboration becomes more difficult as the number of stakeholders increases. Therefore, if they collaborate, one would expect externalities to be reduced in regions with fewer separate firms.

We research this hypothesis for salmon, the second largest aquaculture species globally by value and an industry that has experienced major disease challenges. Here we show that the prevalence of the most harmful negative externality in Chile and Norway, sea lice, is significantly reduced in regions with a higher degree of ownership concentration. As shown in Figure 1, regardless of the region (south – blue, central – green, north – red), the sea lice count increases as the biomass diversity increases, i.e., more farmers are holding salmon stocks. This result is significant in countries with limited governance capacity as it suggests that self-governance concerning environmental challenges can be facilitated by how production sites are divided between producers. Furthermore, there is often a strong focus on developmental policies geared toward small-scale farmers, which unintendedly increases firm heterogeneity. As a result, our research suggests that this may not be environmentally optimal.

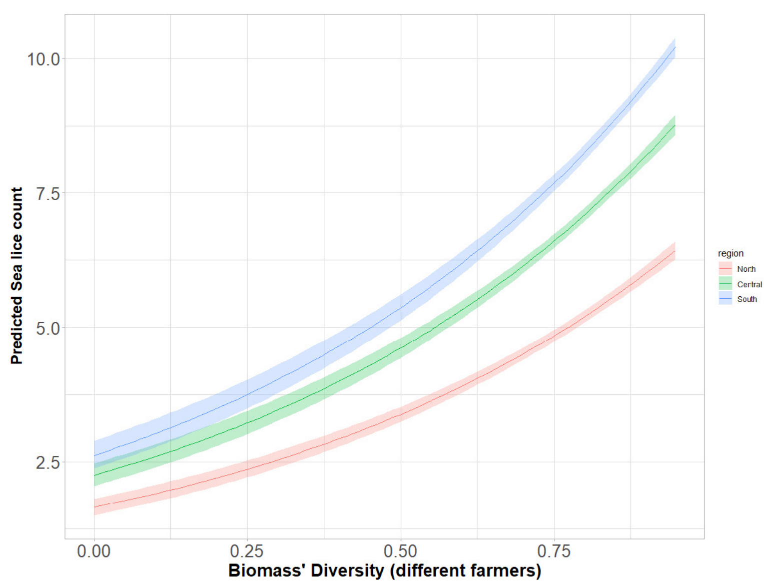

Figure 1. Predicted sea lice count for different Biomass diversity index and regions in Norway.

NOVEL INTEGRATED MULTITROPHIC AQUACULTURE PROJECTS IN THE GULF OF MAINE, GULF OF MEXICO, PUERTO RICO, AND THE NORTHERN MARIANA ISLANDS

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Interest in integrated multitrophic farming technologies is growing in the US, Caribbean, and Pacific. This type of aquaculture incorporates lower trophic species that extract inorganic and organic nutrients from a fed species. This technique has been demonstrated by the University of New Hampshire with steelhead trout, blue mussel, and sugar kelp from a floating sea structure called the AquaFort. This small-scale farming approach reduces nitrogen to the environment and produces additional species to be sold thus increasing the economic viability of a farm. Currently, several fishing groups in Maine are exploring IMTA sites to diversify their income due to concerns with the future lobster fisheries. The Gulf States Marine Fisheries Commission has funded an AquaFort project near Dauphin Island, AL to culture red drum, oysters and gracilaria. Other projects are being considered in Puerto Rico with tropical species such as conch, lobster, mangrove oyster, and ulva. More recently, interest is emerging in Micronesia near Saipan to adopt IMTA practices to culture rabbit fish, pearl oysters, sea grapes and sea cucumbers. A status of these projects will be provided during the IMTA session.

GLOBAL DEVELOPMENT OF IN-POND RACEWAY SYSTEMS: AN ENHANCED EFFICIENCY POND AQUACULTURE PRODUCTION APPROACH

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This presentation will describe the theory and history of In-pond Raceway development and how it has become a globally adopted aquaculture technology capable of culturing a wide range of fish species. From cold water to warm water, In-pond Raceways have elevated the opportunity for competitiveness using soy-based extruded diets. We will discuss a sample of the numerous species cultured in raceways and why they are a popular aquaculture approach.

THE DEVELOPMENT AND ON-LINE AVAILABILITY OF THE IN-POND RACEWAY SYSTEM MANUAL

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This presentation will briefly introduce the In-Pond Raceway System Manual to the conference participants and provide a tour of the manual and several of its features including interactive elements users will find highly beneficial as they consider adoption of the technology or in making management decisions after they have made the decision to adopt as the manage their systems.

ECONOMICS OF IN-POND RACEWAY SYSTEMS - A GLOBAL PERSPECTIVE

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An introduction of In-pond Raceway System economics as found across several species around the world will be presented. Many examples are found in the manual which is accessible on-line and some will be briefly described in this presentation. This presentation will be useful to any who are interested in In-pond Raceway Sytems either academically or as actual practitioners in the commercial sector.

PRODUCTION AND ECONOMIC EFFECTS OF CATFISH FEEDING FREQUENCIES

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Daily feeding to apparent satiation is the most common feeding strategy followed in U.S. catfish industry as it maximizes growth and fish yield. However, under conditions of tighter credit or higher feed prices certain farmers have to feed catfish less frequently than daily in order to adjust to available capital. In this study we detail the production and economic effect of various feeding frequencies employed in research ponds from three different decades (Li et al. 2006; Nanninga and Engle 2010; Kumar et al. 2021) under varying management conditions. The feeding frequencies analyzed included every day, every other day, and every third day feeding to satiation. Economic benefit associated with every other day and every third day were analyzed using a partial budget equilibrium evaluation. All three studies reported every other day feeding to have better feed conversion ratio (FCR) and subsequent 32% reduction in feed costs relative to every other day feeding. However, this was at a cost of 18% reduction in fish yield. Under current conditions, every day feeding is more economically beneficial than every other day feeding as the total reduction in feeding cost does not offset the revenues lost from reduced fish yield. However, the study found that this recommendation is sensitive to catfish prices and feed prices (Table 1). Hence careful consideration of market conditions needs to be incorporated into farm management decisions while selecting and implementing reduced feeding frequency on catfish operations. Under current economic conditions (feed price =$605/MT and fish price =$2.85/kg), if available capital is sufficient feed, it is economically beneficial to feed every day to satiation to take advantage of fish growth and gross returns.

Table 1. Sensitivity of net benefit ($/ha) from feeding every day as opposed to every other day at various fish and feed prices

		Fish prices ($/kg)					
		$2.42	*$2.53*	*$2.64*	*$2.75*	*$2.86*	*$2.97*
Feed prices ($/MT)	*$440*	$1,785	$2,113	$2,440	$2,768	$3,095	$3,423
	$495	$1,153	$1,480	$1,808	$2,135	$2,463	$2,790
	$550	$520	$848	$1,175	$1,503	$1,830	$2,158
	$605	-$113	$215	$543	$870	$1,198	$1,525
	$660	-$745	-$418	-$90	$238	$565	$893
	$715	-$1,378	-$1,050	-$723	-$395	-$68	$260

ECONOMIC RISK OF COMMERCIAL CATFISH PRODUCTION PRACTICES

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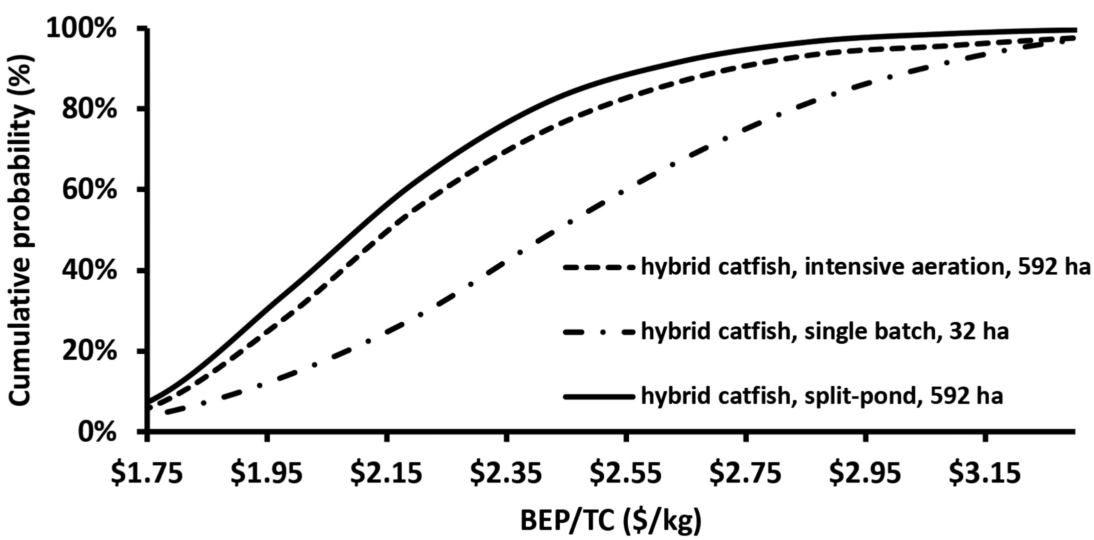
The U.S. catfish industry has adopted numerous production practices that encounter varying degrees of economic risk. This study utilized commercial farm data to quantify the economic risk associated with common production strategies while identifying production parameters contributing most to economic risk. Stochastic Monte Carlo simulations employing established enterprise budgets found fish yield, feed price, and FCR contributing most to variations in BEP/TC. Multiple batch farming of channel catfish was the least risky production strategy with greater probabilities of lower cost of production. However, split ponds and intensively aerated ponds were stochastically dominant (second-order) strategies and have a greater probability of maximizing returns. Among channel catfish production strategies, multiple batch and intensively aerated production were stochastically dominant to medium intensity single batch production. For hybrid catfish production strategies, split pond and intensively aerated production were stochastically dominant to medium intensity single batch production. Multiple-batch and intensively aerated culture of channel catfish were more susceptible to price (market) risk while hybrid catfish production was more susceptible to yield (production) risks. Price risk was not a significant contributor to economic risk for any production strategy in short-run (1-year) as fish prices remained relatively high and less fluid. First order stochastic dominance of split-pond technology on larger farms as compared to low intensity culture on smaller farms suggested that yield increasing intensive production practices supersede low intensity technologies and help achieve economies of scale (Figure 1). The results of this study will provide critical information on relative risk associated with varying catfish production strategies under varying economic or price conditions.

Figure 1. Cumulative probability of breakeven price ($/kg) of hybrid catfish production strategies at different scales exhibiting first order stochastic dominance



FACTORS LIMITING FISH PRODUCTION IN FRESHWATER PONDS

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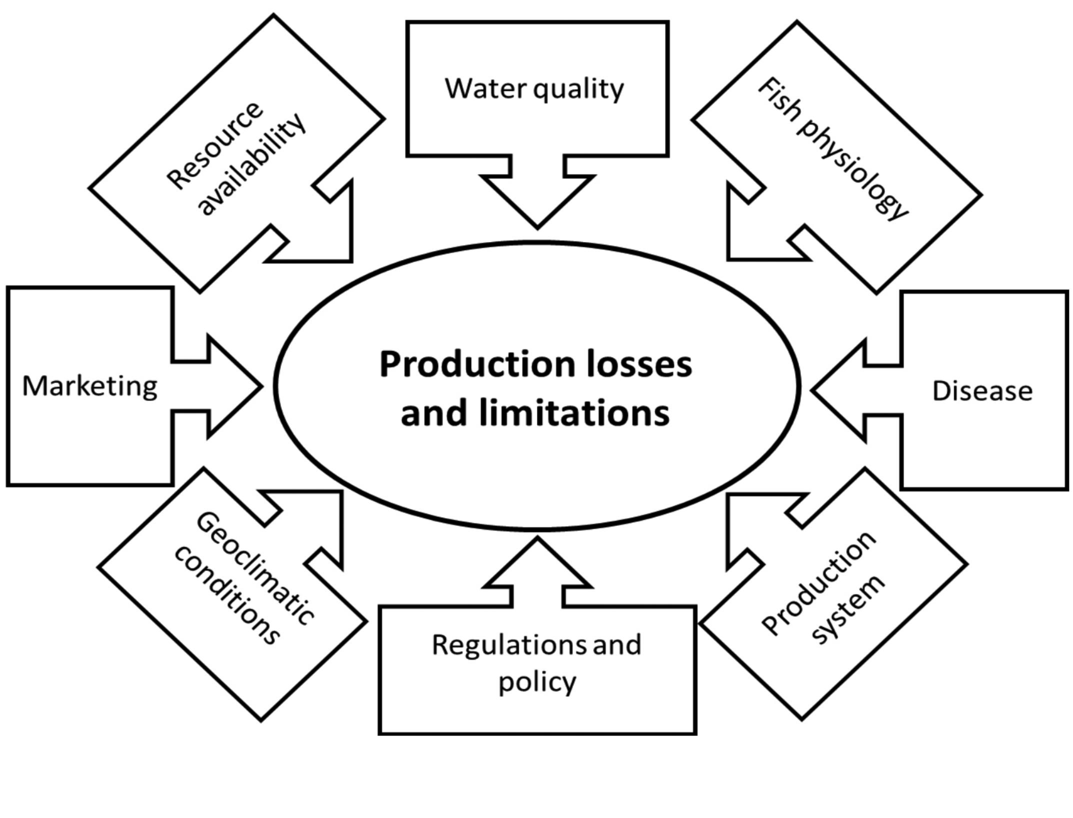
Fish production in ponds is the most dominant production strategy followed globally, especially in freshwater inland aquaculture. Various species of carp, tilapia, and catfish are commonly produced in pond aquaculture systems with production levels varying from subsistence farming level to large-scale commercial farms. As intensity of production increases, production in freshwater ponds is limited by several factors. This review highlights some of the most important limiting factors influencing freshwater pond aquaculture. Productivity in ponds is largely limited by the carrying capacity of ponds, availability of quality diets, animals, and capacity of the pond to process waste products. Specific limiting factors reviewed here include water quality parameters, geoclimatic conditions, fish physiology, diseases, resource availability, regulations and policy, type of production system used, and marketing bottlenecks (Figure 1). Technological innovations and modifications of the traditional earthen pond have been researched and adopted in efforts to increase the carrying capacity of freshwater ponds. Examples of these include improved pond aeration, partitioned pond systems, bacterial augmentation, and pond lining. Although high-tech aquaculture production systems, such as recirculatory aquaculture systems, are gaining greater attention in modern aquaculture, pond culture will likely remain the most widely used form of aquaculture production globally for the foreseeable future. Understanding the critical factors impacting these pond systems is absolutely necessary for the successful and sustainable expansion of the aquaculture industry.

Figure 1. Production factors contributing to fish loss or limiting fish production in freshwater ponds



RESEARCH VERIFICATION OF 4 COMMERCIALLY UTILIZED SPLIT-POND DESIGNS

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Split-ponds systems are emerging as a potential catfish production system with capabilities of improving productivity in the catfish industry. However, adoption of these systems requires a significant understanding of design and involves significant capital outlay. Hence production performance of these designs under controlled conditions over multiple years on commercial scales provides fundamental information for the continued adoption of these systems. The Thad Cochran National Warmwater Aquaculture Center (TCNWAC) was at the epicenter of design, development, and testing of four design variants of commercial-scale split-pond systems. These four designs are primarily differentiated by the mechanism of water circulation between the fish culture pond area and the waste treatment pond area. The water moving devices include the slow rotating paddlewheel (SRP), modified paddlewheel aerator (MPA), screw pump (SP), and axial-flow pump (AP). This study investigated the economic and investment feasibility of these four split-pond designs using data collected over various years of research at the TCNWAC. An economic engineering approach using standard enterprise budget analysis was used to develop estimates of annual costs and returns for producing food size hybrid catfish from four split-pond systems. The additional investment capital required for converting a traditional open pond into any of the four split-pond designs ranged from $54,400 to $71,150 per 4-ha pond and involves the cost of earthwork, installation labor, water circulation devices, generators, inlet structures, additional 7.5-kwh paddlewheel aerators, and an automated oxygen monitor. Cost of production ranged from $2.03 to $2.37/kg. However, the breakeven yields required to cover total costs were also high showing financial risk associated with high intensity production systems (Table 1). Net present value (NPV) was highest for the MPA design at $226,397 and lowest for the SP design at $76,520. Similarly, the modified internal rate of return (MIRR) was highest for the MPA design at 31% and lowest for the SP design at 27%. All four designs were found to be economically feasible under current conditions with modified paddlewheel design showing greater potential.

Table 1. Breakeven analysis of four commercial scale spilt pond designs in research settings

Parameters	Units	Slow Rotating Paddlewheel	Modified Paddlewheel Aerator	Screw-Pump	Axial-flow Pump
Fixed costs	$/ha	6,519	6,256	5,681	6,142
Variable costs	$/ha	27,950	28,139	24,847	27,003
Total costs	$/ha	34,468	34,395	30,528	33,145
Breakeven price to cover variable costs	$/kg	1.75	1.66	1.93	1.79
Breakeven price to cover total costs	$/kg	2.16	2.03	2.37	2.20
Breakeven yield to cover variable costs	kg/ha	10,703	10,775	9,515	10,341
Breakeven yield to cover total costs	kg/ha	13,199	13,171	11,690	12,693

CONCLUDING THOUGHTS: FRESHWATER POND CULTURE OF GULF KILLIFISH *Fundulus grandis*

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Due to their interest as a live baitfish, production protocols for Gulf killifish, *Fundulus grandis*, have been evaluated and modified since the late 1970s. However, they have not been widely adopted by producers, due in part to limited understanding of commercial-scale grow-out techniques to market size (> 6 cm). Recent studies have shown that Gulf killifish can be acclimated to fresh water just seven weeks after hatching and grown out to market size in freshwater ponds. Following initial freshwater grow-out trials, it was apparent that further information was needed to guide potential inland killifish producers in making more informed decisions about stocking.

Two studies were conducted to test whether Gulf killifish cohorts can be stocked throughout the warmer months and at greater densities. In the first study, the effects of stocking time on growth rate and survival were evaluated over a 12 week period. Two juvenile cohorts, 7-9 weeks old, were stocked at 25,000 fish/ha, one month apart. While the first cohort had lower survival, both cohorts across all ponds grew rapidly, reaching market size in 22-32 days (Table 1). In the second study, density effects on growth rate and survival were evaluated over a 12 week period. Juveniles, 7-9 weeks old, were stocked at 25,000 fish/ha or 50,000 fish/ha. Survival did not differ between densities and fish at both densities grew to over 7.5 cm in length on average. While individual growth in length was lower in the higher density (Table 1), killifish at both densities reached market size in 29-31 days. To meet market demands consistently, inland producers may need to stock ponds multiple times over a growing season at a stocking density of at least 50,000 fish/ha in freshwater ponds.

Table 1. Effects of stocking time and density on growth and survival of Gulf Killifish in freshwater ponds.

Treatment	Mean Growth/week (cm)	Mean Survival (%)
Year 1: Cohort 1 (June)	0.71	40
Year 1: Cohort 2 (July)	0.60	79
Year 2: 25,000 fish/ha	0.76	75
Year 2: 50,000 fish/ha	0.71	72

YOU ARE WHAT YOUR PARENTS EAT: BROODSTOCK DIET INFLUENCE ON EGG QUALITY AND PRODUCTION IN CALIFORNIA YELLOWTAIL, *Seriola dorsalis*

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Diet fed to broodstock during a spawning period influences the quality and quantity of egg production. Furthermore, it can be extremely costly to provide a high-quality diet to broodstock year-round. By understanding the timeline of nutrient incorporation from parent to offspring, a more efficient diet plan can be established. This will help reduce cost as well as increase feed efficiency. The objective of this study is to determine the duration required for the broodstock to transfer nutrients obtained from their diet into eggs using an alternating diet plan of cut bait and commercial pelleted feed. In 2021, nine California yellowtail, *Seriola dorsalis,* broodstock at Hubbs-SeaWorld Research Institute in San Diego, CA were fed two alternating diets from April to September: Vitalis Prima pellets (World Feeds Ltd, UK) and a cut-bait diet comprised of sardine and squid. Each diet was switched after every 6 weeks within the spawning season, which lasted for a total of 24 weeks. Egg samples, along with biometric data from each spawn, were collected and processed for proximate analysis and fatty acid content. Data from samples collected were compared for varying nutritional incorporation that occurred during each diet switch. Proximate analysis results of eggs were statistically similar throughout each feed and diet switch as seen in Figure 1.

This study will provide a greater understanding and insight into nutritional assimilation on a temporal scale. Findings from this study will contribute towards the development of a cost-effective and reliable broodstock diet for farmers, which will further improve the economic viability of the California Yellowtail as a staple aquaculture species.

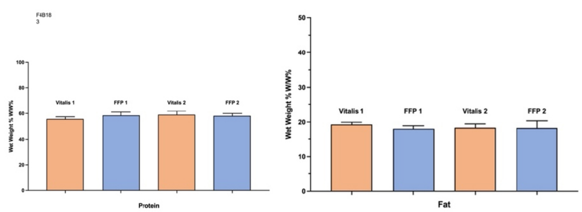

Figure 1: The mean ± SD wet weight percentage (W/W%) of protein and fat from a minimum of 5 spawns and a maximum of 15 spawns within a given diet switch period obtained from proximate analysis.

AMMONIA-INDUCED INHIBITION TO ANAEROBIC DIGESTION OF FISH WASTE SOLIDS (SLUDGE) FROM RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Anaerobic digestion (AD) is a biological process occurring in the absence of oxygen, where microorganisms degrade organic matter to produce biogas, a mixture of methane (CH_4), carbon dioxide (CO_2), and trace gases like hydrogen sulfide (H_2S) and ammonia (NH_3). In assessing AD of RAS sludge, understanding how aquacultural sludge characteristics affect the digestion process and the microbial community inside the digester will determine the feasibility of treatment. The breakdown of abundant nitrogen-rich organic compounds, such as proteins, in fish sludge leads to excess ammonia formation inside an AD system. Ammonia can exist in two forms (ammonium ion, or NH_4^+, and free ammonia, or NH_3) inside the digester, depending on the pH of the sludge. As the pH increases, ammonium ions are rapidly converted to toxic free ammonia, which might destabilize the AD process and inhibit desirable CH_4 production.

In this study, RAS sludge from a Steelhead trout grow-out tank was mixed with deionized water varying the initial concentration of total nitrogen (five treatments). Subsequently, the sludge was mixed with an inoculum source in lab-scale batch reactors. The ratio of microorganisms to organic matter (inoculum-to-substrate ratio or ISR) was kept constant in each treatment. The results showed an increase in the lag phase period for CH_4 production as the concentration of total N increased in the treatments. However, at the end of the study, the cumulative CH_4 production values were similar for most treatments, signifying that the microbial consortia may have acclimated to the high ammonia conditions. While not significantly different, it was noted that the mixture with 40% sludge and 60% deionized water resulted in the highest normalized cumulative CH_4 production (397 ± 3 mL CH_4/g VS).

Microbial analysis of the final digested samples was also conducted using high-throughput sequencing (HTS) to understand the acclimation process of the microbial community. The results showed that the increase in the relative abundance of the ammonia-tolerant genus *Methanosarcina* and a simultaneous decrease in the ammonia-sensitive genus *Methanosaeta* corresponded with the increase in the initial total nitrogen concentration of the treatments. Due to the increase in the ammonia concentrations, the methanogenic archaeal community gradually shifted towards genera that were more resistant to high ammonia concentrations, which may have resulted in the extended lag phase observed for the treatments with high total nitrogen concentrations.

A STUDY ON US CONSUMERS' PERCEPTIONS, PREFERENCES, AND WILLINGNESS TO PAY (WTP) FOR CELL-BASED SEAFOOD AT GROCERY SETTING.

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Cellular agriculture is a novel food production system that uses the advances in muscle tissue engineering techniques for recreative medicine for food production. Although in its infancy this technique has incredible potential for creating sustainable seafood sources and can help with addressing the growing global demand for seafood globally. This alternative seafood production system is also crucial for relieving the pressure on our ocean and reducing the need for the high amounts of energy, water, and feed required for the aquaculture production of seafood. However, the cell-based seafood industry needs to address numerous technological, societal, challenges before the realization of affordable, scalable, and ubiquitous cell-based seafood production. Amongst these challenges are consumer acceptance, perceptions and willingness and to pay and try novel seafood products. Hence, this study aimed to address this question through an online survey targeting 1500 U.S. residents, ages 18 to 65, with the samples representing nationwide population to understand the influence of personal and family's seafood consumption patterns, conventional and cell-based seafood perceptions, and demographic factors on their cell-based seafood attitude and WTP. Single bound dichotomous choice experiment (DCE) was the model that assesses consumers' WTP for cultured seafood. The three main attributes of this DCE were 1) Fish species (salmon, tuna, and shrimp); 2) Production method (farm-raised, wild-caught, and cell-based seafood); 3) Price levels (6 levels for each species). Mixed logit (MXL) was the statistical model in this experiment. A focus group with28 participants was conducted to test pre-conceived findings and uncover ideas that may have not been considered. The result showed that sensory was the main motivation for consumers to purchase cell-based seafood, followed by environmental impact, toxin-free, cost, and health benefits. Hence, this study utilized a between-subject approach to examine the effects of various information types on consumer preferences for cell-based seafood. All 1500 respondents were randomly assigned to one of the three treatments (control, sustainability, toxin-free). The results of the study will contribute to the current literature on consumers' perceptions, preferences, and WTP on consuming cell-based seafood, and provide insights into the potential price point of cell-based seafood as an alternative product in the market.

FURTHER DEVELOPMENT OF LIPOSOME-CONTAINING COMPLEX PARTICLES FOR THE DELIVERY OF WATER-SOLUBE NUTRIENTS TO MARINE FINFISH LARVAE

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With the global population just surpassing 8 billion people, there has never been a greater demand for seafood. There is great potential for marine finfish raised in aquaculture systems to help meet that demand. However, domestic production of marine finfish and long-term success of the industry is limited by the industry's ability to produce sufficient quantities of high-quality larvae and juveniles. Existing commercial microdiets fail to supplant inconsistent and expensive cultured live feeds in marine finfish hatcheries. Currently available microdiets are subject to high rates of nutrient leaching when in suspended in water and rapid sinking rates which result in poor feeding efficiencies and may not meet the nutritional requirements for marine fish larvae. Ultimately, these factors manifest in poor larval growth, low survival outcomes, and elevated malformation rates in larvae. Recent studies have found liposomes to be a novel and successful method of delivery of water-soluble compounds to marine finfish larvae via enriched live feeds. Building upon this research, we have incorporated liposomes into larger alginate-based particles, resulting in liposome-containing complex particles (LCP). We hypothesize that these liposome-containing complex particles will show low rates of nutrient leaching when compared to existing commercial-type microdiets while delivering 'complete nutrition' to the larvae. Early iterations of these liposome-containing complex particles containing hydrolyzed casein have been tested in growth trials using Inland silverside (*Menida beryllina*) larvae as a model organism. These early trials show promising results when compared to both liquid- and commercial-type microdiets, as revealed in similar growth parameters between fish fed our LCP diet and those fed a commercial standard.

Despite this progress, further research and development is needed in order to optimize these liposome-containing complex particles to be competitive with existing larval weaning diets. Our next steps in this research project are to: 1) optimize buoyancy of complex particles to increase incidence of larval capture when in suspension, 2) evaluate the leaching of critical nutrients when complex particles are suspended in sea water, and 3) evaluate the success of these diets for commercial species, such as *Seriola sp*., a valuable commercial finfish species. If successful, this research may transform the way marine finfish larvae are fed in commercial hatcheries and enhance larval growth, survival, and overall output in production systems. Moreover, this diet has the potential to reduce the need for live feeds, a sought-after goal of the industry that would spare hatcheries labor and resources. Lastly, this liposome-based complex particles could have broader impacts beyond the scope of larval nutrition and act as a delivery method for vaccines, antibiotics, and other bioactive compounds.

CONSUMER PREFERENCE FOR FISH SPECIES AND COOKING METHODS IN CAMBODIA

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The objective of this research was to determine whether frying or boiling and which fish species were preferred by Cambodian consumers. One hundred consumers in Cambodia evaluated striped catfish, striped snakehead, walking catfish, and fermented striped catfish that were either boiled or fried using a 9-point hedonic scale where 1= dislike extremely and 9 = like extremely. Data were analyzed by the GLM procedure of SAS v9.4 with fish species and cooking method as fixed effect and panelist as a random effect.

Consumers preferred fried fish appearance and aroma ($P = 0.001$) over boiled fish but did not differ in their acceptability ratings for flavor, texture, and overall acceptability of boiled and fried fish (Table 1). In addition, all mean values were between 6.7 and 7.2, indicating that on average, boiled and fried fish were moderately acceptable to consumers. For fish species and preparation method, the appearance and flavor of walking catfish and striped snakehead were preferred ($P < 0.05$) over striped catfish. Aroma followed a similar pattern, with the exception that consumers also preferred the aroma of fermented striped catfish to that of striped catfish ($P < 0.0001$). When cooking method, fish species, and preparation were evaluated together, the acceptability of appearance and aroma was liked more for fried walking catfish (7.2) than boiled walking catfish (6.7) and boiled striped catfish (6.2) ($P < 0.001$). All other treatments were also preferred ($P < 0.05$) over the boiled striped catfish. In addition, for flavor and overall acceptability, the boiled striped catfish was liked less ($P < 0.001$) than all other treatment combinations with the exception of fried, fermented striped catfish. Overall, consumers had a slight preference for the appearance and aroma of fried over boiled fish. Walking catfish was liked moderately for both boiled and fried fish, and striped catfish should be either cooked fried or be fermented before it is boiled. In addition, striped snakehead did not differ in acceptability between frying and boiling methods. This indicates that striped catfish is the only fish that should not be boiled, and that striped catfish is better when being fried than when it is boiled.

Table 1 —Mean scores[1] for consumer acceptability (N =100) of appearance, aroma, flavor, texture, and overall, for three fish species of Cambodian fish, one that was both fresh and fermented, that were either boiled or fried using a 9-point hedonic scale.

Sensory Attributes	Cooking methods			Preparation & Species				
	Fried	Boiled	*P*	Walking Catfish	Stripped Snakehead	Fermented Stripped Catfish	Stripped Catfish	*P*
Appearance	7.0a	6.7b	0.002	7.0a	7.0a	6.9a	6.6b	0.046
Aroma	7.0a	6.6b	<0.001	6.9a	6.9a	6.9a	6.4b	<0.0001
Flavor	7.1a	7.0a	0.230	7.2a	7.2a	7.0ab	6.8b	0.017
Texture	7.1a	7.0a	0.155	7.1a	7.1a	7.0a	6.9a	0.118
Overall acceptability	7.2a	7.2a	0.349	7.3a	7.3a	7.2a	7.0a	0.098

[abc]: Means with the same letter within each row are not significantly different ($P < 0.05$).
[1]: Scores were based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

Table 2 —Mean scores[2] for consumer acceptability (N =100) of appearance, aroma, flavor, texture, and overall acceptability for the combined treatments of fish species, preparation method, and cooking method using a 9-point hedonic scale.

Sensory Attributes	Fried walking catfish	Boiled fermented striped catfish	Fried striped catfish	Boiled striped snakehead	Fried striped snakehead	Boiled walking catfish	Fried fermented striped catfish	Boiled striped catfish	*P*
Appearance	7.1a	6.9ab	7.0ab	6.9ab	6.9ab	6.7b	6.8ab	6.2c	0.001
Aroma	7.2a	7.0ab	6.9ab	7.0ab	6.9ab	6.7b	6.9ab	5.9c	<0.0001
Flavor	7.4a	7.3a	7.2a	7.3a	7.1a	7.0ab	6.7bc	6.4c	<0.0001
Texture	7.3a	7.1a	7.2a	7.2a	7.0a	6.9a	6.9a	6.5b	0.0004
Overall acceptability	7.4a	7.4a	7.3ab	7.3ab	7.2ab	7.2ab	6.9bc	6.7c	0.0003

[abc]: Means with the same letter within each row are not significantly different ($P < 0.05$).
[2]: Scores were based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

NAVIGATING THE REGULATORY FRAMEWORK AROUND SEAWEED MARKETING AND PROCESSING

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Seaweed is a cultivated crop being explored as a sustainable food and ecosystem service provider. With increasing interest in domestic seaweed production, there is a need to identify new and economically viable markets. Given the nutritional benefits of seaweed, the food market is a desirable outlet for potential producers.

Getting seaweed to market as food requires processing and value added production in compliance with existing food safety regulations. Seaweed, on the federal level, is currently categorized as a raw agricultural commodity and in its processed form falls under the Food Safety Modernization Act's Preventive Controls (FSMA PC) for Human Foods rule. Since most facilities in the US are small-scale and meet the definition of a qualified facility, they are exempt from some aspects of the FSMA PC rule. These facilities must then abide by state requirements for processing seaweeds, and some states are turning to the seafood HACCP rule to regulate them. Harvest and marketing of raw materials are currently unregulated at the federal level, thereby falling to states to determine production and marketing requirements for raw materials.

Since 2022, NY Sea Grant, in collaboration with CT Sea Grant and the National Sea Grant Law Center, has coordinated a group of food safety professionals from across the country and internationally to discuss seaweed food safety and regulations, to provide clarity and guidance on how to safely and effectively bring seaweed to market as food. This workgroup identified four major needs which may support the viable commercial expansion of this new industry. The first was to develop guidance to clarify the regulatory framework surrounding seaweeds. The newly developed regulatory guide describes the similarities and differences between two regulations (FSMA PC and Seafood HACCP) that have been applied to the sale of seaweed as foods on the federal and state levels. It provides guidance on how those operating under the HACCP system at the state level could transition into FSMA PC compliance as they expand their operations and no longer meet the definition of a qualified facility or farm. Future efforts will focus on developing additional resources and exploring the best approach to developing training to support this emerging industry.

CHANGES IN CONSUMER PREFERENECS FOR SEAFOOD PRODUCTS DUE TO THE COVID-19 PANDEMIC

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The public health crisis created by the COVID-19 pandemic led to the shutdown of restaurants and nonessential businesses throughout the United States. With the majority ($69.6 billion, 68%) of seafood expenditures prior to the COVID-19 pandemic occurring at food service establishments, this has resulted in an unparalleled shock to U.S. fisheries and aquaculture producers (NOAA 2018). Furthermore, the USDA Census of Aquaculture reported that for shellfish farms only 4% of their first point of sales were direct to consumers (USDA 2019). As farms and businesses attempt to respond to the loss of revenue from traditional marketing channels and establish direct to consumer channels, a key question concerned the extent of changes in consumer demand and preferences for seafood products. Thus, the goal of this project was to gather market information on changes in how, when, and where consumers purchase seafood in response to the COVID-19 pandemic.

This presentation will summarize the results of information specific to responses related to seafood purchases at households located within pre-determined regions of New York, Maryland, and Virginia. Survey respondents were asked about seafood purchases prior to (2019) and during (2020) the COVID-19 pandemic.

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GROWTH, FILLET YIELD, AND MUSCLE QUALITY TRAITS ARE NOT AFFECTED BY A GENOTYPE BY DIET INTERACTION IN RAINBOW TROUT CONSUMING DIETS THAT DIFFER IN LIPID CONTENT

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Characterizing how selectively bred fish with improved production traits are affected by environmental factors is essential for ensuring germplasm with superior genetics perform well across different production systems with variable feeding strategies. In this study selectively bred rainbow trout (*Oncorhynchus mykiss)* with improved fillet yield were reared on three commercially available diets that varied primarily in dietary lipid/energy content: low lipid (LL, 18%), moderate lipid (ML, 24%), and high lipid (HL, 29 – 33%). The objective was to determine if a genotype by environment (diet) interaction affects product yield and muscle quality traits in a line of rainbow trout selected for high (HY) and low (LY) fillet yield. Main effects of genetic line ($P < 0.05$) were observed for fork length and viscera yield, for which the LY line was greater, and carcass yield and fillet yield that were higher in the HY line (Figure 1a,b). Muscle quality traits of fillet color, muscle cellularity (Figure 1c) and muscle firmness exhibited main effects of genetic line ($P < 0.05$); findings indicated selection for fillet yield did not compromise fillet quality. A genotype by diet interaction ($P < 0.05$) was observed for viscera lipid content at the 2 kg harvest, for which viscera lipid content was similar between the HY and LY lines for the LL and HL diet, but greater ($P < 0.05$) in the LY line for the ML diet (Figure 1d). Main effects of diet were consistently identified for growth, viscera yield (Figure 1a), and indices of muscle quality ($P < 0.05$, Figure 1e). Collectively, these findings indicate that higher product yields in the selectively bred HY line of rainbow trout will persist across diets with variable crude lipid/energy contents.

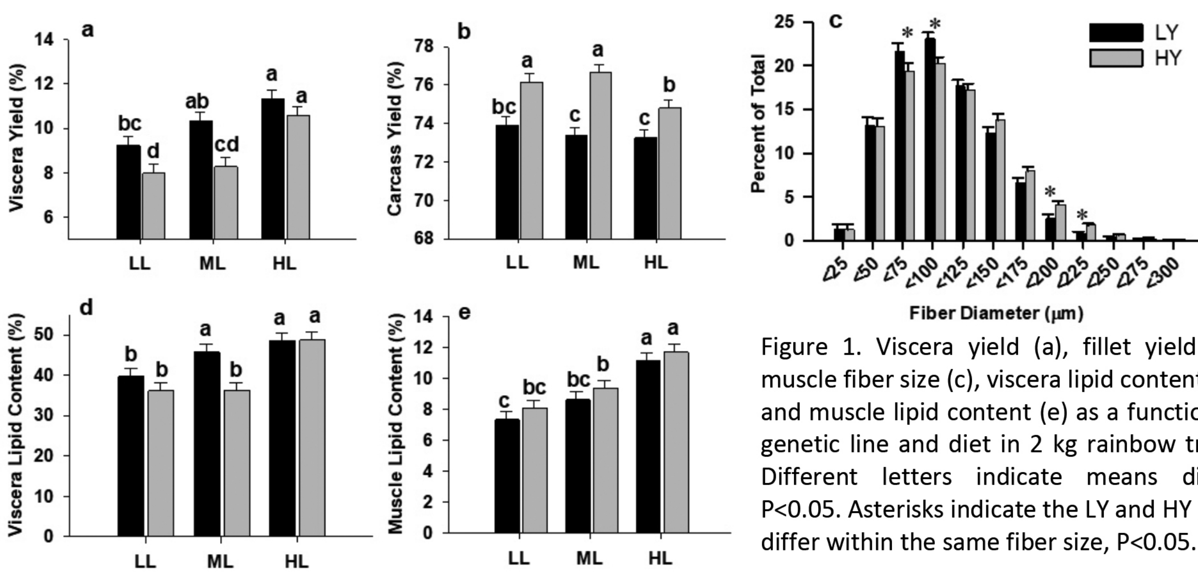

Figure 1. Viscera yield (a), fillet yield (b), muscle fiber size (c), viscera lipid content (d), and muscle lipid content (e) as a function of genetic line and diet in 2 kg rainbow trout. Different letters indicate means differ, P<0.05. Asterisks indicate the LY and HY lines differ within the same fiber size, P<0.05.

BUILDING A BETTER POWER POINT PRESENTATION

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Power point presentations have become the standard for Extension specialists to share information. When was the last time you saw a really good power point presentation? Let's face it, we all know how bad power point presentations can be, but how do you make a really good one? A number of authors have explored this topic and have come up with some very good ideas. Authors Chip and Dan Heath describe a technique in their book "Made to Stick" that can help presenters create a message that will stick with the audience. Others have written entire books on how to best present material using the power point medium. If the story of aquaculture and our research is worth telling then it is worth taking time to learn about some of these techniques.

DEVELOPING A FARMED SEA SCALLOP (*Placopecten magellanicus*) SECTOR IN THE GULF OF MAINE: LESSONS FROM INDUSTRY AND RESEARCH COLLABORATION

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Spurred on by a large domestic scallop trade imbalance on the order of ~$350 million USD annually, fishermen, farmers, and researchers have collaborated to kickstart a burgeoning farmed Atlantic Sea Scallop (*P. magellanicus*) sector in the Gulf of Maine. This nascent industry relies on technology initially developed in Japan, cultivation methods refined through decades of trial and error in Maine, and R&D aimed at understanding how the biology of scallops fits within a successful aquaculture operation. Americans consume ~$1 billion USD of scallop products (a large portion of which is sourced from overseas) annually. The opportunity exists for a Maine farmed scallop industry to feasibly capture a portion of this market share. In the last 10 years, multiple farms in Maine have come online, selling high quality, same-day landed scallops into a competitive U.S. seafood market with great success.

Despite the recent successes of these Maine scallop farmers, challenges remain that may hinder the continued expansion of the industry. Growers rely on wild-caught spat as the sole source of seed in the state, leading to potential supply risks. Similarly, economies of scale dictate that farmers need to increase production levels to reach cost parity with the market, but a lack of process optimization currently poses a labor bottleneck that may lead to cost overruns. Lastly, farmers have the advantage of controlling the size, season, and composition (i.e., whole vs shucked) of their products. However, selling within a market composed of domestically landed day-boat scallops, federally landed scallops, and imported scallop products poses challenges.

Researchers at the University of Maine, members of the scallop industry, and other Maine research institutions are trying to tackle these problems through targeted R&D. Hatchery development could reduce seed risk for farmers and provide a platform for selective breeding and improve stranis. Researchers and farmers are testing novel nursery methods, such as rigid trays, to make more efficient use of lease space, improve growth-rates, and reduce labor costs. Ear-hanging, pinning scallops to vertically suspended “dropper-lines”, may solve many of the challenges related to intensive lantern net handling while also improving growth rates. The scallop research portfolio in Maine consists of field trials supported by biological and economic modeling efforts, with the shared goals of reducing costs and risks for growers and improving the viability of the sector. This presentation will give an overview of the projects underway in the state, key results and lessons learned from collaboration, and future directions.

LARGE SCALE, OFFSHORE KELP PRODUCTION IN NORTH AMERICA: IDENTIFYING SCALING PATHWAYS AND R&D PRIORITIES FROM NURSERY TO HARVEST

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Farmed Kelp has great potential to serve as a low GHG raw material within fertilizer, pharmaceutical, nutraceutical, and biofuel supply chains. Similarly, recent research has focused on the cost and climate change mitigation potential of intentionally sinking kelp to remove atmospheric CO_2 (i.e., Carbon Dioxide Removal, CDR). In emerging kelp farming regions, products are typically processed (e.g., dried or milled) and sold as food. Regardless of the eventual end-use for cultivated macroalgae, current production levels will need to increase to realize economies of scale, reduce per unit C emissions, and accelerate kelp farming down the technological learning curve. The current cost structure of small-scale farms is fundamentally misaligned with the cost structures of raw materials supply chains. The lack of data on the economics of kelp farming at offshore, exposed sites thus represents a critical information gap.

Our research team from the University of Maine, with partners from The University of New Hampshire and Kelson Marine Co., have developed a hyper realistic techno-economic analysis (TEA) and Life Cycle Assessment (LCA) modeling framework for large scale kelp farming in the Gulf of Maine. We quantified the costs, C emissions, and productivity of a 1,000 acre operation across the full production chain, from nursery (Coleman et al., 2022a) through ocean cultivation (Coleman et al., 2022b). We were able to alter key production parameters, such as nursery inputs, kelp yields, and the C content of fuel, to track sensitivities within the model in terms of $ and kg CO_2eq per ton of kelp produced. We also ran a kelp CDR scenario in which farmed biomass was transported to an offshore site and intentionally deposited in the deep ocean.

The results of our analysis provide baseline cost estimates, highlight which elements of the production process may suffer from lack of scalability, identify C “hotspots”, and prioritize R&D areas that would facilitate the continued expansion of the sector. The modeling framework is flexible and can be used in the future to track the potential cost and emissions savings of improved nursery or ocean cultivation, and farm designs that make efficient use of ocean space.

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BUILDING THE DOMESTIC MARKET FOR SEAWEED: LESSONS LEARNED FROM FIVE YEARS OF VALUE CHAIN COORDINATION

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According to NOAA, seaweed is the fastest growing aquaculture sector in the United States. The global seaweed market is expected to surpass \$95 billion by 2027, up from \$40 billion in 2020. While this nascent market represents prospective economic and environmental benefits for coastal communities, and provides a scalable zero-input food source, the realization of these benefits requires collaboration across the value chain. A wave of interest in seaweed has resulted in the misconception that the domestic industry is relatively established. However, seaweed entrepreneurs enter an emerging market, with uncertainty about production capacity, regulations, food safety, and processing capabilities. The industry requires resources and right-sized value chain support to align respective motivations and simultaneously scale supply and demand.

Value chain coordination is the development of relation-based infrastructure for a supply chain, including the creation of networks, information sharing channels, and direct partnerships. Over the past five years, GreenWave has emerged as a connector for seaweed farmers, processors, and buyers. Initial value chain coordination efforts were a natural outgrowth of informal and ad-hoc introductions, which proved difficult to track and resulted in limited outcomes. To address these challenges, GreenWave shifted its programming to incorporate formal high-touch support, intentionally uncovering supply and demand, and working directly with suppliers and buyers to facilitate right-sized connections. Although fruitful, limited access to these services created an unintentional bottleneck. To encourage further collaboration, GreenWave developed Seaweed Source, a web-based application that enables buyers to browse profiles of active seaweed farmers and reach out to request information about their supply. By understanding the needs of stakeholders through interviews and user research, GreenWave is expanding programming to engage farmers, processors, and buyers more effectively. The Market Development Program is focused on building resources and curriculum for buyers and processors, plus piloting accelerator partnerships, a sampling program, and industry cohorts. Through the Kelp Climate Fund, the organization is providing direct climate subsidies to regenerative ocean farmers, incentivizing the industry to scale.

In this session, we'll share lessons learned from the evolution of GreenWave's value chain coordination efforts and how values-aligned partnerships create space and opportunity to build the domestic market for seaweed.

AQUACULTURE EDUCATION THROUGH THE LENS OF HAWAIIAN CULTURE: FISHPONDS IN HAWAII

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The oldest fishpond to be carbon dated is found on the main island of Oahu, on the windward side. Nu`upia fishpond dates back to 1048 c.e. The first to be ever mentioned in legend is Leho `ula on the island of Maui, in Hana. It was estimated that there were 340-360 fishponds in operations prior to the arrival of Captian Cook in 1778. Aquaculture has been in extience in Hawaii for a thousand of years, and serves as a model for idengious wisdom into the future. It is through the eyes of our descendants that we have been given a gift to restore our local ponds through education both in and out of the classroom.

It is through extensive aqualculture, hawaiian fishponds provided a sustainable means of food supply to the community. A care taker as well as his family, was afforded the task of taking care of the pond by managing its resources, people, constructing / maintaining the fishpond wall, channels and gates, and tending to the overall health of the pond and the fish. Hawaiian Fishponds also intregrated the use of irrigation system, stone fishtraps and agriculture. It was the devolpment of the mākāhā (slucie grate), that changed their way of life from hunter gatherer to farmers. This was seen as a huge milestone in the way fish was to be harvested.

Education has been a great pathway to teach our students about the importance of our rich tradtions and its practices. Pacific American Foundation, a non-profit, has written a number of curriculum specific to fishponds that range from elementary to high school. It is through the doing that one is able to learn. Our curriculum gives students the opportunity to learn by teaching theory in class and then being able to apply with hands on activites at the site. We have a number of different stations that students can rotate through; the more popular ones are 1) Land divison: Ahupua`a, 2) Enginering: Slucie Grate (Mākāhā) , 3) Stewardship: Enviro-Cleanup and how to care for the land(Malama Aina). Another station that is popuar is *Life in the pond*. In this station students get to see some of the basic species found in the fishpond thru a microscope and in small fish tanks.

It is through education, in a Hawaiian fishpond context, that we have been able to tell the story of our ancestors to community members, students, and teachers. Addtionally education as helped in restorative aquaculture using different trophic species to help both eviromenatlly and food supply. It is through education we can further understand the enviromental challenages that have prohibited the growth of fish and other native species in the pond. Sediment, D.O. and circulation are the biggest factors contributing to the health of most ponds in the recent years. It is through the learning of our ancestors and we can only now begin to understand how to leverage education to help us revitialze and restock our fishponds.

NURTURING THE SUCCESSFUL GROWTH AND MATURATION OF THE DOMESTIC SEAWEED AQUACULTURE INDUSTRY

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Seaweed aquaculture is a growing industry in the United States. Although the number of farms have increased since 2019, nationally the industry is at varying levels of production and scale. The National Seaweed Hub was established in 2019 to serve as a clearinghouse for science-based, non-advocate resources about domestic seaweed aquaculture. Through a comprehensive needs assessment, seaweed stakeholders identified four common challenges preventing the industry's expansion: Market Opportunities, Post-harvest and Processing Infrastructure, Regulations, and Production Systems. Facilitated by Sea Grant and National Sea Grant Law Center staff, diverse stakeholder-driven work groups were formed, based on these common challenges, with the goal of developing strategies with achievable outcomes. With the extension of the National Seaweed Hub, additional resources and engagement opportunities will be developed to further foster collaboration of seaweed stakeholders across the nation.

An update on the National Seaweed Hub activities will be provided. More information can be found on the Seaweed Hub's website (www.seaweedhub.org).

CULTURE OF *Francisella noatunensis* subsp. *Orientalis* AND IN VITRO RESPONSE OF NILE TILAPIA LEUKOCYTES

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Francisella noatunensis subsp. *Orientalis* (FNO) is a facultative intracellular bacterium and the causative agent of franciselliosis, a disease that affects tilapia production in cold water temperatures. Due to the intracellular nature of franciselliosis, it is difficult to diagnose and treat in production operations. Thus, necropsies are a valuable tool for diagnosis. To isolate the bacteria, different methods, media and supplements are utilized. This current trial aimed to optimize the conditions (assay I) and preservation of FNO (assay II) and verify the effects of the inactivated bacteria on the proliferation of leukocytes *in vitro* (assay III). In assay I, treatments groups were two nutritional supplements, Nile tilapia blood serum (TBS) and fetal bovine serum (FBS); two concentrations of these supplements (3 and 10%) and bacteria cultured at four different times (12, 24, 48 and 72h). In assay II, the treatments were stored at varying temperatures (-20°C, -80°C, and -196°C) to examine viability. Müller Hinton culture medium was supplemented with 1% glucose and 0.1% L-cysteine hydrochloride monohydrate, along with 3% TBS or FBS. This media was used as a standard, and experimental treatments consisted of different glycerol concentrations in the culture medium (either 20, 30, or 50%). In assay III, FNO was inactivated with formaldehyde, and the impact of this inactivation was evaluated with leukocytes from different organs. No FNO growth performance differences were discerned compared to 72 hours of cultivation. However, hemocytometer counts showed that after 12h of cultivation, the treatment with FBS at a concentration of 10% showed a higher amount of cells/μl than the other treatments (P=0.002). In 24 hours of culture, only the treatment with FBS of 10% showed a significant difference from the treatment with TBS of 3% (p= 0.0196). After 48 h of culture, FBS at a concentration of 3% showed a significant difference with TBS at 3% with the treatment of TBS at 10% (P= 0.622). For cryopreservation, a better performance was found using 20% glycerol in FBS (P = 0.297). However, after 7 d, there was a statistically significant difference in glycerol concentrations. When analyzing the temperature, the TBS with 30% glycerol at -80°C showed a significant difference between the FBS with 50% glycerol at -196°C (P=0.010). Concerning *in vitro* leukocyte responses at different bacterial concentrations, cell proliferation was examined following exposure to inactivated FNO. The leukocyte proliferation values of groups provided Concavalin A and 10^4 cells/μL were different (P= 0.014) compared to the 10^6 cells/μL group. In the spleen cell culture, there was a significant difference between the concentration of bacteria in the 10^5 cells/μL dilution in relation to the unstimulated control group (P=0.046). However, a significant difference was found between multiple comparations in liver cell culture. These investigations have provided additional insight into FNO storage, handling, and viability methodologies for fish health researchers and diagnosticians.

INVESTIGATING THE INTEGRATION OF ROPELESS GEAR TECHNOLOGY FOR OFFSHORE BOTTOM BIVALVE AQUACULTURE TO REDUCE ENTANGLEMENT RISK WITH MARINE MAMMALS

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Large whales are protected under the Marine Mammal Protection Act and several species of whales are listed under the Endangered Species Act. With a dwindling population of less than 400, North Atlantic right whales are one of the most critically endangered species, and entanglement in lines associated with fishing gear remains a significant threat to this species' survival. Reducing whale entanglements is therefore a priority for NOAA fisheries. As marine aquaculture expands, concerns that gear could introduce entanglement risks may limit industry growth. A number of ropeless technologies developed for use by the commercial fishing industry to reduce entanglement risk have conceptual value for integration within the aquaculture industry. This presentation discusses several varieties of ropeless technology for the bottom culture of scallops, mussels and oysters in the Northeastern United States and optimal environmental conditions for each species.

FACTORS INFLUENCING THE HEALTH OF FARMED PACIFIC OYSTERS *Magallana gigas* IN SOUTHEAST ALASKA

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Mariculture is a rapidly growing industry in Alaska, and includes farmed Pacific oysters (*Magallana gigas*). Harmful algal blooms (HABs), which can cause paralytic shellfish poisoning (PSP), are a major challenge for Alaska oyster farmers, along with choosing farm sites for optimal oyster growth. This project seeks to determine environmental factors associated with HABs and oyster health in the high-latitude estuarine environment of southeast Alaska with the goal of predicting HAB events and aiding in site selection for future farms as the industry grows.

We conducted weekly water column, phytoplankton, and oyster tissue sampling at an oyster farm in Juneau, AK. We observed significant seasonal variation in both environmental variables and phytoplankton community composition in southeast Alaska over the four years of monitoring (Figure 1A and 1B).

Preliminary results suggest that higher lipid content in oysters may align with peaks in phytoplankton biomass, and that lipid content tends to be higher at higher water temperatures and lower salinities (Figure 1C). Thus far, toxin levels in sampled oysters have been below safe thresholds for consumption, but analyses are ongoing to identify drivers of toxicity. Determining which factors contribute most strongly to the health of farmed oysters, as well as to toxin levels in oyster tissue, is critical in allowing oyster mariculture to expand in a safe and profitable way in southeast Alaska.

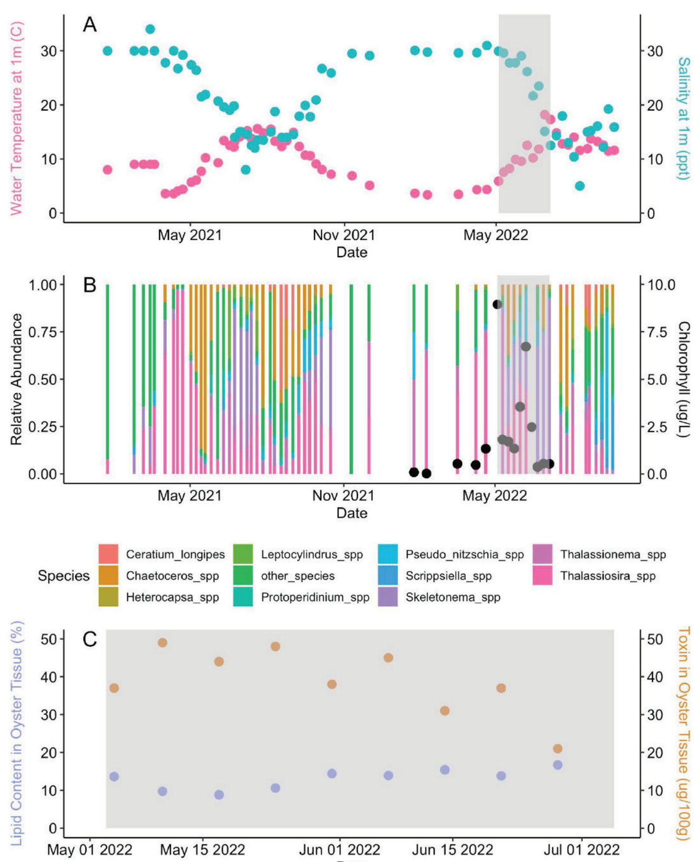

Figure 1. Time series plots of a subset (January 2021-September 2022) of monitoring data, including water temperature (℃) and salinity (ppt) at 1m (A), relative abundance of phytoplankton genera and chlorophyll levels (ug/L) at 1m (B), and lipid content (%) and toxin levels (ug/100g) in oyster tissue (C).

SPERM CRYOPRESERVATION AND IN-VITRO FERTILIZATION OF AXOLOTL *Ambystoma mexicanum* FOR REPOSITORY DEVELOPMENT

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Amphibians draw great attention from biologists because of their importance as biomedical research models and conservation crisis, but housing and maintaining live animals is expensive and risky, especially as numerous transgenic and mutant lines are continually developed. The storage and management of cryopreserved germplasm can provide a safe and cost-effective alternative, reducing the number of live animals that need to be maintained to meet research demands. In collaboration with the *Ambystoma* Genetic Stock Center (AGSC), we are developing a reproducible cryopreservation pathway for the axolotl *Ambystoma mexicanum*. Although some studies have shown initial feasibility of cryopreserving *A. mexicanum* spermatophores, this method of collection is inconsistent, and standardized quality evaluation for *in-vitro* fertilization using thawed sperm has not yet been developed. We tested multiple factors and their interaction on sperm quality during storage and freezing, such as various osmotic pressures of an extender solution (Hanks' balanced salt solution), different cryoprotectants (DMSO, DMFA, glycerol, methanol), and sugar additives (sucrose, trehalose). Based on this we have developed a practical pathway for *in-vitro* fertilization using thawed sperm collected through hormonal induction and abdominal massage. These results provide the groundwork for establishment and operation of an *Ambystoma* germplasm repository to protect, maintain, and distribute valuable genetic resources. This also has relevance to protection of genetic diversity of the small remaining wild populations of this species.

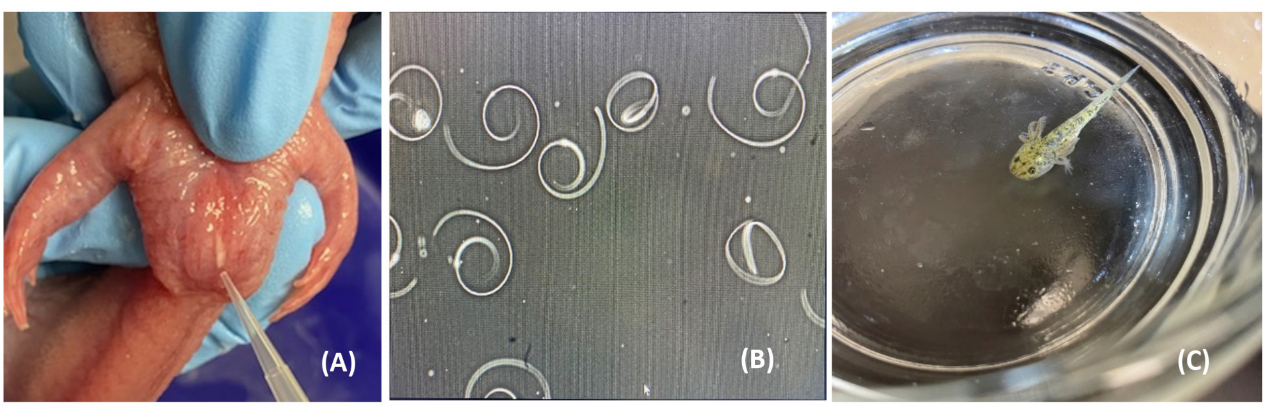

Fig. 1. (A) Collection of *Ambystoma mexicanum* sperm through abdominal massage of an anesthetized adult male. (B) *A. mexicanum* sperm under light microscope. (C) Offspring produced with thawed *A. mexicanum* sperm.

EMPOWERING HISPANIC LEARNING AT CAL POLY HUMBOLDT

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College completion rates in the U.S. continue to be tied disproportionately to race and ethnicity, socioeconomic status, and family history in higher education. Among all the racial/ethnic groups in the U.S., Latinx students have the lowest graduation rate in undergraduate and graduate studies (Figure 1). Latinx enrollment in colleges is 19% nationwide, but there are some places like California where the enrollment is 40%. Of the 766 bachelor's degree holders in agriculture, just 5.6% belong to Latinx (compared with 82% for White). Why there is a disproportionate graduation rate exist for Latinx students? There are myriad contributors to this equity gap, such as Northern American higher education learning environments being biased towards low context or individualistic cultural frameworks. This means that high context collectivistic culture, such as Latinx students, must learn to adapt to survive, or they will drop out of school.

To improve the educational experiences of Humboldt Latinx students, Cal Poly Humboldt began partnering with ESCALA Educational Services of Santa Fe, NM to help Humboldt's practitioners understand how to implement culturally responsive pedagogy and equitable practice. Between 2017 and 2021, 53 Humboldt educators earned the ESCALA's College Teaching & Learning in HSIs Certificate, and 5 earned the ESCALA STEM-mini Certificate. This presentation will review different culturally sustaining educational practices implemented at Cal Poly Humboldt.

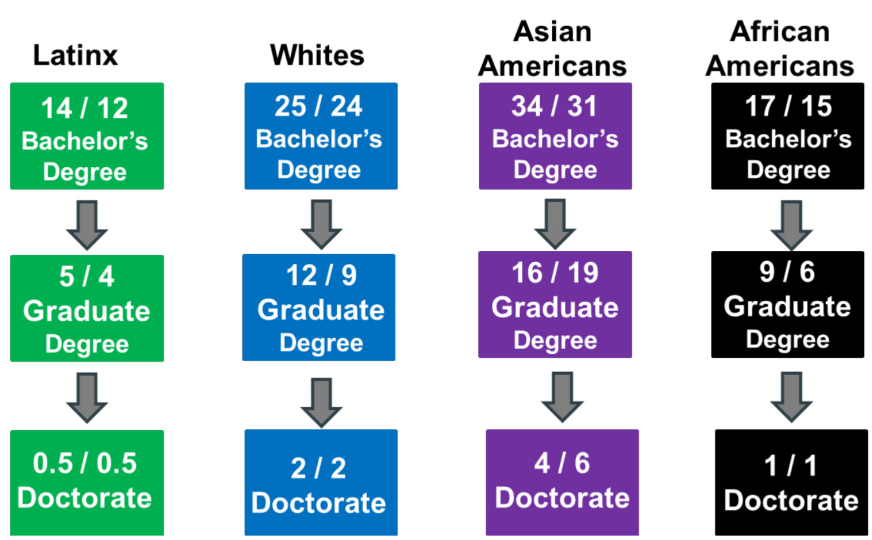

FIGURE 1. Percentage of graduation by racial/ethnic groups. Each square has the percentage of Females / Males. Data from the 2021 U.S. Bureau of the Census

WHERE DO DOUBLE-CRESTED COMORANTS GO AFTER ROOST DISPERSAL

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The Wildlife Services-National Wildlife Research Center's Mississippi Field Station working in conjunction with Alabama WS operations conducted 61 dispersal events on Double-crested cormorant (DCCO) night roosts. Approximately 93,200 DCCO (98 %) 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 dispersal. We deployed a total of 38 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 494,874 DCCO locations in 2020, 2021 and 2022.

Six GPS-DCCOs were present at the night roosts during 14 roost dispersal events with some individual birds being dispersed from 1 to 4 times during February-March 2021 and one DCCO was present at the night roost harassed in early December 2020. These six 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.

The habitat of DCCOs during winters consisted of 14% wetlands, 22% waterbodies, 26% fish ponds, and 38% other types. After harassment we found that 3 of the 6DCCOs 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 6 DCCO did not increase their daily distance after harassment and foraged in the same areas before and after harassment. A DCCO stopped using fish ponds within a week after the night roost harassment. Another DCCO showed an initial reduction in the use of fish ponds followed by fluctuations in the use of fish ponds. We plan to deploy 10 additional GPS transmitters from November 2022-February 2023 to increase our sample size and more accurately assess the impacts of night roost harassment on the night roost use, foraging areas, and daily movements.

COMPARING AVAILABLE DETERRENT METHODS TO REDUCE DOUBLE-CRESTED CORMORANTS ATTEMPTS TO ROOST ON FLOATING OYSTER CAGES

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The Wildlife Services-National Wildlife Research Center's Mississippi Field Station working in conjunction with Mississippi State University conducted a study to test the effectiveness of several commercially available physical bird deterrents available on the open market to reduce roosting time on floating oyster cages. Our hypothesis was that reducing birds loafing time on floating oyster cages would reduce the potential for feces to increase coliform counts in water and oysters which could lead to the closure of oyster farms due to excessive bacterial counts.

Fifteen Double-crested cormorants (DCCO) were captured in night roosts in Mississippi or Alabama using a customized capture boat, flood lights, and dip nets and then transported to the National Wildlife Research Center Mississippi Field Station avian test facility. Five DCCO were released into each of three aviary enclosures containing a 0.1-acre pond stocked with catfish fingerlings.

Each pond contained a floating oyster cage to which one of 6 deterrents was applied and was monitored by 3 motion activated cameras that recorded DCCO positions and movements. After capture DCCO were given 1.5 weeks to acclimate and one additional week of a control period. After that each week consisted of 5 days of a deterrent and 2 days of rest with no deterrent. Pond treatments were randomly reassigned so that each week a different pond was the control pond and the treatment ponds each had a new deterrent method. Deterrents were removed from all oyster cages and ponds for two additional weeks with no deterrent methods to see how DCCO would react.

Deterrent methods that were tested included: Float mounted triangle, Bird B Gone Spinning Bird Deterrent®, Scarem Kite®, Zip ties around floats, Gullsweep Bird and Seagull Deterrent®, and Bird Spikes for Bird, Cat, Squirrel, Racoon Animals Repellent®. Data collected by deterrent method included 1) number of times an individual DCCO successfully landed on floats, 2) number of individual DCCO's on a float, 3) amount of time individual DCCO spent on float, 4) number of times an individual DCCO unsuccessfully attempted to land on floats. Over 184,000 photos were reviewed. Data analysis from this study is underway with preliminary results indicating a variation in the effectiveness depending on the deterrent method tested.

COMPARISON OF INDUCTION AND RECOVERY TIMES UNDER TWO DIFFERENT TEMPERATURES IN THE FALSE PERCULA CLOWNFISH *(Amphiprion ocellaris)* USING THE ANAESTHETIC AQUI-S® 20E

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Anesthetics serve a variety of purposes in commercial aquaculture, including sedation of fish. In the United States MS-222 is the only anesthetic approved for use in aquaculture. MS-222 is a suspected carcinogen and can cause serious retinal damage to humans. Aqui-S® 20E is an alternative anesthetic derived from clove oil. Here, we tested the use of this anesthetic on the False Percula clownfish (*Amphiprion ocellaris*), a popular marine ornamental, under two different temperatures, 30°C and 25.5°C. Our goal was to assess the effect of rearing temperature on induction and recovery. A total of 48 fish were divided evenly between two treatment groups. Twenty-four fish were assigned to the 25.5°C tanks, and twenty-four fish were assigned to the 30°C tanks. For each temperature treatment, 12 fish were kept as a control group, in order to observe and compare post-recovery effects. Upon arrival to our facility, fish were held for at least two weeks at the specified temperatures and at a salinity of 35 ppt. Fish were induced using an Aqui-S® 20E concentration of 200 mg/L. Every 30 seconds, the fish's reactions were recorded until they were considered to be fully induced. Induction was attained when the fish was stationary for 30 seconds and when the fish did not respond to pulling of the caudal peduncle. Once a fish was fully induced they were then placed in a recovery tank where they were observed every 30 seconds, until they fully recovered. Full recovery was defined as when a fish was able to swim freely, and reacted to the presence of a net in the water by swimming away from the net. Induction time took an average of 153 seconds at 25.5°C and 165 seconds at 30°C. Recovery time took an average of 155 seconds at 25.5°C and 174 seconds at 30°C. Fish did not show any adverse post-recovery effects at either temperature. In general, recovery and induction times seem to increase with higher temperatures.

USING FILM TO CONNECT CONSUMERS AND CHEFS WITH SEAFOOD PRODUCERS: A FLORIDA PERSPECTIVE

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Most Floridians, both consumers and chefs, are unaware of the breadth of the aquaculture industry in the state. Sereia Films has created videos featuring a clam farm, a land-based pompano farm, a land-based shrimp farm, a sturgeon farm, and an oyster farm. Our work introduces Floridians, and the greater public, to the diverse types of aquaculture taking place in Florida and the methods used on those farms. We give viewers an overview of these industries, while highlighting specific farmers and their stories.

Many chefs are interested in where the food they're serving comes from and love to visit farms in person. As this isn't always possible, these films are a way to still create that connection. During this session, we'll share clips of the films, an overview of our process, and the impacts these films have had in South Florida.

SUSTAINABLE MARICULTURE DEVELOPMENT FOR RESTORATION AND ECONOMIC BENEFIT IN THE EVOS SPILL AREA: AN INTRODUCTION TO THE RECON

Alysha Cypher*, Katrina Hoffman, Ginny Eckert, Julie Decker, John Whissel, Rob Campbell, Quenin Fong, Melissa Good, Jordan Hollarsmith, Amanda Kelley, Brenda Konar, Christopher Long, Alexei Pinchuk, Michael Rehberg, Anne Schaefer, Schery Umanzor.

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The Mariculture Research and Restoration Consortium (Mariculture ReCon) is a multi-year investigation that takes a multifaceted approach to grow the mariculture industry in Alaska. The ReCon will address five-farm centered (Fig. 1) and integrative components that focus on the restoration and economic development potential of mariculture in Kodiak, Kachemak Bay, and Prince William Sound, AK. Species of interest include Pacific oysters (*Crassostrea gigas*), sugar kelp (*Saccharina latissima*), bull kelp (*Nereocystis luetkeana*), and ribbon kelp (*Alaria marginata*). The objective of this program is to support restoration, habitat enhancement, and economic development through research and partnerships between scientists and seaweed and shellfish farmers. Our program will support this growth by bringing farmers, scientists, and economists together to evaluate environmental conditions that enhance crop yields, effects of farming on biological communities, and mariculture product and market development.

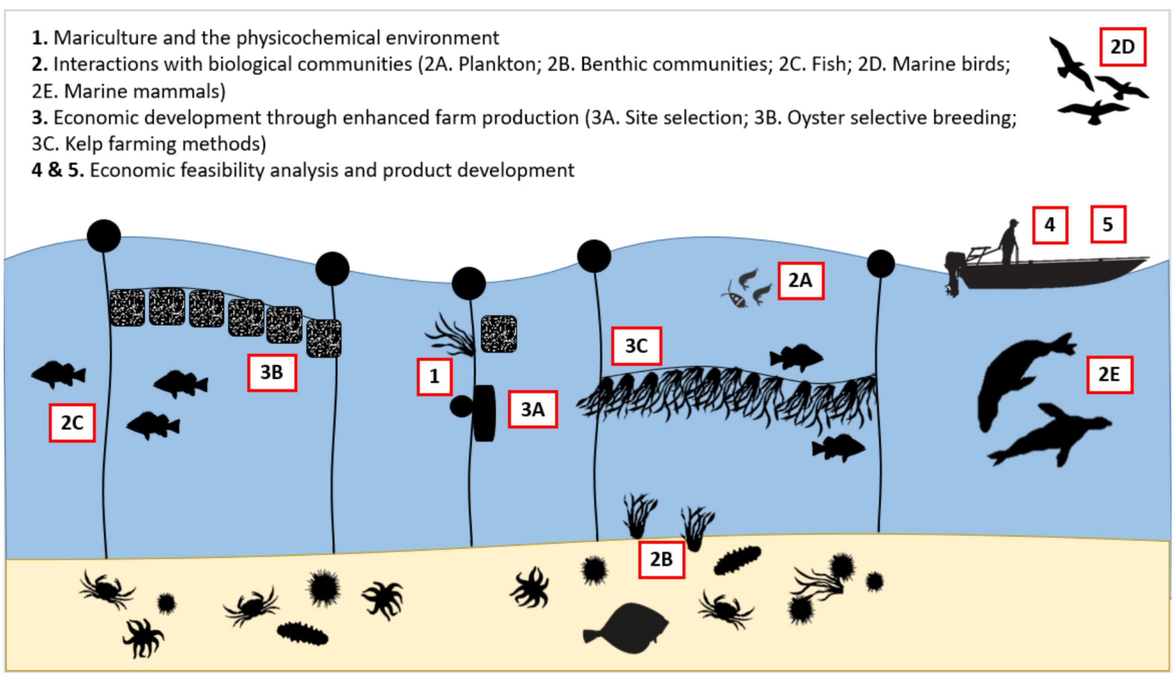

Figure 1. Research components for the Mariculture Recon Project that will evaluate 30 hypotheses concerning the physical environment (1), biological communities (2A-2E), farm production (3A-3C) and economic development (4-5).

PURPOSES OF TETRAPLOIDY AND DISTANT HYBRDIZATION IN FISH: KOI CARP (FEMALE) X GOLDFISH (MALE) EXAMPLE

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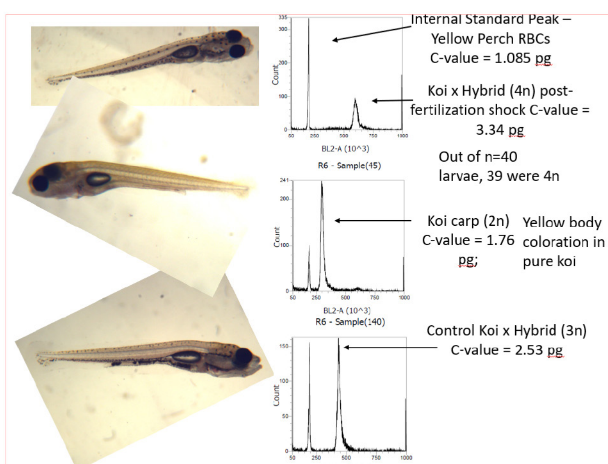
Cyprinid fishes are the most sustainable human food production in aquaculture. However, these fishes in other aquatic systems may contribute to ecological disruption due to their potential as "invasive" species. Polyploidy and distant hybridization are creative genetic tools to establish improvements in growth performance and production efficiency but can also serve conservation goals and act as barriers (sterility) to protect wild fish stocks and their diversity. In other words, these methods are not serving exclusive or opposite research targets but are rather complimentary technologies. Triploidy is recognized and currently utilized as a major tool to eliminate concerns of unintentional release of domesticated fish and to protect the genetic heritage of wild stocks. However, induced triploidy via application of physical shocks also results in high frequency of skeletal deformities and low viability. Therefore, production of meiotic-shock-induced triploids involves inferior survival, compromised marketability (deformities) and significant cost stemming from needs of (individual) confirmation of sterility (polyploidy). Alternatively, crossing diploid and induced tetraploid individuals yields biological triploids that do not undergo physical shocks and thus eliminates the inferior characteristics of induced triploids. In addition, establishment of tetraploid broodstock lines has biological, ethical, and financial incentives.

Tetraploid cyprinids hybrids (goldfish female x common carp male) were obtained (1) by crossing diploid somatic hybrids producing diploid gametes, or (2) by crossing triploid hybrid females with common carp males. We explored the possibility that common carp diploid females fertilized with hybrid producing diploid sperm and progeny subjected to physical shock will result in tetraploidization. Initial outcome of this experiment was highly encouraging as larval stage from this procedure were almost exclusively tetraploids or mosaics (Fig. 1). However, surviving juveniles (4 months old) were confirmed by flow cytometry mostly diploids. We continue to grow this batch of fish and the next generation (diploid gametes) might prove to be the founder of tetraploid linage.



THE HISTORY BEHIND AUBURN UNIVERSITY'S ALABAMA FISH FARMING CENTER

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The Alabama Fish Farming Center (AFFC) was established in 1982 to assist a growing catfish industry. By 1990, the demands on the AFFC's services made the original building located at the Farmer's Market Authority in Greensboro, Alabama, no longer adequate. Producers began working with state legislators and successfully obtained funds through the Alabama Soil and Water Conservation Committee (SWCC). The SWCC then set up contracts with Auburn University Fisheries Department, the Alabama Cooperative Extension System, the USDA Soil and Water Conservation Service, and the Hale County Soil and Water District to hire a Fish Health Specialist, Aquaculturist, Agricultural Engineer, Administrator and a Technician. Richard Avery, a retired probate judge, obtained funds for a permanent home for the AFFC. Due to the continued fiscal shortfalls due to various reasons, Auburn officially took control of the AFFC in 2007. The AFFC is still located in this building today and is currently expanding. The AFFC has helped commercial farmers raising catfish, crawfish, sportfish, tilapia, and marine shrimp in west Alabama with Extension, research, water quality management, fish disease diagnostic programs, and microbiology testing. Providing free and accessible assistance alleviates costs to farmers that would be outsourced and could be time consuming and expensive. The AFFC also serves as a conduit for communication between the fish farming industry, Auburn University's research and Extension community, and state/federal agencies to support the development of the aquaculture industry. With the evolution of the catfish industry through the years, the AFFC has adjusted to the changing needs of the catfish producers in Alabama. Initially, the center focused on technical assistance and has recently since shifted towards a larger research role, while still assisting catfish farmers. The AFFC is presently staffed with two Extension faculty, an accountant, two technical support staff members, a postdoctoral research fellow, a part-time fish health specialist, and several graduate students. Although the team is modest in size, they carry out a large volume of work in support of the Alabama aquaculture industry.

Figure 1. AFFC 1982

Figure 2. AFFC 2022

IMPROVEMENT OF GENETICALLY ENHANCED NILE TILAPIA *Oreochromis niloticus* AT KENTUCKY STATE UNIVERSITY

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Globally, tilapia (genus *Oreochromis*) is the most widely farmed and second most important group of food fishes behind carps. Nile Tilapia (NT) is the most cultured species among tilapia because of its commercially valuable traits. Efforts in advancing these traits have led to genetic improvement through selective breeding programs such as the Genetically Improved Farmed Tilapia (GIFT strain) and genetic sex regulation (YY males). KSU's NT research and Extension work aims to secure, develop, and disseminate genetic, educational, and training resources to advance U.S Tilapia Aquaculture. Objectives pursued include: (1) acquiring various NT strains, (2) crossbreeding YY males with females of different strains, (3) evaluating crossbred progeny for growth rates, sex and color segregation, and (4) identifying genetically improved crosses demonstrating superior traits for local, small-scale production.

Experimental fish were reared in recirculating aquaculture systems (RAS) at the KSU Aquaculture Research Center, Frankfort, KY. Tilapia broodstock were acquired for research studies from Louisiana Specialty Aquaculture LLC (Robert, LA), Miami Aquaculture Inc. (Boynton Beach, FL), and Fishgen Ltd. (Swansea, UK). Two strains of red YY males were bred with three strains of NT females in four crosses (Figure 1). Progeny from the crosses were grown for 167 days in three replicate tanks (Figure 2). Final mean weight, feed conversion ratio (FCR), fillet yield (%), sex proportion, and color segregation were analyzed. The red Til-Aqua YY males crossed with dark GIFT females resulted in significantly improved growth (57 to 75% larger and 1.6 to 1.8 times the daily growth rate) compared to the other crosses. This cross was reproduced in February and March 2022, and the fish were made available to farmers in six Kentucky counties, and one Florida county. Future work will address marketing, and human nutrition of NT produced in Kentucky.

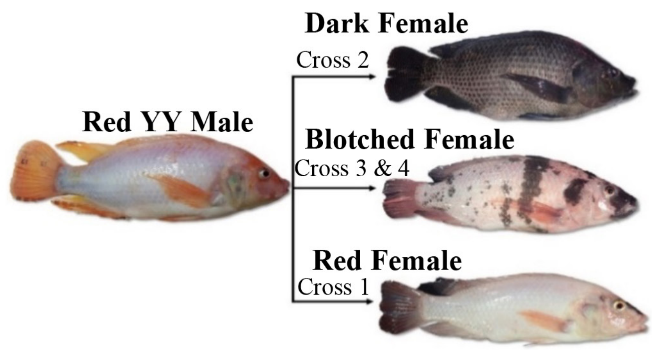

Figure 1. Representative images of each cross.

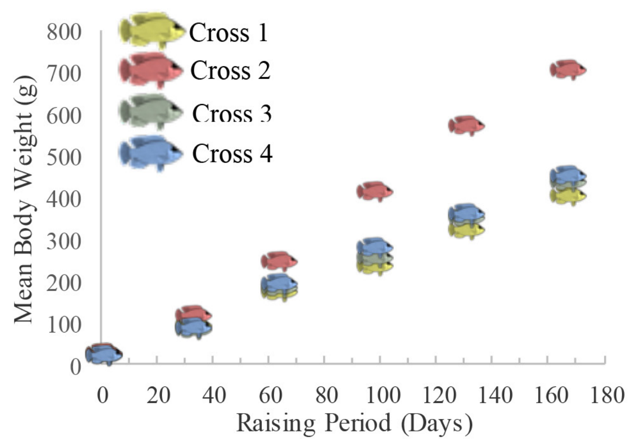

Figure 2. Growth performance of each cross for 167 days. Figure modified from Novelo et al. 2021.

THE INTRA HOUSEHOLD FISH CONSUMPTION PATTERN IN BANGLADESH

Das

Global fisheries and aquaculture industry has been experiencing dramatic change in the demand and supply pattern in terms of fish sources and species during the last three decades. In particular, Bangladesh has been experiencing structural changes with sharp decline in inland capture fisheries and rise in inland culture based fish production affecting fish consumption pattern of different income groups and between rural and urban households. This study examines the fish consumption pattern in Bangladesh based on the Bangladesh National Income Expenditure Survey Data from 2000 to 2016. The study summarizes fish consumption pattern by fish sources, species and households' residential status. In consistent to the earlier studies, the fish consumption has increased over time for every category of household- rural urban and income quantile. However, the lowest income quantile i.e., poorest household has experienced fastest growth in fish consumption. Low value cultured fish species are highly demanded by the lowest income quantile, whereas high value cultured fishes are mainly consumed by the household in upper income quantile. Furthermore, a large share of fish consumption expenditure of lowest quantile households is for low value capture based small indigenous species and culture based catfish. In addition, marine fishes are mainly consumed by the urban and households in the upper income quantile. The findings indicate that culture based fisheries is contributing in improving fish consumption pattern irrespective of income groups and residential status of household especially of lowest income quantile.

ESTIMATING THE EFFECTS OF GENERIC ADVERTISING ON MARKET DEMAND: AN ADL APPROACH

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The advantages of the autoregressive distributed lag (ADL) model for estimating the effects of generic advertising on market demand are evaluated by applying the model and attendant methods to data used in a recent study of Norway's export promotion program for whitefish. The dynamic specification differed greatly depending on model selection criteria (Akaike Information, Hannan-Quin, Schwarz, and Adjusted R2) (Table 1). Despite this there was little to choose between the specifications in terms of the estimated long-run demand elasticities. The estimated short-run elasticities differed among the specifications, with the model selected by the Hannan-Quin criterion indicating a more elastic response to income than the model selected by the Schwarz criterion. The bounds test for cointegration, a special feature of the ADL approach, proved useful in distinguishing between the appropriateness of quantity- and price-dependent specifications of the demand equation. Tests for weak exogeneity of the regressors indicated adjustments in quantity are 5.5 times more important than adjustments in price in resolving dynamic disequilibria caused by random (monthly) shocks to long-run demand.

Table 1: Lag Orders for the Q-Dependent and P-Dependent Specifications of the Export Demand Equation for Norway's Whitefish as Determined by Alternative Model Selection Criteria

Criterion	Lag Order	
	q-dependent model	*p*-dependent model
Adjusted R2	A(4,1,3,3,3)	A'(3,4,4,1,3)
Akaike Information (AIC)	B(1,1,3,1,3)	B'(2,2,0,1,3)
Hannan-Quin (HQ)	C(1,1,2,1,1)	C'(2,2,0,0,0)
Schwarz (SC)	D(1,1,0,0,0)	D'(1,1,0,0,0)
WC's specification	E(1,0,0,0,2)	NA
F-tests of alternative dynamic specifications:		
A vs. B	$F_{(5,141)} = 1.049$ ($p = 0.392$)	Fail to reject B
A vs. C	$F_{(8,141)} = 1.380$ ($p = 0.210$)	Fail to reject C
A vs. D	$F_{(12,141)} = 2.260$ ($p = 0.012$)	Reject D
A vs. E	$F_{(11,141)} = 2.464$ ($p = 0.008$)	Reject E
A' vs. B'	$F_{(7,140)} = 1.332$ ($p = 0.239$)	Fail to reject B'
A' vs. C'	$F_{(11,140)} = 1.594$ ($p = 0.107$)	Fail to reject C'
A' vs. D'	$F_{(13,140)} = 1.958$ ($p = 0.029$)	Reject D'

EVALUATING THE EFFECTS OF NITRATE-NITROGEN (50–100 MG/L VS. 150–200 MG/L) ON POST-SMOLT ATLANTIC SALMON *Salmo salar* GROWTH, HEALTH, AND HEART RATE MEASURED BY BIOMONITORS

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Nitrate accumulates in recirculation aquaculture systems (RAS) when water exchange is reduced and denitrification technologies are excluded from the process flow. Although nitrate is generally less toxic to fish than ammonia and nitrite, several studies have reported sublethal effects to RAS-produced species. During an onsite study, rainbow trout exhibited toxicity symptoms when nitrate-nitrogen (NO_3-N) was maintained at 90 mg/L. On the contrary, another onsite study found that post-smolt Atlantic salmon were unaffected by 100 mg/L NO_3-N. Follow up research is underway to determine if higher NO_3-N concentrations (150–200 mg/L) negatively impact post-smolt Atlantic salmon growth, health, and welfare in low exchange RAS.

To begin the 7-month study, 35 all-female Atlantic salmon (282 g mean weight) were surgically implanted with biomonitors (Star-Oddi, Iceland) that continuously record fish heart rate. These data are expected to elucidate any stress response triggered by accumulating nitrate. Salmon receiving biomonitors were kept in recovery tanks for one month and then divided into six replicate RAS along with equal numbers of untagged fish from the base population. After stocking, feed loading rates were gradually increased causing NO_3-N to accumulate to 80 mg/L. Peristaltic pumps were then used to repeatedly dose concentrated sodium nitrate in three RAS resulting in a gradual increase to 150 mg/L NO_3-N (Fig. 1). Sodium sulfate was added similarly in three control RAS to balance ionic concentrations while maintaining 50–100 mg/L NO_3-N. At the time of abstract submission, salmon in both treatments were ~1.4 kg and adjustments were underway to increase NO_3-N to 200 mg/L. Salmon growth, health, welfare scores, and heart rate data will be reported at the meeting.

Knowledge gleaned from this research could redefine the upper nitrate threshold for post-smolt Atlantic salmon production in RAS. The findings could also lead to reduced water use requirements and minimized necessity for inclusion of denitrification technologies.

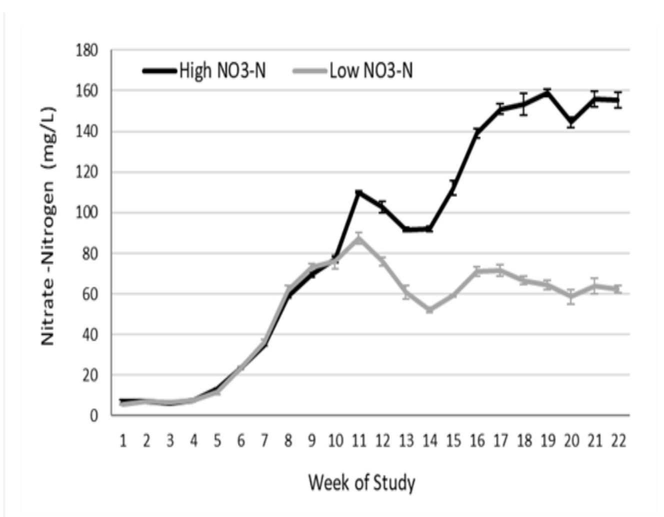

Fig. 1. Accumulating NO_3-N levels in Atlantic salmon RAS (n=3).

SEA VEGETABLE AQUAPONICS IN PUERTO RICO

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Halophyte 'salt-loving' plants also known as sea vegetables have historically been foraged along many coasts for consumption and are now grown as crops in many places in the world. Growing halophyte plants as sea vegetables for culinary dishes and coastal restoration shows tremendous promise to stimulate a new branch of aquaculture for Puerto Rico and other places in the Caribbean. Halophytes are an 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.

Sea vegetables have been grown at Florida Atlantic University in pilot-scale studies, using the excess nutrients from fish, shrimp and other species, in an Integrated Multi-Trophic Aquaculture (IMTA) system. Through technology transfer these methodologies to grow indigenous Puerto Rican halophyte plants for culinary dishes and coastal restoration are being tested in an aquaponic system at the Puerto Rico, Naguabo Aquaculture Center located at the Naguabo Fishing Association. There are three local species that will be grown using a NFT (nutrient film technique) system: sea asparagus (*Salicornia bigelovii*), sea purslane (*Sesuvium portulacastrum*), and saltwort (*Batis maritima*). Queen conch are also grown in the aquaponic system and they provide nutrients for the plants. Additional species to be considered include spiny lobsters, crabs, and West Indian fighting conch and top shells. Although sea vegetables were eaten in Puerto Rico by past generations, today they go largely unnoticed in Puerto Rican cuisine and introducing them to the domestic food industry, targeting restaurants as well as households, will begin to generate a market for a new aquaculture crop with economic and nutritional benefits. This project is funded by USDA ARS and Puerto Rico Sea Grant.

Aquaponic system at Naguabo Aquaculture Center in Puerto Rico (left). Sea purslane is featured on the right.

COMMUNITY-BASED NAGUABO AQUACULTURE CENTER IN PUERTO RICO

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Stimulating the growth of a sustainable aquaculture industry is a top priority for Puerto Rico. This prioritization will help to take pressure off the fishery sector, augment livelihoods for fishers, advance aquaculture technology, offer training programs and workforce development, facilitate food and nutrition security, and provide economic resilience for coastal communities.

In 2019, the community-based Naguabo Aquaculture Center was launched in Puerto Rico in collaboration with Florida Atlantic University, Conservación ConCiencia and Commercial Fishing Association - Villa Pesquera de Naguabo. The Aquaculture Center was designed and built to grow queen conch, halophyte plants (sea vegetables), and other local species for restoration and culinary dishes.

The Center's infrastructure includes: a saltwater system with two 2,000-gallon reservoir tanks on chillers, filtration and UV sterilization (200 ft^2); a temperature-controlled hatchery and microalgae culture area (144 ft^2); a recirculating nursery system for conch; and an aquaponic area for sea vegetables and other species (500 ft^2). The project began culturing queen conch juveniles from egg stage in June 2021, and in October 2021, sea vegetable aquaponics were introduced to the Center. There have been many successes including local job creation, research internships, and additional sources of income for fishers engaging and participating in different aspects of the operation including conch egg collection, infrastructure development, and seawater pumping.

The Naguabo Aquaculture Center is supported through grants from Saltonstall-Kennedy NOAA Fisheries, USDA Agricultural Research Services, and Puerto Rico Sea Grant. This project serves as a model that can be transferred to more fishing communities in Puerto Rico and elsewhere in the Caribbean.

Figure 1. Naguabo Aquaculture Center

OMEGA-3 CANOLA OIL (AQUATERRA®) SUPPORTS ATLANTIC SALMON GROWTH, PERFORMANCE AND ESSENTIAL FATTY ACID TISSUE ACCRETION ACROSS THE LIFECYCLE

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Aquaculture plays an important role in meeting the global demand for protein and fatty acids for human nutrition. The finite supply of marine ingredients has led to increased use of vegetables oils in fish aquafeed, particularly for salmonids including Atlantic salmon. While these oils can provide the fat needed for energy and growth, they lack nutritionally required omega-3 long chain polyunsaturated fatty acids (ω3 LCPUFAs) and cannot completely replace fish oil in fish diets. Development of land-based alternative oil sources to safeguard global aquaculture production, while meeting the nutritional needs of both fish and human consumers, is critical.

This review summarizes data from six studies demonstrating the safe use of a new land-based ω3 canola oil (Aquaterra®) in fish feed to support both total fat and ω3 LCPUFA requirements of Atlantic salmon (Salmo salar), while producing fish for human consumption with adequate essential fatty acid (EFA) content to benefit nutrient needs. Safety, growth and fillet quality of fish consuming this oil were evaluated during each phase of the lifecycle. Additional large-scale trials were conducted with commercial partners to assess on-farm performance of feed containing Aquaterra.

In all cases, growth and performance were comparable to fish fed conventional fish-oil based diets and no adverse effects were attributed to the use of Aquaterra. Fatty acid deposition in muscle (fillet) reflected the feed composition (Fig.1.), resulting in accumulation of EPA and DHA and a low ω6:ω3 ratio. Evaluation of fillets showed fewer melanin deposits in salmon fed diets including Aquaterra vs. standard oils (Fig.2.). Collectively, these studies demonstrate the safe use of Aquaterra in aquaculture feed.

Inclusion of Aquaterra in salmon diets provides a sustainable source of ω3 LCPUFAs for aquaculture and ultimately human nutrition.

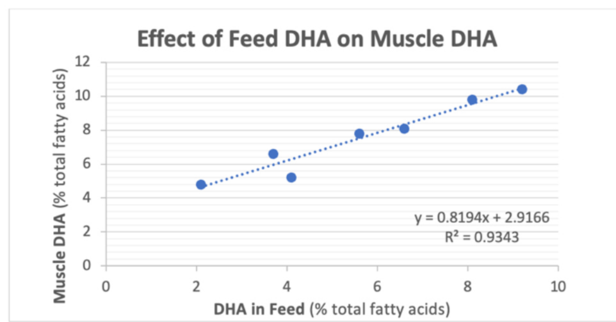

Fig. 1. Relationship between DHA intake (% total fatty acids) and muscle DHA (% total fatty acids) deposition. Each circle represents a feeding group.

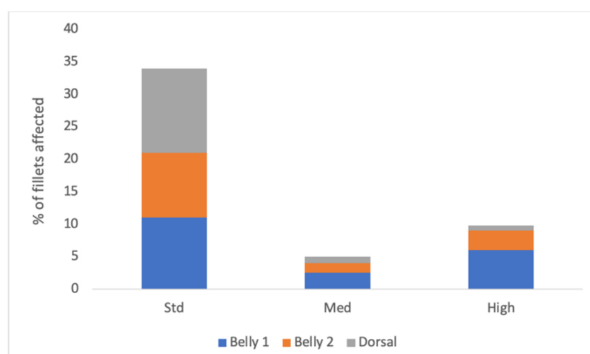

Fig. 2. Percentage of fillets with melanin deposits in the different fillet segments (N=60 fillets examined/cage).

IMPROVING COPEPOD PRODUCTION EFFICIENCY: A BOTTOM-UP APPROACH TO INCREASE MARINE FINFISH AQUACULTURE YIELDS

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Live feeds production continues to be one of the primary constraints to marine finfish aquaculture. Since most marine finfish species require live prey at first feeding, the efficient production of suitable live feeds (quantity, size, and nutritional content) continues to limit commercial-scale production. This limitation is even more significant with species that require copepods as primary prey. Although copepods can significantly improve growth and larval survival in many marine fish species, their inherent production challenges have resulted in limited integration into most commercial-scale rearing protocols.

Over the past decade, Oceanic Institute (OI) overcame many of those challenges and has developed intensive, large-scale copepod production technologies capable of exceeding 100 million nauplii per day. These copepods have been used to successfully rear several coral reef fish species, such as coral grouper, blue-fin trevally, red snapper, flame angelfish and yellow tang. Additionally, fish species that haven't previously required copepods (such as pacific threadfin and mullet) have shown increased survival when copepods were integrated into their culture protocols. However, notwithstanding our recent advances in production methods, the mass production of copepod nauplii is still fundamentally limited due to their inherent biological tendency to produce less nauplii at high adult densities. Specifically, culture densities > 4 adults/mL yield < 5 nauplii per female per day, whereas culture densities < 2 adults/mL yield much greater nauplii production (i.e. > 15 nauplii per female per day). Due to this biological limitation, copepods are currently cultured at low densities (i.e. 1-2 adults/mL) in an effort to produce adequate nauplii for hatchery needs. These low-density, high-volume cultures are labor intensive and relatively costly to maintain, and these restrictions represent a significant obstacle to large-scale copepod production.

One study recently showed that copepods can be selectively bred for increased fecundity, with substantial gains obtained in just a few generations (25% increase in total lifetime egg production within 6 generations of selection). The current study seeks to determine if similar performance gains can be realized when selectively breeding for copepods which remain reproductive at high adult density. If culture density can be increased without negatively impacting reproductive performance or physical characteristics of the copepods, significant production efficiencies can be realized whereby more copepod nauplii can be produced while occupying the same footprint and utilizing the same amount of labor. It is conceivable that this type of significant improvement to large-scale production of nauplii would rapidly advance the commercial-scale application of this technology. If this project objectives are achieved, current capacity of marine fish hatcheries will drastically increase, which ultimately will lead to significant gains in the number of successfully reared fish larvae. Results of the completed project will be presented at the conference.

COMMUNICATING SEAFOOD ONLINE IN A TIKTOK ERA

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The seafood industry is changing. Today, millennials and Gen Z have the largest buying power in the market. This younger demographic is environmentally conscious, tech savvy, and convenience minded. They want information about their seafood that hasn't always been available to them and they want it on platforms where we haven't always made it available.

If we want to reach this important group of seafood customers, then we need to meet them where they're at - social media.

Today, TikTok and Instagram aren't just for pre-teens doing trendy dances. 47% of TikTok's 1 billion users are between 20 and 40 years old - which also happens to be the fastest growing demographic of seafood consumers. And more and more people are turning to these social media platforms for credible information.

There is a need for reliable information on these platforms. Social media content is widely unregulated, making it a breeding ground for fake news and misinformation. The only way to combat misinformation online is to fight back with credible information. If scientists and industry leaders in the seafood space don't flood timelines with the real story, the only narrative that will dominate online is one that isn't true.

The food industry is currently experiencing a once in a generation transition where consumers are actively shifting to their eating habits towards lower impact diets. Right now, for many, a lower impact diet means going vegan or plant based. Most consumers skip right over seafood because they don't even know it's an option. Because we haven't told them it is.

The prior conventional wisdom that consumers thought of fish and seafood as the "healthy choice" is not as prevalent among younger consumers. We need to change that, but this window of opportunity won't be open forever, so we need to act now.

If we can intercept these young consumers during their transition to lower impact eating, we can grow our sustainable blue food system, support ocean literacy, and transform diets for good.

In this session, I'll talk about how seafood marketing has to change to respond to the growing demand from young consumers and share effective strategies for communicating your seafood stories onl

NOCTURNAL NUTRIENT PULSING EFFECTS ON SPECIFIC GROWTH RATES OF HAWAIIAN MACROALGAE IN HIGH CURRENT ENVIRONMENTS

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Seaweed aquaculture has been posited as a multifaceted solution to addressing global food and energy demands without using arable land or freshwater, and working to transform carbon, nitrogen, and phosphorous pollution in our atmosphere and marine environment. While the majority of the US macroalgae production occurs in temperate zones, the US Pacific Islands' exclusive economic zones, including the state of Hawai'i, constitutes 513 million hectares, roughly half of the total US EEZ.

The offshore environment surrounding the US Pacific Islands offers opportunities and challenges for macroalgae cultivation. High rates of insolation and low levels of turbidity offer high levels of photosynthetically active radiation (PAR) to great depths. While arrays will be present in nutrient poor surface waters during day light hours, deep cycling of arrays to nutrient rich waters may permit nutrient acquisition for the macroalgae during nocturnal hours.

Five Hawaiian macroalgae species (*Caulerpa lentillifera, Gracilaria parvispora, Halymenia hawaiiana, Ulva ohnoi,* and *Ulva lactuca*) were grown in varying levels of current velocity, and deep-sea water (DSW) pulsing concentrations (Figure 1). Six 1,200L partially recirculating flume-tanks exposed macroalgae to current velocities ranging from 0 cm/s to 30 cm/s. DSW was nocturnally pulsed at concentrations between 0% and 10% for 12 hour intervals. All trials were conducted in 60% shade coverage to mimic PAR at 10m depth. The feasibility of nutrient pulsing was determined by weekly analysis of specific growth rates (SGR) for each trial.

Results indicate that *U*lva *ohnoi* grown in 1% and 10% DSW exhibited both higher SGRs and higher average mass in current speeds of 20 cm/s versus no current treatments. Likewise, *C. lentillifera* showed average SGRs of 3.5% and 1% in 20 cm/s and 0 cm/s current respectively.

This study assesses the feasibility of deep-cycling macroalgae arrays in oligotrophic environments for nutrient acquisition. Developing new technologies for offshore production of macroalgae has the potential to expand the aquaculture production of the U.S. Pacific Islands EEZs, while enhancing food security and providing valuable ecosystem services.

Figure 1: Current velocity, DSW percentage and duration of pulsing trials

Species	Current (cm/s)	DSW%	# weeks
Caulerpa lentillifera	0,10,20,30	0,1,2.5,5,10	3
Gracilaria parvispora	0,10,20,30	0,1,2.5,5,10	3
Halymenia hawaiiana	0,10,20,30	0,1,2.5,5,10	2
Ulva spp.	0,10,20,30	0,1,2.5,5,10	2

LOW-COST MICROHAPLOTYPE DISCOVERY AND ALLELE FREQUENCY ESTIMATION USING POOLED SEQUENCING DATA

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As the cost of obtaining genetic information has decreased, more applications for this information have been created. Genotypes are now used by breeding programs in a variety of aquaculture species for parentage inference and genomic selection. For parentage inference, low-density panels of highly variable loci are used to maximize statistical power when determining pedigree relationships. For genomic selection, a typical strategy is to apply a low-density panel of highly variable loci to the majority of individuals and a high-density panel to a subset of individuals. The missing genotypes are then imputed to yield high-density genotypes for all individuals. In both these applications, using a low-density panel often lowers genotyping costs sufficiently to make the application economically sustainable.

For commercial-scale breeding programs, low-density amplicon sequencing panels targeting biallelic single nucleotide polymorphisms (SNPs) are typically used due to the cost-efficiency and accuracy of amplicon sequencing. However, these panels are limited in statistical power because each locus only expresses two alleles. Microhaplotypes are loci that contain multiple SNPs close enough together to be genotyped in the same sequencing read. These loci can therefore display more than two alleles, which increases their statistical power above that of SNPs, and genotyping a microhaplotype locus via amplicon sequencing uses the same resources as a locus with one SNP. Panels of microhaplotypes therefore have the potential to be more cost-effective than panels of SNPs for parentage inference and imputation.

Development of microhaplotype panels is hindered by a lack of cost-effective methods for allele frequency estimation of candidate loci. Currently, candidate microhaplotypes for a low-density panel can be discovered by either a reduced representation technique (e.g., RAD-seq), which only surveys a small fraction of the genome, or whole genome sequencing of many individuals, which is typically cost-prohibitive. To address this barrier, we developed new computational methods for estimating candidate microhaplotype allele frequencies from pooled sequencing and low-coverage whole genome sequencing (skim-seq) data. We validated these methods using datasets from three different species: Pacific oysters *Crassostrea gigas*, Atlantic salmon *Salmo salar*, and Pacific lamprey *Entosphenus tridentatus*. Across all three datasets, allele frequency estimates were unbiased and mean square error plateaued at a depth of 20 – 30 reads / locus. This demonstrates that the developed methods will allow cost-effective pooled sequencing or skim-seq data to be used for discovery and evaluation of candidate microhaplotypes. In turn, this will facilitate the creation of low-density microhaplotype panels for parentage inference and imputation, thereby lowering genotyping costs for aquaculture breeding programs.

FROM ONSHORE TO OFFSHORE AQUACULTURE: HOW NUMERICAL STUDIES CAN HELP BLUE MUSSELS (*Mytilus edulis*) SHELLFISHERIES?

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Coastal areas are submitted to lots of anthropic pressure, which is raising with the increase of demand for aquaculture and renewable energy. Coastal habitats are fragile and can be preserve by relocating aquaculture offshore with offshore renewable energy (ORE), which could help to: 1) manage space availability efficiently; and 2) decrease the risk of contamination due to the proximity between aquaculture sites and source of pollution (e.g., estuaries). Blue mussels industry (*Mytilus edulis L.*) in North Wales represents one third of the UK production and could be impacted by the development of other industries. Here, we used a Lagrangian particle tracking model (PTM) coupled to hydrodynamic model to study the best area to catch mussel larvae during their pelagic larvae duration. Furthermore, we showed the connectivity between the main aquaculture and ORE sites to evaluate the feasibility of Multi-use platform at sea (MUPS).

Telemac-2D (V8p2r0) model representing surface and mid-water depth currents was developed during the month of April (i.e., time of main spawning event) for 10 years (from 2012 to 2022). The domain covered an area of 165,000 km2, which correspond to the whole Irish Sea as previous studies show that larvae can potentially travel up to 300 km. To resolve the complex coastal current, the grid resolution varied from 50 m on the coast to 5,000 m at the offshore limit of the domain. A Lagrangian PTM was developed to predict mussel larvae dispersal from 6 sites for a pelagic larvae duration (PLD) of 45 days. Simulations were performed for two larvae behaviour (i.e. dispersal at the surface and at mid-water depth), then

Connectivity showed the same results every year when larvae travel at mid-water depth as expected. However, connectivity is site and PLD dependant (p-value < 0.0001). Connectivity varied inter-annually when larvae travelled at the surface and especially during windy contrasted years (i.e., westerly wind in 2014 and easterly wind in 2018; p-value < 0.0001), but release site and PLD remain significantly important. One ORE site is the most connected with aquaculture sites, however this connectivity varied through mussel PLD. During April 2014, aquaculture sites are connected with ORE sites located in the eastern Irish Sea (i.e. North Wales and North of England) whereas during 2018 they are connected with ORE located in the western Irish Sea (i.e. Dublin and North of Ireland).

The simulated larvae behaviours results showed that when larvae travel at the surface, they encountered stronger currents (e.g. wind driven currents), which increased their dispersal. Results observed when mussel larvae travel at the surface are correlate with observations made by mussel farmers during the year 2014 and 2018. The results highlight the importance of vertical position of larvae in the water column to study potential multi-use platforms at sea. However, the potential ORE site called leasing site 1 is shown to be the best area to catch mussel larvae in the Irish Sea. This study shows the importance of considering larvae dispersal for the development of MUPS, especially in the Irish Sea, where ORE will occupy approximately 14% (6,564 km^2) of the space in a close future.

ENHANCED BENEFITS OF BIOCHAR FOR USE IN RECIRCULATING AQUACULTURE SYSTEMS

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Since the oceans can no longer be looked at to provide us with all the fish we need as a population, we must rely on aquaculture to supply the rest. Recirculating Aquaculture Systems (RAS) are getting larger and are quickly becoming one of the most common techniques used in raising fish for food. The increase in technological advancements and efficiencies have enhanced our abilities to raise more fish in a smaller volume of water than ever before, and every step we can take to improve on water quality benefits the productive capacity of these systems. Biochar is a carbonized product that comes in a variety of sizes can be made from a variety of organic substrates under high heat and low oxygen. Under these conditions the organic material is carbonized, preserving its cellular structure, and creating a unique, chemically stable and versatile product. When used as a substrate for a trickling filter within a RAS, biochar's properties allow it to surpass any current available substrate for nitrification while at the same time provide the ability to virtually eliminate all suspended solids from the flow, enhancing water quality, disinfection and oxygen use efficiencies, fish health and fish feed efficiency. The use of a trickling filter, composed of bamboo biochar, as part of a RAS facility is discussed for its benefits and limitations.

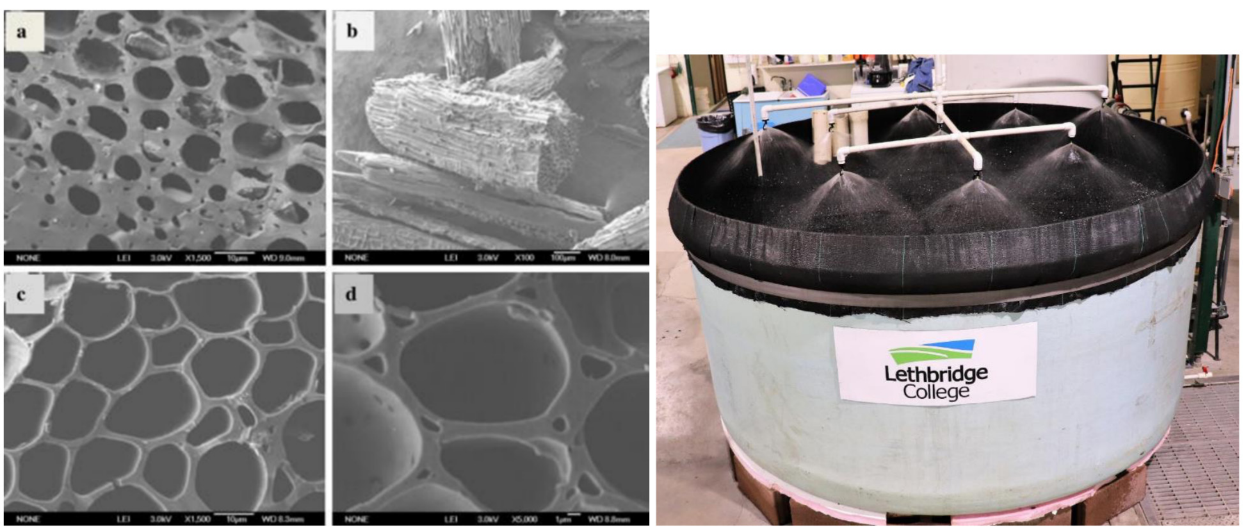

Figure 1: The microscope appearance of bamboo biochar at 100x (b), 1,500x (a, c) and 5,000x (d) magnification. The working biochar trickling filter at Lethbridge College's Aquaculture Centre of Excellence.

ZINC IS A LESS EXPENSIVE SUBSTITUTION FOR COPPER AS AN ANTIMICROBIAL

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The use of copper to control fungus and bacteria in aquaculture has been in use for many years; however, the price of copper has risen > 650% since the year 2000. Additionally, copper has become less available due to government-controlled monopolies of mineral mines outside the US. Zinc is ~1/3 the price of copper yet the two elements share many chemical properties and behave competitively for protein binding sites. We evaluated the ability of Zinc sulfate to kill several bacteria relevant to finfish aquaculture: *Streptococcus iniae*, *Flavobacterium covae*, *Flavobacterium columnare*, and *Aeromonas veronii*. Using minimum inhibitory concentration (MIC) assays to compare the antibacterial effect of copper and zinc, we found the MICs of copper and zinc to be similar with copper being slightly more potent against Gram negatives and zinc being more potent against Gram positives. We additionally compared the acute toxicity of $CuSO_4$ and $ZnSO_4$ in Largemouth Bass fry and found zinc to be slightly less toxic. Follow up research is underway to develop on-farm usage procedures.

WILLINGNESS TO PAY FOR SAFER FOOD: EVIDENCE FROM AN EXPERIMENTAL AUCTION OF FISH IN BANGLADESH

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This study examines the consumers' willingness to pay (WTP) for safer fish produced under controlled feed management and without antibiotics in Bangladesh. In this study, we used tilapia and pangas considering that these species are commonly consumed fish species in Bangladesh. Data were collected from 135 fish consumers of Mymensingh, Patuakhali, and Narayanganj districts through experimental auction (Vickrey auction) sessions conducted in June and July 2022. The bidders were purposively chosen from rural (36%) and urban (64%) areas. Among the respondents, 28% were female consumers, and the remaining 72% were male and selected from different income groups. We presented two items with varied qualities for each variety of fish throughout the auction. One was purchased from the neighborhood wet market, and the other was cultured with controlled feed management under the supervision of the Bangladesh Fisheries Research Institute. Each bidder bid two prices for each fish in a particular session.

TABLE 1. The WTP per kg. of safer tilapia

Auction location	Bids ($ per kg)				Premium for safer fish (%)	Local market sell ($ per kg.)		Premium for safer fish (%)
	Control		Trial					
	Session							
	1st	2nd	1st	2nd		Control	Trial	
Mymensingh	1.32	1.31	1.53	1.77	**35**	1.54	1.92	**25**
Patuakhali	0.99	0.96	1.42	1.67	**75**	1.54	---	---
Narayanganj	1.32	1.26	1.70	1.92	**52**	1.63	2.31	**41**
Average	**1.20**	**1.17**	**1.55**	**1.79**	**35**	**1.57**	**2.12**	**33**

Note: $ 1 = BDT 104 (Bangladesh Bank, November 2022)

TABLE 2. The WTP per kg. of safer pangasius

Auction location	Bids ($ per kg)				Premium for safer fish (%)	Local market sell ($ per kg.)		Premium for safer fish (%)
	Control		Trial					
	Session							
	1st	2nd	1st	2nd		Control	Trial	
Mymensingh	1.28	1.24	1.30	1.59	**38**	1.15	1.56	**35**
Patuakhali	1.07	1.01	1.39	1.54	**33**	1.15	---	---
Narayanganj	1.39	1.19	1.40	1.66	**24**	1.35	1.44	**7**
Average	**1.24**	**1.14**	**1.37**	**1.60**	**31**	**1.22**	**1.50**	**21**

COMPARISON OF REAL-TIME PCR ASSAY PERFORMANCE FOR DETECTING *Decapod penstylhamaparvovirus* 1 IN PENAEID SHRIMP

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Decapod penstylhamaparvovirus 1, commonly known as infectious hypodermal and hematopoietic necrosis virus (IHHNV), remains an economically important viral pathogen for penaeid shrimp aquaculture. Since the initial report of the virus in 1982, the virus has spread in all major shrimp farming regions of the world. The World Organisation for Animal Health (WOAH, Paris, France) recommended methods for the detection of IHHNV include both conventional and real-time PCR. However, published reports and anecdotal evidence suggest the occurrence of non-specific amplifications when testing for IHHNV using the WOAH protocols. The accurate detection of IHHNV was further complicated when a viral genomic fragment was found to be integrated with the host genome as an endogenous virus element (EVE).

Studies were designed to develop a sensitive, robust TaqMan PCR method for detection of IHHNV in the three commercially important penaeid shrimp: *Penaeus vannamei, P. monodon* and *P. stylirostris*; and the method can differentiate between the infectious form of IHHNV and the EVE. We compared the performance of the WOAH-recommended real-time PCR method to several published as well as in-house designed primer/probe sets spanning the entire genome of IHHNV. Our results show that (1) more than one primer/probe set is needed when testing for the infectious form of IHHNV in all three species of shrimp; and (2) primers/ probe: qIH-Fw and qIH-Rv/3072-IH-Probe, and 3144F and 3232R/ 3187-IH-Probe have diagnostic characteristics that would enable IHHNV detection in all three shrimp species with high diagnostic specificity and sensitivity. These findings are valuable for large-scale screening of shrimp using a TaqMan real-time PCR assay.

VALIDATION OF A REAL-TIME PCR ASSAY IN DETECTING INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV) IN PENAEID SHRIMP

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Infectious hypodermal and hematopoietic necrosis virus (IHHNV), also known as Decapod *Penstylhamaparvovirus* 1, is a World Organisation for Animal Health (WOAH, Paris, France) listed pathogen. In the early 1980s, infection with IHHNV caused large-scale mortalities in blue shrimp (*Penaeus stylirostris)* in the Americas. In Pacific white shrimp (*P. vannamei)*, the predominant farmed penaeid shrimp worldwide, IHHNV infection does not cause any mortality but results in growth retardation and deformities. The virus has been reported in all shrimp farming countries around the world. IHHNV is vertically transmitted and hence routine screening of broodstock and post larvae are critical to ensure specific pathogen free (SPF) status of a population.

IHHNV challenge experiments were performed simultaneously at the Aquaculture Pathology Laboratory (APL) at the University of Arizona and at the National Veterinary Services Laboratories (NVSL) using a single lot of *Penaeus vannamei* SPF shrimp. Infected shrimps were sacrificed over a 30-day period and archived at -80°C. Unchallenged SPF *P. vannamei* served as negative controls. Both IHHNV challenged and unchallenged animals from APL were sent to the NVSL, where duplicate samples containing gill and pereopod tissue from each animal were dissected and coded. The blinded panel was evaluated at both laboratories using TaqMan real-time PCR with newly developed IHHNV primer/probe sets (qIH-Fw and qIH-Rv/3072-IH-Probe, and 3144F and 3232R/ 3187-IH-Probe), and EF-1 internal control gene. We are currently comparing assay performance and reproducibility that are necessary for IHHNV screening.

RESEARCH AND EXTENSION PROGRAMMING AT THE UNIVERSITY OF FLORIDA TROPICAL AQUACULTURE LABORATORY: PAST, PRESENT, AND FUTURE

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The Tropical Aquaculture Laboratory (TAL) is a research and extension facility operated by the University of Florida. The TAL, located in Ruskin Florida, was founded in 1996 to better serve the stakeholders of the ornamental aquaculture industry. This initial venture was made possible through collaboration with the Hillsborough County Board of County Commissioners, the Florida Tropical Fish Farms Association, and the Florida Department of Agriculture and Consumer Services. Early programming focused primarily on fish health and applied aquaculture production. Since that time the TAL has expanded its research and extension programming to include reproduction, aquatic ecology, physiology, non-native species ecology and management, youth education, and restoration aquaculture. A full scale diagnostic laboratory is also located on site, catering to a wide variety of aquatic animal health issues. Partenerships have also grown to include Hillsborough Community College, USDA APHIS Wildlife Services and USDA APHIS Veterinary Services, who has stationed the Senior Staff Veterinarian in Aquaculture Health at the TAL.

The TAL has always been committed to a mission of serving Florida's aquaculture industry by solving problems and creating opportunities. Over 20 UF faculty, staff, and students now operate out of the TAL. Moving forward the TAL has established a strategic plan to once again broaden its programs to include new commodities and clientele and improve its resources and capacity as it continues to support the broad diversity of aquaculture in Florida.

THE EFFECTS OF SOCIAL ISOLATION ON STRESS, NEUROCHEMICAL SIGNALING, AND FEED INTAKE IN ZEBRAFISH *Danio rerio*

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Many organisms exhibit social behaviors and are part of some scheme of social structure. Because of this, social isolation can act as a stressor and influence the way the brain and body behave. While stress is typically thought to reduce appetite and inhibit feed intake, prolonged (chronic) isolation can result in increased feed intake and frequency of binge eating. This may be useful when utilizing lower quality and less palatable diets, such as those based on plant meals. Changes in production and release of signaling molecules, namely cortisol, dopamine, and serotonin, may help to understand the effects of social isolation on feed intake and feed utilization. The objective of this study was to evaluate the effects of social isolation on feed intake, stress response, neurochemical signaling, and intestinal health of juvenile zebrafish fed a high-inclusion soybean meal (SBM)-based diet.

At 20 days post-fertilization (dpf), zebrafish were randomly assigned to chronic isolation (1 fish per 1.5L tank) or social housing (6 fish per 9.0L tank). Fish were allowed to acclimate to treatment tanks for 15 days while being fed a commercial diet to apparent satiation. At 35 dpf, zebrafish were switched to a high inclusion SBM diet and fed to apparent satiation for 16 days. Water samples for future cortisol analysis were taken before separation into treatment groups (20 dpf), 24 hours after separation (21 dpf), after acclimation period (35 dpf), 24 hours after SBM introduction (36 dpf), and at experimental termination (51 dpf). Brain and gut samples for future analysis of dopamine and serotonin related genes and histology were taken to assess levels of stress and intestinal inflammation, respectively.

At experimental termination, the mean weight and weight gain were not significantly different between treatment groups ($t_{17} = -1.4035$, $p > 0.05$). The mean total body length was also not significantly different between treatment groups ($t_{17} = -1.4219$, $p > 0.05$). Feed intake ($t_{17} = 4.7258$, $p = 0.00019$) and feed conversion ratio ($t_{17} = 4.2046$, $p = 0.00059$) were significantly higher in chronically isolated fish compared to those in social housing.

These results show that chronic isolation did not negatively affect growth parameters of juvenile zebrafish and suggest that isolation may be useful in promoting feed intake of less-palatable diets such as soybean meal. However, increased feed intake in that group did not translate to better growth, resulting in poor feed conversion ratios. Analysis of cortisol release, gene expression, and intestinal morphology will be presented during oral presentation and may provide insight into the associations between chronic isolation, stress, increased feed intake, and gut health in fish.

EFFECTS OF OREGANO ESSENTIAL OIL ON GROWTH PERFORMANCE OF ZEBRAFISH *Danio rerio* FED A HIGH INCLUSION SOYBEAN MEAL DIET

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Soybean meal (SBM) has become a common dietary replacement for fishmeal in aquafeed. However, at high inclusions, SBM has been shown to have negative impacts on fish such as reduced feed intake and intestinal inflammation. Medicinal plant extracts, namely essential oils, have been used to promote growth performance and immune response. One notable plant extract is the essential oil from oregano (*Origanum vulgare*) as it is composed of two health-promoting compounds: carvacrol and thymol. These compounds have shown antimicrobial, antioxidant, and anti-inflammatory properties. The objective of this study was to investigate the effects of oregano oil on utilization of a high-inclusion SBM diet using zebrafish as a model.

At 20 days post-hatch (dph), zebrafish were randomly separated into 15 tanks (3.0 L) with 11 fish per tank. Five diets were used in this study: **reference** – fishmeal based diet, **control** – SBM based diet, and three experimental SBM based diets **OEO1**, **OEO2**, and **OEO3** that were supplemented with 1, 2, or 3% of oregano oil respectively. After 28 days, the FM group had overall better growth performance when compared to the other treatment groups. However, the OEO3 mean weight was significantly higher than that of SBM control ($p = 0.0478$) and not significantly different from the FM group ($p > 0.1$). Similarly, the FCR in the OEO3 was significantly lower than the FCR in the SBM group ($p = 0.0490$) and not significantly different from the FM group ($p > 0.1$). The OEO2 mean total length was significantly higher than that of the SBM group ($p = 0.0478$) but not significantly different than the FM group ($p > 0.1$). The OEO1 mean weight, FCR, and total length were not significantly different from those of the SBM group ($p > 0.5$).

Furthermore, the OEO2 group showed a numerical downregulation of inflammation related genes *tnfa*, *il-1b*, *mmp9*, and *il-10* when compared to the other groups, but most notably in comparison to the SBM group. However, there was only significant downregulation in the *il-10* gene expression for anti-inflammatory cytokines when compared to the FM group ($p = 0.032$). While the FM group had higher numerical expression of nutrient absorption related genes (*pept1* and *fabp2*), the OEO1 group showed numerical upregulation of these genes when compared to the other SBM-based groups. The OEO1 group had significantly higher expression of *fabp2* than the SBM and OEO2 groups ($p = 0.055$, $p = 0.040$). These results suggest that inclusion of oregano essential oil at or above 2% inclusion improved growth performance of zebrafish fed a high-inclusion SBM diet and might potentially have beneficial anti-inflammatory effects on the gut.

AQUACULTURE PROGRAM DEVELOPMENT AT OREGON SEA GRANT: NEEDS, TOOLS, AND OPPORTUNITIES

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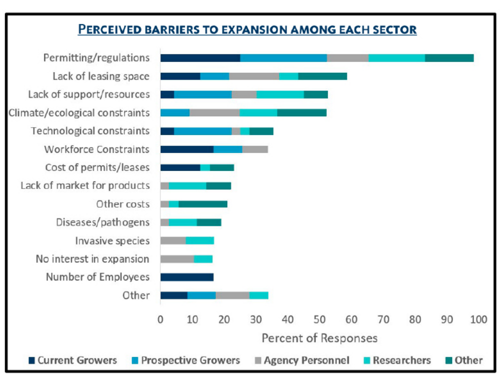
There has been a recent resurgence of interest in aquaculture within Oregon, as in much of the U.S. Over the past twenty years, Oregon Sea Grant's (OSG) support of aquaculture has been intermittent, with the last full-time OSG Aquaculture Extension specialist retiring in the mid-2010s. A search for a new Aquaculture specialist was indefinitely delayed in 2020 due to a COVID-related hiring freeze, and the fate of the position is unclear. However, OSG recognized the need to support research and industry efforts following renewed interest by coastal communities. We hired an Aquaculture Fellow in Nov. 2020, serving in a temporary Extension position until March 2023, and a Marine Resource Extension faculty member partially supports aquaculture. These efforts have enabled OSG to take on a larger role.

Since 2020, OSG has focused on addressing current needs and establishing partnerships with other organizations supporting sustainable aquaculture expansion. In early 2021, we conducted a needs assessment that revealed strong interest in aquaculture expansion, and several barriers, including permitting challenges, lack of a regulatory framework for novel species, limited leasing space, workforce constraints, and climate/ecological limitations. OSG has been working to address these challenges over the past year by providing informational resources, leading a workforce development grant, and working with partners to support their outreach efforts. We produced a white paper that provided recommendations for addressing regulatory and policy barriers, and a StoryMap focused on current aquaculture production in the state. Additionally, OSG partnered with Oregon Aquaculture Association to plan an aquaculture development conference and develop online business planning and siting tools.

In this presentation, we will give an overview of the past and current efforts of OSG to support aquaculture expansion, our plans for the future – such as workforce development planning and permitting guidance – and seek advice from attendees on building a state aquaculture program.



AN ASSESSMENT OF SEA LICE IN COASTAL NEW HAMPSHIRE AND EXPLORING THE USE OF LUMPFISH *Cyclopterus lumpus* AS CLEANERFISH OF STEELHEAD TROUT *Oncorhynchus mykiss* IN EXPERIMENTAL AQUACULTURE CAGES

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Using cleanerfish for sea lice biocontrol is becoming a common practice in salmonid aquaculture in the North Atlantic, however, it is relatively new in the US and there is still much to learn. At the University of New Hampshire (UNH), lumpfish (*Cyclopterus lumpus*) cleaning behaviors are being assessed with steelhead trout (*Oncorhynchus mykiss*), a salmonid important to the future of New Hampshire's finfish aquaculture industry.

In 2020 and 2021, we utilized an experimental steelhead trout aquaculture pen off the NH coast, operated by NH Sea Grant and UNH. Weekly from November to June, we assessed sea lice populations collected from a subsample of steelhead trout and documented lice species, life history stage, sex, and abundance. Lice loads (mean lice per fish) peaked in January 2020 at 3.60 lice per fish, and the dominant species observed was *Caligus elongatus* (99%; n=930) though some individuals of *Caligus curtus* were observed (n=9). Female lice and adult lice made up 74% and 87% of the lice population, respectively, throughout the assessment. The lice loads of gravid females peaked in February 2021 at 2.20 gravid lice per fish.

Using small microcosm cages stocked with steelhead trout, we also evaluated whether lumpfish presence and hide design (fake kelp, PVC panels) affected sea lice loads. Two 10-week trials were conducted from October to December 2020, with all fish sampled biweekly for lice. At the end of each trial, all lumpfish were euthanized, and gut contents analyzed. Hide design affected mean lice loads on trout in both trials, with lower lice loads in cages containing kelp hides (Trial 1: GLM, p=0.01; Trial 2: GLM, p=0.02). Lice loads were lower in cages containing lumpfish versus no cleanerfish (GLM, p=0.04), but only in one trial. There was no evidence of sea lice within any lumpfish stomachs. Water temperature and lumpfish size differed between the two trials suggesting that cleanerfish size, hide design, and water temperature are key variables for steelhead farmers to consider for effective sea lice control.

These foundational studies contribute towards developing best practices of lumpfish use for sea lice mitigation, leading towards the goal of increasing the sustainability and production of steelhead trout aquaculture in NH waters.

TWO YEAR EVALUATION OF FOOD SAFETY HAZARDS IN A COMMERCIAL RECIRCULATING AQUAPONICS SYSTEM

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Aquaponics is a sustainable agricultural method for the propagation of fish, microorganisms, and produce by harnessing the byproducts of one component as a nutrient source for the other. Recently, aquaponic farms have undergone increased scrutiny regarding the food safety of their products. This is due to limited research on the association of foodborne pathogens from fish and aquaponic water and the potential for produce contamination. The purpose of this study was to perform an initial evaluation of a commercial aquaponics farm to determine potential food safety hazards present within the system.

Over 2 years, a microbial evaluation was performed on a controlled environment aquaponics farm containing 3 recirculating systems. The fish tank contained Nile tilapia (*Oreochromis niloticus*) and lettuce (*Lactuca sativa*) was grown hydroponically in a deep water culture (DWC) grow bed. Samples (n = 1,044) were collected bimonthly from each system, including lettuce, roots, fingerlings (0-3 mo), fish (>3 mo), water, and sponge samples from the tank interior. Total plate count (TPC) was enumerated on Tryptic Soy Agar, while total coliform and generic *Escherichia coli* most probable number (MPN) were determined using IDEXX Colilert Quanti-Tray. Enumeration and enrichment were used to detect Shiga-toxigenic *E. coli* (STEC), *Salmonella enterica*, *Listeria monocytogenes*, *Aeromonas hydrophilia*, and *Pseudomonas aeruginosa*.

The average TPC in water (n = 351) and sponge (n = 351) samples were 4.59 and 6.06 log CFU/ mL, respectively. Generic *E. coli*, STEC, and *L. monocytogenes* were not detected from any of the collected samples. *P. aeruginosa* was isolated from water (4/351; 1.14%), sponge (3/351; 0.85%), fish gut (1/108; 0.93%), lettuce (2/99; 2.02%), and root (1/99; 1.01%) samples. Sponge (9/351; 2.56%) and water (7/351; 1.99%) samples collected from the fish tank and DWC were positive for *S. enterica*. *A. hydrophila* was isolated from all sample types (544/1044; 52.1%; Table 1). These results indicate that although common produce associated foodborne pathogens were not identified or had low presence in the aquaponics system, *A. hydrophila* was identified as a hazard in the system and may pose a risk for fish disease and produce associated foodborne illness.

Table 1. Frequency of *Aeromonas hydrophila* (+/total) detected in each sample type by the age of the aquaponics farm, 0-24 months

	Age of aquaponics farm (months)				
	0	1-6	7-12	13-18	19-24
Sponge	1/27	48/81	45/81	39/81	58/81
Water	0/27	34/81	47/81	56/81	50/81
Lettuce	*	9/18	8/27	11/27	6/27
Root	*	15/18	17/27	20/27	18/27
Fish gut	*	14/27	11/27	16/27	14/27
Fingerling	*	6/9	0/9	2/9	4/9

* indicates no samples collected

ADVANCES IN DIET DEVELOPMENT FOR CALIFORNIA YELLOWTAIL *Seriola dorsalis* – AN OVERVIEW

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Over the years, Seriola spp. has emerged as an important economic fish in several parts of the world. Notably, the Japanese Amberjack (*S. quinqueradiata*) has been cultured in Japan commercially for decades. In the United States, *S. dorsalis* and *S. dumerili* are grown in small net-pen operations. In addition, RAS-based farms in Europe, and more recently, in the United States have been operating for a few years. California yellowtail *S. dorsalis* has been the subject of research efforts at the Hubbs SeaWorld Research Institute for the past 20 years, which were focused on reproduction and nutrition.

We present an overview of key nutrition results obtained over the years, as well as recent findings toward the refinement of practical diets. Notably, feed formulations were modernized to reduce the inclusion of fish meal and oil, through the systematic evaluation of terrestrial and algae-based ingredients. This work was also supported by projects refining the dietary requirements of key nutrients such as taurine and methionine, and their interaction with the diet matrix. Additionally, a series of trials focused on the balance of carbohydrates and lipids as energy sources.

Overall, considerable progress has been made to the diets for the California yellowtail which should help facilitate species specific feed formulations.

PERFORMANCE OF *Ulva lactuca* IN A LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) SYSTEM WITH WHITE SEABASS *Atractoscion nobilis*

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Nitrogenous compounds such as ammonia, nitrate, and dissolved organic nitrogen are the main waste components of marine fish aquaculture effluents. These compounds are also regarded as the primary nitrogen sources for macroalgae. In order to understand how sea lettuce (*Ulva lactuca*) could be used to scrub dissolved nutrients from the effluent of flow-through white seabass (*Atractoscion nobilis*) raceways, we designed a series of 3-week experiments to assess the benefits of the fish nutrients, shading, algal density, and water exchange rates. All *Ulva* cultivation trials were conducted in 175 L tanks run in triplicate, with water quality and growth measurements taken weekly. Seawater was supplied by gravity from seabass raceways unless otherwise noted. For the shading trial, *Ulva* was stocked at 0.56 kg/m^2 with either 0%, 30%, and 60% shade with seawater supplied at 7.6 L/min for each tank. The removal efficiency of total ammonia nitrogen (TAN) decreased with increasing shade levels, and the peak exceeded 80% at 0% shading. Growth rate and productivity of *Ulva* under 0% shading was 12.09±3.13 %/d and 13.28±4.66 g DW/m^2/d, which were both significantly higher than other shading levels. For the stocking density and flow rate trial, the stocking density of *Ulva* was 0.56 and 1.12 kg/m^2, and seawater effluent was supplied at 0.5, 1.5, and 7.6 L/min in a 2-factor design. In this trial, TAN removal efficiency was 100% across all treatment combinations. Growth rate of *Ulva* was highest (20.36±2.36%/d) at 0.56 kg/m^2 under the flow rate of 7.6 L/min, and the productivity reached up to 30.89±6.53 g DW/m^2/d which was not different than that of *Ulva* stocked with 1.12 kg/m^2 at the same flow rate (p=0.47). In a trial comparing raw, sand-filtered seawater (RAW seawater) with seawater used to grow 278 kg of white seabass (FISH seawater), TAN removal efficiency by *Ulva* was consistently nearly 100% when supplied with FISH seawater with a TAN concentration ranging from 0.11 to 0.18 mg/L. Growth rate and productivity of *Ulva* supplied with FISH seawater were 21.36±2.25 %/d and 33.83±7.29 g DW/m^2/d, which was not different than that for *Ulva* supplied with RAW seawater. However, the protein content of *Ulva* (22.24±2.49%) in FISH seawater was significant higher than that of *Ulva* (9.98±1.08%) cultured with RAW seawater (p<0.01), and the C/N ratio of 8.37±0.87 in *Ulva* tissue cultured with FISH seawater was significantly lower than that of the *Ulva* (15.94±2.01) when supplied with RAW seawater (p<0.01). This indicated that *Ulva* was in a nitrogen-limited situation when supplied with RAW seawater. In light of the better understanding of *Ulva* performances achieved in our study, the optimum *Ulva* and white seabass combinations can be established to maximize the nutrient assimilation efficiency, biomass production, and diversity of seafood production.

IMPROVING THE ACCURACY, PRECISION, AND CAPACITY OF OFF-FLAVOR ANALYTE DETECTION IN FISH TISSUE, FEED, AND FECES

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The highly cited method of Lloyd and Grimm[1] for off-flavor analyte detection in fish has seen wide-spread use over the past two decades. The method relies on microwave heating of fish tissue to generate steam; analytes with high vapor pressures are distilled with the steam, which is cooled and captured as an aqueous sample. The quick sample preparation method, coupled with solid-phase microextraction (SPME), is an attractive option for multiple reasons. SPME use with gas chromatography-mass spectrometry (GCMS) provides enhanced sensitivity compared to traditional liquid injections. Automated SPME interfaces the sample concentration step with introduction into the GC inlet.

Despite its popularity, certain aspects of the microwave-SPME-GCMS method are not optimal. Mass spectral detection of sub-ppb concentrations of MIB and GSM requires the use of selected ion mode (SIM) sampling; this provides sensitivity for targeted compounds but excludes information from other taste and odor compounds that may be present in a sample. Furthermore, the microwave and glassware are configured for single-sample processing, limiting the ability to perform multiple analyses and analytical replicates.

Building upon the benefits of the Lloyd and Grimm method, an improved method was developed which provides the option of analyzing the MIB and GSM content of 20 samples simultaneously. The method uses a repurposed GC oven as a heating source, with PTFE tubing and septa connecting gas flow to distillation and collection vessels. The opportunity to perform concurrent distillations of multiple samples facilitates quality assurance measures to be taken (such as blanks, standards, and spiked replicates). The SPME Arrow, which offers a stronger core and thicker adsorbent phase, was used in place of traditional SPME. Additionally, synchronous SIM/scan detection provided sensitive signal with complete scan data from each acquisition.

GSM levels in fish tissue (trout and catfish), fish feed, and fish feces were determined using the improved method. Recovery values of 45(±7)% and 21(±6)% were obtained for GSM distilled from water and trout, respectively. Linear analyte recovery (r^2 = 0.991) over a wide concentration range (0.1 to 10 ppb) was achieved through simultaneous distillation of 20 samples of spiked trout. Semi-quantitative information from over 30 volatile compounds (aldehydes, ketones, and alcohols) was also obtained during off-flavor analysis by SPME-GCMS.

1. Lloyd, S. W.; Grimm, C. C., Analysis of 2-Methylisoborneol and Geosmin in Catfish by Microwave Distillation−Solid-Phase Microextraction. *Journal of Agricultural and Food Chemistry* **1999,** *47* (1), 164-169.

USING A NEW DATABASE TO ENHANCE FISH HEALTH MANAGEMENT

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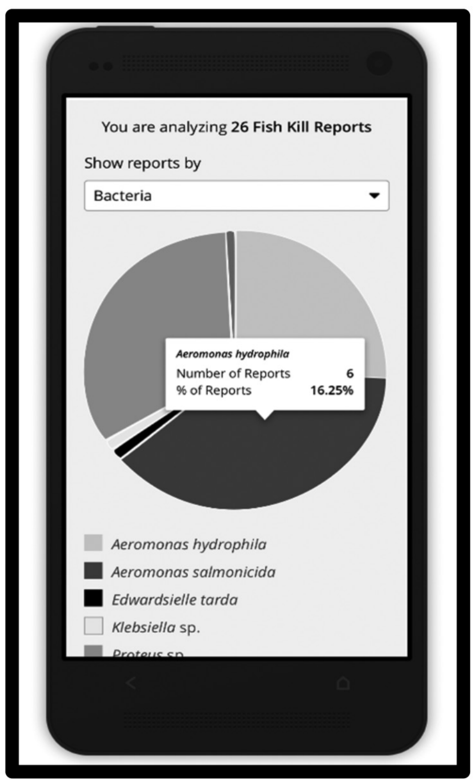
A digitized Mobile Responsive Clinical Fish Health Database (for computers and mobile devices) completed in April 2022 enables more accurate disease case record-keeping and timely identification and remediation of fish pathogens by fish health professionals, enhancing the effectiveness of fish health services to the aquaculture industry. Several fish disease diagnostic laboratories will enter their historic and current fish disease diagnostic data into the Database and will provide beta-testing feedback in a survey designed to improve the effectiveness of the Database. Laboratories doing beta testing will include the Kentucky State University Fish Disease Diagnostic Laboratory, itself, and approximately 40 other similar laboratories (both public and private). 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. The Database also serves as a reference source and teaching tool for fish disease diagnosticians and students.



COMPARISON OF GONADAL GROWTH IN PURPLE URCHINS *Strongylocentrotus purpuratus* USING THREE DIFFERENT DIETS

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Purple sea urchins (*Strongylocentrotus purpuratus*) are one of the most abundant species of echinoderms that occur in the Pacific ocean. Sea urchins are a great species to raise in a mariculture setting because they are dioecious, meaning they have both male and female reproductive organs in separate individuals; Their reproductive systems are highly sought after in the restaurant industry creating a high demand for quality product. The gonads, otherwise known as 'uni', are a delicacy for their buttery texture and salty flavor when they are served –usually uncooked. The color of the uni usually sets the bar for how expensive the product will be since it is visually pleasing to the consumers. The California Sea Urchin Commission (CSUC) describes that the best quality will be a "California gold" color, and this was formerly known as grade A uni.

As a group, we decided to test the difference in uni growth between three feed types; Bull kelp (wild harvest), spinach from the grocery store, and Urchinomics commercial feed. Bull kelp, *Nereocystis luetkeana*, is the most abundant species of kelp along the California coast and an important species for many local organisms. We chose to use bull kelp as the control of our experiment because it was easily attainable and a preferred diet by sea urchins. We chose spinach because of its easy accessibility and how inexpensive this produce is. Using an agricultural feed will help us design more sustainable food resources to utilize, grocery stores and restaurants can donate their unused leafy greens for these echinoderms. From the many options of commercial feed, we chose urchinomics since the company claims that their product would grow adequate uni in less than 5 weeks. From these three feeds, we predict that Urchinomics would grow the best uni in a short amount of time.

Our conclusion was interesting, after two months we found that the spinach feed was producing grade A uni and had the "California Gold" hue that is sought after. The sea urchins receiving Urchinomics had less desired results, their uni was significantly less than spinach and had a light yellow/orange hue. The bull kelp produced barely any uni, at the two month mark they were barely starting to produce gonads.

Figure 1. Gonad growth in Purple Urchins after 2 months of changed diet. (Left)Bull Kelp tank, (Middle) Urchinomics tank (Right) Spinach tank.

INVESTIGATING THE PREVALENCE OF BASKET COCKLE *Clinocardium nuttallii* BIOFOULING ON GEODUCK FARMS IN PUGET SOUND, WA

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Intertidal shellfish operations are often hindered by biofouling. In Puget Sound, an estuarine fjord in Washington State which hosts many commercial shellfish farms, a particularly interesting form of biofouling can occur. Basket cockles (*Clinocardium nuttallii*), a medium-sized bivalve, accumulate in aquaculture cultivation equipment such as PVC piping and mesh tubes (see Figure 1) used for planting geoduck (*Panopea generosa*) seed. Farmers sometimes remove and discard cockles from these tubes as they worry that the density of cockles may limit the growth and yield of their target crop, geoduck. However, this 'nuisance' species has been a preferred food for many Native American tribes in the Puget Sound region for thousands of years. In fact, some of the earliest forms of aquaculture were "clam gardens" which indigenous people would actively cultivate and care for. Recently, cockles have become increasingly difficult for tribal members to find in their usual and accustomed fishing areas. This presents an opportunity to create a mutually beneficial relationship between shellfish farmers and tribal communities, providing an outlet for this unwanted byproduct of geoduck aquaculture while restoring a preferred indigenous food.

Here we discuss our work to investigate the prevalence and patterns of cockle biofouling on geoduck farms across Puget Sound. We conducted surveys on two large commercial farms in the summer of 2022 to estimate the potential harvestable biomass of cockles per cultivated area. We then looked at cockle abundance and biomass in relation to factors such as geoduck presence, tidal elevation, geoduck tube condition, and geoduck planting date. By developing a more comprehensive understanding of these relationships, as well as the abundance of this fouling species across geoduck farms, we seek to maximize efficiency and success in potential future efforts to harvest and utilize these cockles.

Figure 1. Basket cockles biofouling inside a mesh geoduck tube, Case Inlet (Photo by Puget Sound Restoration Fund)

SUSTAINABLE OCEAN FARMING AND APPLIED FISHERIES EDUCATION IN ALASKA

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University of Alaska Southeast - Sitka Campus has a robust Applied Fisheries Program that provides career driven education to prepare individuals for jobs in fisheries management, diving, aquaculture, and mariculture. Degrees offered include Associates of Applied Science, Certificates, and occupational endorsements in these fields. Our distance learning options have been an integral part in educating individuals across the nation, with options for in-person labs and courses in Sitka, AK. In addition, we offer semester-long intensive programs, such as the Alaska Aquaculture Semester or the Alaska Dive Semester, which are in-person and driven by hands-on practical learning, preparing students for multiple occupations. As part of these programs, specialized industry training and experience is gained by completing a for-credit internship in partnership with a relevant organization.

Our programs promote and research sustainable ocean farming alongside current and developing aquaculture and fisheries industries. We are the first farm in our local community to outplant Sugar kelp (*Saccharina latissima*) and Ribbon kelp (*Alaria marginata*) near rearing chum (*Oncorhynchus keta*) and pink (*Oncorhynchus gorbuscha*) salmon to help determine the feasibility and measurable benefits of this form of Integrated Multi-Trophic Aquaculture (IMTA). Kelp growth, nitrite, nitrate, ammonia and dissolved phosphorus as well as dissolved oxygen and salinity are measured bi-weekly. As part of this work students not only learn about kelp ecology and sustainable aquaculture but they also gain skills using oceanographic equipment, operate small vessels and work closely with industry professionals.

Future work will support aquaculture workforce development, increase the sustainability of salmon enhancement aquaculture in Alaska, improve food security and provide industry with a proof of concept important for demonstrating the potential economic and environmental benefits for IMTA in Alaska.

AQUAPONICS IN GRENADA: HEALTH SURVEILLANCE OF FERAL TILAPIA TO ASSESS PATHOGEN AND DISEASE RISK

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The Caribbean island of Grenada is rich in fisheries and agricultural history, but also faces challenges to support fish farming. The Grenada government, in partnership with the United Nations, has launched a "Zero-hunger Initiative" and "Climate Smart Agricultural Program" to provide opportunities for sustainable tilapia (*Oreochromis mossambicus, O. niloticus, O. aureus)* aquaponics. The goal is to improve food security, provide rural employment, and reduce exploitation of natural fisheries. The Aquatic Animal Medicine Research Laboratory at St. George's University, School of Veterinary Medicine has developed a collaborative program to enhance the productivity and sustainability of aquaculture in Grenada through capacity building and training, disease diagnostics, veterinary support, and applied research.

A key component of this program is to identify infectious diseases of concern that may limit the industry. This study investigated pathogens of feral tilapia from three waterways (n=60 fish per waterway) known to be sources of fish for farmers, and documented diagnostic findings from seven aquaponics farms (n=25). Diagnostic methods used in this study included wet mount microscopy of gill and skin, aerobic bacterial culture of spleen and kidney, virus isolation, and pathogen-specific PCR. Pathogens identified in feral tilapia are similar to those identified in aquaponics systems. Results showed a relatively high incidence o parasites including *Ichthyopthirius multifilis*, *Dactylogyrus* spp., motile and sessile peritrichs, and encysted metacercaria. Bacterial pathogens thus far have been opportunistic including *Aeromonas* spp. and *Plesiomonas* spp. No viral etiologies have been identified to date. These results indicate that aquaculture in Grenada is largely affected by parasitic and opportunistic bacteria with relatively little impact by primary pathogens.

This emphasizes the importance of appropriate husbandry and biosecurity practices within local farms and international biosecurity for importation of fish and fish products into the country. Results additionally provide a baseline list of endemic pathogens to guide disease outbreak investigations.

Figure 1. Trichodina spp. isolated from 56% tilapia from waterways

Identified Bacteria	Prevalence
Plesiomonas shigelloides	4.4% (8/180)
Aeromonas spp.	2.7% (5/180)
Klebsiella spp.	1.1% (2/180)
Bacillus spp.	1.1% (2/180)
Pantoea spp.	0.5% (1/180)

Figure 3. Bacterial prevalence in tilapia from waterways

OREGON MARINE AQUACULTURE BARRIERS AND POLICY RECOMMENDATIONS

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Oregon's small commercial aquaculture industry consists of estuarine shellfish mariculture, tank-based seaweed culture, and public and private finfish hatcheries. While there has been interest in expansion among the industry, the state has been slow to progress. In 2021, Oregon Sea Grant conducted a needs assessment to determine major barriers to expansion from the perspectives of multiple sectors (Figure 1). Results of the needs assessment indicated strong interest in expansion from current and emerging growers, but highlighted several barriers, such as permitting/regulations, lack of leasing space, lack of support/ resources, climate/ecological constraints, and technological limitations.

Permitting and regulations were identified as major hindrances, particularly for novel species and technologies. Specific challenges expressed from industry included a lack of guidance on the current processes, lack of regulatory framework for species other than oysters, and support for new growers and changes to existing operations. To begin to address these challenges, Oregon Sea Grant produced a white paper that summarized the current regulatory framework for marine aquaculture in Oregon and provided recommendations for addressing regulatory and policy barriers. This presentation will summarize the needs assessment, outline recommendations for policy change in Oregon from the white paper, and seek feedback on ways to support industry expansion with limited capacity within the state.

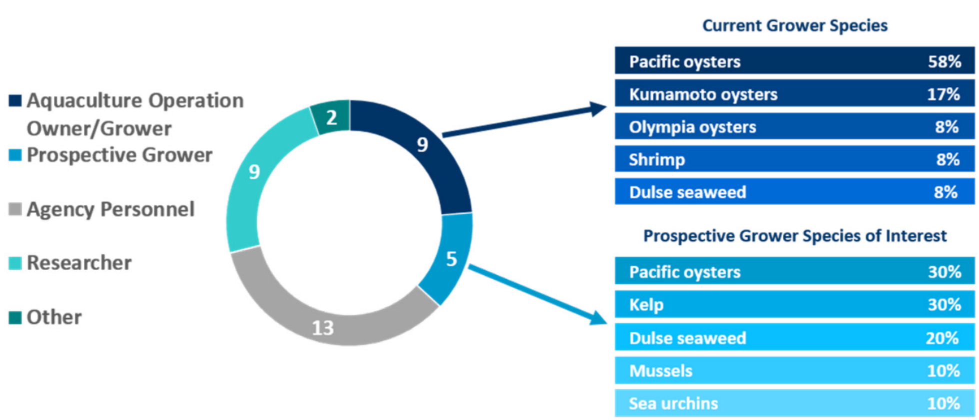

Figure 1. Number of respondents to Oregon aquaculture needs assessment and types of aquaculture species currently grown and of interest.

FEASIBILITY ASSESSMENT OF A LOCAL FEED MILL FOR AQUACULTURE IN HAWAI'I

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In Hawaii, seafood consumption per capita is about twice of the national average. Although Hawaii is surrounded by pristine seawater, Hawaii imports 63% seafood annually. One of the key obstacles for aquaculture development is the high cost of feed. To overcome this barrier, the Center for Tropical and Subtropical Aquaculture, one of five regional aquaculture centers funded by the National Institute of Food and Agriculture, has supported several projects to locate alternative protein sources from other available resources in Hawaii. However, there were yet studies that economically assess the potential of using available local ingredients to produce feed in Hawai'i.

This study aims to assess the feasibility of manufacturing feed using existing feed mill in Hawai'i to meet current demand. First, we conducted a statewide survey on the feed demand from aquaculture and other livestock farms in Hawaii. Then, we developed a comprehensive economic model using Oceanic Institute (OI) research feed mill in Hilo, Hawai'i to estimate the cost of producing feed for tilapia and Pacific threadfin in Hawai'i. We calculated construction cost, fixed cost including annual depreciation, and variable costs. Our preliminary results indicated that feed ingredients are among the highest cost component in the total production costs. Using two scenarios of using locally and imported feed ingredients on two different fish species, we found that local feed ingredients have lower production cost but the significance depends on, among others, fish species. In this report, we will detail the challenges and findings we have encountered from survey to the economic model developed as a reference for any future similar studies.

Table 1. Estimated total production cost to produce 5,635 tons of feed annually in Hawai'i

	Tilapia with 100% imported feed	Tilapia with a combination of locally produced feed	Moi with 100% imported feed	Moi with a combination of locally produced feed
Average Total Annual Cost Per Tons	$1,397.26	$1,267.63	$2,029.34	$1,641.74
Total Annual Cost	$7,873,556.29	$7,143,072.88	$11,435,346.46	$9,251,210.37
COST BREAKDOWN				
Total Construction Cost	$4,009,647.50	$4,009,647.50	$4,009,647.50	$4,009,647.50
Total Fixed Cost	$700,752.54	$700,752.54	$700,752.54	$700,752.54
Total Variable Cost	$7,172,803.75	$6,442,320.34	$10,734,593.92	$8,550,457.84

ACCUMULATION OF METAL IONS IN CLOSED LOOP AQUAPONICS

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We have been operating two aquaponics systems continuously for nearly three years, one with Koi and another with Tilapia. Water quality has been monitored on a regular basis using the API freshwater test kits and drinking water tests strips. Note, we use aged municipal water sourced from Lake Superior, so the source water has very few ions. Although all water quality parameters appeared within optimal levels, we suddenly lost two Koi fish. Ammonia levels had increased slightly, but other metrics appeared normal. Our analytical chemistry colleagues offered to run water samples using their Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipment, and were surprised to find high levels metal ions at least an order of magnitude higher that the source water. We decided to shut down the system and moved all fish to another location (where they are doing well). We recently restarted the system with yellow perch, and take regular water samples to see if the metals are accumulating from the food or other contaminants such as condensation from overhead pipes.

Six months after the Koi mortality event we started losing our large Tilapia. Although the pH was low, no other parameters looked out of line. Water samples were again run on the ICP-MS and high levels of metals were found again. Although not as high as those from the Koi samples, several were in the range of chronic toxicity. Comparing our data to chronic and acute levels from the NOAA Screening Quick Reference Tables (SQuiRTs) suggests levels of Aluminum and Copper may be a factor in the fish mortality (Figure 1). However, we did not see any evidence of fish stress, such as gasping at the surface or erratic swimming prior to the mortality.

Our results suggest that caution should be taken with closed loop systems and recommend testing water for metals on a regular based. Although not all aquaculture facilities have access to an ICP-MS; a number of labs will analyze samples. We are still not sure of the sources of these metals as the Tilapia food is different from the Koi food, and our source water has very few ions. Copper levels may be high due to copper pipes, and vary with residence time of the water in copper pipes. We regularly remove water from the system with the solid waste and replace with aged water, so are surprised that we have an increased concentration of these metal ions. We plan to test all feed samples for contaminants, and will also look for other contaminant sources.

Ions	Ca	Mg	Na	Fe	Pb	Al	Mn	Ni	Cu	Ar	Zn
Units	ppm	ppm	ppm	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
Source Water	18	3.1	1.6	5	<1	23	1	1	~	<1	9
Koi Water	188	15.5	27.7	465	0.084	838	316	15	54	2.6	296
Tilapia Water	88	10	16	50	0.15	500	35	7	30	1.8	47
Acute Toxicity***					65*	750**	2300	470*	13*	340	120*
Chronic Toxicity***				1000	2.5*	87**	80	52*	9*	150	120*

* hardness dependent; **pH dependent; ***NOAA SQuiRT Tables

Figure 1: Measured ion concentrations and toxicity levels

ENHANCING THE SOYBEAN UTILIZATION IN ATLANTIC SALMON, *Salmo salar* DIET VIA USING INSECT MEAL AS A COMPLEMENTARY INGREDIENT

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Aquaculture is the fastest growing sector of agriculture and supplies over half of global fish consumption. Alternative aquafeed options have been explored to improve efficiency and lower production and environmental costs. Soybean meal (SBM) is a major ingredient in aquafeed industry and high inclusion of SBM exhibit soybean induced enteritis in carnivore fish including Atlantic salmon (*Salmo salar*). The black soldier fly (*Hermetia illucens*) is an efficient, nutritionally sound ingredient. Using black soldier fly meal as a complementary ingredient to SBM based diets has been shown to improve performance of rainbow trout in terms of growth performance and mitigate the enteritis. Therefore, our goal was to evaluate the effects of whole black soldier fly larval meal (WBLM) as complementary feed ingredient in soybean meal based diets on growth performance and gut health in Atlantic salmon.

Seven experimental diets were isonitrogenous (42% crude protein) and isolipidic (20% lipid): fish meal based diet (FM), low level SBM based diets (LS), SBM+5% and 10% WBLM (LS-WB5 and LS-WB10) and high level SBM based diets (HS), SBM+5% and 10% WBLM (HS-WB5 and HS-WB10). A total of 630 Atlantic salmon (15 g) were distributed in 21 tanks (triplicates) in recirculatory aquaculture system.

Results revealed that supplementation of WBLM in soybean meal based diets improved the growth performance of salmon. WBLM exhibited significant positive effects in low level of soybean meal based diets whereas high inclusion (10%) of WBLM in high soy bean meal (40%) based diet exhibited negative effects on growth performance of fish. Gene expression pattern related to enteritis and histology distal intestine are being analyzed.

Conclusively, inclusion of whole insect larval meal in low level of soybean based diets improved the production performance of Atlantic salmon for sustainable salmonids production.

AQUACULTURE WORKSHOPS: TEACHING THE TEACHERS

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The Aquaculture Challenge is a competition designed to stimulate high school student interest in aquaculture. However very early on, team mentors asked for help, finding that their students quickly surpassed their knowledge of aquaculture. In response, we have developed and hosted a variety of aquaculture workshops for the teachers to better prepare them to mentor their students. Our most recent workshop was targeted broadly to K-12 teachers, and we had attendance by teachers from second grade through high school levels.

Aquaponics is a very effective platform for teaching STEM in the K-12 classroom, and may also be the best approach to supply the aquaculture industry with an adequately skilled workforce. However, in areas such as the Midwest where commercial aquaculture is less common, many teachers do not have any background in aquaculture.

We have tried several approaches to support the teachers. Initially we gave each Aquaculture Challenge team a kit, cost free, but many teams lacked skills to incorporate all components. Later we offered intensive workshops, but with little hands on activity. We then offered intensive hands on opportunities which teachers found difficult to implement back home. The most recent workshop hosted by LSSU, combined several of these approaches. We offered intensive background on key aspects of aquaponics, but also engaged teachers in a number of hands on activities, with a final project building an aquaponics system they could take home. Most were excited to get their systems started ASAP.

The barriers faced by the individual teachers varied considerably. Some had substantial funding but faced issues with system maintenance. Others had to find ways to convince their administrators to fund a startup system. Response from the teachers indicated they were very happy with the content of the workshop, and enjoyed the tours of LSSU aquaculture, but wished there had been more time. Forcing them to build a small aquaponics system they were able to take with them was key to their enthusiasm for continuing the process with their students. We have all attended a conference and/or workshop where we were initially very motivated to apply the new concepts. However, returning home, many face barriers that reduce the enthusiasm to continue. Our observations from this workshop suggest that getting aquaculture curricula established in the school system requires both teacher training and access to working systems.

CONTROLLED ENVIRONMENT AGRICULTURE INNOVATION CENTER – A TRANSDISCIPLINARY PROJECT TO PROMOTE CEA

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The Controlled Environment Agriculture Innovation Center (CEAIC) is a transdisciplinary center developed and managed by the School of Plant and Environmental Sciences and the Virginia Seafood Agricultural Research and Extension Center at Virginia Tech and the Institute for Advanced Learning and Research (IALR). The CEAIC is located at IALR and has greenhouse- and indoor vertical-based research facilities equipped with various hydroponic and soilless production systems including nutrient film technique, strawberry gutters, dutch buckets and aquaponic systems. Research facilities are outfitted to control, monitor and record environmental parameters such as temperature, light, humidity, airflow and CO_2 concentration. The CEAIC has several analytical labs as well as an engineering lab to support research activities. Currently, research is focused in the areas of new crops development, waste water processing and use, optical sensing, beneficial endophytes and microbiome of tank solutions. Future programming will also incorporate aquaponics. In addition to helping solve problems and create opportunities for the CEA industry, the CEAIC was designed to work closely with industry partners and to develop CEA as an economic development engine. As such, numerous projects are conducted in collaboration with CEA industry partners and the CEAIC is active in extension and work force training.

PHAGOCYTIC AND LYSOZYME ACTIVITY OF SEA URCHINS (*Arbacia punctulate*) IN DIFFERENT PHYSICAL AND CHEMICAL STRESSED CONDITIONS

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Sea urchins perform essential ecological functions in the marine environment. Over the past decades, the entire world has undergone rapid environmental changes and transformations. These changes have affected and caused severe stress to marine species including sea urchins, therefore, it is important to study the effects of environmental stressors on their physiology to save them from extinction. The aim of this study was to investigate the impact of acute and chronic stressors on phagocytic and lysozyme activity of sea urchins (*Arbacia punctulate*) in three different stress conditions that include increased temperature as a physical stressor, lipopolysaccharides (LPS) as a chemical stressor, and both increased temperature and LPS. The coelomocytes of Sea urchins contain phagocytic cells along with other immune cells that circulates freely in the body fluid of coeloms and build the immune system. Coelomic fluid also contain lysozyme, an antibacterial enzyme which helps to protect them from infections agents. We investigated phagocytic activity by using formalin killed- *Bacillus megaterium* and lysozyme activity (*Micrococcus lysodeikticus* as a substrate) of three different stressed group (as mentioned above) and compared them with control. We observed that chemical and physical stressors have a significant impact on phagocytic and lysozyme activity of *Arbacia punctulate*. Each experimental group responded differently with physical and chemical stressors (acute and chronic). Detailed information will be presented at the conference.

CLEANERFISH AQUACULTURE IN THE UNITED STATES, WITH AN EMPHASIS ON LUMPFISH, *Cyclopterus lumpus*, RESEARCH IN NEW HAMPSHIRE

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Lumpfish, *Cyclopterus lumpus,* a species endemic to the northern Atlantic Ocean, has become the focus of the salmonid cleanerfish industry. Commercial or large-scale research production of lumpfish now occurs in many countries including Norway, UK, Iceland, and Canada. American aquaculture researchers are making steady progress in transferring lumpfish rearing technology from their Canadian and European counterparts to their own facilities to catalyze the use of cleanerfish for Atlantic salmon and steelhead trout ocean farms in the United States, specifically in Maine and New Hampshire.

Since 2019, lumpfish have been reared from egg to adult at the University of New Hampshire's Coastal Marine Laboratory. In Maine, at the University of Maine's Center for Cooperative Aquaculture Research and at the United States Department of Agriculture's National Cold Water Marine Aquaculture Center, lumpfish culture and research also occur. Further, the US Lumpfish Consortium, made up of additional research institutions and aquaculture businesses, is working collectively to address some of the barriers that limit cleanerfish use, in general, and to transfer all known technology to the US aquaculture sector.

An overview of ongoing lumpfish research in the US, with an emphasis on NH-based studies, including a variety of projects focused on lumpfish hatchery needs, using lumpfish in salmonid farms, and wild lumpfish population dynamics, will be presented. These studies are funded by the National Oceanic and Atmospheric Administration's Saltonstall-Kennedy Program, New Hampshire Agricultural Experimental Station, New Hampshire Sea Grant, Northeastern Regional Aquaculture Center, and the United States Department of Agriculture's National Institute of Food and Agriculture.

EFFECTS OF VARYING PROTEIN AND LIPID CONCENTRATIONS ON JUVENILE LUMPFISH (*Cyclopterus lumpus*) GROWTH

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Very little is known about lumpfish nutritional requirements even though there are now commercial diets available that are either suitable or specifically marketed for lumpfish. Researchers at the University of New Hampshire (UNH), the USDA National Cold Water Marine Aquaculture Center, and the University of Maine are conducting a series of juvenile lumpfish feeding strategies studies. The first of these feed studies focused on juvenile lumpfish nutrition and was conducted in 2020-21 at the UNH Coastal Marine Laboratory.

A common garden experiment was conducted using 6- to 8-month-old lumpfish, stocked into 0.01-m^3 (10 liter) tanks at initial densities of approx. 10g/L in a flow-through, ambient temperature sea water system. This protein to energy study examined two different crude protein levels (50, 55%) and three different fat levels (10, 15, 20%) in feeds. Lumpfish were evaluated using six experimental diets and a control diet. Experimental diets were formulated by the USDA Aquaculture Research Service at the Bozeman Fish Technology Center in Bozeman, Montana with animal ingredients (fishmeal and poultry-by-product meal) as the main protein source and fish oil as the main lipid source. The control diet consisted of a standard commercially available diet (Skretting Europa, 55/15) that is used in US facilities. In addition, a salmonid diet, BioTrout, consisting of 47% plant-based protein, 24% lipid, was tested too. Fish were fed at 3% body weight/day for 10 weeks and survival, growth, and aggression measured.

In general, varying animal protein and lipid concentrations did not negatively affect growth, survival, or fish aggression of juvenile lumpfish. In all treatments fish survival was >98%. Overall mean percent growth ranged from 633 to 781%, and FCR ranged from 1.10 to 1.23. The use of plant-based protein (and a diet formulated for salmonids), however, suppressed juvenile lumpfish growth; overall mean percent growth was only 394% and FCR was 1.54.

DEVELOPMENT AND FIELD TESTS OF HYBRID AERIAL UNDERWATER ROBOTIC SYSTEM (HAUCS) PAYLOAD FOR AUTONOMOUS DATA COLLECTION

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Fish farms are an important aspect to food security and are currently the source for the majority of fish consumed globally. An important aspect to maintaining a fish farm is tracking water quality, especially dissolved oxygen (DO) levels. The current practice to monitor the DO levels is labor intensive and error prone as it requires manual sampling of every pond in a farm multiple times a day.

The Hybrid Aerial/Underwater Robotic System (HAUCS) is an innovative Internet of Things (IoT) approach to data collection on aquaculture fish farms. The system consists of robotics systems to support automated data collection to the cloud, an AI prediction model, and an app to serve as a user interface between the system and the farm manager. This system also scales well with larger farms as the more expensive components (DO probes and drones) can be used on multiple ponds with the maximum range limited only by the flight time of the drone.

This presentation will discuss the effort to develop a *platform-independent* HAUCS payload that can be easily integrated into different drones and ground vehicles. The prototype was first tested at HBOI campus. Subsequently the system was tested at Touch of Nature (ToN) research aquaculture farm at Southern Illinois University (SIU). These tests will also be discussed in the presentation

Figure 1. (a) Illustration of our platform-independent payload attached to a waterproof drone. (b) The payload underwent test in our laboratory (c) Field test of the HAUCS system at SIU ToN site.

EVALUATION OF WINTER-FEEDING STRATEGIES FOR CHANNEL CATFISH *Ictalurus punctatus* USING COMMERCIAL CATFISH FEEDS

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West Alabama (AL) catfish farmers employ various approaches for feeding catfish during the winter months with no set protocol regarding winter feed management. Farmers typically use daily satiation feedings during the growing season to maximize fish growth and maintain efficient feed conversion ratios. However, fish metabolism is greatly reduced during winter, drastically decreasing feed intake and growth rate. Catfish farmers use feed with 28% or 32% protein or a combination to feed their fish. Because of the uncertain winter feed management, this study aims to investigate different winter feed management strategies using the two feeds most used by the west AL catfish industry.

Two completely randomized trials were conducted with Channel catfish, *Ictalurus punctatus*, at two different sites. Trial A consisted of four treatments with three replicates, totaling 12 pond-based tanks of 2,495 L located at E.W. Shell Fisheries Center, Auburn University. Trial B comprised four treatments and four replicates, totaling 16 pond-based tanks of 800-L located on a Greensboro, AL, farm. The study treatments consisted of a 28% or a 32% crude protein feed fed either 4 times/month or 8 times/month throughout the winter. The feeding protocol consisted of checking the weather forecast, and fish were then offered feed on the warmest day(s) of a given week. A feed response was observed to feed the set rate of 1% of body weight/day. Water temperature and dissolved oxygen concentrations were measured daily; alkalinity, hardness, ammonia, and nitrite were measured weekly. Channel catfish with an average weight of 36.64 ± 0.23 g (trial A; mean ± *SE*) and 36.65 ± 0.39 g (trial B) were stocked in mid-January with water temperatures ranging from -3.3 to 23.2 °C (5.5 ± 1.6 °C). No feeding activity was recorded until mid-February, when water temperature ranged from 10.8 to 15.4 °C (12.9 ± 0.1 °C).

Preliminary results showed differences among treatments in some growth performance and proximate composition parameters. Trial A revealed individual final body weight was higher in fish fed the 28% protein diet 8 times/month (0.58 ± 0.02 kg) compared to fish fed the 32% protein diet 4 times/month (0.52 ± 0.02 kg; $t_{(362)} = 2.9$, $P = .0203$). Whole body crude protein retention in trial A was higher in fish fed the 32% protein 8 times/month (58.8 ± 0.56%) than in fish fed the 28% protein feed 4 times/month (55.1 ± 0.67%; $t_{(8)} = 4.1$, $P = .0140$). Proximate composition showed higher flesh phosphorus retention in trial A in fish fed either diet 8 times/month compared to those fed 4 times/month ($F_{3,8} = 14.2$, $P = .0014$). Trial B presented significantly higher flesh amylase enzyme for fish fed 8 times/month than those fed 4 times/month ($F_{3,12} = 12.7$, $P = .0005$), regardless of feed protein content. Preliminary results suggest changes in production, digestion, and modulation of nutrients from diets containing different percentages of crude protein. Further blood biochemistry could potentially corroborate biological processes and provide more conclusive results.

PHENOTYPIC CHARACTERIZATION OF DISEASE RESISTANCE IN WHITE BASS (*M. Chrysops*) AND STRIPED BASS (*M. Saxatilis*) AND THEIR HYBRID STRIPED BASS OFFSPRING

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Hybrid striped bass (*M. saxatilis* ♂ x *M. chrysops* ♀) (HSB) and its parental species, white bass (WB), and striped bass (SB) were evaluated for their susceptibility to three detrimental bacterial diseases: Columnaris disease, motile aeromonad septicemia, and streptococcosis. Using mean survival time in days post challenge (± SEM) as the measurable phenotypic trait, we found WB to be the most resistant and SB to be the most susceptible with the hybrid offspring in the middle (n = 225, 5-10g per species). Mean survival in days for three diseases was 5.1 (± 0.48), 4.6 (± 0.68) and 2.5 (± 0.67) for WB, HSB and SB respectively which were significantly different ($P \leq 0.05$). The mean percent survival for the three diseases was 66.1 (± 33.1), 34.5 (± 31.9) and 1.1 (± 1.1) for WB, HSB and SB respectively. The culmination of the data generated by these experiments indicate that the striped bass is the most susceptible of the three species and thus the target of selection if improvement of disease resistance phenotype is to be achieved.

EFFECT OF REPLACEMENT OF SOYBEAN MEAL BY BLACK SOLDIER FLY LARVA MEAL (*Hermetia illucens*) ON GROWTH AND AMINO ACIDS PROFILE IN *Labeo rohita*

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Aquaculture sector is progressing at fast pace in Pakistan, so aqua-feed industry has a vast prospect to flourish. The most commonly used protein sources are fish meal and soybean meal. Considering the sustainable protein sources, alternative protein sources are being searched. Use of insects in fish feed has proved its value due to fast fish growth and high feed conversion efficiency. Insects contain high quality protein (up to 74.4 % dry matter), balanced amino acid profile, minerals, and vitamins. Black soldier fly larvae (*Hermetia illucens*) are particularly promising among insects because they are good source of proteins for fish diets.

Present study was carried out to investigate the effects of replacement of soybean meal by black soldier fly larvae meal on growth and amino acids profile in rohu (*Labeo rohita*). Trial started in April, 2022 and continued for four weeks. Four isonitrogenous (23% crude protein) diets were prepared replacing soybean meal with black soldier fly larvae meal (0%, 10%, 20%, and 30%). Fingerlings were (average initial weight= 27.50±0.30 g) reared in replicates and fed at the rate of 2 % of their total body weight. At the end of the trial, survival rate, total body weight, total body length, condition factor, weight gain, specific growth rate, feed conversion ratio was calculated. Profile of amino acid was determined in fish muscles. It was observed that replacement of soybean meal by black soldier fly larvae meal improved growth and amino acid profile of rohu. Essential amino acids like arginine, leucine, isoleucine, valine, and lysine were higher in group with 30% soybean meal replacement. It can be concluded that black soldier fly meal can be an alternative protein source for fish feed of rohu.

Table 1 Summary of growth parameters in four different experimental diet groups of rohu (*Labeo rohita*)

Parameters	C		T1		T2		T3	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Labeo rohita								
TBW (g)	27.00±0.29	35.33±0.53	27.20±0.30	42.00±0.49	27.60±0.33	52.60±0.45	27.60±0.33	58.40±0.39
TBL (cm)	11.10±0.15	13.26±0.18	11.17±0.14	14.05±0.17	11.25±0.11	14.90±0.16	11.27±0.11	15.65±0.12
K (%)	2.00±0.08	1.54±0.07	1.99±0.08	1.54±0.06	1.96±0.06	1.61±0.05	1.95±0.06	1.53±0.03
Weight gain (g)	-	99.96	-	296.00	-	500.00	-	616.00
Specific growth rate	-	0.84	-	0.95	-	1.72	-	1.96
Wt. gain/fish/day (g)	-	1.26	-	1.50	-	1.88	-	2.09
Survival rate (%)	-	100	-	100	-	100	-	100
FCR	-	1.38	-	1.43	-	0.56	-	0.45

THE EFFECT OF WATER TEMPERATURES ON *Nereocystis luetkeana* DEVELOPMENTAL STAGES

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Bull kelp (*Nereocystis luetkeana)* is a predominant canopy forming macroalga along the California coast, creating one of the most diverse ecosystems. Unfortunately, due to a series of anomalous warm seawater events, there has been a 95% decline in these kelp forests along the California coastlines, that have yet to recover. Due to its sensitivity to thermal stress, our goal is to evaluate the temperature tolerance among *N. luetkeana* found at different locations (Humboldt, Monterey and Mendocino) and how the early stages in their life cycle respond to such extreme temperature variations.

An 8-week temperature trial was conducted to evaluate the growth of *N. luetkeana* at three different temperatures: 10°C, 18°C and 19 °C. Each temperature treatment had four 5 L tanks containing five glass slides that were seeded with a spore suspension obtained from reproductive sori tissues from Humboldt County, CA. Tanks were exposed to a 12:12 photoperiod and a light intensity of 15 µE/m2/s. Growth was measured every 10 days by counting the number of attached spores and gametophytes, as well as the presence/absence of sporophytes. To test how increasing temperatures affect the success of spore settlement and development, an ANOVA test was conducted. Statistical difference was considered significant when $P<0.05$.

After 8 weeks, germination occurred at all temperatures; however, sporophytes only developed at 10°C and 18°C. By the end of the trial, slides at 19°C were highly contaminated with other algae, which could have impacted the further development of the gametophytes. Previous publications showed no germination at 18°C, indicating that the upper temperature limit could vary across geographical regions. In future trials, the same experiment will be repeated with reproductive tissues collected from Monterey and Mendocino, to evaluate if there is any significant difference in growth performance across locations. Findings from this study could be beneficial for restorative approaches, such as green gravel, that are currently being implemented to enhance kelp beds.

Figure 1. Gametophyte growth comparison 10 days post-spore-release of *N. luetkeana* collected in Humboldt County, CA when exposed to 10°C (A), 18°C (B) and 19°C (C).

LIFE CYCLE OF AN ANCIENT OYSTER FARMING BUSINESS. THE CASE OF LAKE LICRINUS (85 BC – 79 AD)

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Lake Licrinus, near Pozzuoli in the coast of Campania, was the focal point of a successful oyster farming private business supplying the market in Rome for around one century, from the collapse of the Republic to the consolidation of the Imperial period. Ancient writers and archeological findings provide abundant information about oyster farming, in general, and about the business in Lake Licrinus, in particular. Modern knowledge in economics and oyster biology allows organizing the available evidence into the framework of a theoretical model for explaining the causes of business success and the evolution of the business as a celebrated key actor of the Roman oyster market.

As today, oysters were a superior good, with a positive income elastic demand, and with low or non-significant price elasticity. Transactions took place in a free market framework, with prices changing as a consequence of the shifts in demand and supply, with no significant intervention of the authorities until the early IV Century. Innovation and differentiation appear as the keys for success in the market positioning of the Lake Licrinus oysters. Proximity to the main destination market added another competitive advantage for the producers in Campania.

The business life cycle is described along the different stages, from the start-up to decline. External and internal events and factors affected the revenues and profits. Demand size, economic growth, increasing competition, political instability and environmental conditions are among the events and factors driving the Lake Licrinus industry. The consequences of these factors on demand, prices and profitability are presented along the different stages of the business life cycle.

6
SUBSTITUTION ACROSS WHITE FARMED FISH IN THE MEDITERRANEAN MARKETS

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The market for farmed white fish in the Mediterranean countries is dominated by European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). Generally, both species are farmed and sold by the same companies, since they share the same technology and environmental conditions. Greece and Turkey lead the market in a framework of price competition and low product differentiation. Substitution across country of origin for the same species has been tested and confirmed in previous research, but there is no evidence so far regarding substitution across the two different species. Price integration is tested with the two species and countries in the Spanish and Italian markets. Both countries account for the biggest consumption of seabass and seabream.

All the price series were found to be non-stationary, excepting Spanish imports of Greek seabass and seabream. A VAR system rejected any causal relation across the two species and, by definition, substitution across Greek and Turkish farmed white fish must be rejected given the latest were found non-stationary. However, it cannot be rejected in the case of Turkish seabass and seabream, in which a weak exogeneity test found seabass prices as a causal influence of seabream prices.

All Italian import prices are non-stationary, allowing combined substitution analysis across countries and species. Johansen tests show one single cointegrating vector in which Turkish imports cause changes on the price of Greek imports of both species. Turkish seabass substitutes Greek seabass and seabream in the Italian market, as well as Turkish seabream, which is, indistinctly, a substitute for Greek bream and bass.

Substitution across the two species can not be rejected in the Italian market, as well as in the Turkish imports of seabass and seabream in Spain. On the opposite, Greek imports appear to be differentiated in the Spanish market, both in terms of country of origin and across the two species. Despite the strong similarities in consumer preferences in the two countries, the involvement of Greek and Turkish companies in the domestic value chains differs and may explain any differences in the properties of the price series and the results of the analysis.

INDUCTION OF OUT-OF-SEASON SPAWNING OF AN INTENSIVELY REARED WALLEYE (*Sander vitreus*) BROODSTOCK

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For walleye (*Sander vitreus*) to be a commercially viable aquaculture species, walleye eggs, fry, and juveniles must be available year-round from a domestic broodstock so that producers can bring multiple cohorts of walleye to market throughout the year. Walleye spawn once per year during spring and therefore are not naturally suited to year-round egg production. One potential solution to this problem is to induce out-of-season spawning by manipulating photoperiod and temperature regimes for different cohorts of walleye broodstock thereby providing spring spawning conditions for each cohort at multiple points throughout the year. This approach has been successfully applied to other aquaculture species including zander (*Sander lucioperca*), Common Carp (*Cyprinus carpio*), Channel Catfish (*Ictalurus punctatus*), and Eurasian Perch (*Perca fluviatilis*), but is a novel approach for walleye. The aim of this study was to apply photoperiod and temperature manipulations to three groups of walleye broodstock to induce out-of-season spawning and assess the viability of this approach for developing a year-round supply of walleye eggs and fingerlings. We manipulated thermal and photoperiod regimes for 3 groups of walleye broodstock to simulate spring spawning conditions in February (early), April (in-season), and July (late season). Broodstock from each group were evaluated weekly for egg and milt expression and injected with hCG to initiate ovulation during their respective spring periods. Fecundity, egg size, fertilization success, and eye up success were recorded and compared among spawning seasons. Fecundity was significantly lower for late season broodstock and egg size was significantly smaller in the early-season treatment. Fertilization success was similar for the early and in-season treatments but was 0% for eggs from the late season treatment resulting in no viable offspring. After fertilization, all eggs from the early and in-season treatments were incubated in hatching jars, hatched, and reared in larval systems for 30 days at which point larval growth, survival, and deformity rate was recorded and compared. Larval growth did not differ significantly between early and in-season treatments, but survival was significantly lower for larval walleye from the early-season broodstock. Our results suggest that early out-of-season spawning for walleye is possible, but comes with tradeoffs of smaller egg size and lower larval survival. Going forward, out-of-season spawning efforts may be more successful if broodstock are phase shifted to continue spawning out-of-season for multiple years and broodstock nutrition and environmental conditions are further optimized.

INTENSIVE PRODUCTION OF WALLEYE *Zander vitreus* IN FLOW-THROUGH AND RECIRCULATING WATER SYSTEMS FROM START TO FINISH

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Walleye *(Zander vitreus*) is one of the most culturally important and sought-after species of freshwater sport fishes in North America. Walleyes are native to North America and can be found from the Arctic down to the Mississippi river basin. They inhabit waters across the mid-eastern and central parts of the US and Canada. They have also been introduced to some of the western and northwestern regions of the US. Within this region, Walleye inhabits many varying environments including rivers, lakes, and reservoirs. Walleye were first cultured in the late 1800's and early 1900's as recorded by James Neven in Ontario. In 1948, 44 public hatcheries in the US distributed over 600 million fry and fingerlings of various sizes. Jurisdictions that culture and stock walleye in North America have increased over 410% since 1984.

Walleye stocking is a major program of many fisheries agencies in North America; at least 32 state, federal, and provincial agencies reported stocking walleye between 1986 and 1991. Based on information collected from 36 states and five provinces, it was estimated that almost 869 million Walleyes (all life stages combined) were stocked in North American waters in 2006. More than 1.2 billion walleyes are stocked into North American waters each year because of their popularity as a sport fish. For example, in Wisconsin, walleye is estimated to attract 1.8 million hours of targeted angling effort annually and are the individual species with the highest level of interest from the angling public. The average number of fingerlings stocked annually by each agency during this time was 32.5 million fish for an investment over $19 million US.

All lifestages are cultured intensively and extensively including; Egg, Fry, Small Fingerlings(summer), Extended Growth Fingerlings(fall), Yearlings and Adults-Broodstock. Typically eggs and fry are cultured indoors and the other lifestages are reared utilizing external outdoor ponds of various types. More recently, indoor, intensive systems are being utilized for culture of all lifestages with new and improved equipment and feeds. This presentation will discuss the application of intensive systems for conservation, enhancement, and sportfish management programs as it relates to Walleye and the continued interest in husbandry and culture of various lifestages to support stocking and conservation programs.

INVESTIGATING THE POTENTIAL OF DISTANT HYBRIDS BETWEEN KOI CARP *Cyprinus carpio* AND BIGHEAD CARP *Hypophthalmichthys nobilis*

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Hybridization between different fish species is frequently performed to enhance production in fish culture. Previous work has examined the feasibility and morphological characteristics of hybrids between common carp (*Cyprinus carpio*) and bighead carp (*Hypophthalmichthys nobilis*) primarily in pond culture. Due to the differences in chromosome numbers between common carp (n=100) and bighead carp (n=48), this cross has asymmetric viability only producing surviving offspring when common carp eggs are utilized. Recent investigations identified four different morphotypes and three different ploidy outcomes (diploid, triploid, and tetraploid) in 10 months old fish produced from a cross of these two species. The present work produced Two progenies to evaluate production metrics of the intergeneric hybrid of Common carp (Koi) x Bighead carp from early development up to 6 months old. A half-sibling Koi carp control group was reared in parallel to compare growth performance of the first hybrid progeny. The ploidy of fish produced were examined at 5 days post fertilization (dpf) using whole larvae and after 67 dpf from fin clips using flow cytometry.

The first progeny of Koi carp x Bighead carp was produced from 50 g of eggs from a single Koi carp fertilized with a mixture of sperm from 5 bighead carp males. Only swimming larvae (n = 260) were initially stocked to 10L tanks in four replicates at 6dpf. Survival (**Table 1**) was significantly lower compared to controls during the first two weeks of feeding with live prey items (*Brachionus plicatilus* and *Artemia* nauplii). Large size variability was observed at 14 days of feeding (dof), which led to each initial tank being split into small and large size categories before each was randomly restocked. Significant differences (Welch's t-test) in weight (**Table 1**) were only observed for Koi carp and Koi carp x Bighead carp hybrids between small size category fish at 35 dof. Analysis of ploidy for larvae produced found that almost all larvae examined (n = 40; p = 0.976) were true hybrids parent (c-value = 1.41 ± 0.03 pg) with one chromosome set from each parent. A single larva (p = 0.024) was identified as triploid (c-value = 2.23 pg) having two chromosome sets of Koi carp (2n c-value = 1.75 pg) and a single bighead carp (2n c-value = 1.00 pg) chromosome set. Ploidy analysis of surviving fish at 56 dof identified a relationship between size of fish and whether they were true hybrids (0.84 ± 0.39 g) or triploid individuals (8.35 ± 6.24 g) with most fish which were classified as large being triploids and those categorized as small being true hybrids. The second progeny was produced several months after the first progeny and data are still being assessed. However, initial flow cytometry results of 5 dpf larvae (n = 30, p = 1.0) were similar with those from the first progeny with almost all larvae being true hybrids (c-value = 1.39 ± 0.02 pg).

Table 1 Weight and survival of Koi carp (control) and Koi carp x Bighead carp progenies for 14 and 35 days of feeding (mean ± SD) are presented.

	Weight (mg)		Survival (%)	
	14 dof	35 dof	14 dof	35 dof
Koi carp			71 ± 9	
Small	21 ± 7	295 ± 38		56 ± 1
Large	56 ± 3	638 ± 146		90 ± 11
Koi carp x Bighead Carp			31 ± 4	
Small	23 ± 9	130 ± 23		61 ± 17
Large	95 ± 39	726 ± 182		91 ± 10

F3: ACCELERATING THE RACE TO REPLACE THE FISH IN FISH FEED

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Fishmeal (FM) and fish oil (FO) have historically been the ingredients of choice to provide nutritional advantages in aquaculture feeds. While these ingredients represent efficient nutritional packages, the uncertain future of forage fish stocks means that feed formulators need more options in their pantry to adapt to supply chain disruptions and ensure global food security. To address the growing concern about the availability of FMFO in the future, Future of Fish Feed (F3) was formed as a collaborative effort between NGOs, researchers, and private partnerships to accelerate the commercialization of innovative, substitute aquaculture feed ingredients to replace wild-caught forage fishes.

F3 focuses on three areas:

- F3 Challenge, a series of industry contests to develop and sell fish-free feeds and fish oil replacements within the aquaculture sector.
- F3 meetings and webinars to facilitate networking and collaboration between ingredient suppliers, feed companies, farmers, and investors.
- Feed Innovation Network (FIN) to support the innovation and widespread adoption of fish-free feed ingredients by providing experimental protocols, open formulas, and connections to testing facilities and ingredient providers.

Through these major areas of focus, the F3 team has brought together the aquafeed industry to address the challenges facing the sector and provide opportunities for alternative ingredient providers and fish farmers to connect. Industry feedback on the technical difficulty of developing fish oil substitutes and replacing FMFO in carnivorous fish diets led to the F3 Fish Oil and Carnivore Challenges. F3 meetings brought together emerging alternative ingredient suppliers, investors, and some of the world's largest feed manufacturers to spark new ideas to facilitate FMFO replacement among members of the aquaculture industry. The Feed Innovation Network was launched in response to requests for a repository of alternative ingredient information. F3 feed research trials continue to address knowledge gaps in fish-free ingredient research. Since marine fishes and shrimps claim a disproportionately high value in the seafood market and include some of the biggest users of FMFO, these species are priorities for F3 research.

F3 continues to evolve as the needs of the aquafeed sector change and new opportunities for alternative ingredients arise. New ingredients are coming online every day, but the race to replace fishmeal and fish oil will only advance through collaboration across the seafood industry. The responsiveness of the industry will be a key factor in its ability to feed the growing population and protect the world's oceans.

2022 GLOBAL REVIEW OF TILAPIA PRODUCTION AND MARKETS

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2022 appears to have shaped up as a banner year for tilapia production and consumption. The minor exception seems to have been China, where the continued Covid restrictions hurt production from farms, sales in stores and consumption in restaurants. Otherwise, the global covid recovery has seen bounce backs in production and consumption in most countries. The industry has noted a significant increase in feed prices globally, mostly due to commodity shortages in grains and oilseeds related to Russia's invasion of Ukraine, along with the overall global inflation rate.

However, global inflation has a silver lining for the tilapia market. As one of the lower cost protein sources in many countries, tilapia demand increased considerably. As the pandemic abated, the major importing countries reported increasing grocery sales of fresh and frozen fillets to be prepared for home consumption. High fuel costs also led to considerable price increases for most wild caught seafood, leaving even more space for tilapia (and some of the other farmed white fish) to gain market share. The unraveling of supply chain bottlenecks for refrigerated containers and port facilities was especially helpful for tilapia as so much of the product is transported 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 were able to increase production to supply increases in international demand.

Another bright spot has been the increase in positive press for tilapia. As consumers search for lower cost protein and especially healthy seafood, many writers are suggesting tilapia as a high quality and safe product. The false claims filling the internet from years past, seems to have finally been replaced by truthful and accurate information.

Brazil and Bangladesh have probably been the brightest spots for increased tilapia production (and consumption) in 2022. Correspondents from both countries have predicted that 2022 will see production exceed 400,000 mt. each, while their domestic demand and export markets increased. Vietnam also expects to well exceed 300,000 mt. in production, with most staying in domestic markets. Egypt and Indonesia also expect to see moderate increases in production and demand. Besides the slowdown in China, Ecuador was reported to have reduced tilapia production, switching some production from tilapia back to more lucrative shrimp farming. While US consumption has increased in 2022, domestic production has been static. One of the largest US farms shifted production to barramundi, with the production barely replaced with increases from other farms.

Overall, the best estimates suggest that global production increased in 2022 to 6.8 million mt. 2023 is looking positive and should see increasing US domestic demand and international production and demand. Prices will increase due to overall inflationary pressure, but much less than wild-caught seafoods.

SALINITY TOLERANCE OF BELL PEPPER *Capsicum annuum* IN BRACKISH WATER

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Inland aquaculture systems using brackish-water often accumulate high levels of nitrate and phosphate due to limited water exchange. The effluent from these systems is often difficult to dispose of, as the high levels of salt make it unsuitable for fertilizer and many municipalities do not allow saline discharge into municipal sewer systems. Aquaponics may allow these aquaculture producers to remove nitrogen and phosphorous through plant production. However, there is a limited number of plants that have been shown to be successful in brackish water, many of which are halophytes that may be difficult to market due to consumer preference or unfamiliarity. Bell peppers (*Capsicum annuum*) are one of the most consumed peppers in the United States, with an average of over 5kg of peppers consumed per person in 2018. Bell peppers are also subject to a rapidly increasing trade deficit; in 2006 35% of bell peppers were imported and in 2021 75% were imported. Successfully producing peppers in brackish water may allow producers to reuse water while producing a high demand, locally grown product.

To test the salinity tolerance of bell peppers, fifteen 18L tanks were assigned in triplicate to 5 treatments: 0 ppt, 5 ppt, 10 ppt, 15 ppt, 20 ppt. Two plants were grown in each tank and were susupended using a polystyrene raft. LED grow lights provided 12-16 hours of illumination per day. Crystal Sea® Reef Crystals©, a commercial sea salt mix, was used to reach the taget salinity in each treatment, and each tank was fertilized with 50ml of a commercial hydroponics fertilizer. Water quality parameters (temperature, DO, pH, salinity, conductivity, total ammonia nitrogen, nitrite, nitrate, phosphate, potassium, and iron) were measured weekly.

As of this writing the study is still ongoing, however data was collected recently on day 24 of the study. CCI, height, number of buds, flowers, and peppers all decreased as salinity increased. Several instances of plant mortality have occurred at 15 and 20 ppt. No flowering has occurred at 15 or 20 ppt, however peppers at 5 and 10 ppt have flowered with no peppers currently present. Nitrate and phosphate uptake is significantly higher in 0 ppt compared to all other treatments. Iron uptake is significantly higher in 0, 5, and 10 ppt compared to 15 and 20ppt. Plants are also showing signs of light stress as salinity increases, along with increased cell lysis along leaf veins on the underside of the leaves. Future analyses will include ICP testing of both water and plant tissues to document nutrient dynamics.

FROM GUTS TO GLORY: RECIRCULATING AQUACULTURE OF BLACK GROUPER *Myceteroperca bonaci* AT THE COLLEGE OF THE FLORIDA KEYS

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As a prime target species for the fishing industry in the Florida Keys, pressure is put onto the grouper family. The effects of overfishing fecund adults and the premature harvesting of developing juveniles, takes away from the total annual population and is not a sustainable or responsible practice within the fishery. Despite concerns for the future species populations, the pressure on the grouper family will continue to rise. To combat the intense fishing pressure on such an essential Florida Keys apex predator species, our work with aquaculturing and raising our own grouper through in-vitro fertilization will develop into a one-of-a-kind Florida Keys stock enhancement initiative. Our results, through this in-vitro fertilization process, has the potential to spark a revolution in the marine finfish hatchery industry as well as the aquaculture community worldwide.

As part of 'From Guts to Glory' through the College of the Florida Keys, we are specifically looking at gonads of the black grouper, *Mycteroperca bonaci*. We will harvest male black grouper gametes, cryopreserve the samples, and use the sperm to fertilize female black grouper eggs in an effort to aquaculture a 'College of the Florida Keys black grouper'. We will be working with Dynasty Marine to collect our 9 female broodstock, where they will be housed in a 2,700-gallon indoor recirculating system specially designed to help induce spawning. Our goal is to induce female egg production through simulations of natural spawning conditions; temperature regulation, photoperiod control via lunar cycle simulation, and the use of audio stimulus with male courtship calls.

As data on the aquaculture of grouper species on a semi-commercial scale is limited, our efforts to analyze behavioral interactions of female brood and attempt invitro-fertilzation with the local species faces challenges. Maintaining water quality parameters and deterring aggression among the broodstock are two of the most notable challenges we have faced in the beginning stages of this project. As an aggressive species capable of displaying high levels of dominance, balancing territory through the installation of various pipe structures deters aggression which would otherwise influence hormonal egg production during a simulated spawning event.

EXAMINING THE POTIENTIAL TRANSMISSION OF *Enterocytozoon hepatopenaei* (EHP) BETWEEN *Penaeus stylirostris* AND *Penaeus vannamei*

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Enterocytozoon hepatopenaei (EHP), the etiologic agent of Hepatopancreatic Microsporidiosis (HPM**),** continues to affect shrimp production worldwide. The parasite is known to infect two commercially important marine penaeid species, Pacific white shrimp (*Penaeus vannamei*) and blue shrimp (*Penaeus stylirostris*), as well as the freshwater prawn (*Marcobrachium rosenbergii*). As farmers continue to practice polyculture, it becomes increasingly important to investigate the possibility of HPM transmission among farmed species of crustaceans.

We describe here a simple challenge method to determine the susceptibility of *P. stylirostris* to EHP and its potential for horizontal transmission of EHP to *P. vannamei*. To determine the susceptibility of *P. stylirostris*, EHP inoculum was directly injected into the hepatopancreas of Specific Pathogen Free (SPF) *P. stylirostris*. Seventeen days post-injection, the EHP-injected *P. stylirostris* were divided into two groups. In Group 1, ten *P. stylirostris* were cohabitated with fifty SPF *P. vannamei*. In Group 2, the hepatopancreas was excised from two *P. stylirostris*, minced, and used to orally challenge ten SPF *P. vannamei*. The data gathered provides insight into the possibility of EHP susceptibility in *P. stylirostris*, and transmission potential of EHP from *P. stylirostris* to *P. vannamei* via cohabitation as well as oral challenge.

PACIFIC REGION AQUACULTURE AND COASTAL RESOURCE HUB: REVITALIZING AND EXPANDING AQUACULTURE DEVELOPMENT IN THE PACIFIC

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In 2019, with funding from the National Oceanic and Atmospheric Administration, the University of Hawai‘i Sea Grant College Program (Hawai‘i Sea Grant) established the Pacific Region Aquaculture and Coastal Resource Hub (The Hub) to revitalize and expand aquaculture development by building strategic partnerships throughout Hawai’i and the U.S. Pacific region. Through The Hub, Hawai‘i Sea Grant seeks to improve and support Indigenous and contemporary aquaculture practices, including the restoration, and success of traditional Hawaiian fishponds, management of coastal resources, local and regional food sustainability, related education in Science, Technology, Engineering, and Math (STEM), and to contribute positively to the economic condition of the state and region. The Hub aims to be a major catalyst for aquaculture and coastal resource-related research and products, and has been encouraging participation and contributions across academia, state and local government, industry, not-for-profits, and community groups to achieve its mission of linking the aquaculture industry with the sustainability of natural resources, promotion of healthy coastal ecosystems, and respect for indigenous people. This presentation will highlight The Hub’s research, extension, and education efforts over the past three years and provide some insight into future plans and endeavors.

GENOMIC SELECTION APPROACHES TO DEVELOP DISEASE-RESISTANT SHRIMP

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A successful breeding program depends on the accurate identification of genetically superior individuals that will parent the next generation. Factors that affect accuracy include the number of animals enrolled in the program, the heritability and genetic architecture of the trait, and the method used for prediction breeding values. In addition to that, selection intensity and generation interval are factors that will affect genetic gain.

Traditionally, shrimp breeding strategies rely in selection within family. In such approach, individuals are selected according to their pedigree, and random animals from the best families are kept for reproduction. This approach is limited by its low accuracy, especially for traits with low heritability, which is usually the case for disease resistance. In addition, such methodology cannot identify the difference between animals of the same family, which may lead to an inaccurate selection of candidates.

In the past decade, the inclusion of dense genetic marker chips in genetic evaluations became a commonplace in livestock breeding programs. This technology can be used in shrimp aquaculture resulting in higher accuracies and shorter generation intervals, which leads to higher genetic gains. The single-step Genomic BLUP methodology has the ability to include phenotypes, genotypes, and pedigree of multiple animals, which makes it the method of choice for aquaculture commercial applications. Such method uses the same models as used in pedigree/family selection programs, with little change of statistical models. Moreover, this method allows inclusion of multiple traits and animals without genotypes in the evaluation.

Another challenge in breeding programs for disease resistance is the accuracy of phenotypes. Phenotypes are usually collected after challenge studies, in which individuals are exposed to pathogens and their survival is observed over time. Common phenotypes are a binary survival status at the end of the challenge, or survival days. While progress can be achieved with this information, better phenotypes would optimize the time and resources dedicated to challenge studies. Alternative and novel phenotypes include PCR for quantifying pathogen load, ELISA for toxin levels, microbiome for bacterial community analysis, among others.

Finally, shrimp breeding programs struggle to optimize the number of animals without reducing profitability. There are many questions regarding the number of animals, genotyping strategies, and chip density for shrimp. Simulations show that the current chips with 50k markers are adequate for accurate breeding programs. Moreover, data collection and genotyping must be carried out for at least 3 generations. Finally, collecting data on more animals per family will yield higher accuracies then studying the same number of individuals across several families.

Genomic selection programs can be implemented for accelerating the pace of genetic gain for disease resistance in shrimp. Genetic gains are cumulative and there are no concerns about pathogen resistance or environmental impacts. More importantly, genetic gains are spread to the whole population, which facilitates the observation of the results in commercial implementations. Finally, information about the ideal number of animals and markers will only become available once programs are implemented and those variables tested.

MULTI-COUNTY ASSESSMENT ON LOCAL PERSPECTIVES AND SITE SUITABILITY OF COMMERCIAL RAS OPERATIONS IN MARYLAND, MAINE, AND BEYOND

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The success of commercial RAS production of Atlantic salmon and other high-value marine species will depend on our ability to break industry bottlenecks to flourish as a sustainable and viable option in finfish production. The Recirculating Aquaculture Salmon Network (RAS-N) helped to identify high-priority constraints in need of support and solutions. Our newest project, Sustainable Aquaculture Systems Supporting Atlantic Salmon (SAS^2), is a new iteration of RAS-N, designed to address these needs through research, education initiatives, and extension programming. RAS-N feedback on community perspectives was broad, but there was a strong focus by the industry on public aquaculture literacy and marketing needs. We were left with one question, "What is the role of Extension in all of this?". In SAS^2, Extension chose to focus on needs assessments in rural areas experiencing RAS siting, leasing, and development, starting with the state of Maryland, where an incoming RAS company had begun the permitting process.

As the landscape of commercial RAS production is constantly shifting, our Extension collaborators have had to pivot and reassess programming. Our latest question has been, "How do we shift our programming so that it's less dependent on changes by industry?". Our new focus is to assess local support and opposition to commercial RAS facilities to identify suitable counties for siting and leasing. This presentation will cover our proposed work and generate discussion and feedback from our Extension Community, whose collective experience and expertise are invaluable.

ASSESSING FATIGUE LOADS FOR KELP FARM MOORING LINES

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The farming of brown macroalgae of order *Laminariales* (kelp) has grown tremendously, especially in temperate waters of the United States like in the Gulf of Maine. As sites move from protected near shore waters to more exposed conditions, engineered approaches will be necessary to assess mooring components for fatigue loads. In this study, a set of comprehensive measurements of forcing, biomass and mooring line response were obtained from a kelp farm in the Gulf of Maine exposed to northeast conditions to assess cyclical tensions. Wave and current profile forcing parameters were measured with two Acoustic Doppler Current Profilers. Mooring line tension was measured on each of the two mooring legs with custom made load cell instruments. The mooring leg components examined in this study consisted of 25 mm (1 inch), 3-strand nylon rope connected to the load cells. From the field study, a relationship is presented correlating the waves, currents, and biomass to the cyclical tensions in the nylon mooring rope. Both operational and extreme wave conditions for periods of maximum biomass were estimated by comparing in-situ measurements to those obtained from the National Data Buoy Center station 44007. The fatigue life of the nylon mooring rope was then examined by estimating the number of cycles to failure with techniques described in Huang and Pan (2010), Mandell (1987), and Leeuwen (1981), that included extreme tension values from St. Gelais et al. (2022).

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SUSTAINABLE DEVELOPMENT THROUGH CO-LOCATION: OFFSHORE AQUACULTURE AND MARINE RENEWABLE ENERGY

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As ocean-based development continues to increase, sustainable growth and solutions from marine activities offer opportunities to mitigate climate change and other impacts on the environment. The possibility to co-locate marine infrastructure, or bring together compatible activities, will provide solutions for efficient and sustainable use of the ocean. Marine renewable energy (MRE; energy from the waves, tides, currents, and salinity or temperature gradients) and offshore aquaculture are two industries that are likely compatible for co-location. Synergies between these industries arise as MRE can provide power for offshore aquaculture and can also decrease the environmental impact of aquaculture operations by providing power at sea and replacing the reliance on diesel.

This study aimed to understand the potential for co-location of MRE and offshore aquaculture and provide recommendations to advance this opportunity for sustainable marine development. A review of the different types of MRE technologies and their application to aquaculture operations was first performed. While each MRE technology presents both advantages and challenges for aquaculture, the most promising for offshore aquaculture are wave, ocean current, and thermal gradient technologies due to their ability to be used in environments that are suitable for offshore aquaculture operations. Then, several case studies were highlighted, representing research or pilot projects combining and co-locating aquaculture and renewable energy. Some examples include wave energy devices for nearshore and offshore finfish aquaculture and for offshore seaweed aquaculture and tidal turbines for oyster aquaculture. Using the information and lessons learned collected in this study, opportunities and challenges for co-location were identified (Table 1) as well as recommendations for advancement. These were categorized into three common themes: technical and operational processes, regulatory processes (including environmental and social aspects), and economic impact. Overall, key recommendations include the need for more information on energy demands of aquaculture operations or creating partnerships between MRE and aquaculture industries.

Through a related study, spatial analyses have also been carried out off California, Hawaii, and Puerto Rico to further understand the feasibility for co-location of MRE and offshore aquaculture. Results highlighted the feasibility for co-location in the US by identifying specific areas where these industries align. These studies show the possibility of bringing these marine-based industries together for sustainable marine development.

TABLE 1. Example of opportunities and challenges for co-location, categorized by three main themes.

Theme	Opportunities	Challenges
Technical or Operational	-Shared operations, maintenance, and equipment -Improved energy security & reliability	-Limited energy storage -Unknown interactions between aquaculture and MRE system
Regulatory	-Reduced environmental effects (carbon emissions, fuel/oil spills) -Sustainable/efficient use of marine space	-Unclear and long licensing processes -Need for political/policy support
Economic	-Cost savings with shared equipment and operations -Possible increased market value of products grown using MRE	-More financial support needed (funds, investments) -High upfront capital expenditure

TECHNOLOGIES FOR SUSTAINABLE AQUACULTURE DEVELOPMENT IN AFRICA: WHAT NOW?

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Fish farming or aquaculture is the fastest growing food industry in the world, producing half of global fish production. In Africa, aquaculture is crucial to both the continent's economic growth and meeting food and nutrition security. Though the continent is blessed with abundant natural resources, major challenges to aquaculture development include poor quality fish seed supply, high cost of fish feed production and poor postharvest handling. Technologies for African Agricultural Transformation (TAAT) program, funded by the African Development Bank (AfDB), is a knowledge- and innovation-based response to the need to scale up proven technologies across Africa. In meeting the Feed Africa strategy implementation for aquatic foods, an Aquaculture Compact led by WorldFish was created, with the aim to boost production and productivity. Both soft and hard technologies were deployed with activities implemented through capacity building, demonstrations, outreaches, hatchery and genetics improvement, technology and innovation dissemination, and efficient project management. Aquaculture technologies towards mass production of monosex tilapia and quality catfish seed were produced in hapa, in addition to low-cost fish feed production and fish value development.

Results and lessons learnt in 12 African countries (Benin, Burundi, Cameroon, Côte d'Ivoire, Democratic Republic of Congo, Ghana, Kenya, Malawi, Nigeria, Tanzania, Togo and Zambia) provide opportunities for scaling up. For example, adoption of the technologies disseminated produced 177,358,220 quality fingerlings for dissemination to small and medium enterprises (SMEs) and increased the survival rate of fingerlings production to 90% at92 demonstration centers. Production of Mono sex male tilapia table-sized increased average production from average weight of 200g to 500g in five-month production cycle, while catfish increased from 500g to 1kg on the average. Hence, several African governments, notably those of Zambia, Cameroon, Malawi and Benin have secured loans from multilateral donors to scale aquaculture technologies. Despite the fact that the TAAT Aquaculture Compact has been effective in overcoming the difficulties associated with the tilapia seed production system in Africa, financing for long-term genetic improvement programs is still required to keep the route toward greater production and productivity of farmed fish especially herbivorous species.

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.

BEHAVIORAL RESPONSES OF PACIFIC HERRING *Clupea pallasii* TO POTENTIAL DETERRENCE STIMULI

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Nearshore kelp farming is a rapidly growing industry in the United States, presenting powerful opportunities for economic growth, sustainable food sources, and environmental benefits. However, her-ring spawning events can threaten to undermine the efforts of kelp farmers. A serious need exists for a singular study which would explore the relative effects of different stimuli. We chose to investigate whether certain stimuli could be used to physically contain a group of Pacific herring away from a given region of a net pen.

We subjected Pacific Herring (n ≈ 400) to six different stimuli: a bubble curtain, a 2x2 array of strobing lights (SafetyNet Technologies), an acoustic pinger (Fishtek Marine whale deterrent pinger), a 1x6 array of static fishing flashers, a 1x6 array of moving fishing flashers, and a 2x6 array of moving fishing flashers. We exposed the fish to the stimuli in blocks of 30 minutes on and then 30 minutes off. We recorded fish locations and movements using sonar (Flexview multi-beam; Fig. 1)

We measured the distance between the fish and the stimuli, comparing the distances when the stimuli were on versus off. The fish were largely unafraid or influenced by the flashing lights, acoustic pinger, or fishing flashers. Only the bubble curtain deterred the herring. Our analysis (a multiple linear regression) confirmed our observations. We hope these findings can lead future re- search and attempts to protect kelp farms.

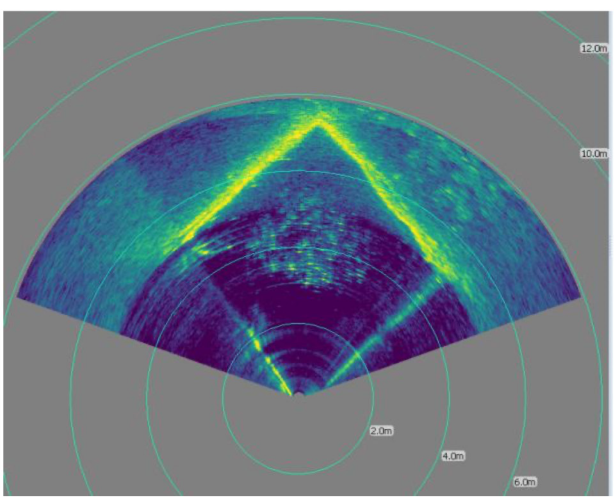

Figure 1: Example sonar image; net pen visible as square while individual herring are visible as smear signals inside the middle of the pen

Figure 2: Results show exposure to bubbles were the most effective at moving herring away from the center of the pen

DIFFERENTIAL GENE EXPRESSION RELATED TO MALPIGMENTATION IN SOUTHERN FLOUNDER *Paralichthys lethostigma*

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Hatchery-produced flatfishes often have a high incidence of malpigmentation. This malpigmentation is generally observed to be irreversible and thought to result from the interaction of genetic and environmental factors, such as larval nutrition and rearing conditions, during early development. The physiological pathways that are altered to produce malpigmentation are not well known.

We sampled skin from the ocular and blind sides of 10 normally pigmented juvenile Southern Flounder and 10 juvenile pseudoalbinos (ocular side almost entirely unpigmented). We used the Tag-Seq method to measure mRNA expression, and then identified differentially expressed genes (DEGs) in all combinations of pigmentation and side of the body. There were 216 DEGs between the ocular and blind sides of normally pigmented fish, but only 7 DEGs in the same comparison of pseudoalbinos (Fig 1). There were no DEGs between the blind sides of normal and pseudoalbino fish. These results suggest that the ocular side of pseudoalbinos has essentially the same gene expression as the blind side.

The comparison of ocular sides of normal and pseudoalbino fish revealed 35 DEGs. Gene set enrichment analysis (Metascape) showed that these 35 DEGs were associated with pigment cell differentitation and signaling pathways involved in pigment production. Similar studies of two other flatfish species found many more differences in gene expression between the ocular sides of normal and malpigmented fish (235 DEGs for Olive Flounder *Paralichthys olivaceus* (Wang et al., 2017) and 271 DEGs for Senegalese Sole *Solea senegal-ensis* (Pinto et al (2019)). The three studies shared only two DEGs (*GCH1* and *MREG*) but the two *Paralichthys* shared 18 DEGs.

We plan to use this information to select specific genes that can be monitored at earlier stages of development in experiments designed to identify the causes of malpigmentation or to screen batches of larvae to estimate malpigmentation rates.

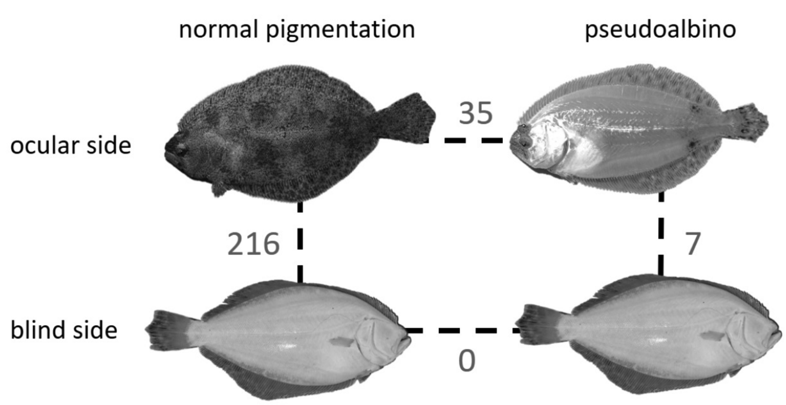

Fig. 1. Comparisons of gene expression in normally pigmented and pseudoalbino Southern Flounder. Numbers are DEGs for each comparison.

COFEEDING LARVAL HYBRID STRIPED BASS WITH LIVE FEEDS AND MICRODIETS SIGNIFICANTLY IMPROVES GROWTH TO THE FINGERLING STAGE

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One factor limiting the growth of the HSB industry is year-round spawning, which requires refined methods to rear larvae in tanks with minimal time spent on live feeds while maximizing growth and survival. The most cost-effective way to accomplish this would be using manufactured microdiets (MDs) in lieu of live feeds. Traditional tank rearing methods for this species involves up to 3-weeks of live feeds followed by weaning onto a high protein starter meal. Our lab has previously established the optimal feeding duration for live feeds to maximize growth and survival. In the current study, we looked at two methods of replacement of live feeds with: 1) direct replacement (substitute live feeds with MDs with no overlap); or 2) cofeeding replacement (live feeds then live feeds cofed with MDs before feeding MDs alone) using six different commercial or experimental MDs measured at two different developmental time points (18 days of feeding and 36 days of feeding).

Significant differences in growth were found for diet, replacement strategy, and developmental time point ($P < 0.0001$ for every comparison). When comparing diets x replacement strategy at 18 days, the effects on growth was not clearly defined, possibly due to the short cofeeding duration (Figure 1). However, after 36 days all diets showed significant improvement in growth under the cofeeding strategy, but still showed inferior growth to an all-live feed strategy (Figure 2).

This study demonstrated that when transitioning larval *Morone* onto manufactured feeds, cofeeding replacement maximizes growth. Further experiments to shorten the period of cofeeding while still maximizing growth will further reduce the costs of live feeds, with the ultimate goal of completely eliminating live feeds from *Morone* indoor larval culture.

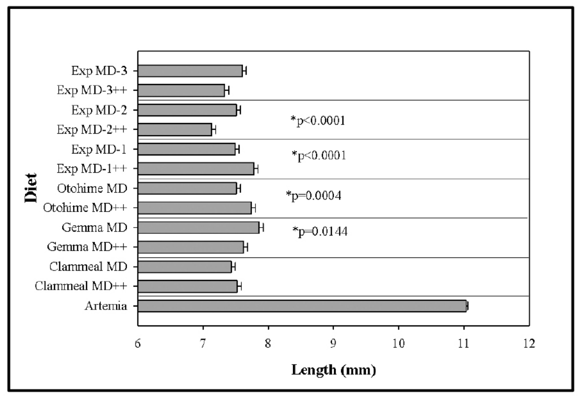

Figure 1. Comparison of traditional versus cofeeding live and MDs for 18 days. Traditional feeding – 8 days live feeds then 10 days of selected MDs
+ indicates cofeeding treatment – 3 days live feeds, 5 days live plus MD, 6 days MD.

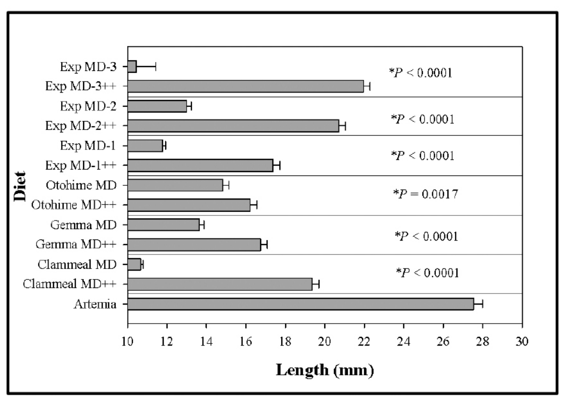

Figure 2. Comparison of traditional versus cofeeding live and MDs for 36 days. Traditional feeding – 8 days live feeds then 24 days of selected MDs
+ indicates cofeeding treatment – 4 days live feeds, 12 days live plus MD, 16 days MD.

NORWEGIAN SALMON EXPORT – DO NORWEGIAN EXPORTERS PRICE DISCRIMINATE BETWEEN BUYERS?

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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 to what degree Norwegian exporters of salmon offer quantity discounts to foreign buyers.

Variation in unit value within narrowly defined HS-codes has most commonly has been associated with quality in the trade literature. For example, a typically result from the widely-used gravity models is that exporters charge higher prices in more distant markets, explained by quality upgrading to reduce the importance of transportation costs. However, in business-to business transactions observed differences in prices between buyers might also be explained by quantity discounts, or second-degree price discrimination. This aspect is often neglected in standard gravity models used to investigate main determinants of exporters' performance.

We provide evidence for price discrimination in transaction-level export data for Norwegian salmon. Within narrowly defined exporter-good-year categories we utilize observed variation in unit value among importers to estimate the elasticity of prices with respect to purchased quantities. The size of the quantity discount is estimated for different goods and different transport modes.

MANIPULATING POST-HARVEST RICE FIELDS FOR MAXIMUMIZING SEASON-LONG FOOD SUPPLY FOR POND RAISED RED SWAMP CRAWFISH *Procambarus clarkii* IN LOUISIANA

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Crawfish producers in south Louisiana create a suitable wetland habitat for crawfish to survive, reproduce and grow to harvest size. These shallow water impoundments are used for both crawfish and rice production. In fields where a crawfish crop follows a rice crop, it is the stubble, harvest debris (straw) and ratoon rice growth that create the food web for the duration of the crawfish season. Rice is typically planted in March or April for harvest in July or August. When the rice is harvested, several thousand kilograms of straw per hectare is left scattered in the field. If fertilized and reflooded, the ratoon growth will result in several thousand more kilograms per hectare of live biomass in the pond by October when ponds are flooded deeper for crawfish production. The live biomass has very low Biological Oxygen Demand (BOD), but the dead stubble and straw have a very high BOD in water. This can result in hypoxic water conditions in the pond during the fall, and reduce crawfish survival, and retard growth. Hypoxic conditions require frequent flushing of the pond at considerable expense.

The persistence of the live and dead biomass contributes to the food web that crawfish need throughout the season. Most of the dead stubble and straw decomposes over the winter with little persisting into the spring. The ratoon growth will eventually die during the winter but will persist in the colder water later into the spring.

Four different stubble management schemes were evaluated at the LSU AgCenter Rice Research Station in Crowley, LA during the 2021-2022 crawfish season. A one-way analysis of variance (ANOVA) was performed to assess the effect of common post-harvest management methods on crawfish food supply during the season. The treatments included bushhogging the stubble, rolling the stubble, burning the straw off the top of the stubble and the control was scattering the straw and not manipulating the stubble in any way. In addition, fertilizer was added to one set of treatments and not to the other set. Total biomass was measured in each treatment monthly from October 2021 to May 2022. Overall, there was no significant difference between the stubble treatments. The only significant difference was due to the addition of fertilizer after the rice crop was harvested. The results of this study suggest that crawfish farmers should consider applying fertilizer to the stubble rather than spending time and resources on manipulating the stubble vegetation.

DEVELOPMENT OF REARING METHODS FOR JUVENILE PACIFIC LAMPREY (*Entosphenus tridentatus*) TO BE USED IN RESEARCH

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The goals of lamprey research at the Abernathy Fish Technology Center are to develop rearing protocols for fish held in captivity, for research and for captive rearing. Conservation aquaculture is a potential technique to help restore Pacific lamprey populations. Lamprey are new to culture and have a unique life history. Research has been done to increase our understanding of lamprey biology which will improve our ability to raise lamprey through their larval stage. Recent work at Abernathy has indicated the importance of water source/temperature, clean sand as well as larval density in raising these fish.

By providing thermal cues in the heated well water treatment (14.7/15.1 ^{o}C), we were able to increase the number of juvenile lamprey produced (30%) compared to Well Water (12%) at 12.2/13.1 ^{o}C. The percentage of juveniles in the Creek Water treatment (27%) at 12.0/13.1^{o}C was comparable to the Heated Well Water treatment (BY15 fish).

The objective of using aged sand versus unused or new sand in a larval lamprey growth trial was to get at the cause for the lamprey growth plateauing in culture. Results indicate clean sand is a better culture medium for lamprey than aged sand. Average weight gain of the fish in the clean sand treatments was significantly higher than the fish in the aged sand treatment (BY18 fish).

For the density study the fish at 34 fish/^{2}m performed significantly better ($P<0.05$) than the fish at the higher density, 76 fish/m^{2} in final average weight, final average length average weight gain and condition factor. Well water was used in this trial and may have been a factor in the number of transformers produced (BY16 fish).

SUSTAINABILITY ASSESSMENT OF MARINE FISH AQUACULTURE IN SEMI-CLOSED BAYS BASED ON AN INDEX

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Marine aquaculture has enormous potential in providing humans with food and high-quality protein. However, with the rapid development and expansion of marine aquaculture, excessive nutrient loading from the farm causes the deterioration of the water environment, resulting in eutrophication and red tide. Therefore, a suitable cultural density within the ecological carrying capacity should be understood for the sustainable development of marine aquaculture. The present study was conducted to propose an index to assess the sustainability for fish culture within semi-closed bays in Japan.

The target area of this study are semi-closed bays in Japan where fish farming is currently carried out. Waste nutrient loading from fish farms was calculated based on the average annual biomass of farmed fish. Fish biomass was calculated based on the number and size of fish cages detected from satellite imagery. The nutrient load released from watershed of bays was also calculated. Based on analysis of factors affecting water quality within the bay, an evaluation index is proposed that integrates the water depth of the bays, area, closure degree, fish farm location, nutrient loading from fish farms and watersheds.

The results of factor analysis show that the nutrient loading, topography, closure degree and the allocation of fish farms are important factors affecting the water environment within the bay. The result of the correlation analysis between the proposed index and the frequency of red tides (Fig. 1) shows that the greater the index value, the higher the probability of red tides and the lower the sustainability for fish aquaculture.

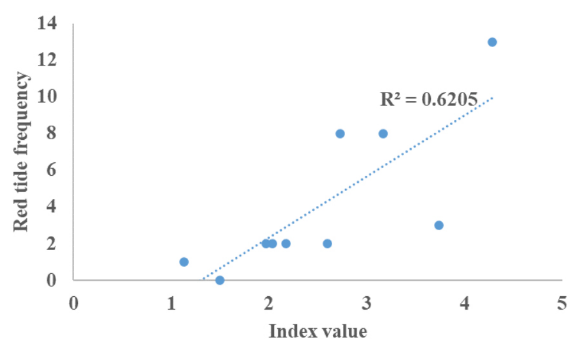

Fig.1 Correlation analysis.

OPTIMAL BIOMASS AND BROMOFORM PRODUCTION IN *Gracilaria parvispora:* A PILOT SCALE EXPERIMENT USING ONSHORE AQUACULTURE

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One promising strategy to reduce climate-polluting methane emissions from livestock is the recent application of seaweed as feedstock in ruminant animals. Bromoform (CHBr3), is a relatively common chemical constituent of seaweeds and has been shown to be the likely active ingredient responsible for methanogen inhibition in cattle and sheep. Most notably this observation has been found in the red alga *Asparagopsis taxiformis* where bromoform concentration is relatively high compared to other seaweeds. However, *Asparagopsis sp*. has thus far proven difficult to culture due to some intrinsic traits of the genus. This study investigates an alternative seaweed *Gracilaria parvispora* as a viable option for scaled production of bromoform containing seaweed. Given the apparent importance of bormoform in seaweed for reducing methane emissions this study expanded on benchtop *Asparagopsis* experiments assessing bromoform emissions to *G. parvispora*. This pilot scale study uses twelve 350 liter conical bottom tanks with untreated flow-through seawater exposed to natural light. Independent variables of interest were irradiance, water temperature, culture time, and immersion time. The series of experiments lasted 6 weeks each with tissue samples taken throughout the experiment. Tissue samples were freeze dried and bromoform extracted using methanol quantified with gas chromatography.

We found that bromoform, a photosynthate, has a positive relationship with temperature and irradiance with a maximum of 503 µg/mg $DW_{biomass}$. However, growth (%/day) was negatively impacted by increased light and temperature. A principal finding in this study was the time needed to increase bromoform concentrations. We have shown that bromoform content can increase as much as 4X from sunrise to the irradiance maximum (< 5 hours). These findings will help inform seaweed culture efforts for the purpose of methane mitigation in ruminant livestock by outlining growth and harvesting conditions that maximize biomass and bromoform content intended for animal feed.

ECONOMIC POTENTIAL OF ON-GROWING LOBSTERS IN FLORIDA

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Spiny lobster is one of Florida's most important commercial fisheries with a value of $42 million. The economic viability of the Florida spiny lobster fishery is inextricably linked to exports of spiny lobster to China. China is the major market for frozen and live spiny lobster, the two major product forms, with about 70% of frozen exports and about 90% of live exports of spiny lobster going to China each year. The high demand for live lobsters in China has put upward pressure on dockside prices, and the price of live lobster is nearly double the price of frozen lobster. Thus, the live export market provides an economic opportunity to enhance the value of the fishery. However, the live market remains underutilized. In recent years, the industry has been challenged by Chinese tariffs and disruptions associated with COVID-19. More importantly, the current management regime has resulted in the majority of spiny lobsters being harvested in the first few months of the season (i.e., August through October) when price and demand in the live market are at its lowest. Harvested lobsters are also susceptible to higher mortality rates during this time due to high water temperatures. On-growing of wild legal-sized lobsters in tanks prior to export is being explored as a means to grow lobsters to a more profitable size and align supply with peak demand associated with Chinese holidays. We conduct a hedonic price analysis to explore how price varies seasonally, by gear, and market segment. We also develop a harvest model combined with biological and economic data collected from on-growing experiments to explore the economic feasibility of on-growing lobsters for the live export market.

Kyphosis vagiensis (AKA. NEUNE) A FISH WITH POTENTIAL FOR COMMERCIAL AQUACULTURE, ON-LAND OR OFFSHORE

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To diversify the types of marine food-fish cultured in the US, Ocean Era has been investigating *Kyphosis vagiensis* (*aka* Nenue or Brassy Chub) as a candidate species for aquaculture. Our results indicate that this fish has tremendous potential for culture in both land-based and offshore systems.

Kyphosids are warmwater marine herbivores found around the globe, but to our knowledge they are not farmed anywhere. The commercial fishery for wild Nenue in Hawai'i is relatively small, but they are often caught recreationally and are consumed both raw and cooked.

This presentation will outline the techniques Ocean Era has used for Nenue broodstock management, larval production and grow out. The results of several feed trials will be discussed, as will the fish health issues we've experienced.

Nenue seem to be well-suited for culture in offshore or land-based systems, and we believe that this species is commercially ready. Initial taste tests and local sales show that the community has an appetite for farm-raised nenue. These fish will be appreciated by markets that demand high quality and sustainable products.

DISTRIBUTION AND PATHOGENICITY OF TWO CUTTHROAT TROUT VIRUS GENOTYPES IN CANADA

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Cutthroat trout virus (CTV) was first isolated in 1988 in spawning trout from the West Coast of the United states. Due to its similarities with hepatitis E virus it has been officially classified as the only aquatic member of the *Hepeviridae* family. Since the discovery of CTV, surveillance of salmon and trout in Eastern and Western Canada isolated and sequenced numerous viruses resembling CTV. Phylogenetic analysis of eight full genomes delineated the Canadian CTV isolates into two genotypes (CTV-1 and CTV-2). Hepevirus genomes typically have three open reading frames but an ORF3 counterpart was not predicted in the Canadian CTV isolates. *In vitro* replication of a CTV-2 isolate produced cytopathic effects in the CHSE-214 cell line with similar amplification efficiency as CTV. Likewise the morphology of the CTV-2 isolate resembled CTV, yet viral replication caused dilation of the endoplasmic reticulum lumen which has not been previously observed (Fig 1). Controlled laboratory studies exposing Sockeye (*Oncorhynchus nerka)*, Pink (*O. gorbuscha*), and Chinook Salmon (*O. tshawytscha)* to CTV-2 resulted in persistent infections without disease and mortality. Infected Atlantic Salmon (*Salmo salar*) and Chinook Salmon served as a host and potential reservoir of CTV-2. The data presented herein provides the first *in vitro* and *in vivo* characterization of CTV-2 and reveals greater diversity of piscihepeviruses extending the known host range and geographic distribution of CTV viruses.

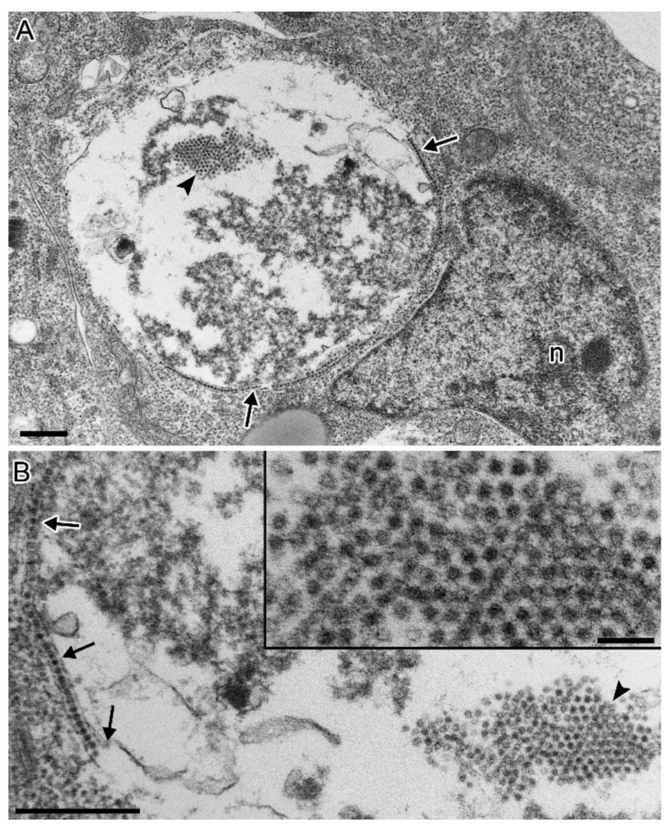

Figure 1. CHSE-214 cells infected with CTV-2. (**A**) A cell with severely dilated ER, making a vacuolar space containing replicating virus. Endoplasmic reticulum (ER) containing viral capsids delimits the vacuole (arrows) and viral capsids are arranged in a crystal array within the vacuole lumen (arrowheads). (n, nucleus) (bar = 500 nm). (**B**) Higher magnification from (**A**) showing viral capsids in a single row within the ER lumen (arrows). Note the inner ER membrane opens to the dilated ER lumen making up the vacuolar space (bottom arrow). Viral capsids arranged in a crystal array (arrowhead and inset) (bar = 500 nm; inset bar = 100 nm).

THE TEXAS A&M AQUACULTURAL RESEARCH AND TEACHING FACILITY

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The Texas A&M Aquacultural Research and Teaching Facility (ARTF) was established in 1973 by the Texas Agricultural Experiment Station and the Department of Wildlife and Fisheries Sciences as the Aquaculture Research Center. The facility originally consisted of 24 earthen ponds of 0.10- and 0.25-acre size, and a laboratory/office building. In 1980 another building was constructed to accommodate fish culture systems for research purposes. Currently the facility has three enclosed buildings containing over 200 individual culture chambers consisting of glass aquaria and fiberglass tanks to support research in various aspects of fish nutrition, physiology and genetics. Three other buildings accommodate culture systems used for induced spawning of marine fish and live foods production. The name of the facility was changed to Aquacultural Research and Teaching Facility in the late 1980s to reflect not only its research but teaching function. The facility supports the teaching of laboratory portions of several aquatic courses as well as extension/service activities.

In 1993 the pond complex was renovated again and expanded to 36, 0.1-acre ponds each with concrete harvest basins. In the most recent renovation (beginning in 2010 and completed in 2012), the ponds were re-shaped and re-sloped with rubber liners installed to minimize soil erosion and maximize water retention. Thus, the ARTF is now comprised of modern pond and laboratory facilities to support research, teaching and outreach activities related to aquaculture and aquatic resource management which have all been continuously conducted at the facility over the past 45 years. During that time, faculty, former students and staff have made numerous contributions in advancing the scientific and technological bases of aquaculture for seafood production and fish stock enhancement. Many have also held leadership roles in various state, national and international aquaculture organizations. Some of the ongoing research and extension projects at the facility will be reviewed.

DIETARY FORTIFICATION OF TURKESTAN COCKROAHES *Shelfordella lateralis* FOR FEEDING ENDANGERED TOADS

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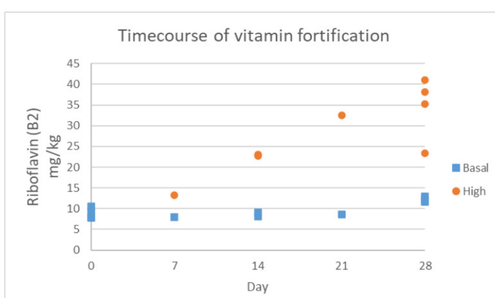
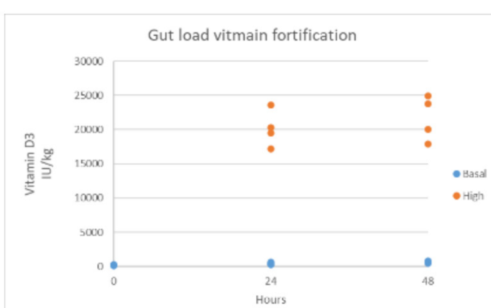
The Wyoming toad (*Bufo baxteri*) and Houston toad (*Anaxyrus houstonensis*) are endangered species reared for restoration by the US Fish and Wildlife Service and partners. Classically defined nutritional requirements for toads are not established. In the wild, toads benefit from a diverse prey base fortified with naturally occurring nutrients. When in captivity, it becomes increasingly difficult to meet the nutritional needs of toads, and a variety of prey items are utilized to provide nutrition to toads. This has led to presumptive nutritional health-related issues, including malnutrition, developmental and reproductive problems, disease, and death. To meet nutritional needs, it is important to understand the organism's nutritional requirements then administer these key nutrients effectively at the appropriate levels.

Three experiments were conducted at the Bozeman Fish Technology Center to quantify dietary fortification levels in Turkestan cockroaches. The first experiment was to determine a time course of vitamin fortification utilizing the current feeds for roaches and a complete vitamin supplement to boost tissue stores in feeder roaches. The second experiment quantified the efficacy of gut loading on vitamin composition of roaches. The third experiment tested the efficacy of dusting insects with vitamin supplement and the residual time of fortification.

The first experiment showed that dietary vitamin fortification was time dependent and diet dependent. Riboflavin and pyridoxine demonstrated linear increases in body concentrations over the 28-day trial for the high supplement diet. The fat-soluble vitamins cholecalciferol (D3) and retinol (vitamin A) plateaued by day 7 in roaches consuming the high supplement diet. The second experiment tested the ability of gut loading to increase vitamin fortification in roaches. The gut load was able to increase roach vitamin levels in by 67-97% for beta carotene and up to 2900% for vitamin D3 (Fig 2), dependent of gut load formulation. The efficacy of dusting will be presented.





PHYTASE CAN REDUCE THE NEED FOR PHOSPHORUS SUPPLEMENTATION IN RAINBOW TROUT *Oncorhynchus mykiss* FEEDS

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Phosphorus is a required nutrient for rainbow trout and is supplemented as a mineral phosphate to meet fish's dietary needs. As plant-derived ingredients are increasingly utilized in trout feeds, increased P supplementation is often needed. This is due to the inability of trout to digest phytate bound P commonly found in plants. Increasing the efficiency of digestion and retention of P from the plant-based feed for rainbow trout will have both economic and environmental benefits for producers. Phytase has been shown to be effective in releasing phytate-P in aquafeeds and in other animal industries supra-dosing phytase has shown benefits other than improving P digestibility.

To test the efficacy of phytase to release phytate-P in trout feeds to meet fish requirements and reduce P discharge, a 2 x 3 factorial experiment was conducted with rainbow trout to test phytase (Quantum Blue, AB Vista) at 0, 2500 and 7500 FTU/kg in a fishmeal-based and plant-based feed. These formulations were also compared to positive control formulations with monocalcium phosphate supplying dietary P requirements. Fish were reared in 15 ^{0}C in triplicate tanks of 40 fish weighing on average 31.9 g each for 12 weeks. Feeds were extruded, floating 4mm pellets with phytase top-coated prior to final oil top-coating.

Rainbow trout grew less when no P was supplemented as monocalcium phosphate and no phytase was added. Interactive effects were observed for growth, feed conversion ratios and feed intake with phytase addition between fishmeal-based and plant-based diets. In plant-based feeds, final fish weight increased form 153 g when no P or phytase was supplemented to 208 g with supplemental P and an average of 213g when phytase was utilized. Phytase improved FCR from 1.14 to 0.90 in plant-based feeds with no effect on fishmeal-based feeds. Phytase addition reduced water-soluble P waste loads by 43% from the fishmeal-based feeds and 56% from the plant-based feeds.

Table 1. Rainbow trout performance on fishmeal-based and plant-based feeds with phytase addition.

Diet	Final Fish Mass		Percent Increase		FCR		Water P	
	g		%				mg/L	
FM Control w/ P	211	ab	570		0.87		0.033	a
FM Control w/o P	193	b	518		0.91		0.018	b
FM w/2500 FTU	216	a	570		0.87		0.018	b
FM w/7500 FTU	207	ab	564		0.89		0.019	b
PP Control w/ P	208	y	551	y	0.93	y	0.040	a
PP Control w/o P	153	z	384	z	1.14	z	0.019	b
PP w/2500 FTU	206	y	550	y	0.91	y	0.016	b
PP w/7500 FTU	221	y	590	y	0.88	y	0.017	b
Pooled S.E.M.	15.92		50.95		0.07		0.004	
Diet type	0.1264		0.1010		0.0257		0.7927	
Phytase level	0.0014		0.0017		0.0132		0.0290	
Diet type X Phytase level	0.0139		0.0237		0.0272		0.4730	

PRODUCTION OF MICROALGAE AND THEIR POTENTIAL USE AS HATCHERY FEEDS IN AQUACULTURE

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Nutritionally complete feeds are necessary to nourish fish to the desired level or form of productivity. The main applications of microalgae for aquaculture are associated with nutrition as sole component or as food additive to basic nutrients and for inducing other biological activities. Microalgae are required as live prey fed to small fish larvae. Microalgae production systems for aquaculture can be grouped in to open and closed culture systems. The technologies needed for cultivation, harvesting, and processing are considered as leading factors contributing to the high costs of producing micro-algal biomass. Although microalgae are feasible sources for hatchery feeds in general, some limitations and challenges remain, which must be overcome to upgrade the technology from pilot-phase to industrial level. A high microalgae concentrations in fish feed could reduce the growth rate and the fish weight. Open culture systems such as large open ponds, circular ponds, raceway ponds and tanks are the least expensive ones; however, issues of vulnerable species contamination, low productivity, high harvesting cost, and large volume of water loss have to be addressed. Closed culture systems are most commonly tubular and 'big bags' in configuration or less commonly flat panel photobioreactors. Microalgae species commonly used in aquaculture include *Chlorella* sp., *Dunaliella* sp., *Spirulina* sp., *Tetraselmis* sp., *Chaetoceros* sp., *Arthrospira platensis*, *Skeletonema costatum*, *Thalassiosira pseudonana* etc. Microalgae also contain immune-stimulants, compounds that modulate the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens. The most challenging and crucial issues are enhancing microalgae growth rate and production costs, seasonal variation, microbial contamination, and low digestibility. The increasing needs for protein and high cost of fish meal has led to the need to search for new alternatives animal and plant sources of protein for aquaculture. Careful species selection and evaluation of growth in various environments are required to reduce the production cost. It should be emphasized that the productivity of any hatchery is directly related to the quantity and quality of the food source used. For microalgae to become a successful alternative to fish meal, it has to overcome number of obstacles.

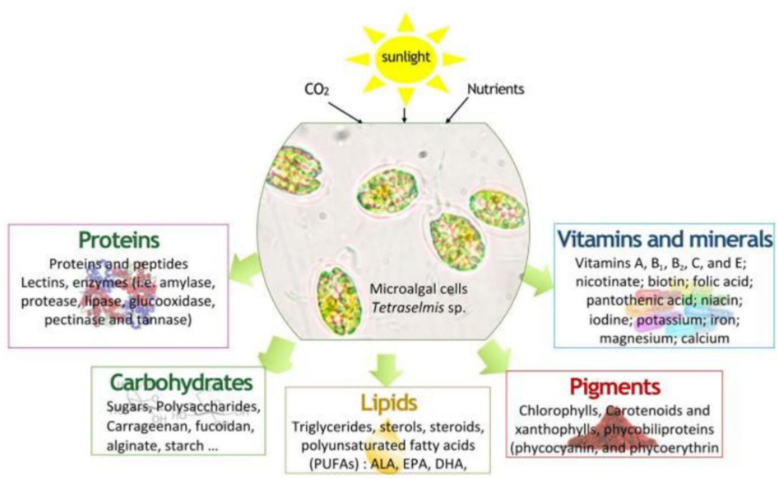

Figure 1: Microalgae metabolites produced during photosynthetic activity

AN INVESTIGATION OF THE POTENTIAL FOR WHALES TO BREAK FIBERGLASS REBAR WHEN USED TO REPLACE ROPE IN MARINE AQUACULTURE STRUCTURES

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Since 2019, the University of New Hampshire has led an investigation into the feasibility of using composite materials to replace rope in kelp aquaculture systems to mitigate the risk of marine mammal entanglement in such structures. The substantial bending stiffness of composite rods should help prevent entanglement of marine mammals.

Entanglement in rope-based fishing gear led to 27 of the 54 known deaths or serious injury events in North Atlantic Right Whales (NARWs) since 2017. NARWs are listed as critically endangered by the International Union for the Conservation of Nature. Entanglement risk reduction has been highlighted as a key priority by NOAA's protected animal resource division.

Tests comparing the performance of various types of composite rod are underway as are lab-based experiments simulating the potential for the composite line to break when interacting with NARW morphologies. Analysis of the degradation of strength and modulus of elasticity of fiberglass as a function of outdoor exposure and use in kelp farm applications has also been completed.

These experiments use the morphology of various of NARW body parts in which the whale contact surface is represented by a circular arc oriented perpendicular to the composite lines.. The applied force, resultant deformed geometry and breaking limit was recorded. This testing will indicate whether the fiberglass rods will break as intended when interacting with different body parts of a NARW at pushing forces achievable by these animals. If successful such breaking behavior will release marine mammals who might otherwise get entangled, while retaining high tensile strength during normal operation.

This research has been conducted using fiberglass rebar from one manufacturer. To widen the scope of investigation, two specimens of fiberglass from two other manufacturers made with different resin-fiber ratios are currently undergoing ocean exposure experiments to evaluate their degradation characteristics in ocean water. If shown to be sufficiently durable, these materials will also be evaluated for their breaking limits using trials similar to those described previously.

	Unused Fiberglass	Fiberglass Deployed in Kelp farm	% Difference
Strength (MPa)	796	753	5.8
Modulus of Elasticity (GPa)	37.6	37.5	0.14

Table1 - Effects of aquaculture structure deployment on fiberglass rods.

POTENTIAL FOR SALMON AQUACULTURE DEVELOPMENT IN THE UNITED STATES

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The salmon market in the USA is mainly import-based, and the country's large-scale domestic producers are just emerging. Atlantic salmon is the most consumed salmon species.

Whole chilled salmon and its fillets are highest in demand on the market. They account for the main part of the import.

Today, only a small amount of salmon is raised in seawater cages; there are several emerging major players in land-based aquaculture, such as Atlantic Sapphire and Aquabounty (both are in the development stage).

The huge potential of the market, which has been import-based so far, provides new players with an opportunity to take on the niche of farming and producing chilled salmon. From technological perspective, the most relevant technology for the US market is farming salmon in land-based aquaculture systems. In terms of technology, there are several solution providers that are currently market leaders in producing equipment for land-based aquaculture; they are located in Israel and Denmark.

Once their projects were launched, some companies faced high fish mortality or failed to reach declared capacity. That is why choosing the right farming technology and a proven concept is a key factor for implementing new investment projects.

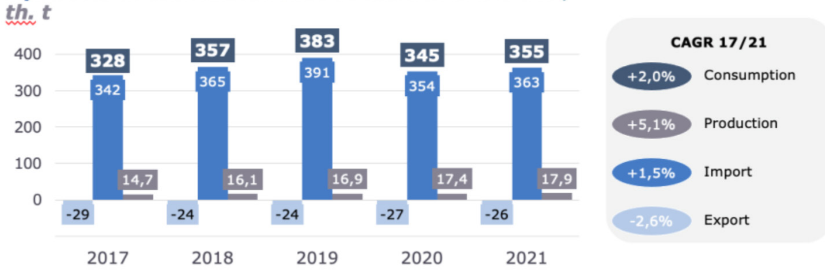
Today, most of the new projects based in the US face problems in obtaining farming permits, which impedes industry development. In addition, required permits vary greatly from state to state.

Another significant problem is access to debt financing for faster project implementation. The majority of ongoing projects were initiated by people from Scandinavia who received funding from investors and banks in their countries.

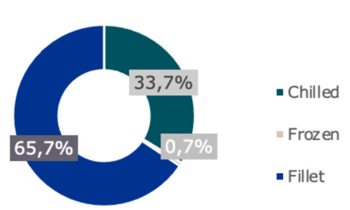
For the market to develop faster, it is necessary to:

- shape project development concepts and select an effective and proven technology
- introduce a less complicated permitting process or find areas for which permits have already been obtained
- have access to debt financing.

Dynamics of the Atlantic salmon market in the USA, *th. t*



US Atlantic salmon import structure, *th. t*



ECONOMIC CONTRIBUTIONS OF THE VIRGINIA SEAFOOD INDUSTRY

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Virginia has a long history and tradition of working waterfronts and maritime economic activity. The seafood industry is organized into different levels of the supply chain from producers, processors, wholesalers, distributors, and retailers. Many of these businesses engage in commercial activities with one another and rely on additional goods produced and services provided by other entities in Virginia for their continued survival and success. The present study is focused on estimating the overall direct, indirect, and induced effects of the Virginia seafood industry in 2019 utilizing primary expenditure data obtained by surveying industry stakeholders.

The primary data on expenditures from watermen, aquaculture farmers, processors, and distributors needed for the economic contribution analysis were collected through an online survey deployed using Qualtrics. The development and execution of the economic impact model used the IMPLAN online system and the analysis-by-parts approach. We created customized expenditure patterns in Microsoft Excel; developing standardized enterprise budgets for watermen, aquaculture farmers, processors, and distributors of the Virginia seafood industry. The expenditures of these activities were converted into spending coefficients and coded by the appropriate North American Industry Classification System (NAICS) sector codes. The coded expenditure patterns were then imported into the IMPLAN online system, relevant models were created, and those models were run and analyzed.

The total economic output effect of the Virginia seafood industry was estimated at $1.1 billion in 2019. The total employment effect of the Virginia seafood industry was estimated to benefit 7,187 people; with a direct effect of 6,051 jobs, indirect effect of 523 jobs, and induced effect of 614 jobs. Retail and restaurant services were not included in this analysis. The Virginia seafood industry supports a wide variety of other economic sectors (64% of 546 industries), from polystyrene foam product manufacturing, boat building, sporting and athletic goods manufacturing, commercial and industrial machinery, and equipment repair and maintenance through direct expenditures by seafood businesses. Non-depository credit intermediation, owner-occupied dwellings, and real estate sectors are supported as wages and salaries paid to employees throughout the seafood supply chain multiply in Virginia`s economy. The estimates presented are conservative and likely underestimating the economic contributions of the Virginia seafood industry in 2019. The response rate was the primary limitation of this analysis, and a potential cause of under-estimated activity expenditures. In addition, the economic impact estimated in this study was confined to activities and expenditures within the state of Virginia. Despite the conservative numbers, the models generated by the primary data collected directly from the industry are relevant and provide a snapshot of the valuable economic contributions and employment opportunities supported by the Virginia seafood industry in 2019.

RECIRCULATING AQUACULTURE RESEARCH PROGRAM UPDATES FROM THE CONSERVATION FUND FRESHWATER INSTITUTE (SHEPHERDSTOWN, WEST VIRGINIA, USA)

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The Conservation Fund Freshwater Institute (TCFFI; Shepherdstown, West Virginia, USA) has a history of leading edge research in the area of recirculating aquaculture systems (RAS) dating back to the 1980s, thanks to funding support from the United States Department of Agriculture's Agriculture Research Service (USDA-ARS). Over the years, TCFFI has supported the growing RAS aquaculture industry through targeted research projects, open access scientific publications, consultation services, conference presentations, private, state, tribal, and federal outreach, and educational courses, among other things. Our current 5-year USDA-ARS research plan includes projects focused on RAS water quality optimization, preventing early maturation in Atlantic salmon *Salmo salar*, Atlantic salmon and steelhead *Oncorhynchus mykiss* strain evaluation for superior performance in RAS, converting RAS waste to sellable products, and assessing and developing precision agriculture technologies applied to RAS. Future research directions will continue to include significant waste-to-value and precision aquaculture R&D, along with focus on stakeholder-identified issues to support the developing RAS industry. Additionally, expansion into alternative (i.e., non-salmonid) fish species and brackish water RAS research will likely be on the horizon for TCFFI.

ASSESSING THE PERFORMANCE, HEALTH, WELFARE, AND PRODUCT QUALITY OF DOMESTICALLY AVAILABLE U.S. STEELHEAD STRAINS RAISED TO 4 KG IN A SEMI-COMMERCIAL SCALE RAS

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Raising rainbow trout to larger "steelhead" harvest sizes (e.g., 3-4 kg) is a niche production approach in the U.S. trout industry, where a much smaller harvest size has traditionally been the norm. Additionally, there is growing interest in land-based recirculating aquaculture system (RAS) production of steelhead trout, i.e., trout produced in a similar manner to the growing land-based Atlantic salmon RAS industry. To inform land-based RAS production of steelhead, baseline research is necessary, including the assessment of various commercially-available strains in an effort to identify the best performing strains in the novel RAS environment. To this end, we evaluated six separate groups of steelhead trout from three domestic sources: i) USDA-ARS growth & fillet yield-selected strain; ii) Producer #1, who provided both all-female diploid and triploid strains; and iii) Producer #2, who provided all-female diploids and two separate groups of triploids. All fish were initially received as eyed eggs from their respective sources, cultured and raised in flow-through tanks until approximately 150 g in weight, and then stocked into a semi-commercial scale RAS and raised to a target harvest size of 4 kg. Additionally, all strains received either constant photoperiod or a winter signal photoperiod (i.e., 6 weeks of 12h light, 12h dark, followed by a return to constant photoperiod) to induce smoltification, to determine whether this process might confer additional performance benefits during growout. A portion of each group was PIT-tagged, with repeated data collection on tagged individuals throughout the growout trial. Final data collection at 4 kg prior to harvest included length, weight, condition factor, coefficient of variation, gonadosomatic indices, deformities, fin condition, fillet yield, fillet color, and fillet proximate composition and fatty acid profiles. At the time of abstract submission, the steelhead strain assessment trial has just been completed, and data are currently being analyzed. Final analyses and recommendations will be presented at Aquaculture America 2023.

THE COLLABORATIVE BENEFITS OF THE NEFSC SEA GRANT CONNECTION: AN EXAMPLE INVESTIGATING POOR GROWTH OF HARD CLAMS IN NEW JERSEY

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The NOAA Fisheries Milford Lab has a strong history of working with industry members to solve issues related to shellfish aquaculture. Meanwhile, Sea Grant has built strong networks throughout the east coast using extension to drive advancements between research, industry, and regulators in the shellfish aquaculture industry. Through the Sea Grant Regional Aquaculture Liaison, there has been a concerted effort over the last year to create better connections between the NMFS aquaculture program and the Sea Grant aquaculture extension network to leverage new partnerships and new funding sources to promote research for sustainable shellfish aquaculture. One project that has come from this effort is an investigation into the slow growth and poor survivorship of hard clams in certain areas of NJ.

The Milford Lab is collaborating with growers, and state regulators in NJ to provide more information to understand the decreasing productivity of Hard Clams in Little Egg Harbor and nearby estuaries. Sampling has begun this past summer collecting water temperature and hard clam condition index. Funding secured from the Northeast SARE program will allow for an expansion of sampling to a second site, collection of additional environmental parameters, and shell age analysis of aquacultured and wild clams. This data combined with existing historical data will lead to better understanding of the growth dynamics of hard clams in New Jersey, and may provide lessons for other regions in the Northeast experiencing hard clam declines.

CONSUMERS' PREFERENCES AND WILLINGNESS-TO-PAY FOR CATFISH PRODUCTS ACROSS THE U.S. SOUTHERN STATES: A CONSUMER BASE SURVEY STUDY

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Increased understanding of the factors that influence U.S. consumers' preferences and willingness to pay (WTP) is critical as it may help restore the economic profitability of the catfish business. Hence, a consumer survey was conducted in 2022 to learn consumers' preferences and WTP in ten U.S. Southern states, including Arkansas, Georgia, Alabama, Mississippi, Louisiana, Texas, Oklahoma, Tennessee, Florida, and Virginia (n=1,360). Preliminary results indicated that most of the surveyed participants (62%) prefer to consume farm-raised catfish products. They also informed us that catfish's source matters when purchasing it from the market. They mostly buy the catfish from the wild-caught (37%), followed by farm-raised (33%) and imported catfish (8%), while the rest were quite indifferent about this matter. Most of these participants prefer to purchase the family-sized catfish fillet packet (4-8 lb), followed by economy size (1-4 lb), large size (8-15 lb), and extra-large size packet (>15 lb) (Figure 1). While asking about their seafood preferences, most participants wanted to see more shrimp items, followed by catfish, tilapia, crawfish, and saltwater fish in the market. With regards to WTP, the majority of the participants want to pay the additional premium price, ranging from 1 to 76 cents per pound, if the seafood products contain information related to USA origin labeling, followed by US-farm raised and certified organic labeling. However, the surveyed participants want to pay fewer premium prices for seafood products containing genetically modified organisms (GMO) labeling.

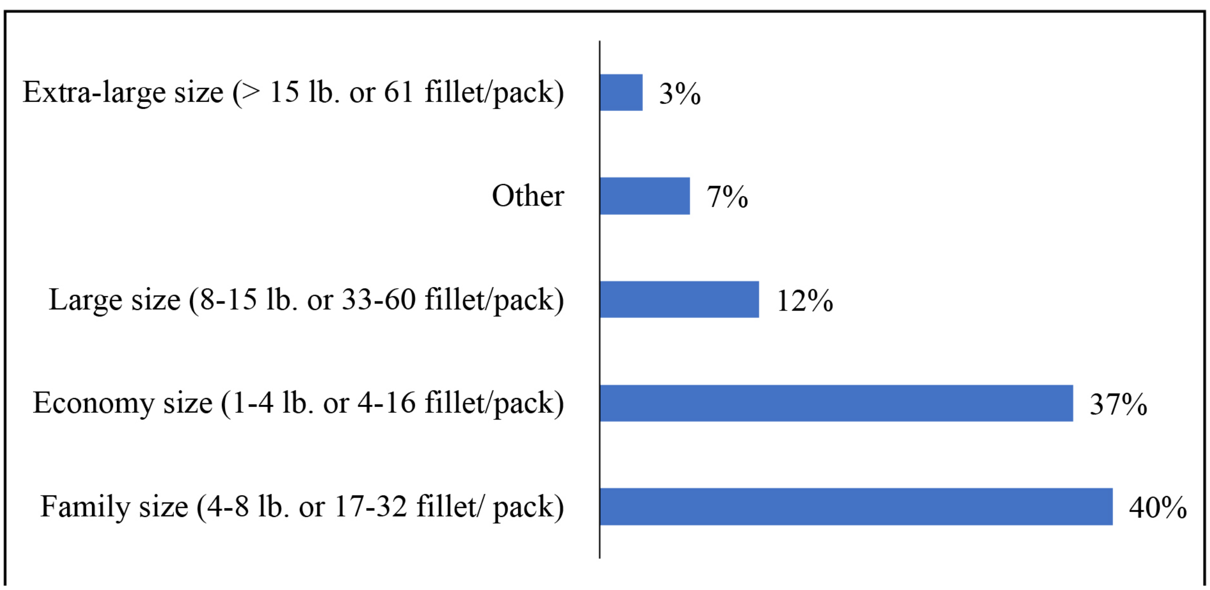

Figure 1: Consumer's preferences for catfish fillet purchase (n=1,360)

PRICE TRANSMISSION ASYMMETRY OF SELECTED FISHES IN BANGLADESH: AN ECONOMETRIC AND VALUE CHAIN ANALYSIS

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Learning the extent of price volatility is critical to ensure the producer and consumer's economic welfare. Hence, we have envisioned assessing the price transmission relationship over time among the farmgate, wholesale, and retail markets for the four major aquaculture products, namely Rui, Catla, Tilapia, and Pangas in Bangladesh. The Granger causality test examined the causal relationships among the farmgate, wholesale, and retail fish prices. The Houck/Ward and error correction approach was also applied to study their price transmission pattern. Results indicate that the retail price leads the wholesale and farmgate price. In most cases, the coefficient of variation decreases as the fish handover from the farmgate to retail levels. Out of 15 pairs of farmgate, wholesale, and retail price-series analyzed, 14 pairs are co-integrated ($P<0.01$). Current results also suggest that the price transmission pattern is symmetric in the short run but could be a mixture of symmetric and asymmetric in the long run. This asymmetric price behavior indicates that changes in retail prices do not get reflected fully at both farmgate and wholesale prices, and the transmitted prices may vary according to the retail prices based on their rising or falling pattern (Figure 1).

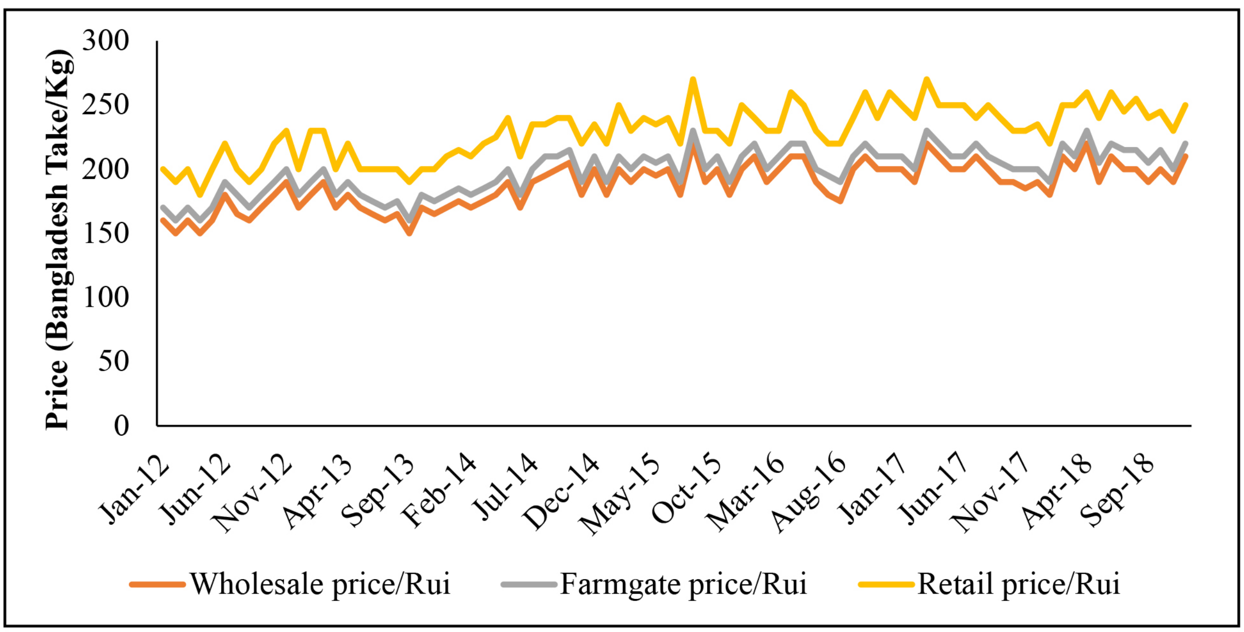

Figure 1: Monthly farmgate, wholesale and retail prices (taka/kg) of Rui for Dhaka.

CONSIDERING PROTECTED SPECIES' STATUS AND HABITAT USE AS ENVIRONMENTAL CRITERIA FOR AQUACULTURE SITING AND PLANNING

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Within a multi-pronged approach to ensuring marine aquaculture production expands in an environmentally responsible manner, the National Oceanic and Atmospheric Administration (NOAA) must avoid or minimize potential adverse impacts to protected species. Various types of aquaculture may cause direct impacts through entanglement or entrapment, and indirect impacts through displacement from important habitats, and changes in trophic dynamics via attraction and depredation. Proactive avoidance of protected species' distributions and habitats during spatial planning and siting of marine aquaculture offers an important opportunity to reduce potential adverse impacts to the populations occurring in the planning area.

In response to a 2020 Presidential Executive Order, NOAA identified the Gulf of Mexico as a primary region to evaluate for an Aquaculture Opportunity Area (AOA), which could support multiple commercial aquaculture farms in the open ocean environment. We developed a generalized scoring model for protected species data layers that captures their relative vulnerability using each species' conservation status and trend (Table 1) and applied this approach to data layers for eight species listed under the Endangered Species Act, including Rice's whale, smalltooth sawfish, giant manta ray, and five species of sea turtle. We evaluated several possible methods for combining overlapping data layers and created a single protected species data layer that was used within a multi-criteria decision-making model for the AOA marine spatial planning process. Results indicated the product method was the best approach as it provided the most logical ordering of and the greatest contrast in site suitability scores. This process identified aquaculture areas with reduced potential for adverse impacts to protected species. This approach is transferable to other regions, to other sensitive or protected species, and to spatial planning for other ocean uses.

Table 1. Generalized scoring system for protected species.

Status	Trend	Converted scores for model
ESA Endangered	declining, small population[a] or both	0.10
ESA Endangered	stable or unknown	0.20
ESA Endangered	increasing	0.30
ESA Threatened	declining or unknown	0.40
ESA Threatened	stable or increasing	0.50
MMPA Strategic	declining or unknown	0.60
MMPA Listed	small population*	0.70
MMPA Listed	large population	0.80

THE EFFECT OF BACTERIAL BASED SINGLE-CELL PROTEIN ON THE GUT MICROBIAL COMPOSITION, PREDICTED FUNCTIONAL PROFILES, AND TAXONOMIC CO-OCCURRENCE NETWORKS ON ZEBRAFISH *Danio rerio*

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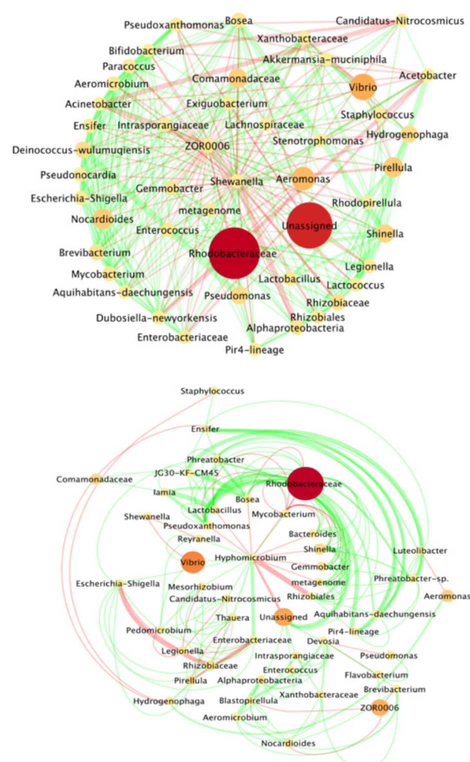
We report the gut microbial composition, taxonomic co-occurrence networks, and predictive functional profiles of zebrafish, *Danio rerio*, fed a bacterial-based Single-Cell Protein diet (SCP) against a standard reference diet via targeted amplicon sequencing of the V4 region of the 16S rRNA gene. The microbial communities of SCP-fed *D. rerio* displayed increased abundances of *Rhodobacter,* Firmicutes *ZOR0006*, and *Planctomycetia*. In contrast, *Vibrio* and *Aeromonas* were dominant in the microbial community of *D. rerio* fed a standard reference diet. The co-occurrence network analysis revealed distinct differences amongst the microbial interactions in the SCP-fed and standard reference fed *D. rerio*. Taxonomic organizations in SCP-fed *D. rerio* were interconnected via *Deinococcus* and *Ensifer* and were determined as key taxa in the microbial network. In contrast, Rhizobiaceae and *Rhodobacter* were revealed to be key taxa in the standard reference diet. Predicted function of microbial community (PICRUSt2) of SCP-fed female *D. rerio* revealed significant upregulation in primary and secondary bile acid synthesis. The SCP-fed male revealed a significant upregulation in predicted metabolism attributes of xenobiotics by cytochrome P450. In contrast, the standard reference diet revealed an increase in cell-motility pathways. These data support future investigations to understand key interactions between the gut microbiome and diet, an essential component to maintain metabolic health in *D. rerio*, an important animal model of human disease.

Figure 1. The network represents the microbial gut ecosystem of female *D. rerio* (above) and male (below) fed the bacterial-based protein diet. The edges are represented via q-value. The edges displayed are coded as green (indicating co-presence) and red (indicating co-exclusion). The nodes represent taxa identified as part of the network, which were scaled via size and color according to abundance.



WATER CHARACTERIZATION AND BIOFILTER SIZING CRITERIA FOR OLIGOTROPHIC RAS HATCHERIES

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Nitrogenous and carbonaceous waste associated with decaying eggs, eggshell debris, and hatching-related fluids, can overload egg incubation systems. In this work, BOD_5, nitrogen, and protein are measured and compared across decaying eggs of 8 species to determine similarity in loading behavior. A statistical analysis shows that marine and freshwater species display similar dead egg loading. Results suggest that a unified sizing criteria developed for application across many species would suffice for incubation biofiltration sizing. A conservative loading of 120 $mg_N d^{-1} g^{-1}$ is estimated for the egg masses of species investigated, assuming a 100% loss and decay over a 24-hour period. Biofilter sizing criteria of 0.8 and 1.6 L g^{-1} for floating bed filter and moving bed filter, using typical media, are recommended.

Table 1: Levels from BOD5, nitrogen, and protein and Tukey's Studentized Range (HSD) test for the eggs of all 8 species tested

Scientific Name	Water Type	BOD_5 (g/g)	BOD Grouping	Nitrogen %	Nitrogen Grouping	Protein %	Protein Grouping
Balantiocheilus melanopterus	Freshwater	0.64±0.00	C, D	10.97±0.06	B	68.55±0.36	B
Thunnus atlanticus	Marine	0.62±0.01	D	11.67± 0.12	A	72.84±0.60	A
Ictalurus punctatus	Freshwater	0.66±0.01	C	10.92± 0.09	B	68.30±0.28	B
Rachycentron canadum	Marine	0.64±0.01	C, D	10.83± 0.06	B	67.74±0.22	B
Lutjanus campechanus	Freshwater	0.71±0.02	B	9.24± 0.15	D	61.41±0.92	C
Cynoscion nebulosus	Marine	0.71±0.01	B	10.13± 0.29	C	68.40±0.55	B
Oreochromis niloticus	Freshwater	0.75±0.01	A	11.67± 0.31	A	73.10±0.42	A
Thunnus albacares	Marine	0.63±0.01	D	11.70± 0.00	A	73.17±0.04	A

EFFECT OF BIOFOULING AND STOCKING DENSITY ON MICROCLIMATE IN OFF-BOTTOM OYSTER CULTURE GROW-OUT BAGS

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Oyster farmers growing C*rassostrea virginica* are increasingly utilizing off-bottom culture practices as there is a potential to improve growth, survival, shell shape, meat quality, and product consistency relative to traditional methods. Despite this potential, sudden spring/summer mortality events impacting both on and off bottom farms, occurring without apparent connection to disease or harmful algal blooms, have been observed along the East and Gulf Coasts, with mortality reaching 85% in 2014 at some Virginia farms. These mortality events could not be explained by changes in ambient water parameters (e.g., a sudden drop in salinity) and typically, the mortalities do not affect every farm in a given waterbody. This pattern suggests that the seed stock and/or the farming practices may be key factors in these mortality events. For the latter, farming practices can lead to substantially different grow-out conditions for oysters.

Conditions inside an oyster bag (dissolved oxygen, pH, turbidity, and chlorophyll-a) may vary from ambient conditions due to a spatio-temporal lag induced by reduced water exchange rates which may be exacerbated by biofouling and oyster stocking density. This ongoing project addresses three questions: 1) Do biofouling control (air-dried vs. not air-dried) and oyster stocking density (high, normal, and empty) decisions affect the microclimate (water quality parameters) inside grow-out bags; 2) Do any observed differences in the water parameters correlate with *C. virginica* performance, including oyster health and disease prevalence; and 3) What combination of farm practices can growers employ to maximize oyster performance while minimizing costs?

Significant effects of both stocking density (typical grow-out density and highly stocked at 150% of typical) and biofouling control (air dried periodically or not) on water parameters within floating bags have been observed in work to date. In a sampling on September 19th, 2022, which occurred two days before a split (decreasing the density of oysters within each bag), stocking density significantly affected dissolved oxygen ($p \leq 0.01$), where dissolved oxygen was significantly lower in stocked bags, regardless of stocking density, relative to empty bags. The interaction of stocking density and biofouling control significantly impacted pH ($p \leq 0.01$), chlorophyll-a ($p \leq 0.01$), and turbidity ($p \leq 0.02$). pH was lower in stocked bags relative to empty bags, while turbidity and chlorophyll-a were also lower in stocked bags, with the exception of highly stocked, non-air-dried bags where the values were not significantly different from bags in empty treatments.

Results from this ongoing study suggest that farmers can influence the water parameters within their floating bags through various husbandry decisions, which could correlate to oyster performance. We hope to provide producers with better data so farmers can make more informed husbandry decisions while also identifying factors that could be driving these spring/summer mortality events.

OYSTER FARMING RESILIENCE INDEX

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Commercial oyster farming has many challenges even during the best of times. To increase the opportunity for success and recovery during extraordinary events the Oyster Farming Resilience Index (OFRI) was developed recently by a team at the Mississippi Alabama Sea Grant Consortium (MASGC). The OFRI is a self-assessment tool developed for oyster farmers to serve as a simple and inexpensive method of predicting if individual businesses are prepared to maintain operations during and after disasters. In recent years, the industry has experienced everything from hurricanes, significant environmental events to COVID which closed restaurants for an extended period of time.

The OFRI is designed to be completed by farmers. However, Sea Grant staff are willing to meet individual farmers to assist in completing the OFRI and all conversations are confidential. When the OFRI is used, each farm should consider the farm's level of preparedness for both large- and small-scale events. Being able to withstand and adapt to events before they happen has become a focal point for businesses and industries. Recognizing the vital role that planning, reparation and collaboration play in developing and executing strategies is essential in building a resilient business.

This brief presentation will cover the major sections of the tool and provide contact information for those that would like to learn more or have questions about any of the indices.

PEPTIDOGLYCAN HYDROLASES TO TREAT *Staphylococcus aureus* INFECTION

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Staphylococcus aureus is a gram-positive, highly pathogenic, opportunistic, multi-drug resistant, and virulent bacteria that cause infections in animals and humans. It can also colonize fish and be maintained in the environment. Possible transmissions from animal to human or vice versa increase the potential spread of infections. Treating those infections is increasingly compromised as *S. aureus* has a great capacity for acquiring new antibiotic resistance, methicillin-resistant *S. aureus* (MRSA) being a significant concern. MRSA has been found in fish and shrimp from aquaculture origin and has emerged as a significant animal health problem worldwide, resulting in an economic burden. In humans, *S. aureus* predominantly causes osteomyelitis, an inflammatory bone disease that leads to progressive bone destruction. Most treatments fail due to the increased resistance of *S. aureus* against antibiotics, thus raising a global challenge for alternative therapeutic strategies that can be used alone or in combination with conventional antibiotics to treat the disease. One alternative is bacteriophage therapy, which showed promise on an individual case basis but remains limited by the strain specificity of phage infection. Phage endolysins are cell wall degrading peptidoglycan hydrolases (PGHs), enzyme antimicrobials that digest peptidoglycan, the major structural component of the bacterial cell wall. We identified two PGHs that can potentially treat *S. aureus* infections. We used the pET21a (+) vector to express PGH-6x His tag in BL21 (DE3) *E. coli*, purified the proteins, and tested against *S. aureus* clinical strains. Our preliminary data suggests new potential alternatives to antibiotics to treat *S. aureus* infections.

HIGH THROUGHPUT CRYOPRESERVATION TO SAFEGUARD ALGAE GENETIC RESOURCES

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Microalgae and cyanobacteria provide the basis of the food chain in most aquatic environments. Currently described microalgal species are around 40.000-50,000, and it is estimated that the total species may be approximately one million. In aquaculture, filter feeders represent a quarter of the world production. Bivalves, fish and early life stages of many aquatic organisms, consume microalgae as their main nutrient source. Thus, hatcheries for these organisms rely on a steady supply of microalgae. Additionally, microalgae are an important feedstock for marine biotechnology products, a sector estimated to be worth over $3 billion dollars. Microalgal strains have been traditionally maintained as live cultures. Due to the time, cost and risk of maintaining these live cultures, as well as the routine loss of traits, only a few thousand strains are maintained in culture collections. To overcome these problems, culture collections have turned to cryopreservation to maintain strains that are less frequently used. The protocols used to this end, have been developed from those used for animal sperm preservation, and are not universally suited for microalgae.

Most microalgal strains preserved are obtained from active cultures and preserved in 0.5 to 1.0 mL containers. This small volume is enough to start cultures in small volumes (1-20 mL) , and it is useful for the preservation of the genetic material. The recovery of these strains for aquaculture use, requires careful planning, as it will be necessary to expand the cultures from this small volume to those needed for hatchery operations. As there is no standardization regarding cell counts or volume and cryoprotectant dose, the time to recover strains from cryopreservation may vary amongst preparations.

In this work, we explored the possibility of using high throughput cryopreservation, with standardized, low-cost containers (0.5 mL French straws), that can be labeled, packed and frozen at the rate of tens of thousands of samples per hour, in a standardized, repeatable way. Microalgal samples of different species, including *Tetraselmis chuii*, *Chaetoceros muelleri* and *Tisochrysis lutea*, were cryopreserved at the culture density, and after concentration to 10^8-10^9 cells mL^{-1}. The concentration of the cells was done by two methods: centrifugation and water removal trough superabsorbent polymers, and combinations of these two methods. The results show that microalgae can be successfully recovered after cryopreservation under standardized, high throughput conditions. The efficiency of the superabsorbent polymers is affected by the salinity of the culture media. Sodium-based polymers are more affected by salinity than the potassium-based polymers. Cryopreserving concentrated samples, a 0.5 mL straw can be used to start cultures 50-500 times larger than samples without concentration, saving weeks of wait for the culture to grow. Concentrating algae for cryopreservation maximize the use of available resources, reduce space, and labor, and preserve higher density biomass for research, conservation and animal feeding.

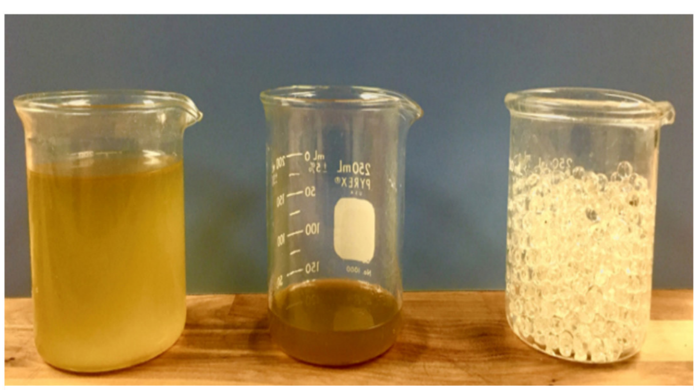

Figure 1. Dewatering of *Tisochrysis lutea* with sodium-based hydrogel. Culture as collected (left). Culture after dewatering (center). Hydrogel after the dewatering process.

THE EFFECTS OF INSECT MEALS SUBSTITUTION ON THE GROWTH, PHYSIOBIOCHEMICAL RESPONSE, GUT MICROBIOME AND IMMUNE RELATED GENES EXPRESSION OF ATLANTIC SALMON *Salmo salar*

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Atlantic salmon (*Salmo salar*) farming in marine waters and in emerging land-based systems is a vital industry globally for a high demand product. As this industry continues to grow, fish nutrition research is the key factor for sustainable feed development and ultimately for sustainable aquaculture production. The purpose of this study was to evaluate the potential of two insect-based feed ingredients as alternatives to fish meal (FM) in the diets of Atlantic salmon using growth, physio-biochemical, gut microbiome, and nutrigenomics approaches.

A 12-week growth trial was conducted in an indoor recirculating system at the University of Maine, Aquaculture Research Institute. Twenty Atlantic salmon parr (38.5 ± 0.1g initial weight) were stocked into each of 16 experimental tanks. A control diet (FM-based diet), and three test diets [substitution of either 50% or 100%FM by defatted mealworm meal, *Tenebrio molitor* (50%DMM and 100%DMM) or by whole mealworm meal, *Alphitobius diaperinus* (50%WMM), on a crude protein basis] were evaluated as a completely randomized design with four replicates.

The study results indicated that Atlantic salmon showed high final growth, survival rate (greater or equal to 98.8%), as well as high feed efficiency ratios (FE) and condition indices when fed the experimental diets. Whole-body proximate and amino acids composition not statistically different between any treatments, while essential fatty acids (including EPA and ALA) were lower in fish fed 100%DMM. Plasma total protein, alanine aminotransferase, alkaline phosphatase and total iron-binding capacity were not significantly affected by dietary substitutions whereas immunoglobulin M showed significantly higher levels in fish fed 50%DMM and 100%DMM when compared to the control. Neither liver peroxides (malondialdehyde content) nor antioxidant enzymes (SOD and GPx activities) were significantly different between treatments. Dietary insect meal inclusion significantly impacted the beta-diversity of Atlantic salmon gut microbiomes. The most common genus in all treatments was *Pseudomonas,* which has been shown to have both pathogenic and commensal members in the literature. The relative expressions of growth (IGF-I) and immune related genes (TIPRL, IFN-γ and IL-1β) were not significantly different between fish fed the different dietary treatments. Overall, this study concluded that the insect meals tested have similar benefits to fish meal in the diet of Atlantic salmon.

SODIUM ALGINATE PRODUCTION FROM IRANIAN MARINE BROWN SEAWWED (*Sargassum illicifolium*) - ANALYZE CHEMICALLY AND TOTAL PRODUCTION COST

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A huge resources of brown seaweed *Sargassum illicifolium* is in coastal boarder of Sistan and Baluchistan province which a sample was harvested from Oman Sea- IRAN, during autumn season, 2019 for analyzing minerals, vitamins, macro nutrients, essential amino and fatty acids, ash content and sodium alginate yield. Seaweed cleaned and rinsed after harvesting, dried under sun light, chopped and cut into small pieces and apart of powdered by grounder to measurement crude protein, lipid, carbohydrates using standard methods. Alginate was extracted from chopped and cut seaweed chemically, using 0.5% formalin for 2 hours, rinsed with fresh water then placed in 0.2 N sulfuric acid for 5 hours, rinsed again to obtain pH 7, and using carbonate sodium for 6 hours, then was filtered. After adding ethylic alcohol, the viscous mixture was separated from its residue by centrifuging at 14,000×g. So a paste form sediment which has been dried to produce clod form, was powdered by blender to obtain sodium alginate and finally total production cost was estimated.

Results revealed that this Chabahar bay brown seaweed has 9.8±1.15 percentage total protein(TP) with 0.77 ± 0.1 % DW of total EAA, 2.09±0.28 percent total lipid (TL) with DHA/EPA ratios of 5, carbohydrate 33.04±2.08 %, , and minerals with cobalt minimum 0.04 ± 0.01 mg/100 g DW, and Magnesium maximum 78.90 ± 9.11, Phenolic content was 28.66±3.05 , antioxidant activity was 36.66±9.8 $mg.g^{-1}$ and alginic acid content was between 12.6-15 percent 648.2% , sodium alginate with molecular weight, Mw, of 8.06×10^{5} g mol^{-1}. During this procedure, total production price was calculated for one kg sodium alginate extracted from amount of wet Sargasso seaweed.

Tab. 1: Production processes of one Kg Alginate sodium powder and cost

	Procedure ()	**Cost**
1	Collecting, Cleaning, Washing and drying	**3.00 $**
2	Chemicals	**4.50 $**
3	Bleaching, Powdering and Packing	**0.40 $**
4	Electricity consumption	**0.60 $**
5	Water consumption	**0.20 $**
6	Labor force	**1.30 $**
7	Total production cost	**10.00 $**
8	Price in Market(2020) Feed grade	**15.00 $**
9	Benefit	**5.00$**

ASSESSING OYSTER GROWTH AND THE HABITAT VALUE OF OYSTER AQUACULTURE GEAR FOR ESTUARINE FISHES AND INVERTEBRATES

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The harvest of Hard Clam (Mercenaria mercenaria) and Eastern Oyster (*Crassostrea virginica*) from Delaware's Inland Bays (Rehoboth Bay, Indian River Bay, and Little Assawoman Bay) has been an important resource for the people of southern Delaware for centuries. From the post-war period to 1979, bottom ground in Rehoboth and Indian River bays was used for the cultivation of Eastern Oyster. Unfortunately, natural oyster populations within Delaware Bay and the Inland Bays have been greatly reduced due to historical overharvest, and disease vectors among other factors. In order to augment natural harvest and support the demand for Eastern Oyster, commercial oyster aquaculture was reinstated in the Inland Bays in 2017. In multiple studies along the US east coast and within the Inland Bays, fishes and invertebrates have been observed around oyster cages and other types of gear used to grow oysters. Oyster farms may therefore be supporting fisheries through ephemeral habitat provisioning for juvenile fishes. Research on oyster aquaculture gear as habitat for fishes within Delaware's Inland Bays has thus far focused on oyster gardening programs and the relative value of artificial oyster reef habitat. Our project aims to further our scientific understanding of the ecological services rendered by shellfish farming by examining the habitat value of 1) traditional on-bottom commercial aquaculture gear and 2) an experimental gear type actively used by a commercial oyster farmer through a single growing season. Preliminary results suggest variability in diversity and evenness are present between two different gear types assessed with higher species richness and diversity observed in bottom gear compared to an experimental floating cage.

TREATMENT WETLANDS FOR TREATING MARINE AQUACULTURE EFFLUENTS: ENGINEERING, SCIENCE AND EMERGING BEST PRACTICES

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Treatment wetlands, taking ideas from constructed wetlands and engineered wastewater treatment systems, are emerging as an engineering approach to sustainably treat a range of effluent, including aquaculture wastes. Marine wastewater adds an extra challenge in that there is salt in the effluent making it difficult to land apply on freshwater plants. This project, a multi-institution collaboration, takes advantage of macrophytes (plants) whose roots and affiliated bacterial communities can remove ammonia, nitrite, nitrate, carbon, and phosphorus from marine aquaculture wastewater. By using salt tolerant plants (e.g. *Juncus sp; Spartina sp; Distichlis sp*) these systems seem effective at removing nutrients in salt water and then reusing or redirecting treated water.

The current study was done in triplicate with three different species of plants (*Distichlis spicata*, *Juncus roemerianus*, and *Spartina alterniflora*) in combinations (by themselves; with one other plant species; or all three plant species). Once plants begin to grow and bacterial communities are developed, the nutrients were removed quite effectively. Preliminary findings indicate approximately 91% ammonium reduction in treatments containing *J. roemerianus*. Phosphate reductions of 22% occurred in the treatment consisting of both *J. roemerianus* and *D. spicata*. The treatments consisting of all three species achieved 95% reduction of combined nitrate and nitrite.

A number of key engineering parameters are needed to predictably design these systems: Temperature effects (on both plants and bacteria); an understanding of the 'warm up' time; rates of nutrient removal and thus effective hydraulic retention time; as well as optimum plant selections (single species or mixed). Data from this study will contribute to our understanding of how to optimally design floating treatment wetlands to remediate nutrients from marine aquaculture wastewater.

Figure 1

6
CAN THE DIVERSIFICATION OF RICE FARMING WITH FISH SUPPORT COMMUNITIES AND COUNTRIES IN ACHIEVING THE SUSTAINABLE DEVELOPMENT GOALS? INSIGHTS FROM NIGERIA, LAO PDR AND P.R. CHINA

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Introduction

Diversification of rice farming with fish allows farmers to harvest the combined yields of the rice and fish crops and this increased production contributes to improved food security. Beyond the production function, however, integrated rice-fish systems may also stimulate and catalyse the achievement of at least nine Sustainable Development Goals (SDGs) and fourteen SDGs targets. This presentation examines how rice-fish farming as a farm diversification strategy can contribute to achieving the SDGs, based on research as well as insights from farmers' field experiences.

Contribution towards the Sustainable Development Goals

The promotion of low-cost integrated rice-fish* farming systems (RFS) leads to higher yields and sale of surpluses reducing the proportion of population living below the national poverty line (SDG 1: No poverty - targets 1.1, 1.2). At the same time, fish from rice fields provide poor families with a constant supply of nutritious food: A "poor man's fridge". Fish provide an emergency meal when other foods are too expensive or unavailable (SDG 2: Zero hunger - targets 2.1, 2.2). The farm diversification with fish helps farmers make the transition to healthier food production, promotes an ecological perspective in food production, and reduces the incidence of diseases in farming communities (SDG 3: Good health and well-being - target 3.3). Women and younger family members in particular support the diversification towards RFS as it lessens their burden to forage food (SDG 5: Gender equality - target 5.1). Also, farmers pay more attention to water management and water-use efficiency increases in RFS. Avoidance of harmful pesticides allows farmers to grow other foods safely at the same time (e.g. aquatic plants) (SDG 6: Clean water and sanitation - targets 6.3, 6.4, 6.6). RFS provide decent work on the farm and can kick-start a farming transition as economic benefits increase (SDG 8 Decent work and economic growth - targets 8.2, 8.3), reducing the proportion of poor people living below 50 per cent of the median income (SDG 10: Reduced inequalities - target 10.1). Farmers pay more attention towards the ecological use of resources in the watershed (SDG 12: Responsible consumption and production - target 12.2). Finally, experimenting with diversification leads to enhanced knowledge. Modification of rice fields e.g. with a deepened refuge can enhance resilience to effects of climate change (SDG 13: Climate action - target 13.3).

Conclusion

The transition from rice farming to integrated rice-fish systems can contribute to the achievement of several SDGs in all three dimensions – economic, social and environmental. Governments and international development agencies should promote rice farm diversification with fish and support its upscaling, for the benefit of farming communities and the environment.

*The term "fish" includes finfish as well as other captured and cultured aquatic organisms.

GENETIC IMPROVEMENT OF ROHU (*Labeo rohita*) IN BANGLADESH

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Rohu carp (*Labeo rohita* Hamilton) is a globally significant aquaculture species with over 2.0 Mt produced annually. In Bangladesh, it is the most abundantly cultured carp species. However, suboptimal genetic management of hatchery broodstock and a lack of genetically improved strains has historically resulted in the dissemination of rohu seed exhibiting poor performance. To address these issues, the family-based WorldFish Rohu Genetic Improvement Program (WFRGIP) was initiated with the spawning of a base population in 2014 (Figure 1). The WFRGIP aims to improve growth rate by 10% per generation.

In 2020, a multiplier population comprised of highly-ranked WFRGIP Generation 3 (G3) families was released to hatcheries in Bangladesh for development into broodstock. These G3 multiplier broodstock were spawned in commercial hatcheries for the first time in mid-2022. On-farm performance trials revealed that the 2020 G3 multiplier outperformed a well-regarded commercial strain and the WFRGIP unimproved control line (by 37%, on average) across 19 semi-commercial farms (Figure 2).

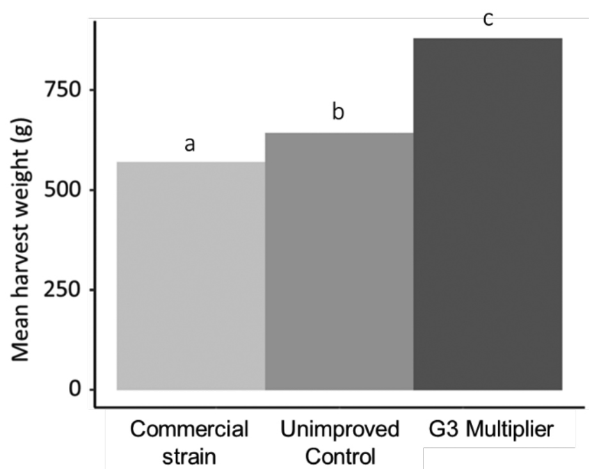

FIGURE 2. Mean harvest weight across 19 semi-commercial farms in Bangladesh.

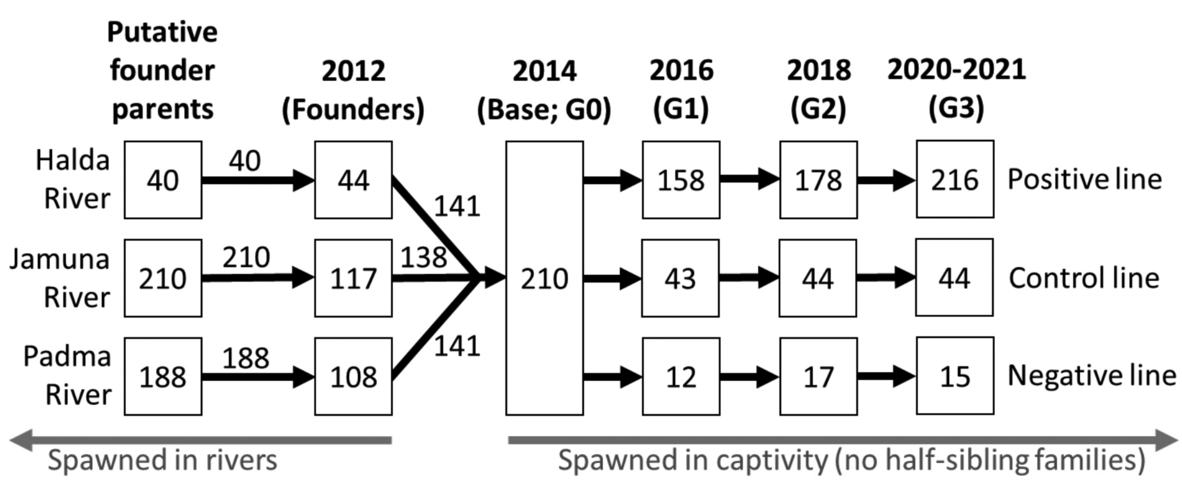

FIGURE 1. Families generated in the Worldfish Rohu Genetic Improvement Program. The number of full-sibling families by line and generation are shown in boxes and the number of parents used to generate families are shown adjacent to arrows.

UNDERSTANDING AQUACULTURE BIOSECURITY TO IMPROVE CATFISH DISEASE MANAGEMENT IN OGUN AND DELTA STATES, NIGERIA

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Nigeria is now one of Africa's largest aquaculture producers with catfish and tilapia being the dominant farmed fish. Yet the lack of a clear aquatic animal health strategy has resulted in substantial disease-related production losses. There is little or no biosecurity management practiced at the production level, except by a few large-scale commercial farms. This study aimed to better understand epidemiology and risk factors leading to mortality and production losses of catfish in a regional model using Ogun and Delta States. WorldFish and partners developed a Fish Epidemiology and Health Economics digital survey tool to collect baseline data from 399 farms.

Descriptive statistics were classified by state identity and unusual level of farm mortality, were calculated for production system, biosecurity, management, and other potential risk factors on 220 farms, which only raised table size catfish. Mixed model logistic regression was used to assess the association between the occurrence of high mortality and these potential risk factors. State identity was included as a random effect.

Key findings:

- A significant association between unusual farm mortality and state identity wasn't detected (p=0.314) but it was included as a random effect to account for it as a source of variation.
- 10.45% farms experienced unusual fish mortality
- Farms having unusual fish mortality (n=23) had a higher baseline mortality compared to farms without unusual fish mortality (n=197), 15.1% and 6.6% respectively
- No biosecurity procedures were followed at stocking (96.8%), or between production cycles (30.9%)
- 14.6% of farm kept paper records of mortality losses, while other farms estimated losses from memory
- Farms that dried ponds between productions had less unusual mortality (8.47%) compared to farms that didn't (11.18% unusual mortality) but the difference was not significant (p=0.856)
- Farms that did not share equipment/staff with other farms had lower unusual mortality (8.28%) compared to farms that shared with one (15.38% or two or more farms 16.00%) but the differences were not significant (p=0.443)
- Farms that added fish to the stock after the main stocking event reported higher unusual mortality (42.86%) than farms that did not (only 9.39% unusual mortality) (p=0.045)
- Only 1.36% of farms reported using the services of a veterinarian

Conclusions:
Biosecurity can be improved. Risk factors analysis allow a better understanding of the industry and can further inform development of interventions in the form of better management practices, guidelines for national aquatic health strategies and farm level biosecurity plans for sustainable aquaculture in the targeted regions.

HARNESSING MACHINE LEARNING TO ESTIMATE AQUAUCULTURE'S CONTRIBUTIONS TO THE ECONOMY OF SOUTHWEST BANGLADESH

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Aquaculture in Bangladesh has grown quickly over the past three decades, becoming a major contributor to the country's rural economy. National systems for collecting aquaculture statistics have not kept pace with these changes, so more accurate, up-to-date information is needed to inform policymakers. We used machine learning techniques to extract information from freely available satellite images and estimate the area of waterbodies used for aquaculture in seven districts in southern Bangladesh, one of country's most important aquaculture zones producing fish for domestic markets and crustaceans for export. We combined machine learning derived estimates of aquaculture farm area per district with data from statistically representative farm surveys to estimate farm size, productivity, and total output, economic value of production, on-farm employment generation by gender, and demand for formulated and non-formulated feeds. Machine learning estimates returned a total farm area similar to that reported in Department of Fisheries (DOF) statistics, but we estimate that production of crustaceans (shrimp + freshwater prawn production) is 31% lower than officially reported by DOF in 2020, while fish production and total aquaculture production (fish + crustaceans) are 41% and 27% higher, respectively. Aquaculture makes a massive contribution to food production, farmer incomes and employment in southern Bangladesh. We estimate that there were more than 500,000 farms in 2020, producing 787,000 t of aquatic food (89% fish and 11% crustaceans), with a mean yield of 3.1 t/ha. This production was worth a total $1.45 billion (farmgate value) and generated farm profits of $0.67 billion, after subtracting production costs (Table 1). These farms support 430,000 fulltime equivalent (FTE) jobs on-farm, of which 15% worked by women, and created demand for 759,000 t of feed, of which 30% comprised of formulated pelleted feeds. Our findings reveal great potential to combine remote sensing and machine learning techniques with representative surveys to estimate a range of statistics that are difficult to obtain otherwise, with potential to expand the approach to whole of Bangladesh and other countries.

Table 1. Estimates of selected aquaculture statistics in southern Bangladesh by district

Estimate	Bagerhat	Barisal	Bhola	Gopalganj	Jashore	Khulna	Satkhira	All districts
Farm area ('000 ha)	73.6	6.2	11.1	5.8	37.8	72.2	72.1	278.8
No. of farms ('000)	66.8	17.2	29.7	19.1	183.3	130.0	101.8	546.9
Mean farm size (ha)	1.1	0.4	0.4	0.3	0.2	0.6	0.7	0.5
Mean yield (t/ha)	2.4	5.1	5.4	2.8	4.3	2.0	2.6	3.1
Aquatic food ('000 t)	177.4	31.6	60.4	15.9	163.9	147.6	190.1	787.1
Fish ('000 t)	148.9	31.6	60.4	15.6	160.0	120.6	163.6	700.7
Crustaceans ('000 t)	28.5	0	0	0.3	3.9	27	26.5	86.4
Farmgate value (Million USD)	394	39	106	20	220	335	341	1,454
Total farm profit (Million USD)	185	14	36	9	95	172	160	670
Total employment ('000 FTEs)	141.0	9.6	14.9	3.5	31.7	84.7	146	431.5
Female employment ('000 FTEs)	18.2	2.7	0.9	0.96	5.6	17.7	16.0	66.2

EXTRACTION AND DETECTION OF GEOSMIN AND 2-METHYLISOBORNEOL FROM LIPID-RICH FISH TISSUE USING AUTOMATED SORPTIVE EXTRACTION

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Geosmin (GSM) and 2-methylisoborneol (MIB) are naturally occurring volatile organic compounds derived from microbial metabolism. Relatively low concentrations of GSM and MIB can concentrate from water into fish tissues, imparting an earthy and unpleasant flavor. Previous analytical methods focused on detection of GSM and MIB in water because extraction from fish tissue is complicated by the lipid-rich matrix. A method was developed at the University of Maine's Aquaculture Research Institute (ARI) for the extraction and detection of MIB and GSM from fish using a robust metal probe bearing a high-capacity sorptive extraction phase (HiSorb™) containing polydimethylsiloxane (PDMS), carbon wide range (CWR), and divinylbenzene (DVB). This method out-performs other extraction techniques by allowing for full automation, improved throughput, and a reduction in laboratory preparation errors. Robust and quantitative detection limits were achieved even with complex fish tissue matrixes using an internal standard, a matrix match calibration curve, and an increased extraction temperature and time. This method detects GSM and MIB below human tasting thresholds making it suitable for evaluating product quality in aquaculture. This method can be implemented to extract GSM and MIB from various other organ tissues as well, increasing its applicability for researchers and aquaculture farmers.

IDENTIFICATION OF COLUMNARIS DISEASE VACCINE CANDIDATES FOR CATFISH AND OTHER AQUACULTURE FISH SPECIES IN THE SOUTHERN REGION

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Columnaris disease, caused by the gram-negative and yellow-pigmented columnaris-causing bacteria (CCB; formerly under the umbrella of *Flavobacterium columnare*), is a significant ailment in global fish culture. In the southeastern United States, columnaris disease is responsible for significant losses in the catfish industry and control methods rely heavily on antibiotic applications. As of 2022, CCB represents four distinct species: *F. columnare, F. covae, F. davisii*, and *F. oreochromis*, and there are associations between bacterial species and virulence in different hosts. The development of an efficacious vaccine for the prevention and control of columnaris disease has been restricted, partially due to a lack of understanding of the broad genetic diversity of these bacteria. Our team is incorporating these new concepts into strain selection and the optimization of new vaccine candidates. There is a licensed, attenuated vaccine for columnaris disease in channel catfish, but it is no longer available to U.S. catfish producers. This is due to inconsistent vaccine efficacy reported when administered under production settings. One possible reason for the lack of efficacy is the sub-optimal host-pathogen dynamics. Vaccine development has been re-initiated with the newfound knowledge of the genetic diversity of CCB and affinity for different host species. This project aims to identify and modify CCB strains associated with catfish and other cultured fish species within the Southern region to create an efficacious live-attenuated vaccine for columnaris disease. The project team is currently confirming and characterizing strain attenuation to achieve viable vaccine candidates while investigating the protective immune response of the catfish. Safety testing and delivery optimization (immersion or oral administration routes) will be performed on the most promising candidates. Finally, the attenuated vaccine will be tested in channel catfish fingerlings under experimental pond conditions in Mississippi to evaluate efficacy under intensive production conditions. To date, at least one strain of CCB that causes mortality in channel catfish has been attenuated. Results from additional virulence experiments involving attenuated CCB strains from channel catfish and Nile tilapia will also be discussed. The long-term project goal is to produce a viable vaccine to protect catfish and other essential aquaculture species in the southern region against columnaris disease. Together, this project will provide industry stakeholders with new vaccine candidates for the prevention of columnaris disease thus reducing economic losses and the use of antibiotics within U.S. fish farms.

APPLYING NEPA CUMULATIVE IMPACT LANGUAGE TO PROPOSED SHELLFISH LEASES IN NORTH CAROLINA

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Shellfish lease applicants in North Carolina are required to select their own proposed lease site, which is then approved or denied by the NC Division of Marine Fisheries (DMF). Applicant defined areas are required to meet the standards set by NC Marine Fisheries Commission (MFC) rules, NC General Statutes, regulatory closures, and federal permitting requirements. Once the shellfish lease application meets these standards, it proceeds to a public comment period, where only comments specific to the proposed lease, that cite public trust use concerns (excluding viewshed), are considered. As a result of aquaculture industry growth in specific localized areas, the NC General Assembly required the DMF to study and implement MFC rules to address user conflict issues related to shellfish leases. The MFC then adopted rule amendments study aimed at reducing user conflicts, including the addition of cumulative impact language to limit the number of acres leased in any area.

Cumulative impact language is derived from the National Environmental Policy Act (NEPA) to be included in Environmental Impact Assessments of actions by Federal Agencies. The framework for assessing indirect, direct, and cumulative impacts required by the NEPA is outlined in Council of Environmental Quality (CEQ) regulations (40 CFR §§1500-1508).

The inclusion of this language in the MFC shellfish lease rules allows for evaluation of a shellfish lease application for the current and future impacts of shellfish leases on public trust water usage in the surrounding area. However, the development of a cumulative impact implementation strategy that can be applied during the shellfish lease application process posed several difficulties, namely: the method of triggering a Cumulative Impact Analysis (CIA) for an application, the concerning factors to consider, division of waterbodies into CIA managing areas, and the inclusion of CIA results in the application process. Through collaboration with federal and local resource management partners, the DMF has created a cumulative impact framework to assess and apply management to the cumulative effects of shellfish leases in a predefined area. Ideally, this addition to the review process would enable the DMF to further resolve user conflicts and find consensus between shellfish lease applicants and the local public.

THE POTENTIAL FOR OFFSHORE SEAWEED FARMING IN SOUTHERN CALIFORNIA

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Ocean Rainforest, Inc. (ORI) is the prime contractor for the MacroSystems project: an initiative funded by the U.S. Department of Energy's ARPA-E Mariner Program intended to demonstrate the feasibility of offshore cultivation of *Macrocystis pyrifera* (i.e. Giant Kelp).

In June 2020, the team formally launched a three-year demonstration project in the Santa Barbara Channel to test the durability of the cultivation system, as well as to better understand how to minimize its impact on marine industries and ecosystems. Since then, we have developed a hatchery facility, as well as installed a collection of cultivation lines .75 miles off the coast to begin to better understand the functionalities and challenges of the cultivation system.

Among the key innovations of our work in California has been an assessment of the feasibility of direct seeding for Giant kelp. While the traditional method of twine seeding is labor intensive and requires a large hatchery footprint, direct seeding involves the binding of gametophyte and/or juvenile sporophytes onto a substrate (i.e., rope) – effectively reducing space and labor requirements. Using a partial factorial design, we assessed long-term differences in biomass between a traditional twine method and the three direct seeding methodologies developed in partnership with SEAWISER. All direct seeding methodologies produced viable seedlings and we observed no difference in long-term success of the twine, massage, two-step, and spot seeding techniques. These results support the efficacy of using a direct seeding approach to cultivate Giant kelp and suggest the SEAWISER machine is the most efficient and cost-effective methodology.

In parallel to our work in the field, we tackled an extensive permit and application review process for an 86-acre demonstration project in the Santa Barbara Channel. In October 2021, Ocean Rainforest won unanimous approval from the California Coastal Commission for the project, which was quickly followed by final authorization from the U.S. Army Corps of Engineers. The project represents the first offshore facility approved exclusively for seaweed aquaculture in the lower 48.

This presentation will discuss key advancements in seeding techniques and how they relate to the commercial viability of seaweed aquaculture in the U.S. It will also shed light on outstanding barriers to the development of the industry, including but not limited to product/market development, permitting, and social license.

APHIS VS GUIDANCE ON SAMPLING AND POOLING FOR SHRIMP HEALTH INSPECTIONS

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USDA Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) has developed guidance on risk-based sampling and pooling of samples for specific pathogen testing for official *L. vannamei* health inspections. Testing of *L. vannamei* for health status or trade has become overly expensive because of the numerous pathogens of concern and total reliance on molecular techniques, such as PCR. Further, many of the current assays being used are not validated nor is the impact of pooling of animals or tissues known. In effort to minimize the cost of testing, as well as to improve testing consistency and accuracy, APHIS has developed guidance in partnership with industry and laboratory partners. This guidance provides criteria for representative sampling of premises or populations adequately and pooling of tissues or animals at different life stages.

CULTURE MANUAL ACCOMPANIED BY HOW-TO VIDEOS TO HELP GUIDE AN EMMERGING WALLEYE *Sander vitreus* INDUSTRY

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The University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility (UWSP NADF) has experienced substantial success for over ten years raising walleye in recirculating aquacutlure systems (RAS). By using specialized larval rearing systems, enhanced husbandry, indoor closed-loop production, and optimized starter diets we have been able to assemble systematic culture protocol that has advanced walleye for food fish production.

Due to increasing interest of this species, UWSP NADF created an instructional package for the intensive rearing of walleye purebreds and hybrids in tanks. This package consists of two products: a series of how-to videos consisting of chapters for each life-stage from egg to broodstock, and a detailed instructional manual to accompany the videos. Both the instructional videos and manual are based on past success for raising walleye, sauger and saugeye in intensive systems on commercial feeds. Both show examples of systems, techniques and procedures done at UWSP NADF and are comprehensive learning tools to intensively culture purebred and hybrid walleye in tanks.

Educational how-to videos have become increasingly popular and sought after for aquaculture practices. UWSP NADF is continuing to utilize video in several research and demonstration projects to showcase the research, explain the results, and provide future best management practices or techniques that have been learned from the results. Video will continue to be used as a tool for future outreach initiatives and has shown to be a successful and important addition to the current program. These videos and technical manual have become a model for outreaching to the industry about specific fish culture.

As UWSP NADF continues to reasearch walleye for commercial food production, the manual and videos will be updated with new findings. Current research at the facility regarding walleye involves out-of-season spawning of walleye to produce eggs and fry throughout the year, a major bottleneck to the expansion of the industry. At the completion of this and future projects, new information will be shared within the manual and videos, which are currently available on the UWSP NADF website: aquaculture.uwsp.edu

EVALUATING THE DIGESTIBILITY OF TWO INSECT MEALS PRODUCED WITH MEALWORM LARVAE IN THE DIETS OF ATLANTIC SALMON *Salmo salar*

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Finfish aquaculture production has increased dramatically in the past decades and has done so by utilizing wild captured finfish (reduction fisheries) as a primary protein source in fish feeds. At a global level, reduction fisheries appear to be fully exploited and the aquaculture industry now utilizes the majority of this natural resource. The aquafeed industry has been working to identify and utilize an expanding range of alternatives to fish meal but much work is needed to better understand the nutritional value, including the digestibility, of these ingredients. Insect larval meal, whereby insects are cultured on agricultural or food waste products, has gained attention as a potential sustainable protein source in aquafeeds. In this study, we evaluated the digestibility of two insect meals: 1) defatted mealworm meal (DMM) produced from *Tenebrio molitor* and 2) whole mealworm meal (WMM) produced from *Alphitobius diaperinus*.

Three diets were produced to evaluate the ingredient-specific apparent digestibility coefficients (ADCN) of the test ingredients. A basal mix was produced using a standardized formulation used for salmonid digestibility studies. Yttrium oxide (Y_2O_3; 0.1% of the diet) was used as an inert marker. The basal mix was used to produce the reference diet and was also used in a 70:30 mixture (basal mix: test ingredient) to produce the test diets. This approach allows for the estimation of the digestibility of the test ingredients, after accounting for the known digestibility of the reference mix and can be compared across studies using similar methodology. These diets were evaluated in a five-week digestibility trial with 500-700 g Atlantic salmon (*Salmo salar*) reared in a recirculating aquaculture system supplied with brackish (~3 ppt) well water. Crude analysis, amino acid composition and yttrium concentrations were measured in the feeds and feces and used to calculate ADCNs. For protein, $ADCN_{ingredient}$ values were 90.3 ± 2.0 and 90.4 ± 3.9, for DMM and WMM, respectively, and were not significantly different between treatments. These values were in the upper ranges when compared to those previously reported for fish meals (77-94%) fed to Atlantic salmon. Overall, DMM and WMM appear to be highly digestible feed ingredients and, from this perspective, should be suitable alternatives to fish meal in the diets of Atlantic salmon.

IDENTIFICATION AND CHARACTERIZATION OF SEAFOOD CONSUMER CLUSTERS PRE- AND POST-PANDEMIC

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The global COVID-19 pandemic has caused several changes in consumer behavior and preferences for various products in the market including seafood. This study identifies some of the changes in consumer behavior between 2019 and 2020 through a combination of cluster analysis and a multinomial logistic regression to compare the pre and post pandemic consumer survey data collected across 20 major seafood markets in the U.S. Factor Analysis of Mixed Data (FAMD) technique in R software was employed on the survey data to identify most important consumer demographic factors that contribute to the variation in the data. K-means clusters were then formed based on the identified factors. This was followed by a multinomial logistic regression with K-means cluster as the dependent variable and the attribute preferences of consumers for seafood consumed at restaurants as the explanatory variables to understand the attribute preferences of various clusters pre- and post- pandemic. Important demographic factors identified through FAMD for 2019 and 2020 were age, education, household size, number of persons aged above 12 years in the household, ethnicity, household income, and annual expenditure on seafood purchase. Although the principal factors identified for 2019 and 2020 survey responses were the same, there were differences in the resulting k-means clusters for the two years. While six clusters were identified for 2019 through gap statistics, the optimal number of clusters for 2020 were 10. Given that most of the demographic characteristics, except for income and expenditure, remained the same for individual respondents in 2019 and 2020, the variations in clustering can be attributed to changes in household income and seafood expenditure before and after pandemic. Clusters in both 2019 and 2020 exhibited some typical characteristics such as higher seafood expenditures associated with higher income, higher education levels and/or larger household size. On the other hand, clusters with lower seafood expenditures were associated with lower income groups with lesser education and/or higher age groups (50-70 years). A multinomial regression analysis on clusters showed that, in 2019, the highest seafood consuming cluster with an average annual expenditure of $6,045 considered taste, texture, nutritional quality, and U.S. farmed as the important attributes preferred for seafood consumed at restaurants. Similar clusters of people who, on average spent $11,881 on seafood in 2020 placed less importance to texture of the fish but considered price along with nutritional quality and U.S. farmed fish as important attributes for restaurant consumption of seafood. Although seafood prices did not increase in 2020, growing consumer price consciousness can be explained from fears of an economic downturn and potential loss of income. These results provide important insights into changes in consumer behavior with respect to seafood that can guide U.S. seafood industries to develop effective marketing strategies in the post-pandemic U.S. economy.

BIRD PREDATION ON CATFISH FARMS: REGULATORY CONCERNS AND ECONOMIC IMPLICATIONS

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The Migratory Bird Treaty Act (MBTA) protects several piscivorous birds that cause substantial economic losses on catfish farms. In a recent study, problems related to fish lost to migratory birds protected under MBTA was listed by producers as the regulation of greatest concern. The study showed that the cost of bird management emerged as the largest regulatory cost category on U.S. catfish farms. Costs incurred in managing predatory bird on catfish farms amounted to $17.4 million/year with most expenses occurring in manpower, trucks for bird running, and levee upkeep (Fig. 1). When combined, these incurred costs come as the fifth largest cost on catfish operations. Additionally, the impact from direct fish losses to bird predation was estimated at $27.4 million. Thus, bird regulations impact catfish farms by incurring substantial on-farm costs and negatively affecting farm revenues. Federal regulations surrounding the migratory bird species and the prescriptive nature of depredation permits are rendering bird management efforts more difficult on catfish farms. Effective bird management on catfish farms is possible with a combination of lethal and non-lethal activities. More frequent federal roost dispersal activities to avoid congregation of fish-eating birds near fish farms and further efforts to include catfish industry in compensation and relief programs for their losses is necessary to alleviate the burden on catfish farms.

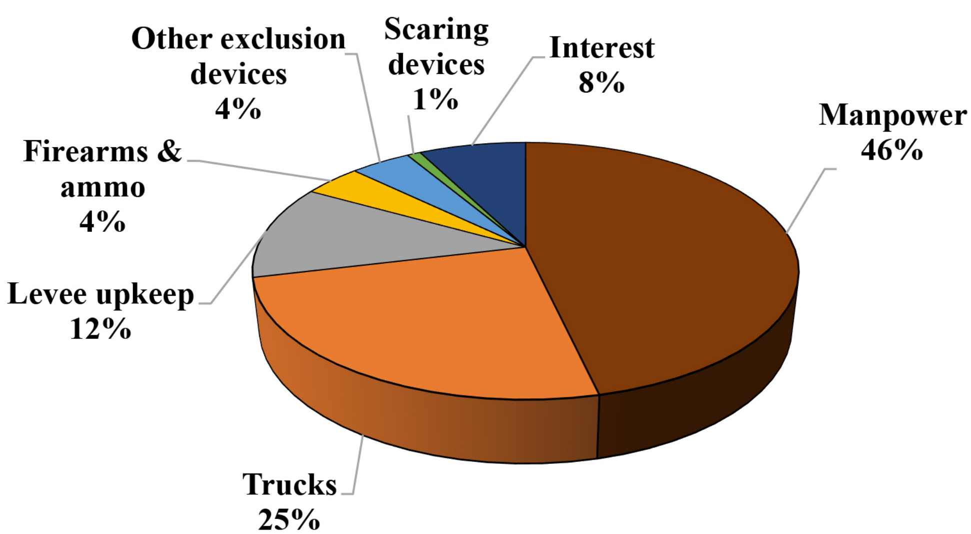

Figure 1. Types of spending associated with bird management on catfish farms, 2019-2020, Hegde et al., 2022.

THE STRUCTURE, CONDUCT, AND PERFORMANCE OF THE MIDSTREAM SEGMENTS OF THE AQUACULTURE VALUE CHAIN IN BANGLADESH

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The rapid growth of aquaculture in Bangladesh over the past 30 years has been facilitated by the proliferation of fish traders and retailers, but comparatively little is known about the organization and behavior of actors in these segments of the aquaculture value chain in Bangladesh. We conducted a comprehensive statistically representative study of 329 aquatic product traders in 31 markets from seven districts in south and southwest Bangladesh between January and May 2021 to address this knowledge gap. Five distinct categories of trader were identified: (1) Wholesalers, who buy fish from farmers and traders for sale to other traders and retailers (N =50); (2) Auctioneers (known as *arotder* in Bangla language) who earn commission from buyers and sellers (N =75); (3) Depots, who receive shrimp and freshwater prawn from farmers and assemblers, for sale to processing factories (N=62); (4) Assemblers, who mainly buy small quantities of crustaceans from farmers and transport them to market (N=42); (5) Retailers, who purchase small quantities of fish from other traders for sale to consumers (N=100). The total number of traders in surveyed markets increased by more than 175% within the past 10 years. Numbers of wholesalers grew faster than any other category of trader. Wholesalers trade the largest average volumes among the five actor types (526 t/year), of which 88% is fish (Table 1). This finding is consistent with results from farm surveys conducted simultaneously in the same zone which show a big increase in fish production over this period. Trading businesses are predominantly family owned and operated, and mainly create employment opportunities for men (99%). Fish trading created an average of 14 days of employment per ton of fish across all trader types, amounting to a total 2,542 full time equivalent (FTE) jobs created by traders in our sample of which 51% family labor and 49% hired. Trader's annual working capital requirements, operating costs and gross margins are substantial, averaging $18,630, $17,000, and $22,722, respectively across all trader types. Average marketing margins earned on each transaction average 6.0% of the sales value, ranging from 2.9% for auctioneers, to 14% for retailers. These are relatively modest margins, indicating that the rate profit extracted by traders is not excessive.

Table 1: Characteristics of aquaculture product traders

Variables	Wholesaler	Auctioneer	Depot	Assembler	Retailer	Total
N	50	75	62	42	100	329
Mean volume traded (t/year)	526	313	250	23	31	235
Total volume traded (t/year)	26,312	23,473	15,524	973	3,115	69,397
Total fish traded (t/year)	23,054	20,367	1370	286	2972	48,049
Total crustacean traded (t/year)	3,258	3,106	14,154	686	143	21,349
Fish share in total volume (%)	88	87	9	29	95	69
Mean labor days per ton fish sold	7	8	13	26	20	14
Total FTE jobs created	659	751	800	96	237	2,542
Working capital (USD/year)	21,159	15,152	41,411	1,737	525	18,630
Mean operating cost (USD/year)	56,493	4,218	19,579	1,039	1,523	16,985
Mean gross margin (USD/year)	43,565	23,795	30,913	5,496	4,975	22,722
Marketing margin (%)	5.3	2.9	4.5	4.6	14	6.0

EFFECTS OF SEX RATIO ON YELLOW TANG *Zebrasoma flavescens* BROODSTOCK MANAGEMENT

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Yellow Tang (*Zebrasoma flavescens*) are one of the most popular fish in the marine ornamental aquarium industry and were the most collected reef species from Hawaii. Previously, around 300,000 fish annually were removed from reefs to supply the aquarium trade until January 2021, when the State of Hawaii suspended the commercial collection of all aquarium species. This fishery remains closed at present, and Yellow Tang are currently only available through aquaculture.

In 2015, Oceanic Institute of Hawaii Pacific University (OI) was the first to be successful in culturing this important species. Methods used to raise Yellow Tang have allowed for various other new reef species to be aquacultured. These advances may provide the aquarium trade a more sustainable future for obtaining marine ornamentals. Although there has been great progress since 2015, commercial production of the species has been difficult with inconsistent results, primarily inhibited by poor egg production and high larval mortality.

To address these bottlenecks, research focusing on broodstock management of the species will be investigated, aiming to obtain best practices for increased egg production and quality, viability of eggs, survival to hatching and first feeding.

An important aspect of broodstock management deals with social environment, in which proper sex ratios and social hierarchies must be achieved to ensure low stress levels and yield maximum spawning success. Maintaining broodstock is costly and optimizing the number of fish required reduces costs and makes commercial production more economically feasible. Observed social behaviors of Acanthurids include both pair formations and group spawning events of 8-10 individuals. Trials will be conducted in fiberglass, 4000L tanks on a flow-through system involving *Z. flavescens* in pairs (1M:1F), trios (1M:2F), and quads (1M:3F) to assess if sex ratio impacts egg production and quality. Daily spawns will be assessed as well as hatch rates and early larval survival to first feeding (3 DPH).

Investigating aspects of broodstock management can allow for greater numbers of high-quality eggs and improved early larval survival, thus improving commercialization for this species. Results obtained from this study can help facilitate the development of techniques applicable to other Acanthuridae species.

LIFE SUPPORT SYSTEMS FOR RELIABLE HATCHERY PRODUCTION OF VARIOUS MARINE FINFISH SPECIES: AN OVERVIEW OF THE UNIVERSITY OF MIAMI EXPERIMENTAL HATCHERY FACILITY

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The availability of marine finfish seedstock continues to be a bottleneck for commercial aquaculture development of high value marine species in the United States and around the world. Given the complex and sensitive nature of hatchery operations, including the captive spawning of broodstock, live feeds production, and larval rearing, it is necessary to employ species- and site-specific technologies to ensure reliable seedstock production. The University of Miami Experimental Hatchery (UMEH) on Virginia Key, Florida has a long track record of successful development of hatchery technology for a variety of high-value marine finfish species. Over the years, a combination of simple and advanced life support systems have been implemented at UMEH to provide optimal rearing conditions for broodstock, larval, and nursery stages for a number of marine finfish species, including cobia (*Rachycentron canadum*), mahi mahi (*Coryphaena hippurus*), red snapper (*Lutjanus campechanus*), yellowtail snapper (*Ocyurus chrysurus*), and olive flounder (*Paralichthys olivaceus*), among many others. The facility operates both flow-through and recirculating aquaculture systems (RAS) utilizing ambient seawater sourced from nearshore waters of the Atlantic Ocean and Biscayne Bay. The life support systems at UMEH are constructed and operated in a manner to enable control over physical and chemical water quality parameters as well as exclude various parasites and microbial pathogens from the rearing systems. Marine finfish hatcheries worldwide face a multitude of issues related to water quality which can serve to inhibit reliable seedstock production. UMEH's efforts to mitigate the potential impacts of such issues through life support systems design and application will be presented.

ALASKA'S NEW FRONTIER! PRESENTING THE FIRST AQUACULTURE STRATEGIC SCIENCE PLAN FOR NOAA FISHERIES ALASKA FISHERIES SCIENCE CENTER

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The cold and nutrient rich waterways of Alaska are ideally suited for the development of shellfish and algae aquaculture. While the industry is currently small (approximately 82 permitted and 24 permit pending farms; current value approximately $1.5 million), the goal is to grow to a $100 million industry in ten years. With such rapid growth comes the potential for both positive and negative impacts to wild populations, other fisheries, subsistence intertidal and subtidal harvest, marine mammals, and other uses of the coastal zone.

The potential for interactions of farms with protected species, Essential Fish Habitat, and other fisheries (e.g. salmon, halibut, crab, geoduck, sea cucumber), and the potential for future expansion into federal waters, all warrant involvement from the National Marine Fisheries Service, and specifically the Alaska Fisheries Science Center (AFSC). To guide AFSC research efforts, a working group of federal researchers was formed to create a strategic science plan to guide the next 5 years of aquaculture research efforts. The strategic plan focuses on seaweed (kelp and red algae), shellfish (Pacific oysters, pinto abalone, king crab), and other invertebrates (sea cucumber).

Research priorities outlined in the strategic plan include:

- Promote sustainable industry growth through monitoring and improved understanding of ecosystem interactions
- Promote economically and socially sustainable growth by expanding the portfolio of species grown in Alaska, focusing on endemic species and multitrophic aquaculture, and building oyster hatchery production through strategic partnerships and selective breeding of Alaska optimized strains
- Enhance the resiliency of aquaculture to climate change through improved understanding of the potential for macroalgae to locally mitigate ocean acidification and sequester carbon, and further modeling efforts to identify future aquaculture locations in Alaska under various climate change scenarios
- Advance outreach and education
- Identify areas with optimal growing conditions for common aquaculture species while protecting Essential Fish Habitat, protected species and other coastal economic and social activities

In order to accomplish the triple bottom line of sustainable aquaculture - environmental, economic, and social sustainability – communication and collaboration across diverse partnerships will be critical. This presentation will provide an overview of the strategic plan to encourage engagement in Alaska from the broader aquaculture and research community.

EFFECTS OF DIETARY ARACHIDONIC ACID ON GROWTH PERFORMANCE, NON-SPECIFIC IMMUNITY, AND INFLAMMATORY RESPONSES IN FRY AND SUB-ADULT RAINBOW TROUT

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Aquaculture's rapid growth and a diminishing supply of fish oil has led to a significant amount of research over the last decade conducted to replace fish oil with alternative lipid sources, primarily vegetable oils, which are more arachidonic acid (ARA) deficient than fish oil. ARA has a variety of physiological functions, one of the most important of which is that it serves as a primary precursor to eicosanoids that play crucial roles throughout the life cycle in several physiological processes including growth and development, immune function, stress and inflammatory responses, and reproduction. The objective of the study was to evaluate the effects of dietary ARA on growth performance, fatty acid composition of whole-body and ovary, nonspecific immunity, and intestinal inflammatory gene expression in rainbow trout *Oncorhynchus mykiss*. Four isonitrogenous (51%, crude protein), isolipidic (15%, crude lipid), and isocaloric (23 MJ/kg) diets were produced: ARA 0 (linseed oil, i.e., the control diet), and three other diets with graded levels of ARA-rich oil (ARA-L: 0.59%, ARA-M: 1.14%, and ARA-H: 2.29% of diets). The feeding trial was conducted in three phases. Phases 1 and 2 were performed to evaluate only growth and feed utilization. Phase 3 was performed to evaluate growth and feed utilization as well as fatty acid composition of whole-body and ovary, inflammatory responses, and non-specific immune responses in response to acute stress. In Phase 1, rainbow trout fry (0.17 ± 0.01) were fed to apparent satiation six times a day for 8 weeks to assess early growth performance and feed utilization. For Phase 2, 50 fish/tank were randomly selected from Phase 1 and continued to evaluate growth performance and feed utilization for another 16 weeks. In Phase 3, rainbow trout (307 ± 3.64) were fed to apparent satiation three times a day for 12 weeks to assess growth performance and feed utilization, then fish were exposed to an acute stress (lowering the water level to 5 cm, just above the eyes, for 30 mins). The growth performance and feed utilization of fish during Phase 1 (fry), Phase 2 (juvenile) and Phase 3 (sub-adult) stages were not affected by dietary ARA levels ($P > 0.05$). In Phase 3, whole-body fatty acid profiles reflected those of the diets except for eicosapentaenoic acid (EPA) content, being significantly higher in the ARA 0 group, demonstrating that rainbow trout are capable of bioconversion of α-linolenic acid to EPA. EPA and docosahexaenoic acid contents in the ovary were less affected by dietary ARA, likely reflecting the selective deposition of those fatty acids in embryonic development. Plasma superoxide dismutase, catalase, and lysozyme activity significantly increased in both pre- and post-stress groups as a result of incremental increases in dietary ARA ($P < 0.05$). The expression of *tnf-α* was significantly downregulated in ARA-M and H relative to other treatment groups suggesting an anti-inflammatory effect. The results of this study demonstrated that dietary ARA did not affect growth performance, survival, and feed utilization indices of fry, juvenile and sub-adult rainbow trout. In contrast, dietary ARA showed significant increases in non-specific immunity parameters pre- and post-stress and downregulation of *tnf-α*, indicating a role in modulating fish health.

ASSESSING PHYSIOLOGICAL AND MOLECULAR RESPONSES OF *Crassostrea virginica* TO WEEKLY DESICCATION TREATMENTS

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Biofouling poses a major challenge to the growing US oyster aquaculture industry and periodic desiccation is a popular husbandry technique for biofouling control. However, desiccation is also associated with reduced oyster growth and elevated mortality, and the duration of desiccation can influence the magnitude of oyster stress response. A clearer understanding of the physiological responses of oysters to desiccation will help to refine desiccation as a husbandry technique. In this study, we measured growth, clearance rates, gametogenic stage, glycogen concentration, and the expression of heat shock proteins (HSPs) among diploid *Crassostrea virginica* desiccated for 0, 4, 8, or 24 hours per week over a three-month period (July – September 2020) in the Choptank River, MD. Oyster clearance rate was reduced immediately after re-submergence in the 8- and 24-hour treatments during the August sampling, but the clearance reduction did not persist after a 4-day re-submergence period or in September (Figure 1). Glycogen content and gonad development demonstrated an expected inverse relationship in July where oysters in the 0-hour treatment had more developed gonad and reduced glycogen, and oysters in the 4-, 8-, and 24-hour treatments had less developed gonad and more glycogen, but these relationships were not present in August. Oysters' expression of HSPs lacked clear treatment effects but trends suggested greater expression during August compared to July in the 0- and 4-hour treatments, while oysters in the 8- and 24-hour treatments mounted a low expression of inducible HSPs during both the July and August samplings. Overall, the 4-hour desiccation interval resulted in less stress to oysters, although the stress response of oysters in the longer desiccation treatments (8- and 24-hour) varied over time. Based on these results, desiccation for 4 hours weekly may be a suitable husbandry tactic with little likelihood of elevated oyster stress.

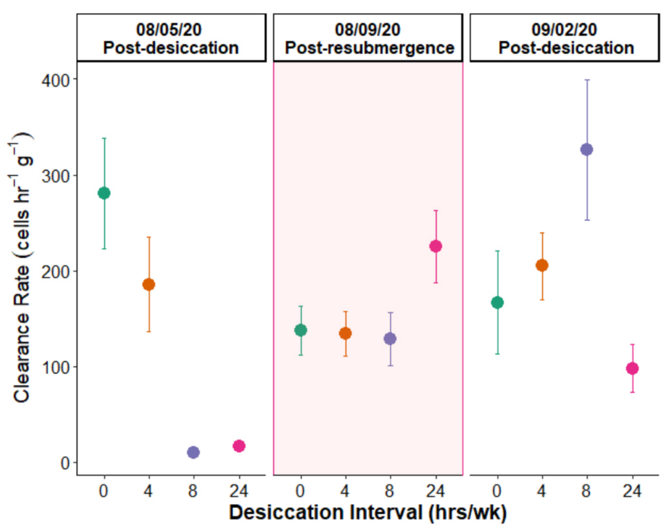

Figure 1. Mass-corrected clearance rates of oysters desiccated for 0, 4, 8, or 24 hours weekly immediately after desiccation (left and right) and after a 4-day re-submergence period (center).

POTENTIAL OF INVASIVE CARP AS A SOURCE OF FISH MEAL AND FISH OIL IN CHANNEL CATFISH *Ictalurus punctatus* AQUAFEEDS

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Invasive carp, a name encompassing black, silver, bighead, and grass carp, are out-competing native fish and causing environmental instability as their abundance continues to rise in the Lower Mississippi River Basin. Harvest by commercial fishermen is the most cost-effective management strategy for invasive carp while offering potential benefits to local economies. However, in Louisiana low demand and dockside prices for invasive carp have reduced incentives for commercial fishermen to target invasive carp, reducing the annual harvest numbers. Expanding and diversifying market demand is one strategy regional managers are implementing to increase dockside prices and incentivize larger commercial catches. Currently, the aquaculture industry relies on fish meal and fish oil from marine fish species, such as menhaden, as primary ingredients in their diets. However, as the industry continues to grow, demand for these marine fish far surpasses availability.

In this study, we assessed the performance of invasive carp fish meal (ICFM) and fish oil (ICFO) as feed ingredients in channel catfish (*Ictalurus punctatus*) diets. Graded levels of ICFM and ICFO (Aquatic Protein LLC, Beardstown, IL) were incorporated into fingerling diets. Diets were formulated on a 35% crude protein 6% fat basis with ICFM substituted for menhaden fish meal or plant and animal protein sources and ICFO substituted for half or all the menhaden fish oil. During 2, 8-week feeding trials, triplicate tanks of catfish were fed one of six treatment diets and growth, feed intake, feed efficiency, proximate composition, and protein- and energy retention efficiency assessed. At the termination of each trial, fish were sampled for length, weight, liver, viscera, and fillet mass. These data were used to calculate condition factor, hepatosomatic index, viscerosomatic index, and muscle ratio. There were no significant differences in growth performance, composition, or overall quality of channel catfish when using invasive carp ingredients in place of industry-standard menhaden or plant-based ingredients. This suggests that ICFM and ICFO are suitable alternative ingredients in catfish aquaculture feeds. The inclusion of invasive carp products in aquaculture diets will diversify market demand, thereby benefiting fisheries and local economies, improving conservation and invasive species management in the Mississippi River Basin, and enhancing overall aquaculture sustainability.

AQUACULTURE PIONEERS: CREATING FARM-BASED, HANDS-ON INTERNSHIPS TO INCREASE ONRAMPS AND PATHWAYS TO CAREER ADVANCEMENT

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Aquaculture Pioneers launched in 2022 to support the growth of Maine's aquaculture industry through a partnership between Educate Maine and FocusMaine. The program addresses a documented and immediate need for talent in a state that is now providing the majority of seafood for global consumers. This paid internship program connects aquaculture businesses to new talent and creates a culture of professional training, building on principles and practices of long-term stewardship of Maine's ocean resources.

Aquaculture Pioneers was launched under the umbrella program, Maine Career Catalyst, an experiential learning program designed to introduce interns, apprentices, and co-op students to the array of career opportunities and the high quality of life in Maine. Students from Maine's community colleges and four-year universities were the initial target audience with a focus on increasing the availability of farm-based internships for students interested in gaining hands-on experience outside the classroom as they build their career path.

The 2022 pilot launched with 10 interns at farms in midcoast and downeast Maine and featured a two-day Aquaculture Bootcamp for interns to level set on industry knowledge, network with leaders, and align important skills needed for job success. Aquaculture Pioneers has also partnered with other emerging programs to create new onramps and pathways to career advancement, including micro-credentialing through the University of Maine and a newly created registered apprenticeship program in Aquaculture. The program is poised to expand across Southern Maine and include placements of 20 interns in 2023 after positive feedback from interns and employers in our pilot year.

INNOVATIONS IN AQUACULTURE: PAST, PRESENT & FUTURE SUCCESSES, CHALLENGES AND R&D PATHWAYS TO COMMERCIALIZATION

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As an aquaculture researcher or entrepreneur, it can be challenging to navigate different business pathways and access the right resources and funding to grow and scale your R&D into a business. Investment in this sector has also changed and evolved in the past 5 years with more finance exploring new opportunities in the blue economies, and concurrently new ocean and aquaculture-specific funds have emerged alongside this trend.

HATCH was the first early stage aquaculture investor globally to apply the accelerator model to develop founders and startups emerging in 2018. Since then, we've applied a unique methodology to select startups within the aquaculture sub-sectors to invest in. Over the last four years, there have been key successes and failures of developing innovations and startups and these will be showcased to highlight the learnings that future startups can apply to their growth plans.

Moving forward, HATCH will adapt and evolve this strategy to support the growth of the aquaculture sector through its global entrepreneur programs in 2023. As the fastest growing food sector in the world, aquaculture is an exciting space to innovate and invest in and there are big opportunities for leaders and finance to play a role.

ADDRESSING THE WELFARE NEEDS OF FARMED LUMPFISH: KNOWLEDGE GAPS, CHALLENGES AND SOLUTIONS

Receiver of the ‘Da Silva award’

Paul N. Howes*, Carlos Garcia de Leaniz, Carolina Gutierrez Rabadan, Sara I. Barrento, Rebecca Stringwell, Ben A. Whittaker
and 24 additional authors made up of academia, lumpfish and salmon producers and welfare advocates.

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Abstract Lumpfish (Cyclopterus lumpus L.) are increasingly being used as cleaner fish to control parasitic sea lice, one of the most important threats to salmon farming. However, lumpfish cannot survive feeding solely on sea lice, and their mortality in salmon net pens can be high, which has welfare, ethical and economic implications. We undertook a knowledge gap and prioritisation exercise using a Delphi approach to assess consensus on the main challenges and potential solutions for improving lumpfish welfare. Consensus among participants on the utility of 5 behavioural and 12 physical welfare indicators was high (87–89%), reliable (Cronbach’s alpha = 0.79, 95CI = 0.69–0.92) and independent of participant background. Participants highlighted fin erosion and body damage as the most useful and practical operational welfare indicators, and blood parameters and behavioural indicators as the least practical. Our study offers 16 practical solutions for improving the welfare of lumpfish and where to target research efforts to generate workable solutions.

The oral presentation will describe the paper in more depth and disseminate the stages of research translation, from CSAR to the industry, that has led to the paper being published. The presentation will additionally discuss the wider research on lumpfish that is helping to address the knowledge gaps, and challenges, within the UK aquaculture sector.

FIGURE 2 Perceived utility on a 4-point Likert scale of 5 behavioural indicators (in blue) and 12 physical indicators (in black) for lumpfish. Shown are the responses of 53 participants in the first workshop on the Welfare of Lumpfish from researchers and producers to buyers and policy advocates.

EXPLORING THE CORE GUT MICROBIOTA FROM HYBRID CATFISH *Ictalurus punctatus* × *I. furcatus* AND SELECTING POTENTIAL AUTOCHTHONOUS PROBIOTICS

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Probiotics have been considered a powerful tool to improve nutrition and health by inhibiting the proliferation of pathogenic microorganisms, biosynthesizing vitamins, and promoting nutrient digestion and uptake for several farmed aquatic species. However, most probiotics were originally isolated from the environment or other host species, which may not present the best candidate taxa for all species due to poor adaptation to the target host and possibly an environmental pollution hazard. In the present study, 3,000 hybrid catfish (~70 g) were distributed equally in three 0.1 ha ponds and fed a commercial feed for three months. Each pond was partially seined (~25% of the population), and a total of 45 individuals underperforming and 45 overperforming were hand selected, euthanized, and their intestinal contents sampled. The DNA from the digesta was extracted for each of these samples and amplified microbial DNA (V4 - 16S rRNA) subjected to microbiota profiling via next-generation sequencing (Illumina MiSeq). Differences in the intestinal microbiota profile was assessed in fish overperforming against the fish with stunted growth. Although diversity metrics were not significantly different between under- and overperforming fish, significant differences in relative abundance were observed.

A total of 40 probiotic candidates were isolated from the digesta of overperforming farmed catfish using *Lactobacillus* selective medium and non-selective medium under both aerobic and anaerobic conditions. To verify the functional potential of the isolates, lipolytic and proteolytic abilities were measured. The selected candidates were further investigated using a biochemical panel, to better understand their nutrient utilization. In the bacterial inhibition assay, pathogenic bacteria that afflict the catfish industry (*e.g.*, *Edwardsiella ictaluri*, *E. piscicida*, and *Aeromonas hydrophila*) were co-cultured with the selected isolates. In this assay, 26 candidates inhibited the growth of these pathogenic bacteria. The hemolytic ability and antibiotic susceptibility were evaluated on the selected isolates to ensure host safety. In summary, a total of 20 samples were identified as potential hybrid catfish autochthonous probiotics with lipolytic and proteolytic ability, which may promote better nutrition absorption for the catfish. In addition, these bacteria inhibited the proliferation of pathogens *in vitro,* and did not present hemolytic activity, which can be a virulence factor.

THE INTEGRATED CULTURE OF FISH, MUSSELS, SEA CUCUMBERS, AND MACROALGAE IN A MODULAR INTENSIVE LAND-BASED RECIRCULATING IMTA SYSTEM: PERFORMANCE AND WASTE REMOVAL EFFICIENCIES

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A modular pilot-scale system for intensive land-based poly-culture of fish, sea cucumbers, mussels and macroalgae was established using a recirculating integrated design aimed at optimizing resource utilization, whilst reducing water requirements and nutrient discharge levels. The first module (Module-1) was included California yellowtail (*Seriola dorsalis*) as the primary "fed" species in one 1,057 L tank and *Ulva lactuca* in three cone-bottom 770 L tanks as the biofilter. Effluent containing solid and dissolved nutrient wastes from the fish tank was passed through a drum filter for solids removal. Seawater from the drum filter entered into a sump by gravity, and a side loop of seawater was pumped from the sump through a chiller and back into the sump for temperature control. Sand-filtered, UV sterilized makeup seawater was added to the sump at 1 L/min. Seawater was pumped from the sump through a UV sterilizer prior to going into the *U. lactuca* tumble culture tanks and then back to the fish tank through central standpipes. The second module (Module-2) housed Mediterranean mussels (*Mytilus galloprovincialis*) and warty sea cucumbers (*Aparastichopus parvimensis*) in separate serial rearing units that were replicated. Backwashes occurred automatically and effluent from the drum filter was directed to a cone-bottom mixing tank. Overflow from this mixing tank flowed into separate parallel 78 L cone-bottom tanks that contained *M. galloprovincialis* as suspension feeders. Aeration was used to keep particles suspended in the mussel tanks. Effluent from mussel tanks was directed to a second mixing tank, and then flowed by gravity into three parallel 339 L rearing troughs that held *A. parvimensis*. Fish feed was the only external nutrient source introduced into this system. Two trials of two weeks each were conducted – one at an initial fish stocking density of 5.2 kg/m^3 (Trial 1), and another at 20.9 kg/m^3 (Trial 2). The stocking densities of other species, seawater flow rates and aeration levels were determined based on earlier pilot studies. Yellowtail grew on average 0.36 %/d and 0.64 %/d with the food conversion ratio of 1.88 and 1.60, respectively, in Trial 1 and Trial 2. *Ulva lactuca* showed reliable growth of 24.39±2.00 and 15.57±2.47 g DW/m^2/d with a protein content of 15.13±3.11 and 30.36±1.41% DW in each trial, respectively, which resulted in nitrogen removal efficiencies of 0.58±0.08 and 0.75±0.12 g/m^2/d. The performance and solid waste removal efficiencies by mussels and sea cucumbers were monitored in the Trial 2. The condition index (CI) of *M. galloprovincialis* was not different between the beginning and end of two-week trial, and the solid waste removal efficiencies ranged from 16.1 to 18.9 % of total produced solid waste by the fish culture tank. The condition index of adult *A. parvimensis* showed no difference between the beginning and the end of the trial, which indicated that the nutritional quality of solid wastes could meet the daily metabolic requirements of adult *A. parvimensis*. The solid removal efficiencies by *A. parvimensis* ranged from 16.4 to 20.4 %. Due to the accumulation of total ammonia nitrogen (TAN) and the surplus of solid wastes inside the system at the high fish density, more tanks of *U. lactuca* and biomass of solids feeders should be introduced into this system to increase the system productivity and enhance resource utilization efficiencies.

AQUACULTURE IN BANGLADESH: RECENT ADVANCEMENT, PROSPECTS, CHALLENGES AND OVERALL IMPACTS OF FIL FUNDED PROJECTS

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In Asia region, Bangladesh is one of the leading countries for aquaculture development to support human nutrition, income generation of rural fish farmers and country's economic growth. Bangladesh is one of the seven countries belonging to Asia-Pacific region among the top 10 global aquaculture producers. The country was ranked in the 5th position being a producer of 2.8 percent of the total global aquaculture production, while India, Indonesia and Vietnam accounted for 7.1 percent, 6.2 percent and 4.5 percent production and were ranked second, third and fourth positions respectively (FAO, 2018). Aquaculture has been attributed as one of the dominant sub-sectors, which alone contributing 56.24 percent to total fish production (2017-18) of the country (DoF, 2018). For last 10 years, average growth performance of fisheries sector is 5.26 percent. Aquaculture sub-sector shows a sturdy and consistent growth, average growth rate is almost 10 percent during the same time frame. Aquaculture alone contributes over 2.81 percent to the GDP in Bangladesh, which is higher than any other counties of Asia Pacific region except the Vietnam (FAO, 2018). Inland freshwater and partly marine aquaculture are presently showing major impact on production, protein supply, economic development and livelihood aspects of millions of value chain actors of the country. Due to innovation of novel aquaculture technologies, rapid advances of aquaculture have been possible, by the national research institutions (viz. BFRI) along with dissemination of such technologies by DoF and NGOs to the end users (ie. Farmers) all over Bangladesh. A total of five research projects are being supported and implement in the Areas of Inquiry (AI) by Feed the Future Innovation Lab for FIL (FIL), Mississippi State University, USA in Bangladesh. Achievement of such projects has been presented and self-assessed in FIL Bangladesh Sector meeting held during 24 – 28 July, 2022. It is expected that successful implementation of these research projects will make a great impact at the end of the project period (2023), while a number of evolved aquaculture and fisheries technologies will be evolved, transferred and used for maximizing human nutrition and poverty alleviation of a large number of vulnerable people in Bangladesh. In this paper, an attempt has been made to address the overall review of advances of aquaculture sub-sector of the country along with Bangladesh Government priorities indicated at the 8th Five Year Plan, Bangladesh Delta Plan 2100 and Blue Economy Development in Bangladesh. Overall aquaculture prospects, future challenges along with status and impact of implementation of five FIL funded projects are also highlighted.

EVALUATION OF DIFFERENT STOCKING DENSITIES FOR NURSERY CULTURE OF THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) IN A BIOFLOC SYSTEM

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There are several potential benefits associated with the use of a nursery phase in shrimp culture, such as stock inventory control, less predation, and greater uniformity of market-size shrimp post-larvae (PLs) at harvest. To optimize the use of energy and space, the density in nurseries should be maximized; although, high density can impact growth performance of the shrimp, survival rate and water quality parameters. Systems should also have environmentally friendly management practices that provide greater biosecurity to the culture system such as biofloc-type systems. Therefore, the objective of this study was to evaluate the effects of different stocking densities on the growth and survival of the Pacific white shrimp post-larvae under intensive nursery conditions in a biofloc system. A four-week nursery trial was conducted at the E.W. Shell Fisheries Center at Auburn University in Auburn, AL. The nursery system consisted of twenty-four 150 L polyethylene tanks recirculating with a sump (~ 800L) and circulation pump run as a common biofloc-type system. Eight different stocking densities were assigned as experimental treatments (0.5, 1, 2, 3, 4, 6, 8, and 10 PL/L). A sub-sample of PLs from each treatment (ie. tank from each treatment) was weighed every five days during the experimental period to determine when and if the growth diverged over time for shrimp reared at the various densities (Figure 1). At day 5, 10, and 15 of the experiment, it was observed that the growth of the PLs didn't diverge between the different densities. At day 20 and 25, the growth of the PL diverged between the various densities. The results of the overall growth of the PLs throughout the trial were significantly different (P= <0.001) between the different densities. As it would be expected, the higher the stocking densities, the lower the growth of the PLs. In practical, a shrimp farmer would want to maximize the use of the nursery space to hold high stocking density, but at same time it's important to find the appropriate stocking density to not inhibit the shrimp growth.

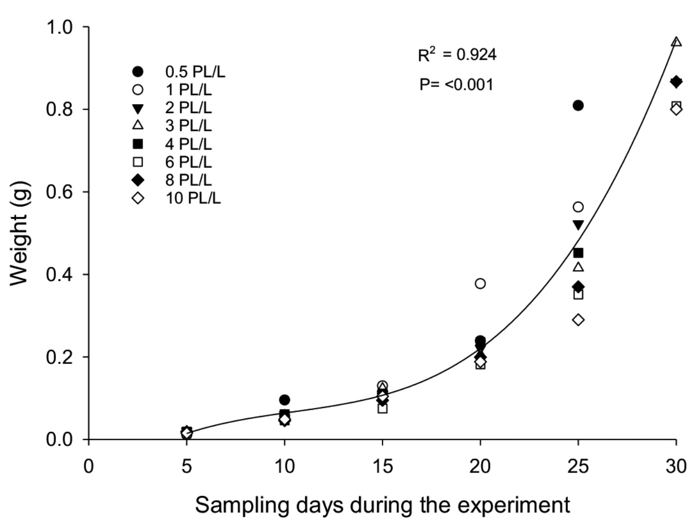

Figure 1: PLs growth in different densities during 4-week nursery trial

MOD YOUR PODS: IMPROVING FEEDING SUCCESS OF MARINE LARVAL FISHES BY MODIFYING COPEPODS' ESCAPE RESPONSES

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Copepods are a critical resource to culturing many species of marine ornamental fish. Unfortunately, culturing marine copepods can be time consuming, difficult, and expensive, and copepods have pronounced escape responses used to avoid predation by larval fish. Methods have been identified to suppress the escape responses of two species of copepod, the calanoid *Parvocalanus crassirostris* and the cyclopoid *Oithona colcarva*, to improve feeding success of two fish species, the Pacific Blue Tang (*Paracanthurus hepatus*) and Golden Domino Damselfish (*Dascyllus auripinnis*).

Copepods' escape responses were suppressed using acute exposures to an extreme temperature and salinity. Previous experiments tested three temperature and salinity treatments on the escape behaviors of these copepods, and the most effective temperature and salinity were used as treatments, in addition to a control, in larval experiments. Four experiments evaluated the effects of the two copepod species on the growth, feeding incidence, and survival of the two fish species. *P. crassirostris* treatments were 1°C for 40 minutes, 60 g L^{-1} for 1 hour, and untreated copepods serving as a reference diet. *O. colcarva* treatments were 40°C for 15 minutes, 80 g L^{-1} for 1 hour, and untreated copepods as a control.

Larval tanks (15L) were maintained at 26°C with 35 g L^{-1} saltwater, a 16h light : 8h dark photoperiod, and the microalgae *Tisochrysis lutea* (150,000 cells mL^{-1} for *D. auripinnis* or 300,000 cells mL^{-1} for *P. hepatus*). The *D. auripinnis* experiment was stocked with 300 larvae $tank^{-1}$ and the *P. hepatus* experiment was stocked with 750 eggs $tank^{-1}$. Starting 3 days post hatch (DPH), copepods were treated and fed to larval tanks (n=6 $treatment^{-1}$) twice daily until the 5 DPH. Fish were sampled at 3 DPH to assess growth and feeding incidence and tanks were fully harvested 5 DPH to quantify growth, feeding incidence, and survival.

A diet of cold-treated (1°C) *P. crassirostris* led to the highest survival of *D. auripinnis* at 5DPH and untreated *P. crassirostris* resulted in the highest survival of *P. hepatus*. However, *P. hepatus* survival in the *O. colcarva* experiment was highest in the high temperature (40°C) treatment. *D. auripinnis* fed *O. colcarva* showed the best survival in the high salinity treatment (80 g L^{-1}). These results showed that suppressing the escape responses of copepods can lead to improved survival at 5DPH but efficacy is species specific.

ENGINEERING AN INFECTIOUS cDNA CLONE of *Laem Singh virus* INFECTING SHRIMP TO DEVELOP A VIRAL VECTOR TO DELIVER ANTIVIRAL THERAPIES

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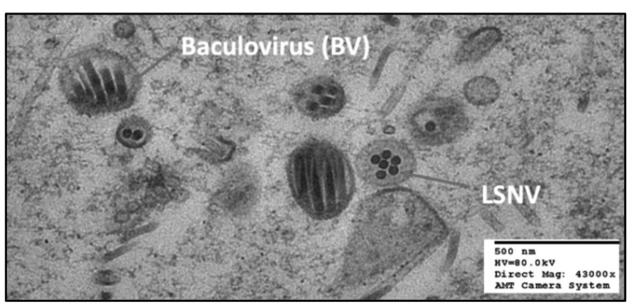
Viral diseases are a bottleneck in the growth and sustainability of shrimp aquaculture worldwide. Despite the enormous economic losses caused by viral diseases, our understanding of the molecular basis of viral pathogenesis in shrimp is often rudimentary. The lack of an infectious viral clone has been a major hurdle to genetically manipulate the viral genome, study viral pathogenesis and develop viral vector to deliver therapeutic molecules like RNAi, microRNA etc. This project was initiated to use reverse genetics approach to construct an infectious cDNA clone of *Laem Singh virus* (LSNV) that can be genetically manipulated to develop a replication incompetent viral vector to deliver antiviral therapeutic molecules. LSNV causes growth retardation in *Penaeus vannamei and P. monodon* and contributed to major economic losses in Thailand. The disease has now spread to other countries in Asia. LSNV genome contains bi-segmented single-stranded RNA of 2206 and 1846 bases in length. RNA1 encodes an RNA-dependent RNA polymerase (RdRp), and RNA2 encodes a viral capsid protein. It was hypothesized that due to a relatively small genome and a simple genomic architecture, LSNV is a good model virus for genetic manipulation, studying viral pathogenesis, and developing viral vectors.

The full-length cDNAs of LSNV RNA1 and RNA 2 were cloned in a dual baculovirus expression vector, and bacmid DNA was transfected to insect cells, Sf9, to produce infectious virions. Upon transfection, LSNV RNA was detected by RT-PCR in Sf9 cells, and a transmission electron micrograph (TEM) revealed both recombinant baculovirus as well as mature virions of LSNV (Fig. 1). In order to prove infectivity, Sf9 cell homogenate containing infectious LSNV was mixed with a commercial diet and fed to juvenile *P. vannamei* at a rate of 7.5% of the biomass, once a day for 7 days. Using the RT-PCR, LSNV could be detected in gill tissue, a target tissue of LSNV at three- and six weeks post-viral feeding in the challenged shrimp.

The data revealed that the reverse genetics approach can be used to engineer infectious LSNV clone using a baculovirus expression vector and Sf9 cells. This opens up avenues to manipulate the viral genome to study pathogenesis at a molecular level and to develop replication incompetent LSNV-based viral vector by replacing the RdRp gene to deliver therapeutic molecules, such as RNAi and microRNA for antiviral therapy.

Figure 1. TEM image of a Sf9 cell showing mature virions of LSNV and baculovirus.



THE ROLE OF AQUACULTURE IN ADVANCING INDIGENOUS FOOD SOVEREIGNTY

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Environmental and safety concerns related to fish consumption can potentially be alleviated with innovative aquaculture production practices. Native Americans tend to consume fish at a higher rate than recreational anglers, and therefore also face greater rates of exposure to bioaccumulated contaminants such as mercury or PCBs.[1] Further, the conversion of lands previously used to grow traditional foods have increased the need for indigenous populations to rely on food aid.[2] Programs that strengthen local food systems can enhance the sustainability, effectiveness, and cultural suitability of food aid.[3] Aquaculture systems on tribal lands that cultivate both fish and produce can improve food sovereignty for Native Americans by decreasing reliance on food programs, while also increasing access to culturally acceptable food to meet the needs of the community.

The objective of this multidisciplinary collaborative project is to understand the needs within the Menominee tribe to establish aquaculture production. Aquaponics training and start-up funds will be provided to Menominee Tribal members interested in raising culturally relevant fish and produce in aquaculture systems. Consumer-oriented research will be conducted to assess how tribal-raised fish will be perceived in markets off-reservation, and whether this value-added fish variety will change certain consumer perceptions about farmed fish quality and safety that impact their purchasing decisions. Outreach efforts of this project will contribute to intertribal research and create more diverse relationships in agriculture as the emphasis is placed on amplifying perspectives of underrepresented minorities.

Preliminary data have been collected to assess community needs and continued work is being conducted using food sovereignty as a measure of community health to capture the social value of the ability to independently grow fish and produce. Establishing local aquaculture production will increase access to nutritious seafood products and produce for the Menominee tribe, while offsetting some of the contamination risks of wild-caught fish as water quality conditions are closely monitored. Results of consumer surveys will be used to effectively communicate the health benefits of aquaculture-raised fish and produce grown in an aquaponic system to targeted populations to improve health outcomes across minority populations.

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LA VIDA CON LOS PECES – LIFE WITH THE FISHES

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Women in aquaculture and fisheries currently account for only 14% of the 59.5 million people engaged in the industry (FAO 2018). During the last decade, women and the diverse groups of minorities have become increasingly represented. However, both groups continue to face professional and personal challenges related to socialization, gender roles, stereotypes, and work-life balances within the industry. Furthermore, data is limited regarding women's roles in aquaculture restraining the reality and real position of women various segments of the industry.

This presentation will represent the status of Latin American women in aquaculture and the journey that has led me to begin career as the new Aquaculture Professor who will assist with the development of an Aquaculture Program at a community college in Northern California. Both my parents came to the United States from Mexico with nothing but ambition for pursuing higher education. My father comes from a line of ranchers and worked hard for his education, while my mother was a dreamer who had four kids. My deep roots of hardworking individuals along with my passion for recreational fishing drove me to the field of fisheries and aquaculture. I chose this field because I found myself in tune with my roots. Although, I am headed in the direction of inspiring and teaching individuals, the farmer inside of me will always be there because it is in my "sangre". As a Latina dipping her fins into the beginning of a career, I hope to inspire and encourage other young women and underrepresented individuals to keep pushing through and follow their dreams. Si, se puede! Aquaculture continues to grow and there is no doubt that the women and diverse minority groups will empower one another because with the right team, a fishbowl of opportunities is possible.

THE GROWING IMPORTANCE OF AQUACULTURE: THE CASE OF NORWEGIAN SALMON

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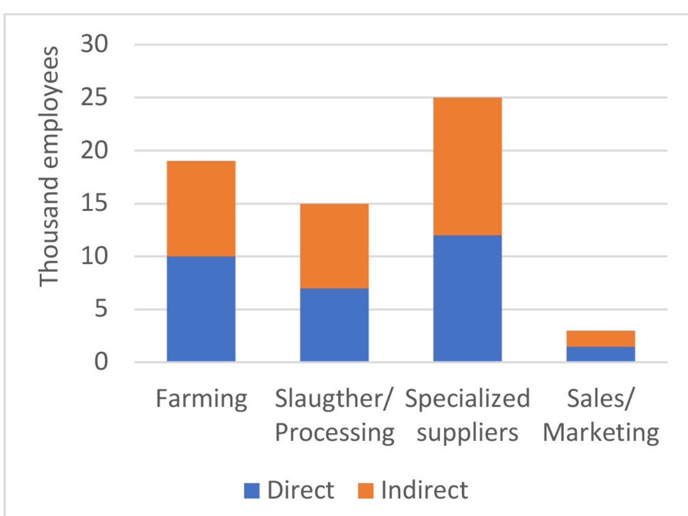
Introduction

Salmon aquaculture has since its modest start about 50 years ago grown to a production of around 3 million tonnes in 2022, to become the second largest aquaculture species by value after shrimp (Garlock et al., 2019). Norway is the largest producer of salmon, with around 55 % of total production (Iversen, Asche, Hermansen, & Nystøyl, 2020). The Norwegian seafood industry has since 2004 grown from 0,8% of Norwegian GDP to around 3%, mostly thanks to this growth in aquaculture. As employment in fisheries decrease, this growth in aquaculture, including its suppliers, has become vital for coastal communities.

The success of salmon farming can be attributed to an increasing degree of control over the production processes that has facilitated innovations in biology, technology, organization, products and marketing (Anderson, 2002; Asche, 2008; M. D. Smith, Asche, Guttormsen, & Wiener, 2010). Much of this innovation is found with suppliers. At the same time, many of the tasks originally performed by the salmon farmer is now performed by skilled and specialized suppliers. Together, these factors explain why salmon farming creates activity and value creation way beyond the industry itself. This paper documents the value creation in salmon farming and explores its importance for other sectors and for different regions.

Methods and results

Salmon farming in Norway employs close to 10.000 people directly, but in total we count 62.000 people directly or indirectly employed by the industry. We have performed an extended and modified IO-model to fully cover the ripple effects of the industry. Firstly, we have used detailed ledgers from a range of firms to establish a detailed pattern of purchase (both by industry and region/municipality), to calibrate the model and secondly, we have included specialized suppliers in the population. This has two main advantages, as it allows us to better cover investments, that are not covered by traditional IO-analyses, and also exports from the specialized suppliers. With Norway providing just over 50%, and salmon technology spreading to farming of other species, export is an important aspect also not previously covered in I/O-models. For many communities along the coast, salmon farming has replaced fisheries as the primary private sector employer, both through the direct activities and its ripple effects. Salmon farming has also become important for its tax contribution, from companies, employees and salmon farming tax schemes.



COMPARISON OF TWO DIFFERENT PRODUCTION STRATEGIES AND TRAPING METHODS FOR CRAWFISH PRODUCTION

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Pacific white shrimp (*Litopenaeus vannamei*) and red swamp crawfish (*Procambarus clarkii*) are currently being produced on small scales in west Alabama. Since 2014 shrimp survival and yields on Alabama farms have been consistently reduced due to several factors. In 2018 crawfish were stocked on one commercial shrimp farm to supplement farm income. Due to the style of ponds available used for shrimp farming, Deep-water crawfish production can be implemented without making any changes to the current infrastructure. In this study we compared economic assessments of traditional and deep-water crawfish production systems at two different farms in west Alabama and evaluated the efficiency of two different trapping methods that can be implemented in deep-water ponds. Traditional crawfish production systems consist of shallow ponds with an average depth of approximately 0.6m that are completely drained from July to October to allow the cultivation of a forage crop. These systems are typically harvested from February to June using small commercial crawfish traps (37-74 traps/ acre). Deep-water systems differ from traditional ponds in that the average depth can be as much as 0.91-1.21m and the ponds are not drained and planted. Instead, these ponds remain filled throughout the year and crawfish are fed some form of supplemental feed. To harvest these ponds, the traps either need to be much taller to accommodate the deeper water or traditional size traps can be placed around the pond shoreline. In this study we found that the traditional systems yielded much higher production and net income than deep-water systems. Production costs/pound were found to be higher for deep-water ponds. The most common commercial crawfish traps used in traditional pond systems in Alabama are pyramid style traps with three entry points around the base and an open top with an 20cm wire neck that is terminated by a plastic ring that prevents escapement and acts as a handle. The total height of these traps measured from the ground is 0.76m. In order to harvest the deep-water systems custom built traps with extended necks were used that were 1.35m in total height. To test the efficiency of trapping methods in deep-water systems tall traps were placed around the pond in water that was 0.91-1.02m deep, while an equal number of the traditional 0.76m traps were placed around the shoreline of the pond at water depths of 0.3-0.61m. A series of harvests were made recording the time required to harvest each type of trap and the catch for each were weighed separately. After multiple runs data indicated that harvest weight differences seemed to be pond dependent while harvest time differences were not significant. Farmer preference favored the traditional sized traps due to ease of handling. While the deep-water systems were profitable, they were far less productive than traditional ponds. In the future these ponds will be run as traditional systems by reducing the water level to 0.61m and planting forage crops during the summer.

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, including how to regulate the sale of seaweed in its whole form as a food product. There is currently no 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. Three components were envisioned for this project: (1) conducting legal research to identify and assess potential models; (2) convening a collaborative learning workshop to engage stakeholders, and (3) developing a model law, regulation, or guidance document for the sale of seaweed in its whole form as food.

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. In March 2021, the NSGLC hosted a virtual workshop over 8 sessions during a two-week time frame. 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 are now available.

On December 8, 2021, the NSGLC hosted a workshop to kick-off Phase 2 of the project. Through discussions with workshop participants, it became clear that before model language could be developed, a better understanding of the existing science was needed. Phase 2 has focused on two main items: 1) developing a webpage that can serve as a clearinghouse for seaweed food safety research and 2) additional outreach materials on the legal framework.

FATE OF EXCESS DIETARY PHOSPHORUS FED TO KOI (CYPRINUS ROBROFUSCUS)

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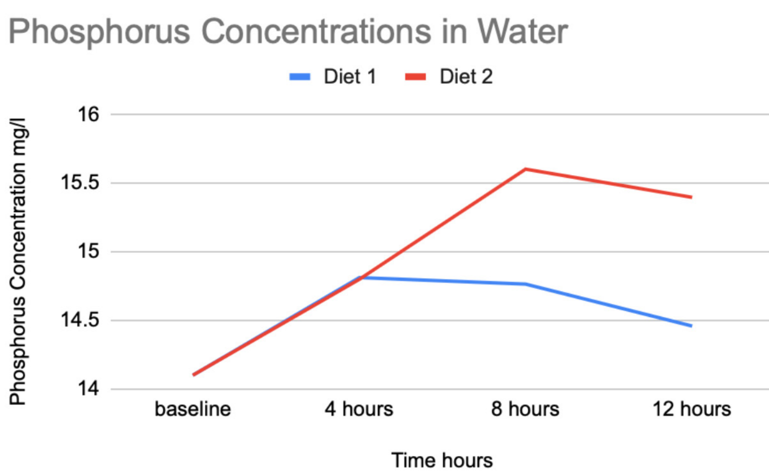
Phosphorus (P) is an indispensable nutrient for fish but when present in diets in unavailable form or in excess, it is excreted to receiving waters. In the aquatic environment, P has been widely recognized as a key driver of eutrophication, which can decrease biodiversity, alter ecosystem dynamics, and result in dead zones.

The objective of this trial was to assess if excess dietary P would be excreted by the fish and detected in the water. To this end, 40 juvenile Koi (*Cyprinus rubrofuscus*) at the Kentucky State University Aquatic Animal Nutrition Lab were fed two different diets: a balanced diet (Diet 1) and a diet with excess P (Diet 2). The Koi were stocked at five fish per tank (110 L) with four replicates per treatment and were fed to satiation once daily. The evaluation was conducted over a 12-h period and P levels were measured every four hours. The results showed that there was a significantly higher concentration of P in the water of tanks assigned Diet 2 (Figure), indicating that the fish did not retain the excess P.



AQUACULTURE LITERACY IN 360: A VIRTUAL TRIP TO INDONESIA FISH FARMS

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Virtual and digital technologies can enhance communication between and among producers, regulators, educators, and consumers, which creates an overwhelming opportunity for education and seafood literacy development. This talk will highlight opportunities we have developed and capitalized on in the Great Lakes region including international partnerships, training, and digital media. More specifically, as an innovative technology, 360-virtual reality (VR) imaging and video brings remote and distant locations and educational efforts to a broader audience while giving them a feeling of 'being there,' and all at a reasonable cost. Currently, 360-VR can be accessible through public platforms (e.g., Facebook, YouTube, Google, etc.) and used in a variety of formats (e.g., smartphone, computer, VR headset, etc.). Various studies indicate 360 experiences can achieve long-term learning impacts. VR technology allows individualized immersive experiences where students engage deeply with content and locations not traditionally accessible and will allow for the sustainability of such programs moving towards the future.

The videos will be integrated into an online educational delivery program (e.g., Desire2Learn) to provide content and context for learners. A great example that we would like to follow is NOAA's lesson plans for virtual dives of their marine sanctuaries (https://sanctuaries.noaa.gov/vr/lessons.html). This effort would provide an open-access platform for students to view, engage, and utilize the 360-VR experience without having to travel, yet experience accessible aquaculture programming through Extension technology. Join us as we guide you across Indonesia on tours of various aquaculture farms and discuss the process of creating and implementing this innovative technology!

THE EFFECTS OF ENDOGENOUS VS. EXOGENOUS CARBON SOURCES AND WOODCHIP MEDIA IN DENITRIFICATION FILTERS COUPLED WITH SHRIMP PRODUCTION SYSTEMS

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Nitrate continually accumulates in most RAS and has been shown to reduce production in high concentrations, thereby limiting long-term water reuse. Denitrification, a process in which facultative anaerobic microbes reduce nitrate to harmless dinitrogen gas, may allow for greater water reuse. Many denitrification filters are media based and may be unsuitable for systems with high solids concentrations such as biofloc-style techniques. Reactors relying on heterotrophic microbes also require organic carbon additions to fuel microbial activity, which increases costs for producers. In addition, some media types can be expensive. Low- cost alternatives such as wood chips for media and utilizing endogenous solid waste for carbon may lower costs while maintaining high rates of denitrification. The purpose of this study was to evaluate the potential for using no media versus woodchips in in-line denitrification bioreactors as well as to evaluate carbon sources, including fermented sludge supernatant, ethanol, and no added carbon.

The study consisted of six treatments with four replicates each and lasted 90 days. Each system included a 1 m^3 shrimp tank stocked at 250 shrimp m^{-3}. Water from each shrimp tank was pumped through a 3 L foam fractionator, an 18 L settling chamber, an 18 L moving bed biofilter, and a 28.7 L denitrification column. Sludge was collected from the settling chambers and allowed to ferment for a week prior to additions. Ethanol was added to maintain a 3:1 C:N ratio based on the daily nitrogen additions through feed. The concentrations of TAN, nitrite, nitrate, phosphate, alkalinity, turbidity, and TSS/VSS were measured weekly. Temperature, DO, pH, salinity and ORP were each measured twice daily.

Results indicate that nitrate was significantly lower in the WC treatments than in the NM treatments and in the ethanol treatments than in the no-C and sludge treatment. Shrimp survival and average weight were greater in the no media treatment than in the woodchip treatment. The ethanol treatment had greater average weight but lower survival than both the no added carbon and the sludge treatments. This experiment indicates that there can be a range of implications based on media and carbon types in denitrification reactors. Some factors seem to benefit water quality but may have negative effects on shrimp. Such considerations are important for producers who wish to balance nitrate remediation while optimizing shrimp production.

	Water		Shrimp	
Factor	Nitrate (mg/L)	TIN (mg/L)	Mean Wt. (g)	Survival (%)
NM	46.4[a]	47.6[a]	16.9[a]	82.6%[a]
WC	23.2[b]	24.1[b]	16.1[b]	75.4%[b]
Ethanol	18.8[a]	20.0[a]	17.1[a]	71.8%[a]
Sludge	43.0[b]	43.9[b]	16.3[b]	81.5%[b]
No C	42.7[b]	43.7[b]	16.1[b]	83.7%[b]

COMPARING NUTRIENT MANAGEMENT STRATEGIES IN DECOUPLED AQUAPONICS SYSTEMS FOR GROWING *Cannabis sativa*

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Through decoupled aquaponics, commercial farms can produce high-quality fish and high-value crops in a controlled environment. They are successful because they can manage the fish and plant components separately. The use of aquaponics has also been a growing trend within the Cannabis industry. When the value of the harvested flower is largely determined by the concentrations of cannabinoids and other secondary metabolites such as terpenes, it makes sense to focus on creating growing conditions that maximize these compounds. However, there has been little focus on the management of nutrients in aquaponics systems for optimizing plant production.

A previous experiment showed that cannabis plants grown with aquaponics had higher cannabinoid and terpene levels than when grown with hydroponics, despite being smaller in stature and yield. While the lower yield is not ideal, the higher value of the harvested flower offsets this shortcoming. Therefore, in subsequent experiments we decided to look at decoupled aquaponics cannabis production with single-pass nutrient flow, as well as various recirculating flow treatments.

In the first experiment, cannabis plants with single-pass flow demonstrated the highest cannabinoid levels and most diverse profile of secondary metabolites compared to recirculating treatments and a hydroponic treatment. The second experiment built off the first and added a hydroponic treatment with 10% aquaponics effluent and a more actively managed recirculating treatment. In addition, supplemental lighting was actively managed to increase plant growth during low light conditions. Results show that aquaponics is not only a viable method for growing cannabis, but perhaps a better method for growing hemp flower with more diverse cannabinoid and terpene profiles and thus potentially higher medicinal value.

WALLEYE *Sander vitreus* CULTURE FOR SPORT FISH ENHANCEMENT STOCKINGS IN IOWA USING RECIRCULATION AQUACULTURE SYSTEMS

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Iowa's Dept of Natural Resources (IDNR) Rathbun Fish Culture Research Facility (RFCRF) has developed pilot scale recirculation aquaculture systems (RAS) for egg incubation, larviculture, and growout to produce 225 mm fingerlings for sport fish enhancement stocking. Few studies have evaluated RAS technology for Walleye production and more research was needed for IDNR to adapt fingerling production to RAS. The first RAS growout system was operational in 2015 with additional systems developed later for egg incubation (2018) and larviculture (2019). This presentation will summarize our modifications and advancements for successful adaptation of walleye culture to RAS.

DIETARY BLACK SOLDIER FLY LARVAL *Hermetia illucens L*. OIL IMPROVES FEED CONVERSION RATIO AND DISTINCTIVELY ALTERS THE FILLET FATTY ACID PROFILE OF NILE TILAPIA *Oreochromis niloticus*

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Black soldier fly larvae *Hermetia illucens* L. (BSFL) oil is being explored as a dietary lipid source in aquaculture. It is a valuable source of energy, as well as characteristic fatty acids such as lauric acid (12:0) with health-promoting properties. Overall, the oil has a desirable balance of saturated and unsaturated fatty acids. In a feeding trial, juvenile Nile tilapia *Oreochromis niloticus* were fed diets top-coated with 2% of one of the following oils: fish oil, soybean oil, coconut oil, poultry fat, or BSFL oil. The tilapia (initial weight ~5.55 g) were fed twice daily to satiation for 10 weeks and had a mean overall survival rate of 98%. The total weight gain was similar across diets. The FCR was lowest in the tilapia fed the BSFL-oil diet and highest in those fed the poultry-fat diet. Fatty acid analysis was conducted on the fillet and liver samples. The results were analyzed using one-way ANOVA and Canonical Discriminate Analysis (CDA). The fatty acid profiles of the livers had many differences, mostly among the individual saturated fatty acids (SFA). Tilapia fed the diet with BSFL oil had lower SFA concentrations than fish fed the other diets. The fillets of the tilapia fed the diets with BSFL oil had the lowest concentration of monounsaturated fatty acids. The tilapia fed the diets with BSFL also had a higher concentration of lauric acid (12:0), which can be beneficial for the health of the fish and human consumers. While we did not measure any immunological parameters, the literature suggests that lauric acid may have antibacterial and antiviral properties even at low inclusion levels (2-5%). Further studies are warranted to identify specific effects of BSFL oil on tilapia health, and to determine the cost-of-gain of diets with this oil relative to other dietary lipids.

DEVELOPMENT OF IMPROVED OYSTER LARVAE FLOWTHROUGH SYSTEM AND METHODS

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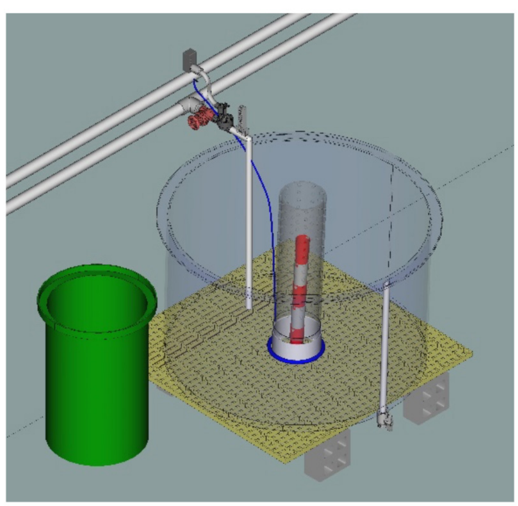
The global population is projected to expand beyond 9 billion by 2040. Current global human consumption of seafood is 157MT and is projected to grow by 1.1% annually for the next decade. Historically, natural fisheries have sustained population growth, but all are now at risk of collapse due to overfishing. Coupled with climate change induced crop instability, global ability to sustain food supply is threatened. Fortunately, aquaculture has been growing steadily since 1980 and is projected to surpass capture fisheries for all fish production by 2025. To further promote growth in the industry, innovation is paramount. Flowthrough rearing systems for oyster larvae were first described in 1977 and offer the advantage of potential improvements in water quality while simultaneously allowing increased larval densities. Oyster flowthrough system designs follow a similar pattern-- cone-bottom tanks, banjo-sieves, and bubble columns for mixing. Continuous flow through is relatively new, with earliest literature mentions around 2011. During veliger and pediveliger stages, and before transfer to downwelling, the cone may concentrate larvae and risks dangerous densities near the bottom due to the exponentially decreasing surface area.

The updated design reduces risk by utilizing a 750L convex bottom fiberglass tank. A bottom drain will be installed peripherally, and the system will be angled 2.5° for complete drainage. A standpipe shall be inserted in the drain during normal operations to serve as overflow protection. The center standpipe will be used for volume control. Height of the center standpipe will be modular to allow rapid reduction in larvae density through incremental volume increases. Larvae will be retained using a 0.5m^2 mesh screen cylinder supported by a ring permanently affixed to the bottom of the tank. Aero-Tube® will revolve around the retention ring to aerate, mix, and reduce larval entrapment. A secondary algae retention tank will provide algae continuously to the culture tank.

Optimal densities will be established and tested using flowthrough protocols developed by Reiner in 2011. Maximum and minimum exchange rates will be determined using data obtained from density trial combined with daily flow rate and NH3-N assessments to develop exchange rate/population curves. Intermittent flow testing will also be performed using an automatic valve and intermittent cycle timer to determine optimal flow and cycle rates to maximize larval survival and growth.



MOBILE IMMERSION FILTER: DECREASING AMMONIUM CONCENTRATION IN LONG-TERM SHIPPING TANKS TO REDUCE AQUATIC STRESS AND NEW TECHNOLOGICAL IMPROVEMENTS ON PRODOCT QUALITY

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One of the fish species that is most frequently transported alive in Europe is rainbow trout *Oncorhynchus mykiss*. Conditions before and during the transport have a significant impact on the fish's health, stress response, and final product quality. Water quality deterioration during transport is unavoidable, despite starvation periods and water changes before the shipment. In transport water, hazardous ammonium and carbon dioxide concentrations increase. Improved water conditions during a transport can be achieved by applying additional filter technology.

Mobile immersion filters developed at AWI have been tested to control ammonia and carbon dioxide concentrations during the transport of fish.

The related scientific research achieved notable results when applying the Mobile Immersion Filter (Patent DE102019122146B3), these results are summarized below.

Initially, three different design concepts for immersion filters were evaluated for the capability to lower ammonia and carbon dioxide in transport water of fish. Results are focused on ammonia reduction with ammonia reduction compared to a control being evaluated as technical proof of concept . Transport boxes for fish were used and prepared by adding ammonium chloride as a solution of 12 mg/L to the water. After 9 hours, a very substantial difference was detected between tanks with a Mobile Immersion Filter (MIF) and controls, with the dissolved ammonium level in the water of the transport tanks decreasing to 3 mg/Liter with a MIF.

Although all models functioned admirably, model A2 (Patented version) was chosen for controlled experiments with tanks for fish transport containing 50kg of live rainbow trout *Oncorhynchus mykiss* and 500 liters of water per tank.

A transport was simulated for four hours with ammonia determined every hour.

Table 1 - Change in dissolved ammonium content in fish transport tanks and comparison of filter performance [mg/L]

	Ammonia concentration in mg/l over time [Interval 1 hour]								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A1	11.8	7.2	5.8	4.8	4.2	3.4	3.4	3.4	3.4
A2	12	7	5	4.6	4	3.6	3.4	3.2	3
MA	12	6.2	6	4	3.8	3.6	3.4	3.4	3.2
C	12.2	12	12.2	12.2	12.2	12.2	12.2	12.2	12.2

Table 2 - Increase in soluble ammonium content produced by rainbow trout in transport tanks [mg/L]

	Time [Interval 1 hour]				
	0	**1**	**2**	**3**	**4**
C1	0.07	0.73	1.37	2.36	3.19
C2	0.06	0.73	1.36	2.38	3.43
C3	0.01	0.53	1.03	1.65	2.47
T1	0.01	0.48	0.83	1.14	1.77
T2	0.02	0.43	0.66	0.97	1.46
T3	0.08	0.66	0.71	1.58	1.79

The results demonstrate that ammonium generation in the control tanks was approximately 50% greater than the experimental tanks with MIF. These results are highly valuable due to the critical role of ammonium in long-term transport.

CONSUMER HEALTH RISK ASSESSMENT FROM SOME HEAVY METAL BIOACCUMULATION IN COMMON CARP (*Cyprinus carpio*) FROM LAKE KOKA, ETHIOPIA

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Lake Koka is one of the Ethiopian Central Rift Valleys lakes, where the absorbance of domestic, agricultural and industrial waste from the nearby industrial and agro-industrial activities is very common. The aim of this research was to assess the heavy metal bioaccumulation in edible parts of common carp (*Cyprinus carpio*) in Lake Koka and the health risks associated with dietary intake of the fish. Three sampling sites were selected randomly for primary data collection. Physicochemical parameters (pH, Total Dissolved Solids, Dissolved Oxygen and Electrical Conductivity) were measured *insitu*. Four heavy metals (Cd, Cr, Pb and Zn) in water and bio-accumulation in the edible parts of the fish were analysed with flame atomic absorption spectrometry. The mean values of TDS, EC, DO and pH of the lake water were 458.1 mg/L, 905.7 μ s/cm, 7.36 mg/L and 7.9, respectively. The mean concentrations of Zn, Cr and Cd in the edible part of fish were also 0.18 mg/kg, ND-0.24 mg/kg and ND-0.03 mg/kg, respectively. Pb was however not identified. The amount of Cr in the examined fish muscle was above the level set by FAO and the accumulation of the metals showed marked differences between sampling sites ($p<0.05$). The concentrations of Cd, Pb and were below the maximum permissible limit. The results also indicated that Cr has a high transfer factor value and Zn has the lowest. The carcinogenic hazard ratio values were below the threshold value (<1) for the edible parts of fish. The estimated weekly intake of heavy metals from fish muscles ranked as Cr>Zn>Cd, but the values were lower than the Reference Dose limit for metals. The carcinogenic risk values indicated a low health risk due to intake of individual metals from fish. Furthermore, the hazard index of edible part of fish was less than unity. Generally, the water quality is not a risk for the survival and reproduction of fish and the heavy metal contents in the edible parts of fish exhibited low carcinogenic risk through the food chain.

MICROBIAL POPULATION DYNAMICS IN PONDS FROM INDIA WITH WHITE FECES SYNDROME COMPARED TO PAIRED PONDS WITHOUT THE SYNDROME

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White Feces Syndrome (WFS) refers to the floating white fecal strings in ponds where shrimp are reared. WFS is associated with shrimp growth retardation, size disparities and reduced feeding and chronic mortalities and has become a serious issue in *P. vannamei* and *P. monodon* farms in Southeast Asia.

Recently WFS has been associated with the presence of *Enterocytozoon hepatopenaei* (EHP) and a *V. parahaemolyticus ToxR*+ PirAB- isolate and a controlled co-infection in shrimp in a laboratory setting with the isolates resulted in WFS.

The goal of this study is to acquire information about the microbial populations in ponds exhibiting WFS and how best to use probiotics to mitigate the syndrome. In this preliminary study we captured microbes from water samples on filters for analysis in the laboratory. Also, water samples from a WFS+ and - pond were plated on TCBS and TSB plates, the CFUs were determined and isolates were identified and characterized (Table 1). Noteworthy was that the WSF- ponds contained more vibrio and more microbes on TSB plates (3.2×10^4 CFU/ml vs 5.3×10^2 CFU/ml data not shown).

Inhibition studies between vibrio's from WFS+ and – ponds and inhibition between vibrio's from WFS+ ponds and potential probiotics will be presented. The implications to pond management from these results will be discussed and future studies will be addressed.

Table 1. TCBS plate results (100ul inoculum)

WFS	Green CFU/ml	*Green	*parahaemolyticus* toxR +/-	alginolyticus	&U	Yellow CFU/ml	$Yellow	*cholera*	&U
+	1.1×10^2	12	7 tox+/ 1 tox-	0	4	8.3×10^1	5	0	4
-	2.4×10^2	9	0	1	8	1×10^3	8	6	2

*green isolates, &unknown, $yellow isolates

THE EFFECT OF SALINITY ON HUMAN SENSORY CHARACTERISTICS OF KALE WINTERBOR F1 HYBRID *Brassica oleracea* GROWN IN DECOUPLED BRACKISH-WATER AQUAPONICS SYSTEMS

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Inland brackish-water recirculating aquaculture systems (RAS) must reuse water as much as possible due to challenges such as saline effluent discharge and inhibitory salt costs. Brackish water aquaponics systems using kale have been shown to reduce nitrate and phosphate that build up over time in RAS. Kale is marketable, but marketability of kale grown in saltwater is unknown. This trial analyzed sensory characteristics and preferences using a panel of volunteers who tasted Winterbor F1 kale grown in a range of salinities (0, 5, 10, 15 and 20 ppt salinity) in decoupled brackish water aquaponics systems containing reused shrimp culture water.

Participants (n=112) were presented with five raw kale samples (one from each salinity) and instructed to taste them in a counter-balanced order and rate their overall liking, taste liking, texture liking, and aftertaste liking on a 9-point hedonic scale anchored by "dislike extremely" and "like extremely". Participants then rated their perceived intensity of basic tastes (sweet, salty, bitter) using a 0-100 line scale anchored by "not at all" and "extremely". Following the sample evaluation, participants were presented with a brief description about aquaponics and asked whether this influenced their opinion and willingness-to-pay. Differences in liking and intensity were assessed using one-way ANOVA followed by Tukey's post-hoc comparisons.

The 5 ppt kale was significantly more liked (overall liking, taste liking, and texture liking) than then 0 ppt kale. Focusing only on taste liking, the 5 ppt was also liked significantly more than both the 15 ppt and 20 ppt samples. No sample was liked less than the 0 ppt control. However, a closer examination of liking ratings revealed a bimodal distribution of the 20 ppt but not 5 ppt samples; in other words, participants were either "likers" or "dislikers" of the 20 ppt sample and very few provided a neutral rating. Aftertaste liking of kale samples grown in any salt concentration was significantly higher than the control 0 ppt sample. Saltiness intensity ratings increased in a dose-response manner, with the 10 ppt, 15 ppt, and 20 ppt samples all rated as significantly saltier than both the 0 ppt and 5 ppt samples. The 5 ppt sample was rated as significantly less bitter than the 0 ppt sample. Together, the saltiness, bitterness, aftertaste, and liking ratings suggest that a low level of saltiness in the kale samples masked the bitter kale flavor, which softened the aftertaste and improved taste liking. Information about aquaponics growing conditions resulted in an average willingness-to-pay of $2.06 per bunch, a 38% increase above the reference price of $1.49. Overall, this study demonstrates that kale grown in brackish-water aquaponics is likely to be accepted by the consumer.

PEKILO®: A MYCOPROTEIN IMPROVES GROWTH PERFORMANCE AND IMMUNE RESPONSE OF ATLANTIC SALMON

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Biotechnology processing of woody side streams from Norwegian spruce trees to produce high-quality microbial ingredients such as fungal single cell proteins can help address the protein deficit of the aquaculture industry, reduce environmental footprint, and improve fish health and welfare. PEKILO®, a β-glucan- and nucleotide-rich fungal single cell protein (mycoprotein), can serve as an alternative protein source in aquaculture feed. In the current study, PEKILO® was evaluated as a protein source in diets for Atlantic salmon at the Norwegian University of Life Sciences (NMBU, Norway). Four experimental diets were formulated to contain an increasing level of PEKILO®, replacing 0, 5, 10, or 20% of the crude protein (CP) content of the diet. The results showed that PEKILO® can successfully replace up to 20% of the CP content without significantly affecting growth rate or feed intake. Moreover, increasing dietary inclusion of PEKILO® resulted in a significant linear improvement in feed conversion ratio (Table 1).

Considering the composition of PEKILO®, its immunostimulant properties were evaluated *in vitro* using Atlantic salmon head kidney leucocytes. Gene expression data (Figure 1) indicated that PEKILO® and its fractions induce a strong immune response in Atlantic salmon by the upregulation of cytokines related to pro-inflammatory (IL-1β), chemoattractant (IL-8) and downregulated anti-inflammatory (IL-10) functions.

These findings suggest that PEKILO® is a high-quality novel protein source that also have potential to stimulate the immune response and improve health status of Atlantic salmon.

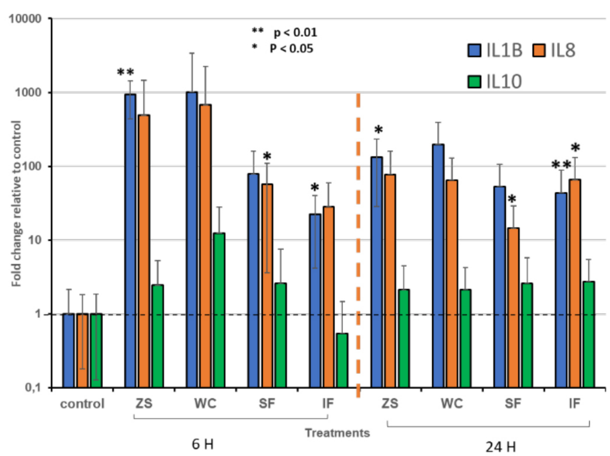

Figure 1: Gene expression of immune-related biomarkers in head kidney leukocytes of Atlantic salmon exposed to Zymosan (positive control; ZYM), the soluble fraction of PEKILO® (SF), the insoluble fraction (IF) of PEKILO® or the soluble + insoluble fractions (SF + IF) of PEKILO® after 6 and 24 hours. (*) indicates statistical significance (P<0.05) between the treatment and control.

Table 1: Growth performance of Atlantic salmon fed the experimental diets.

PEKILO® replacement level (% of CP)	0	5	10	20	P_{linear}[a]	SEM[b]
Parameter	Diet 1	Diet 2	Diet 3	Diet 4		
Specific growth rate (SGR, % day^{-1})	2.37	2.40	2.36	2.46	0.2495	0.05
Feed intake (g fish^{-1})	64.0	64.9	63.1	66.7	0.4750	2.38
Feed conversion ratio (FCR)	0.75	0.74	0.74	0.73	0.0173	0.00

[a]Significance of the linear orthogonal polynomial contrast. [b]Standard error mean.

HOW PRICE AND NON-PRICE FACTORS INFLUENCE THE MARKET PRICE OF MAJOR CARP FISH: A DYNAMIC ARDL SIMULATED MODEL

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Recently, fish prices in Bangladesh have been growing, particularly the market price of major carps, even though the factors that may be contributing to this trend are unexplored. In this milieu, this study considers some price and non-price factors to explore the market price dynamics of major carp species in Bangladesh. To explore the factors impacting the market price of carp fish, we apply advanced time-series econometric modeling, such as the recently devised dynamic simulated autoregressive distributed lag (ARDL) model using publicly available data on fish prices from 2005M1 to 2021M12. This period is attributed to the rapid transformation from mostly low-intensity farming to commercialization due to a rise in productivity and market price. Among the price factors, the findings demonstrate that price of corn, soybean and oilcake, and fisheries wage rate have a favorable significant impact on fish price in both the short and long term. In addition, among the non-price drivers, GDP per capita, inflation rate, and fish consumption all have a significant positive influence on the fish price in the long run, whereas total production has a detrimental effect on fish price in the long run. These results have significant policy ramifications for attaining the UN announced SDGs-1 (no poverty) and 2 (zero hunger) since aquaculture is one of the pertinent occupations of many rural farmers in Bangladesh. For the sake of fish farmers' profit maximization goals and consumers' welfare maximization, the government should have a rational input-output price-fixing policy.

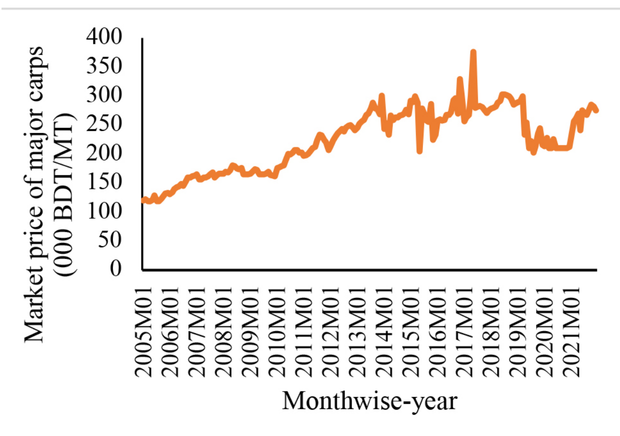

Figure 1. Trend of market price of major

Table 1. Long and short-run coefficient model.

Variables	Coefficient	Std. Error	Prob.
lnCP	0.342[b]	0.138	0.022
ΔlnCP	0.109[a]	0.028	0.000
lnSP	0.320[a]	0.071	0.000
ΔlnSP	0.038[a]	0.011	0.000
lnMP	0.208[b]	0.081	0.016
ΔlnMP	0.298[a]	0.065	0.000
lnFWR	0.492[a]	0.149	0.000
ΔlnFWR	0.147[a]	0.031	0.000
lnFC	0.094[a]	0.022	0.000
ΔlnFC	0.014	0.126	0.569
lnGDP	0.012[a]	0.002	0.000
ΔlnGDP	0.021	0.032	0.453
lnIF	0.019[c]	0.011	0.085
ΔlnIF	0.001	0.002	0.498
lnPRD	-0.219[a]	0.027	0.000
ΔlnPRD	-0.081	0.189	0.631
Cons.	1.170[a]	0.220	0.000

EXPORT POTENTIALITY OF AQUACULTURE FISH SPECIES: EVIDENCE FROM BANGLADESH

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The demand for fish and fish products is rising throughout the world; thus, the fish producing countries has an opportunity to export to consuming countries. In the similar vein, aquaculture production in Bangladesh has increased dramatically over the past years; as a result, after meeting domestic demand, there is potential for export. Therefore, this study tried to assess the export potentiality of three most prevailing aquaculture species of Bangladesh i.e., pangasius, tilapia and rohu. Data were collected from 820 aquaculture farmers in Bangladesh and different secondary sources. The study used Policy Analysis Matrix (PAM) to assess the export potentiality and Domestic Resource Cost (DRC) criteria was used to examine comparative advantage. Results indicated that the producers are not discouraged significantly through the existing policy interventions and average market prices for the inputs are less than the world prices. Regarding the output price, results indicated that the prices of pangasius and tilapia are less than the international price while the price of rohu is higher than the international price. However, producers of pangasius and rohu are unprotected through policy interventions while tilapia producers are protected through the policy interventions. The commodity system of selected three fish species are competitive at producer level. The value of DRC indicates that Bangladesh has comparative advantage in pangasius, tilapia and rohu fish production. The study suggests to provide more output support, given the existing input support or might enhance the input support for aquaculture species.

Table 1. Export potentiality of pangasius, tilapia and major carp

Ratio indicators	**Value**		
	Pangasius	Tilapia	Rohu
Nominal Protection Coefficient on Output (NPCO)	0.829	0.894	0.842
Nominal Protection Coefficient on Input (NPCI)	0.539	0.485	1.429
Effective Protection Coefficient (EPC)	0.920	1.012	0.758
Domestic Resource Cost (DRC)	0.795	0.827	0.497
Private Cost Ratio (PCR)	0.863	0.817	0.656

IMPACT OF CLIMATIC AND NON-CLIMATIC FACTORS ON THE SHRIMP PRODUCTION: AN EMPIRICAL EVIDENCE FROM BANGLADESH

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Aquaculture continues to grow at a rapid pace, making it the world's fastest-growing food production sector, with Bangladesh being no exception. Shrimp is the biggest revenue-generating aquaculture species, with Bangladesh earning a significant amount of revenue from shrimp exports. However, due to the impacts of climate change, which are not just a future but also a present fact, the sustainability of shrimp production is jeopardized. As a result, this study uses longitudinal data from 1990 to 2020 to investigate the impact of climatic and non-climatic factors on shrimp production. The climatic elements include annual temperature, precipitation, CO_2 emissions, and average salinity, whereas the non-climatic components are gross cultivated areas, fisheries credit, and labor force. We have used recently developed novel dynamic autoregressive distributed lag (DARDL) model from a methodological approach. The findings revealed that the variables under examination had a long term cointegration. According to the baseline regression findings, temperature, precipitation, and salinity all have a detrimental effect on shrimp production in the short and long term, whereas CO_2 emissions have an adverse influence on shrimp production in the long run. Cultivated lands, labor force, and fisheries financing, on the other hand, all have a favorable and considerable impact on shrimp production in the long and short term. However, this study also shows that a 10% positive and negative shock in the predictors, as well as a counterfactual shift in the predicted variables. From the standpoint of policy implications, this study has produced far-reaching policy recommendations, including that the government should give farmers with reliable climate predictions so that farmers may take necessary corrective action in the event of a production shortage. Farmers should also be given with prompt extension and finance assistance so that they can respond to any unfavorable weather situations.

Table. Findings of DARDL model

Variables	Coef.	Std. Err.	t-stat
Temperature	-0.092*	0.052	-1.77
∆Temperature	0.045	0.182	0.25
Rainfall	-0.024**	0.012	-2.00
∆Rainfall	-0.051**	0.019	-2.68
Soil salinity	0.52**	0.244	2.13
∆Soil salinity	0.77	0.912	0.84
CO_2e	-0.276**	0.124	-2.23
∆CO_2e	-0.315	0.293	-1.08
Area	0.726**	0.278	2.61
∆Area	0.192*	0.102	1.88
Labor force	0.027	0.041	0.66
∆Labor force	0.093	0.128	0.73
Fisheries credit	0.109**	0.042	2.60
∆ Fisheries credit	0.056***	0.012	4.67
Cons.	1.051**	0.521	2.02
ECT	-0.523***	0.117	-4.47

Note: ***, **, * denotes the significant levels at 1%, 5%, and 10%.

AN OVERVIEW OF SHRIMP SECTOR IN BANGLADESH: WAY TO ENTRY INTO INDUSTRY 4.0

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The shrimp industry is playing significant role to the economy of Bangladesh for the last few decades. The aim of this study is to review the last forty (40) years trend, present status and propose a blockchain based framework to entry into fourth industrial revolution for enhancing export potentiality. In doing so, the study gathers different information from both secondary and primary data sources. A significant production trend and yield with the area expansion were found in shrimp industry. This has been possible due to the adoption of new production and management technologies, and need based policy formulation. Despite substantial progress achieved with shrimp production during the last four decades, growth of shrimp export remains minimal in Bangladesh. Traditional paper- based record-keeping methods for the shrimp supply chain are disparate and, therefore, cannot provide effcient traceability capacity and holistic view of the supply chain. Focusing on the export market, we propose a distributed and accumulative score-based certification approach that will grade packaged shrimps according to the completeness and accuracy of the authenticated data entered during different stages. In this framework, from the post-larva purchasing to the final packaging stage, relevant data for every stage will be entered by the associated actors via mobile/web app or Internet of Things devices to the blockchain network. Such distributed approach of certification will enhance not only food safety but also the quality and compliance to best practices.

TRACEABILITY, FSMA 204 & THE FOOD TRACEABILITY LIST & FRAUD

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As the global demand and market for seafood increases, so does the opportunity for unregulated, fraudulent activity in the sector. The average product passes between at least eight parties before reaching the consumer, each stage of which is widely unregulated and vulnerable to misconduct. While the seafood markets susceptibility to fraud has not gone unnoticed, lack of a unified regulatory system creates a dangerous cradle for the market to grow in.

The solution is simple. An accepted, harmonized set of codified industrial standards coupled with a powerful traceability system used not only to promote transparency within the sector, but one which can compel a clear line of custody. It is indeed encouraging to see organizations such as GDST, MSC and ASC create and implement harmonized standards for the farmed and wild fishing industry. The U.S. FDAs passing on FSMA 204 will also encourage better recordkeeping by industry. By upholding the regulatory standards put in place, a food traceability solution both ensures accountability on part of industry participants, and guarantees a higher degree of quality and communication to customers.

The largest hurdle in instituting a system such as this, is rewriting the stereotype that advanced and effective traceability systems come with a price tag proportionate to its efficacy. Contrary to public perception, modern traceability has become more accessible than ever. Opening the doors for small business and corporations alike to subscribe to a new, higher standard of food safety and security.

I would like to make an in-person or a virtual oral presentation.

INFLUENCE OF DIETARY NUCLEOTIDES SUPPLEMENTATION ON GROWTH, BODY COMPOSITION, AND BLOOD CHEMISTRY OF *Channa marulius* (sole)

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The experiment was conducted to evaluate the effect of dietary nucleotides supplementation on growth, body composition and blood chemistry of *Channa marulius* (sole). The trial was conducted in 12 HAPAs (4 treatments each having 3 replicates) installed in an earthen pond. Four graded levels of dietary nucleotides were added in the isonitrogenous basal diet as 0.0%, 0.33%, 0.66%, and 1.0%, respectively. Results of the study revealed significantly improved survival, final body weight, net weight gain, % weight gain, SGR in treated groups (1.0, 0.66 and 0.33%) nucleotides supplementation compared to control (0.0%). Among treated groups T3 (1.0%) showed significantly higher values followed by 0.66% and 0.33%. In addition to this, the dietary nucleotides (1.0% inclusion) significantly improved the crude protein, crude fat, and dry matter by increasing dietary nucleotides supplementation level. Histological studies showed that inclusion of dietary nucleotides improved villi length, villi width and branch folding in the intestine while a depressed growth of villi and enhanced intestinal lumen has been observed in the control group. Furthermore, increased levels of SGPT, BUN, and TG were observed by increasing the nucleotides supplementation level whereas SGOT and blood glucose were observed opposite. Along with other parameters, the dietary nucleotides improved the nutrient digestibility as ADC (protein) ADC (fats) in the groups having higher concentration of dietary nucleotides while ADC (dry matter) was found higher in the control diet. Conclusion: Dietary nucleotides supplementation upto 1.0% significantly reduced cannibalism, influenced growth performance, body composition, blood chemistry, nutrient digestibility, and gut histology of *Channa marulius*.

EFFECT OF DIET FORMULATION ON AMMONIA PRODUCTION BY LARGEMOUTH BASS *Micropterus nigricans*

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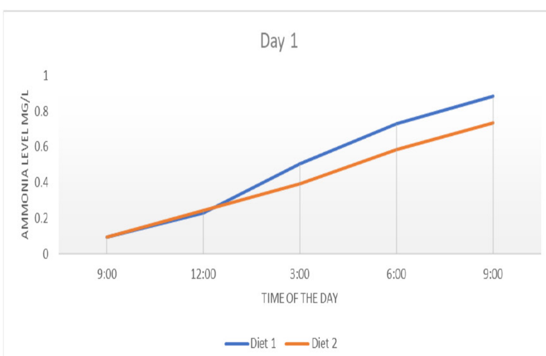
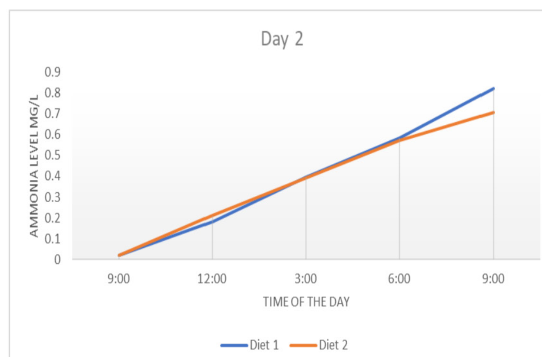
The largemouth bass (LMB) is a high-value fish for aquaculture and sports fisheries in the United States. In teleosts, ammonia is a major component of nitrogen excretion, which is produced from protein metabolism. In feeds, digestible non-protein energy aids in sparing protein use for energy thereby enhancing protein retention efficiency. Reduced catabolism of protein would also reduce ammonia excretion by the fish to receiving waters.

An experiment was conducted in the Aquatic Animal Nutrition Lab (AANL) at Kentucky State University to assess the effects of diet formulation on ammonia production by juvenile LMB. A control diet (Diet 1) was formulated to contain 45% crude protein and 12% lipids and was modified to contain 7% lipids and no supplemental amino acids (Diet 2). After a week of conditioning, each diet was fed once daily to quadruplicate groups of three LMB stocked in 110-L glass aquaria operating as a recirculating system. Water quality was kept within acceptable ranges for LMB and water flow was interrupted during water samplings for total ammonia-N (TAN). TAN readings from each aquarium were performed every three hours for a total of 12 hours. The experiment was repeated once after two days of water recirculation.

No statistically significant differences ($P>0.05$) in TAN levels in the rearing water were found during the study, despite the linear increases in TAN concentration over time (Figures).





SATELLITE DRIVEN OYSTER AQUACULTURE SITE SELECTION IN MAINE: FROM SUITABILITY INDEXES TO COUPLED GROWTH MODELS

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Oyster aquaculture has seen rapid expansion in Maine with a 5-fold increase in harvest value over the last 10 years. As the industry grows, prospective farmers need to choose new farm sites. Most shellfish aquaculture sites are located in narrow estuaries and bays along Maine's convoluted coast. Nearby areas can have vastly different environmental parameters vital for oyster growth such as food, measured through chlorophyll *a* and turbidity, and temperature. These parameters determine the time it takes an oyster to reach market size and ultimately the financial feasibility of the farm. In an effort to reduce the risk of site selection in nearshore environments we use high resolution (100m and under) satellite imagery from Landsat 8 and Sentinel 2 to gather farm scale environmental data across the state. This work highlights previous results validating high resolution products to generate suitability indexes for aquaculture and demonstrates the next phase of satellite site selection with oyster growth models forced with remotely sensed products. We generated daily climatologies of sea surface temperature from almost a decade of Landsat 8 data (2013-2022) while food levels were derived from four years of sentinel 2 data. (2016-2020) An eastern oyster (*Crassostrea virginica*) dynamic energy budget model was validated with in-situ data from 4 farm sites and coupled to the satellite data to explore time to market variability along the coast of Maine. Our work aims to reduce the risk of site selection for oyster industry in Maine, but the framework can be used to site other existing and emerging in the near shore environment.

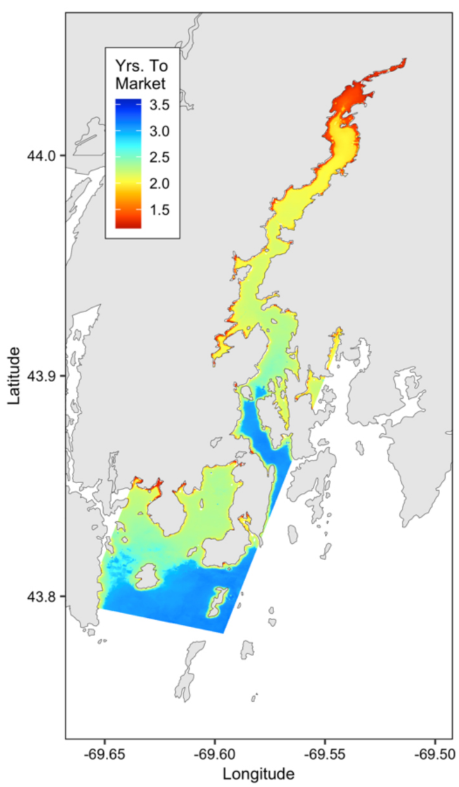

Figure 1. Example of temperature driven time to market map for 4mm *C. virginica* seed planted June 1st in the Damariscotta River, ME

PORT OF SAN DIEGO: CLIMATE RESILIENCY AND NATURE BASED SOLUTIONS INITIATIVES

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This presentation will provide an overview of the Port of San Diego's Blue Economy Incubator portfolio with a spotlight on two companies: Sunken Seaweed and ECOncrete. In addition, we will provide an overview of our climate resiliency and nature-based solutions projects.

The Port's Blue Economy Incubator (BEI) assists in the creation, development, and scaling of new water-dependent business ventures on San Diego Bay focusing on sustainable aquaculture and Port-related blue technologies. The Incubator currently has nine companies in its portfolio, including a shoreline protection project called ECOncrete. The ECOncrete interlocking units provide structural, ecological and community engagement benefits, including the promotion of marine organisms and restoration of local ecosystems. Ultimately, results from the pilot will serve to demonstrate an innovative win-win approach to coastal protection by providing resiliency and adaptation strategies in an urban environment while simultaneously enhancing valuable marine life and bolstering coastal ecosystems.

The Port has also been conducting studies, planning, and pre-development work to support and inform aquaculture opportunities in and around San Diego Bay. Seaweed aquaculture has the potential to store significant amounts of carbon, provide a sustainable source of micronutrients and protein and offer a range of co-benefits and could support the future development of nutrient trading programs. The Port is currently supporting a pilot to grow several species of seaweed native to Southern California and exploring expanding domestic markets for uses in human and animal food production, biofuels, and fertilizers, as well as exploring a variety of ecosystem services applications.

In parallel with the BEI, the Port is building a portfolio of nature-based solutions that advance the ecological health of San Diego Bay including an Eelgrass Blue Carbon Study to look at carbon sequestration potential of eelgrass beds, completing entitlements to create a wetland mitigation bank and restore 75 acres of wetlands and uplands in South San Diego Bay, and a recently installed Native Oyster Living Shoreline Project to prevent erosion and restore native oyster populations.

OPTIMIZING AERATION RATE AND FEED CONCENTRATION FOR THE LARVICULTURE OF PURPLE HINGED ROCK SCALLOPS (*Crassedoma gigantea*)

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Despite continued interest and efforts spanning decades, scallop aquaculture along the West Coast of the United States remains a largely nonexistent industry. With the United States being one of the largest net importers of scallops globally, there remains a need to develop scallop aquaculture to meet this domestic demand. Purple Hinged Rock Scallops (*Crassedoma gigantea*) show a number of traits justifying their development as an aquaculture species including relatively large size, unique life history, and wide geographic range. Unlike other scallop aquaculture industries, the use of wild spat collection is not particularly feasible for the species due to their patchy distribution and unidentified areas for reliable wild spat collection. Reliable and efficient seed production is considered one of the major impediments to the development of the species for aquaculture.

While several hatchery protocols for *C. gigantea* are available, details regarding optimization of culture conditions are sparse and sometimes conflicting. This study investigated some of these aspects including tank water hydrodynamics and microalgae feeding density. One proposed impediment to *C. gigantea* larval survival is the kinetic stress due to aeration and water movement. Microalgae cell concentration is a known critical aspect of scallop aquaculture however the literature on the optimal feed concentration for *C. gigantea* larvae is relatively wide ranging with recommendations varying between 5-80K cells/mL. We conducted a series of range finding experiments to investigate the effect of aeration and feeding rate on larval growth and survival with the goal of determining an optimal range for rearing *C. gigantea* larvae.

The effects of aeration were tested using nine 135L concial bottom tanks that were fitted with airflow meters to regulate aeration rates between 0LPM and 3.2LPM. Scallop larvae shell length increased significantly over time as a function of increasing airflow and by proxy water movement in the tank ($p < 0.001$). We did not detect an upper threshold for aeration within our tested range. Our highest air flow treatment (3.2LPM) yielded the largest and fastest growing larvae over the 42-day experiment.

A second range-finding experiment was conducted in ten 135L concial bottom tanks to determine optimal microalgal feeding rates between 0 – 90K cells/mL. Feed concentrations were quantified and replenished to their prescribed densities daily over the course of the 36-day experiment. Larval growth and survival were significantly associated with feed density ($p < 0.001$). We found the optimal microalgae density to be between 3,000 – 15,000 cells/mL with lower concentrations (3,000 cells/mL) performing significantly better for survival over time and higher concentrations (15,000 cells/mL) significantly better for growth over time.

The results indicate that this species favors aeration rates and hydrokinetic stresses that may be detrimental to larvae of other species of scallops. Similarly, larvae perform better at microalgae densities lower than other commonly cultivated bivalves. Taken together these results show that *C. gigantea* respond markedly different to culture conditions of other farmed bivalves warranting further research and optimization of hatchery practices in order to generate reliable seed production to support industry development of the species.

A SITE FOR SORI: UTILIZING NOVEL AQUACULTURE TECHNIQUES TO TEST BULL KELP (*Nereocystis luetkeana*) RESTORATION TECHNIQUES IN A NORTHERN CALIFORNIA SEA URCHIN BARREN

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Given dramatic declines in bull kelp forests in northern California, restoration techniques such as outplanting of inoculated substrates or spore enhancement have been identified as potential solutions, however, the efficacy of these techniques remain largely untested. An in-situ study was conducted between Summer 2021 and Fall 2022 at Albion Cove in Mendocino County, CA at a restoration site (n=48 1m radius plots) where sea urchins were removed by commercial hand harvest to reduce grazing pressure and paired with a control site.

“Green gravel” substrates were cultured outdoors at Moss Landing Marine Laboratories’ Aquaculture facility under low-cost non-sterile conditions prior to outplanting in the field. A second restoration technique aggregated sori near the benthos in mesh bags, thereby enhancing spore densities. Finally, novel aquaculture techniques were developed for producing large numbers of free-floating sporophytes in small volumes alongside “green gravel” substrates in the lab, which were later outplanted to the field. Ultimately, green gravel and juvenile sporophyte outplants were unsuccessful on the exposed outer coast, despite low sea urchin densities. However, we observed greater kelp recruitment in the soral transplant plots compared to control plots, with a stronger effect at the restoration site where sea urchins were removed. Despite observing kelp recruitment in the survey plots, the only sporophytes that reached sizes larger than 1m were either individuals that recruited to our benthic lines or in locations outside our experimental plots at the restoration site.

FULLY COMPREHENSIVE WARMWATER MARINE BAITFISH RETAILER AND ECONOMIC OPPORTUNITIES SURVEY IN FLORIDA

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There have been many market surveys completed on different portions of the well described large market for warm water marine baitfish retail, however none have been fully comprehensive of all marine baitfish species or been completed on a statistically significant number of licensed retailers until now. We will describe the limitations of previous published surveys and how this one was designed to be fully comprehensive of the entire retail market. This survey fills in many holes, fully describing the current and potential market for all warm water marine baitfish in the state of Florida. This information is invaluable in accurately representing the market, its growth potential, and dispelling myths perpetuated by the limited surveys of the past. As commercial warmwater marine baitfish producers ourselves, it was important to complete this survey accurately and distribute the results rapidly to our current and future hatchery customers in an aim to recruit more grow-out producers into this aquaculture industry. This survey was completed in cooperation with Florida Fish and Wildlife and with funding by the Gulf States Marine Fisheries Council.

PERSPECTIVES FROM A COMMERCIAL MARINE BAITFISH HATCHERY AND FARM

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Founded in 2013 as the first and only marine baitfish aquaculture company in the US, Live Advantage Bait now has a decade of production under its belt. We hatchery produce several different marine baitfish species, in addition to some foodfish species, with pinfish (*Lagoon* rhomboides) and Atlantic croaker (*Micropogonias undulates*) as our most popular sellers. We have customers ranging from grow-out farms to aquaponics facilities to researchers to restoration aiming NGOs. And are at the forefront of R&D for commercialization of these and new species to marine baitfish commercialization; committed to sound science, our community and the ecosystem.

In this talk we will talk about the evolution of the industry over the past decade, our production facility and current research underway, the market, and the many uses of our fish by our customers. In addition, we will talk about the many common pitfalls as well as opportunities producers can face moving forward.

SCALE EFFECTS AND PRODUCTION COSTS FOR MACROALGAE FARMS

Hauke Kite-Powell[1], Erick Ask[2], Simona Augyte[3], David Bailey[1], Julie Decker[4], Clifford Goudey[5], Gretchen Grebe[6], Yaoguang Li[3], Scott Lindell[1], Domenic Manganelli[5], Michael Marty-Rivera[3], Crystal Ng[3], Loretta Roberson[6], Michael Stekoll[7], Schery Umanzor[7], Charles Yarish[3]

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[2]International Flavors and Fragrances, New York, New York USA
[3]University of Connecticut, 1 University Place, Stamford, CT 06901-2315 USA
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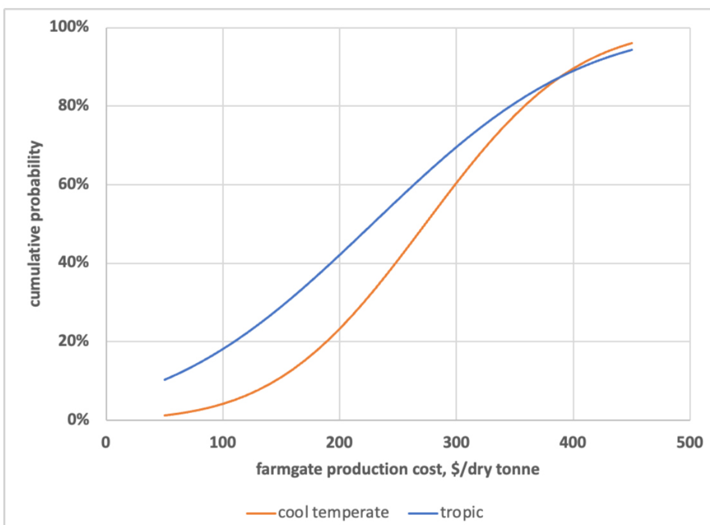
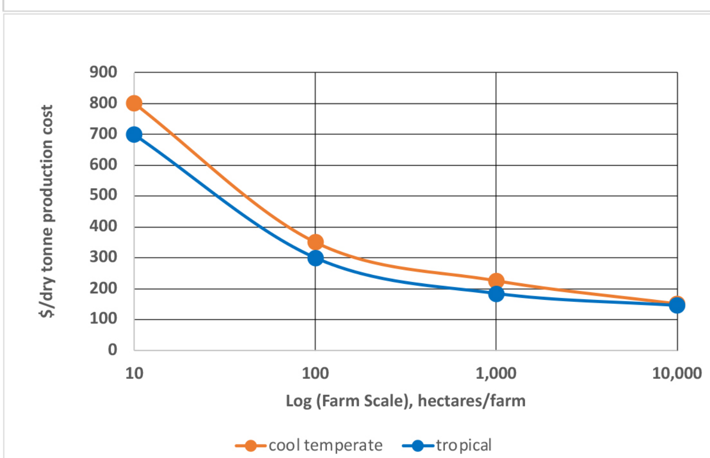
[5]C.A. Goudey & Associates, 21 Marlboro Street, Newburyport, MA 01950 USA
[6]Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543 USA
[7]University of Alaska Southeast, 11066 Auke Lake Way, Juneau, AK 99801 USA

Seaweed farming has the potential to produce feedstocks for many applications, including food, feeds, fertilizers, biostimulants, and biofuels. Seaweeds have advantages over land-based biomass in that they require no freshwater inputs and no allocation of arable land. To date, seaweed farming has not been practiced at scales relevant to meaningful biofuel production.

Here we describe a techno-economic model of large-scale seaweed farms and its application to the cultivation of the cool temperate species *Saccharina latissima* (sugar kelp) and the tropical seaweed *Eucheumatopsis isiformis*. At farm scales of 1000 ha or more, our model suggests that farm gate production costs in waters up to 200 km from the onshore support base are likely to range between $200 and $300 per dry tonne. The model also suggests that production costs below $100 per dry tonne may be achievable in some settings, which would make these seaweeds economically competitive with land-based biofuel feedstocks. While encouraging, these model results and some assumptions on which they are based require further field validation.





MOTE MARINE LABORATORY'S CLIMATE AND ACIDIFICATION OCEAN SIMULATOR; A LIFE SUPPORT SYSTEM DESIGNED TO CREATE FUTURE OCEAN CONDITIONS FOR THE STUDY OF VARIOUS MARINE LIFE

Travis G. Knorr

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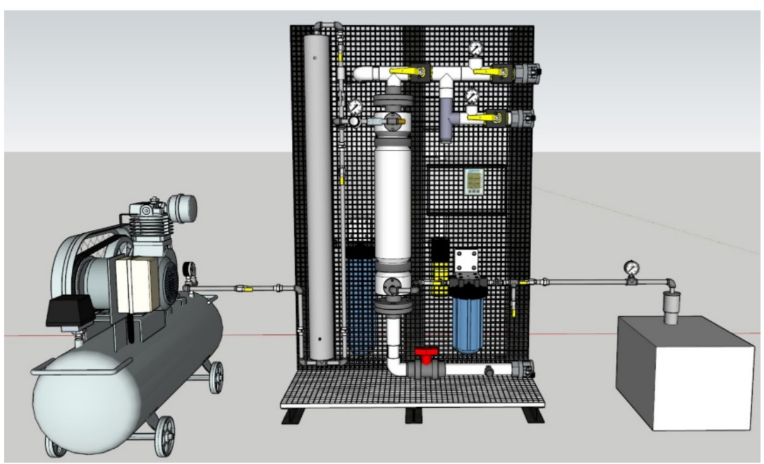
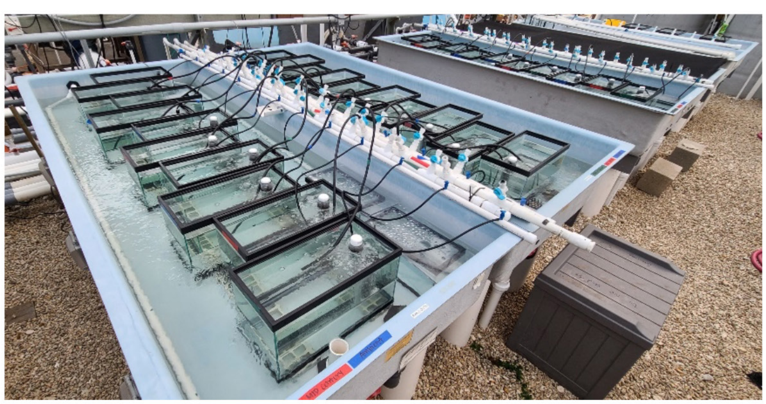
Climate change is altering the chemical makeup of sea water through the increased absorption of CO2 as well as increasing ocean surface temperatures. To facilitate the understanding of how these changes are impacting marine life, Mote has designed and built a unique aquatic research system named the Climate and Acidification Ocean Simulator (CAOS). The CAOS system is capable of manipulating seawater parameters to simulate future ocean conditions predicted conditions of the ocean. This system simultaneously controls and monitors temperature and pH of seawater independently in any of its 18 raceways. The system can be operated in flow-through or static configurations, offering a multitude of options for various types of research including, but not limited to, coral health and disease experiments. At the heart of this system is a specialized membrane filter which effectively strips dissolved CO2 from seawater without altering other constituents. CO2 can then be added back into the seawater at a controlled rate, effectively manipulating pH without the use of buffering chemicals.

The focus of this presentation is to describe the CAOS system's design, applications, and the strategies it can implore in detail. The advantages, disadvantages, limitations, and potential future modifications of the membrane filter within the CAOS system will also be explored through examples of possible research designs.





ASSESSING POLICY AND TECHNOLOGY PREFERENCES FOR PRO-ENVIRONMENTAL SALMON AQUACULTURE IN BRITISH COLUMBIA

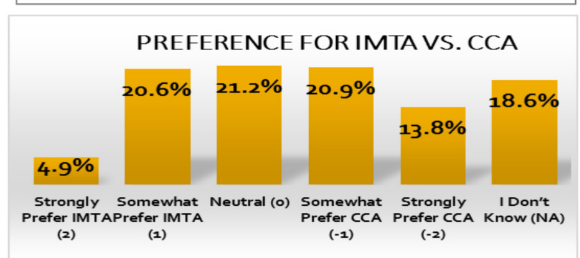
Duncan Knowler[1], Kimberly Irwin, Ryan Trenholm,Wolfgang Haider and Rober Martinez-Espineira

[1] School of Resource and Environmental Management
Simon Fraser University
Burnaby, British Columbia

In recent years, alternative systems of aquaculture, including integrated multi-trophic aquaculture (IMTA) and closed-containment aquaculture (CCA), have been developed to address some of the environmental effects of conventional salmon aquaculture. Industry adoption of these technologies in British Columbia has been tentative, since there is little financial incentive for salmon aquaculture companies to improve their environmental performance. While previous studies have outlined the private economic benefits and costs associated with IMTA and CCA adoption, they did not address the benefits accruing to society from improvements to the environmental performance of the salmon aquaculture industry. Doing so would increase the economic value of these technologies and provide justification for implementing policies that would encourage their widespread adoption. We used a discrete choice experiment (DCE) administered via an online survey of 1321 residents of British Columbia to address three research questions: (i) how do residents of BC value improvements to the coastal environment that could be realized through the adoption of more sustainable aquaculture systems, (ii) how is this valuation affected by using different ‘status quos’ and (iii) are British Columbians supportive of policies to promote alternative aquaculture technologies?

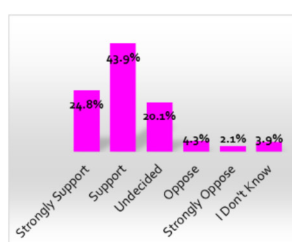
Our results demonstrate that British Columbians are willing-to-pay (WTP) to improve the environmental conditions surrounding salmon farms, but that this WTP varies depending on the status quo conditions. By making assumptions regarding the potential environmental improvements that could arise from widespread adoption of IMTA or CCA technologies in British Columbia, the benefits to society from their adoption were approximated. Based on these assumptions, British Columbians would be willing-to-pay between CDN $77.76 and $159.54 per household per year to support development and fund incentives for adoption of IMTA, and $133.28 to $173.00 per household per year to support development and fund incentives for adoption of CCA, depending on status quo conditions. Opinions regarding IMTA versus CCA are mixed in British Columbia, with 32.4% of residents indicating a preference for CCA, and 25.5% preferring IMTA. Overall, our results indicate that British Columbians are highly supportive of promoting more sustainable salmon aquaculture technologies and using government policies, such as tax-funded R&D or green technology subsidies, for this purpose.

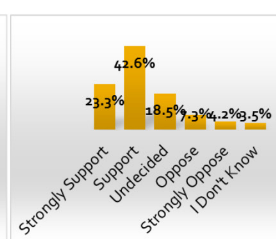
SAMPLE CHOICE CARD FOR THE DCE



SUPPORT FOR TAX-FUNDED R&D

SUPPORT FOR GREEN TECH SUBSIDIES





AN OVERVIEW OF THE UNIVERSITY OF ARKANSAS AT PINE BLUFF'S UNITED STATES AQUACULTURE SOCIETY STUDENT SUBUNIT FOR THE YEARS OF 2021-2022

Knuckles, Hannah*, Jackson, Glen, Stevens, Jeffery, Bhattarai, Sujan, and Duckworth, Ryan

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The University of Arkansas at Pine Bluff has an active and engaging student subunit of the United States Aquaculture Society. Our subunit is made up of 21 students and one faculty advisor. Our subunit is partnered with our university's American Fisheries Society (AFS) student subunit. During the school year, we hold many public service events. These include clean-ups of public waterways and assisting with outreach events. We host guest speakers from different universities and expertise levels during our monthly meetings. Our public outreach programs focus on getting the community involved in wildlife and outdoors and talking to prospective students about our program and careers in Aquaculture and Fisheries. We have partnered with other Subunits to plan group events and socials. When our members are not participating in subunit functions, each individual student is working on research in our respective fields. We plan meetings to help support each other while preparing for professional conferences. We also plan student support workshops including resume workshops. Our Student Subunit engages both undergraduate and graduate level students while harboring an extra learning environment as well as a social environment. Our main focus is creating an environment to not only learn but also network with people who can help support you during your student career.

DEVELOPING GENERALIZABLE AND SCALABLE CRYOPRESERVATION PATHWAYS FOR THE AQUATIC BIOMEDICAL MODEL SEA HARE, *Aplysia californica*: CRYOPROTECTANT TOXICITY AND COOLING RATE STUDIES

Jack C. Koch*, Allyssa M. Oune, Sarah Bodenstein, Lucía Arregui, Yue Liu, M. Teresa Gutierrez-Wing, and Terrence R. Tiersch

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Safeguarding economically important agricultural species has been driven by storing, evaluating, and distributing genetic resources as cryopreserved germplasm maintained in repositories. The value of germplasm repositories is being realized by the broader scientific community, especially for aquatic biomedical model systems. Cryopreservation pathways that are generalizable to a variety of organisms across biological levels of organization can provide a foundation for developing repositories and a means for addressing cross-taxa challenges. In collaboration with the National Resource for *Aplysia* (NRA, University of Miami), we are developing a generalizable cryopreservation pathway, including quality management and economics, that can be applied to the California sea hare, *Aplysia californica*. This is a biomedical model gastropod used to examine behavior, aging, and neural development. Cryopreservation and repository development for this species comes with many challenges, including those related to tissue types and developmental stages (Figure 1). For example, the sea hare produces egg strands with semi-rigid walls that hold capsules, each containing multiple embryos. Cryopreserving these strands presents challenges and is more like cryopreserving tissues rather than germplasm cells such as sperm. We exposed stage-4 embryos and veliger-stage larvae in the egg strand, to several common cryoprotectants at different concentrations and for different equilibration times to evaluate toxicity based on continued development. Cryoprotectants that allowed *Aplysia* to develop to veliger stage and hatch were used in cooling rate range-finding experiments. Frozen strands were thawed, and embryos or larvae were evaluated based on cell integrity and continued development. Assaying cryoprotectant toxicity and conducting cooling rate experiments are useful when developing cryopreservation protocols and are essential preliminary steps for building a cryopreservation pathway. By developing pathways with model organisms, we can leverage existing resources and information to bring much-needed generalization, scalability, and application to other aquatic species.

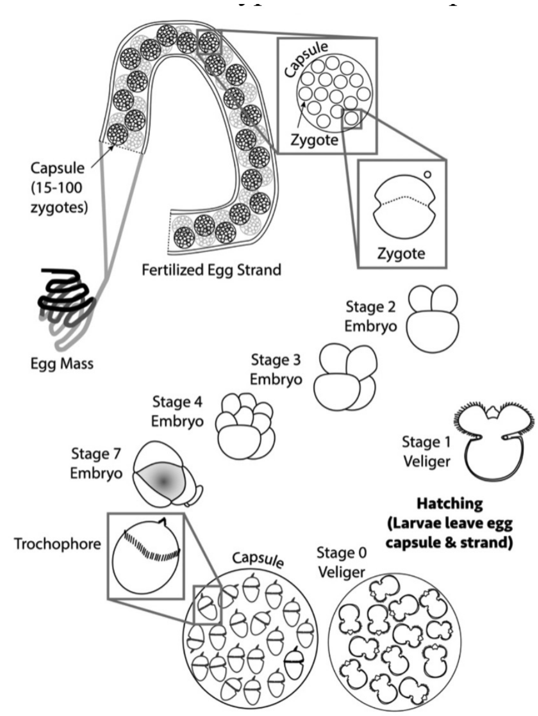

FIGURE 1. Early life stage development of *Aplysia*. Veliger-stage larvae are

AN INVESTIGATION OF ABALONE AND SEAWEED CO-CULTURE TO MITIGATE OCEAN ACIDIFICATION EFFECTS

Noah Kolander*, Scott Hamilton, Luke Gardner, Mike Graham, Andrew Kim, Maya deVries

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Climate change is negatively impacting shellfish aquaculture operations. Ocean acidification (OA) increases shell dissolution rates and decreases calcification rates, which ultimately negatively affects overall growth and development. This study addressed the negative impacts of OA on red abalone (*Haliotis rufescens*), through Integrated Multi-Trophic Aquaculture (IMTA) with the red seaweed Dulse (*Devaleraea mollis*). We tested the hypothesis that IMTA can mitigate the impacts of acidified water by raising seawater pH through photosynthesis. The current study also tested improvements in IMTA system design, by reducing the set-up from two integrated culture tanks to a single co-culture tank with a surplus of algal productivity.

A 5-month experiment was conducted to determine the benefits of IMTA on abalone growth, shell composition, and morphometrics using 4 different treatments: IMTA, Ambient, Sub-IMTA, OA with average pH values of 8.0, 7.9, 7.7, and 7.6, respectively (4 240gal tanks per treatment). To simulate OA and Sub-IMTA conditions, the ambient and IMTA seawater pH readings were reduced by 0.3 pH units by bubbling in CO_2. The upper bound of the pH in each of the treatments was controlled by additional oxygen released from seaweed photosynthesis. A subsample of abalone were measured monthly in each tank and seaweed weight was recorded biweekly to track growth over the course of the study.

After 5 months, a two-way ANOVA, comparing dulse buffering and CO_2 bubbling in each treatment, showed abalone raised in ambient and IMTA treatments had an increase in shell length and density and demonstrated less variation than those raised in low pH treatments (OA and sub-OA). Interestingly, the IMTA treatment had the highest total mass per square centimeter (Fig. 1A). Preliminary analyses of shell structure and function suggest that these growth differences between OA and ambient treatments may translate to differences in shell properties as well. Seaweed from the OA treatment grew faster than those grown in the ambient seawater and IMTA treatments, suggesting that elevated pCO_2 levels may increase seaweed production. Overall, seaweed buffering of acidified water may allow abalone aquaculture to persist in the face of an acidifying ocean, and as an added benefit, it will require less space to yield a better overall product.

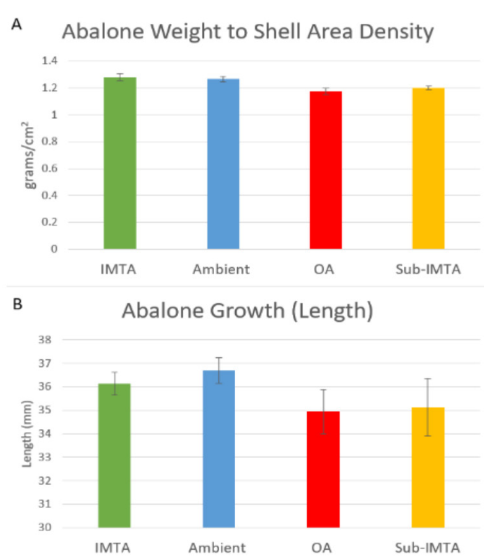

Figure 1. Abalone mass to size ratio (Density = total weight/shell area) (A) Abalone final growth in shell length (B)

GOOD AQUACULTURE PRACTICES; THE COLLABORATIVE USE AND BENEFITS

B.Koonse

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The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is a collaboration between the United States Food and Drug Administration (FDA) and the University of Maryland (UM). JIFSAN has developed an aquaculture program that links food safety and disease prevention. It's called Good Aquaculture Practices (GAqPs). GAqPs are widely used and implemented around the world. This presentation is a short synopsis on GAqPs and a request for to help take GAqPs to the next level where it can be universally used and recognized for a wide variety of purposes. GAqPs could be used or integrated into new or existing programs to show food safety and disease controls are in place by the following:

- Individual aquaculture farms for their buyers;
- Governments for their farm and or processor registration and certification programs;
- Private third-party certification programs to verify food safety and disease prevention;
- Processors in their HACCP or Preventative Control programs to act as their food safety controls for aquaculture related food safety hazards;
- Academia to train future aquaculture professionals in food safety and disease prevention;
- The aquaculture industry and others to demonstrate to the general public that aquaculture products are safe, sustainable, and free of hazardous residues and pathogens.

NEOBENEDINIA PARASITE MANAGEMENT IN OFFSHORE SERIOLA PRODUCTION

Tyler Korte

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Offshore production of Seriola species have proven to be successful ventures across the globe but losses continue to mount and cost of production are inflated due to a small skin fluke with an extraordinarily efficient life cycle.

Blue Ocean Mariculture has been producing Seriola rivoliana for 15 years in warm Hawaiian waters where the monogenean skin fluke, Neobenedinia girellae, thrives. During that time Blue Ocean has instituted several infrastructure, operational, and biological mitigation efforts to reduce the impact that these ectoparasites can have on warm water Seriola production. With new net technology, steady monitoring, and the right tools Blue Ocean has managed to increase survival on cohorts up to 92% while substantially reducing bathing costs per cycle. Discussed here are the mitigation technologies and techniques that have proven successful in offshore culture conditions. While mitigation efforts and success will vary based on location and species further research and investment is needed for the continued success of Seriola culture.

HARMFUL ALGAE BLOOM MONITORING ON SALMON AQUACULTURE FARMS

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Harmful algae blooms present an ongoing challenge on aquaculture farms due to their complex and unpredictable nature. The impacts of climate can lead to more intense algae blooms, and this presents operational challenges that farmers must navigate to protect their livestock that must be overcome to ensure they are achieving more efficient and sustainable production cycles.

The complex nature of the effects of different groups of phytoplankton means that producers must be able track and analyze trends at the species level. Producers must therefore be diligent in their data collection and management strategies both for the real time care of their fish and in the analysis of changes over time.

A data management and visualization system specifically for aquaculture farmers helps derive educated insights from the environmental and water quality data that farms collect. This helps farmers to discover species-specific trends, explore the relationship between environmental data and algae concentrations and discover environmental trends like water currents that might carry species between farms. Sophisticated, map-based visualization software is intuitive and data analytics can lead to forecasting trends and predictions in the future which can help farmers better manage plankton issues on farms.

ENVIRONMENTAL HYPOXIA IN THE INDIAN RIVER LAGOON (IRL) AND ITS EFFECTS ON NATIVE FISH SPECIES DURING EARLY DEVELOPMENT

Alessandra Jimenez Yap*, Bing Ouyang, Paul S. Wills, and Sahar Mejri

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The 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 is well-studied in adult fish, yet gaps of information remain in terms of early life stages, including potential consequences to their development, hatching success, or future recruitment of economically important native species. These include Florida pompano, red drum, snook, Atlantic croaker, and grey snapper. To test effects of severe hypoxia (i.e. 10% saturation, 0.7 mg/L), fertilized eggs will be incubated in two treatments (10 and 20% DO saturation, 0.7 mg/L and 1.4 mg/L, respectively) and one treatment of normoxia (100% DO saturation, 7 mg/L). Treatments will run in triplicates (n=3). Eggs and larvae will be sampled at 10, 20, 30, and 40 hours post-fertilization (hpf) to assess development, survival, and fatty acid lipids use. Lipids will be extracted from each sample and separated into neutral (used for energetic purposes) and polar (used for membranous development) fatty acids. Concentrations of these fatty acids will inform us on the potential effects of severe hypoxia on energy demand and membrane permeability (i.e., homeostatis mechanism). It is expected to see a decrease in hatch rate, survival, and overall development. This project helps 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.

ORAL DELIVERY OF A VACCINE AGAINST STREPTOCOCCOSIS IN NILE TILAPIA

Marie-Eve Koziol[1], Nicolas Versillé[2], Eakapol Wangkahart[3]

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[2] Seppic, Paris La Défense - 50 Boulevard National - 92257 La Garenne Colombes, France
[3]Department of Agricultural Technology, Mahasarakham University - Khamriang Sub-District Kantarawichai District - Maha Sarakham 44150, Thailand

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 local immunity in order to protect 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 Montanide™ GR 01 (GRA) adjuvant tested here is able to provide a matrix protecting the antigen from gastric-route. A first study in tilapia was performed on a *Streptococcus agalactiae* vaccine formulated with GRA adjuvant and mixed at 2% or 20% with feeding pellets. The tilapia were 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).

A second study was conducted at a larger scale on 9000 fishes in farming standard conditions to evaluate vaccine potential. The tilapia were orally vaccinated with *Streptococcus agalactiae* vaccine, whether with antigen alone or formulated with the Montanide™ GR 01 adjuvant and compared to an unvaccinated control group, under two 5 day-periods, 5 days apart for the priming. Oral boost was then given during a 5 day-period at day 95. Immune response of fish was followed by measuring specific IgM antibody levels at different time points (up to day 128). The use of the vaccine did not affect survival rate and appeared to improve economic performance of this fish farming. The group receiving the Montanide™ GR 01 based vaccine also showed significantly higher specific antibody titer than the non vaccinated control group from day 44.

Taken together, these results show that the Montanide™ GR 01 is well adapted for the oral administration of safe and efficient vaccines in tilapia.

COMMUNICATIING FOR IMPACT: THE POWER OF THE PODCAST

Julie Kuchepatov

Seafood and Gender Equality (SAGE)
Julie@seafoodandgenderequality.org

Fifty percent of people that bring seafood to our tables are women and the fishing industry has been described as "male-dominated but female-intensive[1]." Regardless, women are significantly underrepresented in management and decision-making roles, their invaluable contributions to the sector are unacknowledged, and how they participate in seafood supply chains is insufficiently researched. In the seafood sector, women and their contributions, skills, and knowledge are invisible.

To address a lack of gender equality and women's empowerment, Seafood and Gender Equality (SAGE) was launched in 2020 with a mission to uplift and amplify diverse voices in the seafood sector. To achieve our mission, we have implemented a comprehensive communications strategy since our inception to build platforms showcasing the efforts of women and gender minorities in the sector.

The podcast is becoming one of the most popular and accessible formats for sharing information and raising awareness about an issue with huge audiences. Research conducted by Statista in 2019 estimates that the number of Americans who listen to podcasts at least occasionally is 86 million and Edison Research found that about 60% of Americans listening to podcasts use this medium to stay in touch with the latest news[2]. Recognizing this powerful medium, SAGE launched The Conch podcast in 2021 to increase the visibility of women and gender minorities in the sector, with interviews featuring the most inspiring people in the world of seafood, striving to succeed, as well as those already making an impact - all while working to improve the environmental and social responsibility of the planet's most incredible food source - seafood.

This talk will feature reflections on the challenges and advantages of creating a communications strategy for a start-up focused on gender equality in an industry where women and gender minorities are the invisible backbone. We will dive deeper into the podcast as a powerful tool in a comprehensive communications strategy and share lessons learned, emphasizing that everyone can use this tool for effective, far reaching, and impactful communications.

1. https://bit.ly/3AsoYA9
2. https://bit.ly/3724kLA

IS FEED CONVERSION RATIO A RELIABLE METRIC FOR CERTIFICATION? THE CASE OF U.S. CATFISH

Ganesh Kumar*, Nathan Stone, Carole Engle, Menghe Li, Shraddha Hegde, Luke A. Roy, Anita M. Kelly, Larry Dorman, and Matthew Recsetar

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Feed is the most expensive component of U.S. catfish production, contributing over 40% of total production costs. It is well established that improving feed conversion ratio (FCR) at a given level of production improves profit. FCR is also a primary sustainability metric, given the embodied resources in fish feed. Thus, FCR is a key measure of production efficiency and environmental performance. The simplicity and importance of FCR makes it an attractive metric for certification programs, apparently based on the assumption that "good" farmers obtain lower (better) FCRs, and that "bad" farmers can and should improve practices to lower their high FCRs. The reality is far from this assumption. This review focuses on the case of U.S. commercial catfish production and challenges the question of whether FCR is an appropriate certification metric that will accurately reflect better management of pond aquaculture. Although farmers understand the economic importance of FCR, control over production processes is limited for catfish production in open ponds. Major factors affecting catfish FCR include the type of fish (catfish/hybrid catfish), type of feed used, feeding practices, disease, production systems, economic factors, marketing strategies, and climatic conditions. In addition, regulatory stringency of environmental and food safety laws limits producer's ability to control fish losses to piscivorous birds and bacterial diseases. There is a wide disparity in FCR obtained from research studies and commercial conditions. Thus, certification standards stipulated from research studies will not reflect on-the-ground realities of farms. If ponds managed according to the protocol of the university research verification program cannot routinely achieve FCRs of 2.0 or less (Table 1), it is unrealistic to expect farmers to do so. In summary, for U.S. catfish production, FCR is not a reliable, consistent metric that reflects management ability, and thus it is not suitable as a blanket certification standard.

Table 1. Results of research verification trials in commercial catfish ponds in Arkansas, Alabama, and Mississippi, 1993-2020. (Bott 2015; Bott et al. 2015; Dorman 2020; Dorman et al. 2018a, 2018b, 2019; Engle 2007; Hanson et al. 2020; Heikes et al., n.d.; Recsetar 2013, 2014, 2015; SRAC, n.d.). *Split-pond yields have improved over time. This data includes early verification trials when this culture method was new.

Culture System	*N*	Net FCR±SD	Yield ±SD (kg/ha/yr)
Traditional pond	20	2.13 ± 0.45	5,906 ± 1.594
Intensive aeration	48	2.28 ± 0.56	14,081 ± 3,587
Split-pond*	65	2.38 ± 0.31	14,950 ± 3,897

DIETARY INCLUSION OF INSECT MEAL ENHANCES THE SOY PROTEIN UTILIZATION IN RAINBOW TROUT VIA MITIGATING THE GUT ENTERTITIS AND MODULATING THE GUT MICROBIOME

Vikas Kumar*, Mosope F. Abanikannda, Jie Ma, Nicholas Romano, Kenneth D. Cain, Daniel Adams, and Liz Koutsos

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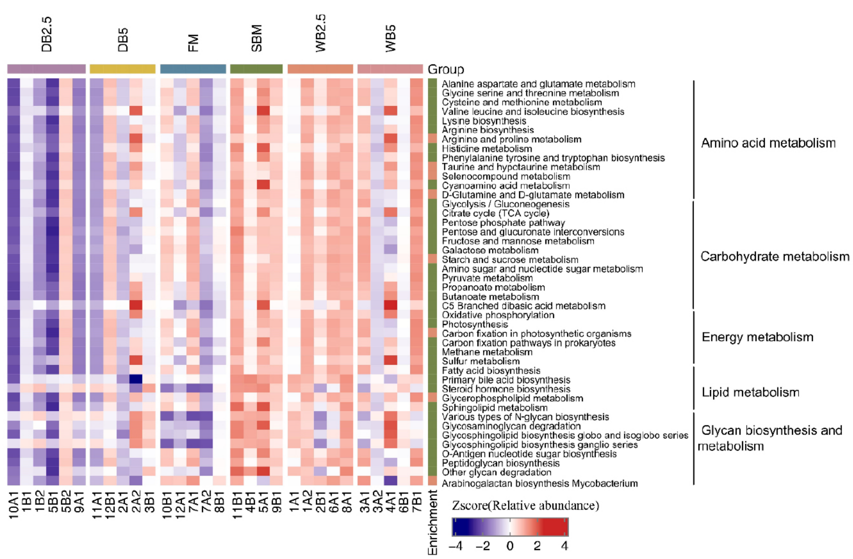
High inclusion of soybean meal (SBM) exhibit soybean induced enteritis in carnivore fish including rainbow trout. Our goal was to evaluate the effects of whole/defatted black soldier fly larvae (WB/DB) as complementary feed ingredient in soybean meal based diets on growth performance, disease resistance, gut histology and gut microbiome 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).

Supplementation of WB and DB improved ($p<0.05$) the performance of rainbow trout. 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 ($p<0.05$). Soy based diets caused enteritis whereas supplementation of insect meal mitigates the enteritis. Gut microbiota at phylum, class, genera and species level were significantly affected by dietary treatments in pre and post challenge study.

The firmicutes was the predominant microbiota in the pre-challenge study however, after post-challenge study the ratio of firmicutes decreased concurrent with the increase in proteobacteria and penericutes in most of the groups. The variation in microbial composition could lead to change in microbial metabolism including carbohydrate, lipid, protein and energy, and glycan biosynthesis. Summary of variation in microbial composition and nutrient and energy metabolism is depicted in figure 1. Conclusively, insect meal could improve the utilization of plant protein in trout via improving the growth and health of fish.



EFFECT OF MICROALGAE OIL ADDITION ON THE OXIDATIVE QUALITY OF SOYBEAN OIL AND FISH OIL BLENDS

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Global production of farmed marine species such as salmon is increasing every year. Consequently, there is a great need for sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are essential for aquaculture feed. Nowadays, the availability of marine oils as a source of these long-chain polyunsaturated fatty acids (PUFAs) is quite limited, so a new interest has arisen in seeking a new renewable source of PUFAs, such as microalgae.

Microalgae oil (MO) contains about 60% PUFAs, of which the EPA+DHA content is usually between 40-45%. This could impact in MO oxidative stability, since PUFAs are the fatty acids most susceptible to oxidation, which could be a disadvantage for quality of oil blends. This study pretends to evaluate the effect of Microalgae oil addition on the oxidative stability of Fish oil and Soybean oil blends.

For study this effect, it was used a Simplex-Lattice Mixture Design, Special Cubic model with 3 aleatory replicates. The variables studied were the soybean oil (SO), fish oil (FO) and microalgae oil (MO) concentrations. The model responses were the induction period (IP) in a Rancimat, measured at 100°C and with a 20 mL/min airflow, and the difference between initial and final PUFAs content of each mixture after 18 h oxidation under these conditions (ΔPUFA).

T he model results showed no significant differences between the effect of fish oil and microalgae oil on the oxidative stability, while the soybean oil is the most stable (Figure 1).

To evaluate the changes in the oxidative stability of different oil blends by adding microalgae oil, the oxidative parameters were predicted using the statistical model (Table 1). It was observed that, the increase in MO content slightly decreases the IP but also decreases the oxidation of the PUFAs, while the desirability did not show significant changes when the FO content decreases and MO increases, maintaining the oxidative stability.

We can conclude that it is possible to add microalgae oil as a sustainable source of EPA and DHA without significantly altering the oxidative quality of the oil blends that are currently used for aquaculture feed.

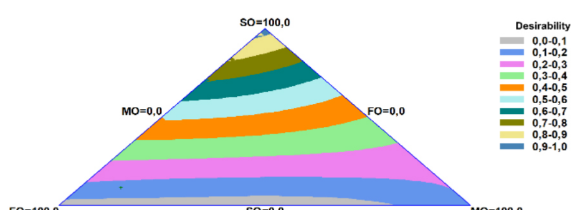

Figure 1. Response surface graphic of the Special Cubic Model. SO (soybean oil), FO (fish oil) y MO (microalgae oil).

Table 1. Comparison of responses of the model for different theoretical blends of oils.

SO (%)	FO (%)	MO (%)	IP (h)	ΔPUFA (%)	Desirability
10	80	10	4.52	19.07	0.16
10	60	30	4.06	18.41	0.15
10	45	45	3.85	17.65	0.15
10	30	60	3.75	16.66	0.15
10	10	80	3.79	14.99	0.18

THE IMPACT OF ANTIMICROBIAL PEPTIDES ON THE BIOFILM FORMATION OF AQUACULTURE-RELEVANT PATHOGENS

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As food fish production continues to increase, the frequency of disease will only continue to rise within the aquaculture industry. Add to this an increase in the regulation of treatments and resistance to available antibiotics means that alternative methods of disease protection will be required. Fish-derived antimicrobial peptides are an important part of the innate immune system because they often demonstrate potent antimicrobial properties. Piscidins are a class of antimicrobial peptides first described in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) but have also been identified in many other fish species. Previous work has shown broad antimicrobial activity of piscidins against Gram-positive and Gram-negative bacterial species. This study sought to determine the extent to which class I and class II piscidins inhibit biofilm formation of different Gram-negative bacteria. In general, the class I and II piscidins demonstrate potent activity against *Escherichia coli* and *Flavobacterium columnare* biofilms. The class II piscidins showed more activity against *Escherichia coli* and *Flavobacterium columnare* isolates than did the class I piscidins. The piscidins in general were much less effective against inhibiting *Aeromonas hydrophila* and *Aeromonas veronii* biofilm growth. Only the class I piscidins showed significant growth inhibition among the *Aeromonas* spp. examined. The potential use of antimicrobial peptides for disease prevention in food fish production will be discussed.

IMPACT OF FLUCTUATING PH ON PRODUCTION OF HARD CLAM *Mercenaria mercenaria* POST-SET IN AN IMTA SYSTEM

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The land based IMTA system located at Harbor Branch (HBOI-LB-IMTA) utilizes various fed, assimilative and extractive components. Good extractive candidates for these systems include bivalves. As filter feeders, they cycle nutrients by removing particulates from the water and storing nitrogen and carbon. The hard clam, *Mercenaria mercenaria,* is the largest food aquaculture industry in Florida, and therefore the natural choice for HBOI-LB-IMTA system. Maintaining a stable environment is important for consistent production, yet this system is subject to large pH swings (e.g., 7.4 to 8.2 over 24 hours). These fluctuations may lead to stress resulting in decreased production, and low pH has been shown to have a detrimental effect on larval bivalves. To better understand the impact of pH on post-set clam production and health, a short-term eight-week study examined the effects of stable (low, high) and fluctuating pH levels in the LB-IMTA system.

Three-week-old clam post-set (1,200±75, 537±85 μm) were randomly assigned to one of three treatments, 8.0, 7.4 or fluctuating (system) pH (N=5 replicates/treatment). The pH in the 7.4 and 8.0 treatments was maintained with Pinpoint pH controllers 120 Vac, a CO_2 tank and MP810 dosing pumps to deliver a soda ash solution. Clams were fed a 1:1 ratio of *Tisochrysis lutea* and *Chaetoceros neogracile* twice daily (50,000 cells/mL initial rate). Dissolved oxygen, temperature, and salinity were monitored daily and pH continuously, via a PASCO wireless sensor. Ammonia, nitrite, and alkalinity were measured weekly. Growth was evaluated bi-weekly, and survival at experimental termination. Condition index, bacterial analysis (Marine agar, TCBS), and shell composition were evaluated at experimental termination.

Significant differences ($P \leq 0.05$) were seen in growth at weeks 6 and 8, with lower growth at pH 7.4 compared to pH 8 and the fluctuating pH treatments. Survival was similar between treatments ($P=0.126$). No difference was seen in the condition index. Total bacterial ($P \leq 0.05$) and *Vibrio* ($P \leq 0.012$) counts were higher at pH 7.4 and the percentage of colony types varied between treatments. Clams from all three treatments showed high levels of calcium, strontium, and sodium, with higher calcium and strontium levels in clams subjected to fluctuating pH levels.

These results indicate that post-set clams can be successfully grown under the fluctuating pH levels inherent in the LB-IMTA system, with no short-term negative effects compared to clams maintained at a stable pH of 8.0. These results likewise have implications for wild or cultured clams in estuarine ecosystems that may be subjected to short-term fluctuations in pH due to natural processes or human impacts and confirm that clams subjected to low pH experience negative impacts as have been reported previously.

IMPACT OF A SYNBIOTIC TREATMENT ON FLORIDA POMPANO PRODUCTION AND HEALTH

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Production of Florida pompano, *Trachinotus carolinus*, has been shown to be commercially feasible based on joint FAU-HBOI and USDA-ARS research projects. Many of those projects focused on nutrition. Probiotics and prebiotics have been shown to result in enhanced health and production of aquatic species compared to probiotics alone. A previous reported study comparing various levels of prebiotics added to larval pompano diets showed no increase in production from 5 to 50 g but did show changes in health indices. The present study was conducted to determine whether an early application of the symbiotic combination that showed promise previously would enhance the production or health of Florida pompano to market size.

The two dietary treatments used consisted of a non-synbiotic basal diet and a synbiotic diet (β-glucan + *Pedicoccus acidilactici)*. The experiment was divided into two phases (Figure 1). Pompano (n=~12,500/tank, 2 replicate tanks/treatment) reared to larval stage received rotifers enriched with Algamac-3050 or Algamac-3050 + the synbiont combination. Following weaning, larvae were transferred to one of 8 tanks in two separate RAS systems (east system 830 L tanks and west system 1260 L tanks) and fed the same dietary treatment in pelleted form (45% crude protein, 12% lipid). In the symbiont treatment β-glucan was substituted for cellulose at 1.0 g/kg^{-1}, and a dried *P. acidilactici* fermentation product (10^6) was applied in the top-coat oil. At ~50 g the two treatments were subdivided into two additional treatments, creating four new treatment groups (n= 175 fish per m^3; 4 replicate tanks/treatment in the two systems [n=145/tank east and n=220/tank west]). At ~100 g fish (total n=400/replicate tank, 3 replicates/treatment; 15 fish from each phase 1 treatment were uniquely tagged in each replicate tank) were transferred into a new RAS system and grown to market size (target size ~680 g).

Production data assessed at the end of each phase included weight gain, specific growth rate and survival. Additional analysis conducted included proximate analysis (whole fish), enzyme analysis (intestinal tissue), and hematological (blood counts, lysozyme) and immune function assays (phagocytic activity, SOD activity). Production and health parameter evaluations for each experimental phase are currently underway and will be reported at AA2023.

EVALUATING NATIVE MACROALGAE SPECIES OF THE SOUTHEAST U.S. AND CARIBBEAN REGIONS FOR USE IN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA)

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Global human population is predicted to approach 10 billion by 2050, prompting an increased need to produce protein to support human dietary demands. Aquaculture food production will help address this increased demand for protein production. Seafood farming has established roots in many Asian and island countries and is becoming a more common form of food production in the United States. The expansion of aquaculture requires social license vetting due to the importance of sustainability to the average U.S. consumer. Integrated multi-trophic aquaculture (IMTA) represents a potentially more sustainable approach to aquaculture than traditional monoculture of a species within a single trophic level. IMTA is a method to produce multiple crops for market across a multitude of trophic levels. The most common IMTA arrangement is carnivorous finfish production coupled with production of secondary products for market, such as algae or bivalves, using the nutrient rich effluent water from the finfish culturing system to grow the extractive species. Research is being carried out at the University of Miami examining the aquaculture potential of four different macroalgae species (*Agardhiella subulata, Caulerpa racemosa, Eucheum isiforme*, and *Ulva lactuca*) in a pilot-scale IMTA system coupled with yellowtail snapper (*Ocyurus chrysurus*) production. Water quality sampling is being carried out over the course of the research trials, while macroalgae ("seaweed") samples are collected every 3 days. The seaweed samples are assessed for compositional (protein, lipid, ash, and carbohydrate) analysis at the beginning and end of the research trials. Additionally, the carbon and nitrogen isotopes of the seaweed are being analyzed from the samples over the course of the project. Results of this work will provide detailed information on the bioextractive capacity of the selected macroalgae species when cultured in IMTA applications. The seaweeds are also being assessed from a market potential standpoint through collaborative work with local chefs and seafood distributors to assess the estimated market value of each species. The results of this project will provide novel information for existing and prospective IMTA operations throughout the Southeast U.S. and Caribbean regions and will allow producers to make well-informed decisions on candidate culture species of macroalgae for IMTA systems. This work has been supported by the Gulf States Marine Fisheries Commission (GSMFC) and the National Oceanic and Atmospheric Administration (NOAA).

EASTERN WHITE CEDAR SAPLINGS GROWTH RATE IN AQUAPONICS

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Silviculture and aquaponics are novel topics that have been intriguing scientists and foresters alike. In theory, by combining the two disciplines, we could use pre-existing fish hatcheries and just add on an aquaponics system to reduce excess nutrients; thereby, reducing eutrophication in local waterways while growing saplings for many purposes. The nutrient waste will hopefully grow stronger saplings faster than conventional agriculture, giving them a competitive advantage over other saplings. These saplings could be used for forest restoration purposes or even landscaping. Increasing the number of native trees in landscaping and restoring natural habitats is a key to reducing invasive species.

In a preliminary study conducted last year, Eastern White Cedar saplings were successfully sprouted from seed, using only damp paper towels and a grow light. Seedlings were transferred into a deep water culture system (DWC) and grown for approximately 8 months before being transferred to soil. The saplings thrived in the deep water culture system and are now successfully thriving in soil. There was a large variation in size of saplings, possibly because half of the sprouts were transferred into the DWC system later than the other half.

A similar study is set to begin late January 2023, this time with a control group and consistent planting date. The study will be a comparison of growth rates between aquaponically grown saplings and soil grown saplings. The aquaponic sapling will be grown in a DWC with no added media for the roots to attach whereas the soil grown saplings will be in medium sized pots of “Dairy Doo” soil. The soil and water chemistry levels will be recorded every 2 weeks and height of each sapling will be measured weekly. Both sample groups will receive the same environmental conditions throughout the study. The predicted outcome of the study is that the aquaponic DWC will lead to faster growth of Eastern White Cedar seedlings while reducing the nutrient load in the effluent. Overall, combining silviculture with aquaculture has potential benefits for protecting the aquatic environment and creating a new field of aquaponics.

DOES THE DECOUPLING OF PELAGIC AND BENTHIC COMPARTMENTS HAS AN IMPACT ON ANOXIA TRIGGERING IN COASTAL LAGOON?

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The Thau lagoon is a Mediterranean lagoon exploited by shellfish farming under oligotrophication and impacted by anoxic crisis. *In situ* experiments using mesocosms were carried out to investigate 1) the triggering and consequences of anoxia on nutrient, sulfides and planktonic organisms in an oyster farming site, and 2) the role of oyster ropes and sediment on anoxia and consequences. The principle of experiments was to confine the lagoon waters in mesocosms, in presence or in absence of reared oysters and bare sediment, to trigger or not anoxia by asphyxia (Figure 1).

In the controls, oxygen concentrations decreased daily but remained above hypoxic concentrations, in presence or in absence of sediment. In contrast, in presence of sediment and oyster rope, anoxia appeared after 54 hours. All the oysters had died between days 6 and 9, after the beginning of the prolonged anoxia and following the appearance of $\sum H_2S$ (up to 295 $\mu mol \cdot l^{-1}$). At 9 d, a peak of nutrients was observed with up to 390 $\mu mol \cdot l^{-1}$ for NH_4^+ and 17 $\mu mol \cdot l^{-1}$ for PO_4^{3-}. These releases favoured a bloom of phytoplankton (11.8 $\mu g \cdot l^{-1,}$ 10^6 cells·L-^{1}), mainly composed of picoeucaryotes. In absence of sediment, the mesocoms did not switch to anoxia, despite the presence of oysters. Although oxygen concentrations decreased during the first days, they then increased to reach supersaturation levels (almost 150%). No H_2S releases and massive oyster mortality were observed in these conditions. Increase of NH_4^+ and PO_4^{3-} were also observed on day 1 with maximum concentrations of 15.6 $\mu mol \cdot l^{-1}$ and 0.4 $\mu mol \cdot l^{-1}$ but 25-fold lower than concentrations observed in presence of oysters and sediment. A phytoplankton bloom, dominated by picoeukaryotes was also observed on day 4 at 9.6 $\mu g \cdot l^{-1}$, corresponding to abundances of 1222 x 10^6 cells·L^{-1}.

Both oyster ropes and sediment triggered oxygen depletion and nutrient releases in relation to respiration, excretion and mineralisation processes. Oyster ropes favoured picophytoplankton bloom by filtration and excretion in normoxia or through flesh decomposition in anoxia. Without sediment, no anoxia and H_2S releases were observed, wondering which is the role of benthic habitat on trigger of massive oyster mortality. These results were completed with a series of *in situ* benthic mesocosms experiment, to highlight the role of several benthic habitat (bar sediment, *Zostera*, *Halopithys*) to trigger or preserve the ecosystems from anoxia and oyster massive mortality.

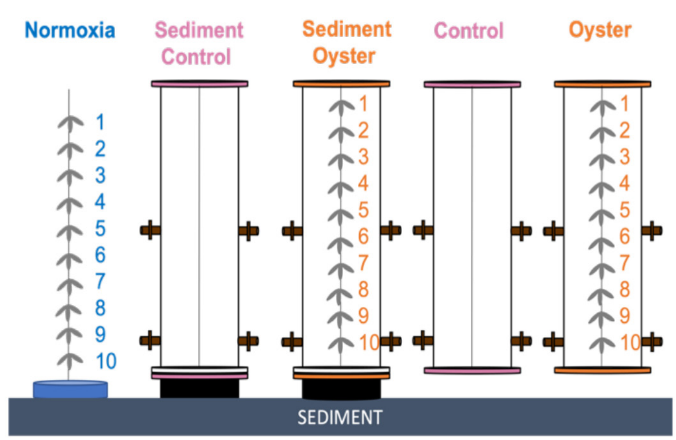

Figure 1: Experimental design of the experiment conducted in the Thau lagoon (September 2020 and 2021)

WHICH BENTHIC COMMUNITY PREVENTS THE DEVELOPMENT OF ANOXIA UNDER LAGOONAL OYSTER FARMING SITES?

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Anoxic crisis are recurrent in the Thau lagoon, a Mediterranean lagoon exploited by shellfish farming. For this experiment, we investigated the triggering and consequences of anoxia in function of the benthic compartment. The use of *in situ* benthic mesocosms made it possible to confine the lagoon waters with or without macrophytes. Oxygen dynamics, nutrient and H_2S concentrations, bacterial abundances and phytoplankton community structure were studied during 11 days near the bare sediment, *Halopithys*, and *Zostera* community. These were the main species found under oyster rearing table.

The responses from the different benthic communities, were highly contrasted according to the community. Near the bare sediment, hypoxic conditions were observed for four days, then concentration went back up. In presence of *Halopithys*, the system switched to anoxia, raising significantly NH_4^+ and PO_4^{3-} concentrations (respectively: 57.8 μmol·l^{-1} on day 7 and 13.06 μmol·l^{-1} on day 11). High bacterial abundances were measured (14.55 x 10^6 cells·ml^{-1}, on days 4, 5 and 6), but the phytoplanktonic biomass lowered (final concentration: 0.33 μgChl*a*·*l*$^{-1}$). In presence of *Zostera*, oxygen levels stayed above hypoxic levels, even during the night. They were the most resistant community among the three treatments.

These results highlight the importance of monitoring and restoring seagrass meadows in lagoonal ecosystems. This is especially important when the lagoon is exploited by aquacultural activities making then more prone to anoxic crisis and mass mortalities.

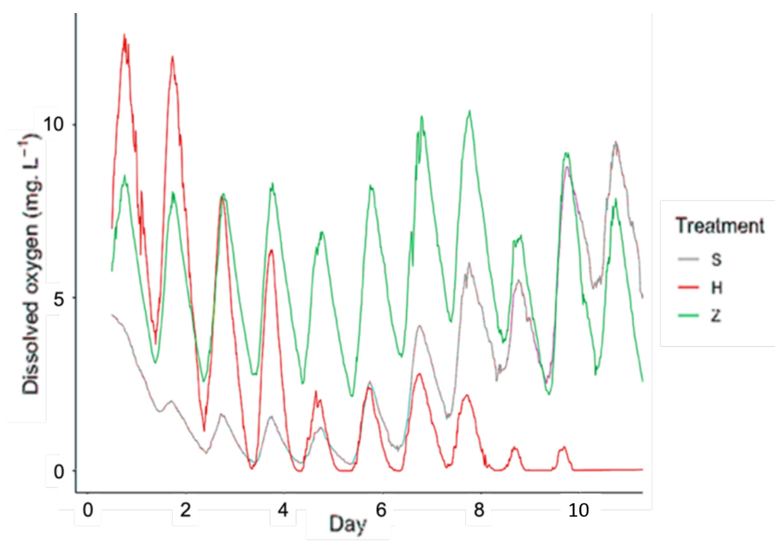

Figure 1: Mean dissolved oxygen (mg·l^{-1}) dynamics in the mescosms: S- bare Sediment, H- *Halopithys* covered sediment, and Z- *Zostera* covered sediment, in September 2021, in the Thau lagoon, France.

THE EFFECTS OF DESICCATION ON DELTA SMELT (Hypomesus transpacificus) EMBRYO DEVELOPMENT AND HATCHING SUCCESS

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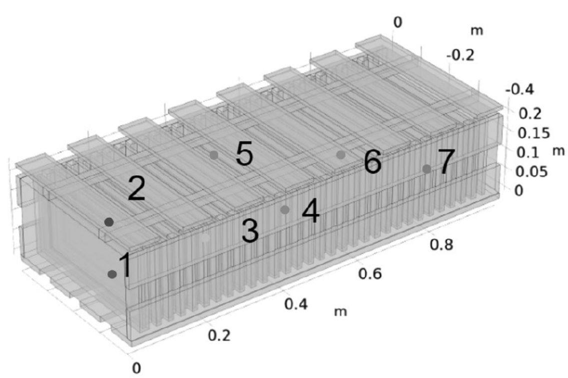
Delta smelt (*Hypomesus transpacificus*) is an endangered fish species endemic to the Sacramento/San Joaquin Delta. A refuge population is maintained in culture by the UC Davis Fish Conservation and Culture Lab (FCCL) in Byron, CA. The FCCL also produces adult delta smelt for supplementation to prevent extinction of the species in the wild. The potential of supplementation at earlier life stages may be feasible, but its potential is understudied. Supplementation at the egg stage via egg frame boxes (EFB, Figure 1), for example, is used to support recreational fishing of the closely related wakasagi in its native habitats in Japan. Adaptation of wakasagi EFB for use in supplementation of delta smelt, therefore, may be a viable method to facilitate conservation of delta smelt in the wild via egg-stage supplementation.

We took a stepwise approach to assess the feasibility of EFB for supplementation of delta smelt eggs. First, we identified Cache Slough as a candidate site for EFB deployment based on annual monitoring survey data, which show that a majority of ripe female delta smelt are captured in this region during the spawning season. We then conducted computational fluid dynamics (CFD) simulations to evaluate if flow conditions in the slough are suitable for EFB deployment during this period. These simulations incorporated USGS flow data obtained from two Cache Slough stations in February. Next, we investigated potential effects of transportation on egg survival. We ran 'transportation' trials at FCCL by soaking EFB in water and then exposing the box to direct sunlight for the approximate travel time from FCCL to the deployment site. Our next step is to investigate delta smelt embryo development and hatching rates based on the range of humidity obtained from the first trial (Figure 2). These studies aim to inform EFB design and deployment conditions that would facilitate egg-stage supplementation of delta smelt.



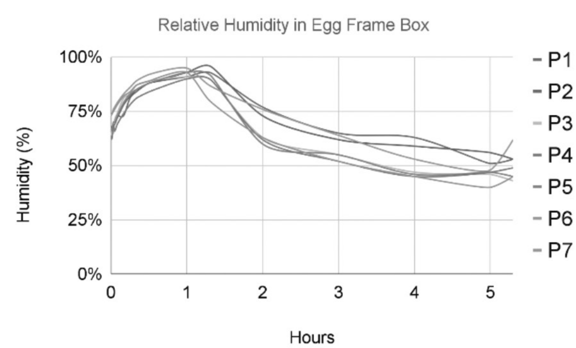


Figure 1 (left): Model of EFB and location of hygrometer probes placed in the EFB.
Figure 2 (right): Humidity recorded on probes placed in the EFB.

USING SKIN MUCUS FOR THE IDENTIFICATION OF ENDOCRINE BIOMARKERS IN NORTH AMERICAN ATLANTIC SALMON (*Salmo salar)*

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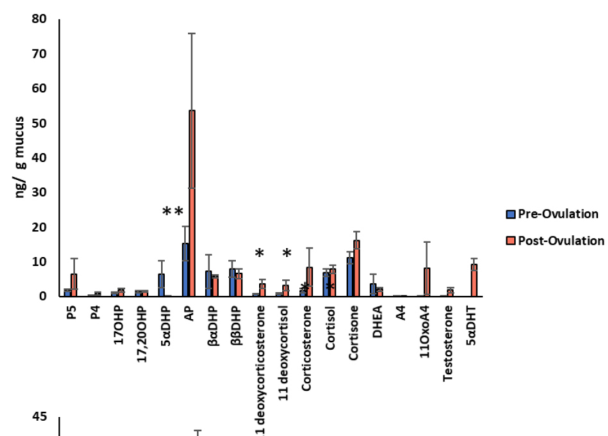
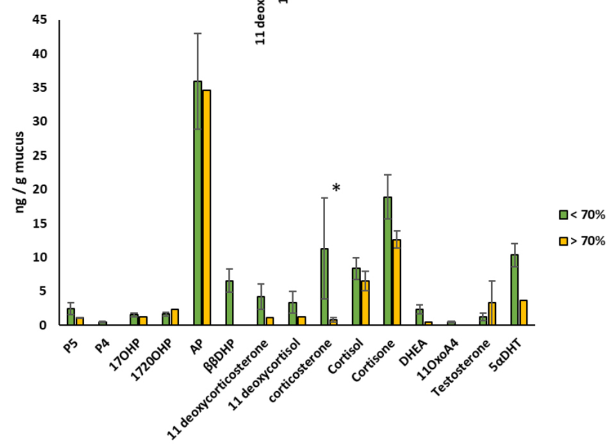
In Maine, North American (NA) Atlantic salmon production has experienced a 20% decrease in the eye-up rate of embryos and the maternal endocrine environment is suggested to be an important factor for fertilized eggs to eye-up. Historically, plasma sample collection and indirect hormonal measurement through immunoassays has made it difficult to complete endocrine pathways in NA Atlantic salmon. Combining new matrices (skin mucus) and advanced technologies (liquid chromatography tandem mass spectrometry (LC-MS/MS)) reduces these historic complications. Mucus was collected from sexually mature NA Atlantic salmon through absorption onto filter paper. Females were sampled pre ovulation (n= 9) and post ovulation (n= 20). Samples from post-ovulatory fish were subdivided into fish with > 70% eye-up rate, n=4 and fish with ≤ 70% eye up rate, (n=14) after analysis. All samples were subjected to a methanol protein precipitation and analyzed with an LC-MS/MS method. The objectives of this study were to 1) identify mucus as a viable matrix to measure steroid hormones, 2) quantify steroid hormone profiles at pre- and post-ovulation of North American Atlantic salmon and 3) compare hormone profiles of salmon with high and low eye-up rates.

Pre-ovulation there was an inverse correlation between plasma pregnenolone and mucosal cortisol ($r^2 = -0.747$, $p \leq 0.05$) and mucosal 17,20 OHP ($r^2 = -0.8314$, $p \leq 0.01$). Post-ovulation, there was a significant correlation between circulating plasma pregnenolone and mucosal cortisone ($r^2 = 0.6037$, $p \leq 0.01$), mucosal cortisol ($r^2 = 0.623$, $p \leq 0.01$) and mucosal 5αDHT ($r^2 = 0.8862$, $p \leq 0.01$). Mucosal allopregnanolone, 11-deoxycorticosterone and 11-deoxycortisol were significantly higher post-ovulation ($p \leq 0.05$). Fish with an eye-up rate ≤ 70% had elevated mucus concentrations of corticosterone ($p \leq 0.05$).



Mucosal concentrations of glucocorticoids significantly elevated post-ovulation combined with significant correlation with hydroxylated pregnanes suggests a synergistic mechanism between glucocorticoids and pregnanes. This combined with the higher corticosterone concentrations in fish with lower eye-up rates suggest a more important role for glucocorticoids then previously suggested.



WORKING ON THE VELELLA EPSILON PROJECT: A GRADUATE STUDENT EXPERIENCE

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As marine aquaculture continues to develop and expand in the United States, opportunities to work, conduct research, or provide outreach in aquaculture are also expanding. In support of sustainable marine aquaculture, environmental literacy, and workforce development, Florida Sea Grant (FSG) has created internships, fellowships, and other aquaculture-based opportunities for students and professionals. In 2020, FSG initiated the Helping Aquaculture Reap Value and Enhance Student Training (HARVEST) program in which students were given internships with aquaculture businesses, offering them the chance to expand their knowledge and professional network in aquaculture while also supporting aquaculture businesses on public outreach, productivity, sustainability, or efficiency. In 2022, FSG started the Aquaculture Outreach and Communications Graduate Fellowship program to provide an opportunity to early career aquaculture specialists to gain professional experience and training related to aquaculture. In this talk, I will describe my experiences as a 2021 recipient of the HARVEST internship for which I worked with Ocean Era to develop a Manual for Aquaculture Permitting Pathway (MAPP), detailing the company's experience obtaining federal permits for the Velella Epsilon demonstration project in the Gulf of Mexico. I will also discuss my role as the 2023 inaugural fellow for FSG's new Graduate Fellowship. For the fellowship, I will be conducting research on perceptions of aquaculture, particularly regarding Velella Epsilon in South Florida, and supporting FSG's aquaculture-based outreach efforts over the next year. These FSG programs provide a model for how cross-sectoral opportunities can offer both mentoring and training to students, and support to government and industry.

WASTE TO VALUE | ENHANCING THE ENVIRONMENTAL SUSTAINABILITY OF RECIRCULATING AQUACULTURE BY TRANSFORMING CULTURE SYSTEM WASTES INTO MARKETABLE COMPOST

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The land-based aquaculture industry has been enhanced by farming methods such as recirculating aquaculture systems (RAS), with its ability to produce more fish in limited conditions through intensive water recycling. However, intensive production also leads to a vast amount of concentrated waste. Typical waste streams include waste solids or sludge from fecal matter and uneaten feed, often treated by municipal wastewater treatment plants at a significant cost to farmers. These wastes, high in carbon, organic matter, and macronutrients nitrogen and phosphorus and low in chemical residues, are then devalued through mixing with lower quality industrial and domestic waste streams. As the RAS industry expands, establishing methods to support a circular economy by reducing or eliminating waste and creating valuable products will reduce environmental impact and increase sustainable global development.

Composting, generally defined as the intentional aerobic decomposition of organic matter, results in a highly stable humus product to be used as a nutrient-rich soil amendment or fertilizer. Finished material also enhances plant growth and development by improving soil porosity, increasing water-holding capacity, and diversifying the soil microbiome. The land-based aquaculture industry has a longstanding interest in composting aquacultural wastes but has not adopted composting practices for various reasons.

In-vessel composting offers an alternative to the traditionally utilized methods, using a tightly controlled and often automated system that is also commercially scalable. Waste materials are fed into a vessel, and operators balance the C/N ratio, moisture content, and aeration, creating favorable temperatures and conditions for optimal microbial decomposition. In-vessel systems are more biosecure and reduce heat loss while reducing odors, leachate, and energy usage. They also minimize the space needed to convert waste into a finished product.

An in-vessel lab-scale batch study at The Freshwater Institute evaluated bulking agent (i.e., carbon) particle size for composting fish waste solids produced from Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), which were dewatered on-site through the facility's settling cones. Additionally, the study provided insights into the volume of carbon needed to reduce moisture content while maintaining an appropriate C/N ratio. Source material was characterized, including a suite of macro- and micro-nutrients, and moisture content (%), temperature (°C), and electrical conductivity (EC) were collected in real-time. Finished product was evaluated using the standard testing assurance for certified compost. Results from this study will be used to develop compost recipes for a commercial-sized industrial drum composter. A description of the industrial composter and preliminary results will also be presented.

PROPAGATION FOR RECOVERY AND RESTORATION: A REVIEW OF TWENTY PRIORITY PROGRAMS

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As extinction risk of imperiled aquatic species continues to increase due to impacts of climate change, anthropogenic habitat degradation, and deleterious interactions with invasive species, the utility of restoration aquaculture will continue to expand. The propagation of aquatic species for the express purpose of recovering threatened or endangered species or restoring unlisted, imperiled populations are priority activities conducted by the United States Fish and Wildlife Service (Service). Developing ecosystem-level benchmarks of success for these programs and continually evaluating and adapting to their outcomes is critical to ensure sustained contributions to conservation.

Framework analysis-based reviews were conducted, for twenty priority aquatic species propagation programs operated and funded by the Service, to evaluate their contributions to recovery and restoration goals. For each program, the authors reviewed details associated with recovery or restoration planning; captive propagation; post-release monitoring and evaluation; and adaptive management. Secondarily, gaps in information required to evaluate each category or assess the overall conservation contribution of the program were highlighted.

Priority propagation profile reports were qualitatively evaluated, and a semi-quantitative factor analysis was performed to identify broad data deficiency categories and the common species attributes among them. Extensive diversities in life history traits, geographic distribution, jurisdiction, conservation status and culture techniques exist for the species evaluated, and trends in data deficiencies among the programs appear to be driven by many of these factors. For instance, propagation programs for recovery of ESA listed species tend to have robust data associated with recovery planning and post-release monitoring and evaluation, while many restoration programs lack the requisite authority structure to ensure consistent application of these activities. Additionally, geographic range is positively corelated with gaps in post-release and monitoring data, as the increasing number of conservation agencies involved leads to challenges in maintaining a consistent approach.

The trends identified in this synthesis can be used to guide the reform of existing conservation aquaculture programs operated by the Service and others to ensure alignment with species recovery and restoration goals. Also, as more species require captive propagation necessitated by increased extinction risk, deficiencies in data required to evaluate conservation success can be preempted by planning those activities with the consideration of these priority propagation framework analyses and this synthesis.

RAPID OUT-PLANTING OF SEAWEED FARMS WITH AUTOMATED LINE SEEDERS

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The seaweed aquacuture industry is rapidly expanding around the world. As coastal and offshore seaweed farms become larger and more complex, new methods of farming are needed to maintain economic viability. Here, we focus on methods and equipment in support of the out-planting process, or the seeding of growlines to support biomass develoment over the growing season. The cost of production needs to drop significantly if the seaweed aquacuture industry is to enter new markets, such as biofuel, bioplastics, and carbon credits. For seaweed farmers, one of the largest costs is labor. Current methods of transferring seed-line from the nursery to the seaweed farm have signifigant drawbacks. The out-planting process is labor intensive and expose juvenile seaweed, which is especially sensitive, to low temperatures, wind chill, and other undesireable environmental conditions. Consequently, there is a need for new out-planting methods that can attach juvinale seaweed to grow-line without requirements to unfasten the grow-line from the farm infrastructure or otherwise manipulate the farm structure.

Researchers at the Woods Hole Oceanographic Institution (WHOI) have developed line seeding equipment for rapidly seeding kelp farms with juvenile seaweed (fig. 1). The farming techniques enabled by the described equipment will allow farmers to out-plant juvenile seaweed to farm structures underwater, without the need to manipulate the farm structure or pull a work boat up and down the grow-lines to apply seed string. The associated reduction in labor is expected to result in significant time and costs saving across the seeding process. This method also reduces juvenile seaweed exposure to air, as the seeding process occurs under water, which is expected to improve successful seeding rates and increasing the weather windows for seeding operations. These improvements reduced stress on the juvenile seaweeds and allow farmers to access to optimal out-planning times which is expected to increase total biomass yield and harvest at the end of the growing-season.

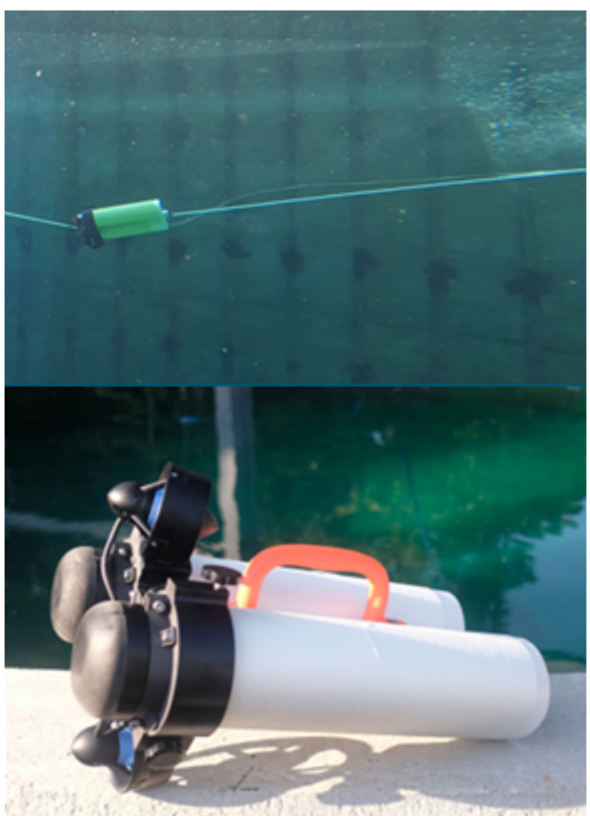

Figure 1. Automated line seeder. Seeding a grow-line (top) and loaded with seed string spools (bottom)

AN OPEN HARDWARE MOVEMENT TO SUPPORT AQUACULTURE INNOVATION

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Aquaculture intermingles multiple sectors that naturally foster development of interdisciplinary technologies, such as bringing together basic biological research and applied engineering. An open hardware movement is emerging as a robust strategy to support community-level scientific research and applications, and can become a catalyst for interdisciplinary innovations in aquaculture and aquatic sciences. Development of open scientific hardware enables formation of user, maker, and developer communities through increasingly assessable consumer-level design software, fabrication techniques (e.g., 3-D printing), electronics, free exchange of designs and modifications, and a shared sense of mission mediated through internet platforms (e.g., 3dprint.nih.gov). This strategy can be illustrated by examples at the Aquatic Germplasm and Genetic Resources Center (AGGRC, www.aggrc.com), where 24 categories of open hardware (Figure 1) have been developed to support development of germplasm repositories for aquatic species. These innovative solutions were created to tackle real community-level challenges encountered in germplasm repository development that otherwise would be difficult to solve by traditional biological research or proprietary commercial solutions. Because of the recognition of the power of the open-hardware strategy, in addition to prototyping laboratories, a 3-D Printing Farm was established at the AGGRC to batch fabricate (e.g., hundreds of duplicates) to facilitate engagement of user communities. With development of open hardware and interactions with user communities, several major applications of open hardware in aquaculture can emerge, including animal husbandry and handling, sample collection and treatment, laboratory tools, sensing and monitoring, quality management, and outreach interaction.

Figure 1. Examples of open hardware developed at the AGGRC for sample production and quality management to support development of aquatic germplasm repositories.

AN OPEN-HARDWARE MOVEMENT TO SUPPORT AQUACULTURE AND AQUATIC SCIENCES: WORKSHOP CONCLUSION AND QUIZ WITH AWARDS

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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 recognized 14 categories of open hardware for a cryopreservation production pathway, and 6 categories for a corresponding quality management pathway to address impediments to establishing repositories among resource centers and research communities. 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. To assist utilization of these new technologies to support aquaculture and aquatic sciences, the Aquatic Germplasm and Genetic Resources Center (AGGRC, www.aggrc.com) is hosting a '3-D Printing and Open Hardware Workshop' at the 2023 Aquaculture America Conference, in New Orleans, Louisiana. The workshop will include an introduction to 3-D printing applications in germplasm cryopreservation and repository development, and provide user interaction (Figure 1) with various cryopreservation devices, 3-D printing process, and outreach kits. Attendees will have the opportunity to receive 3-D printed souvenirs.

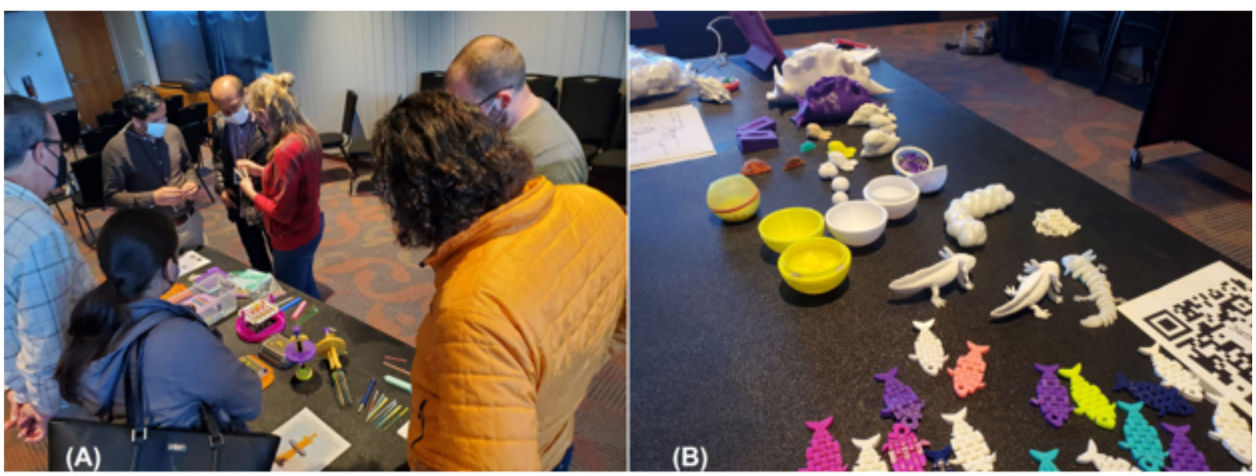

Figure 1. To facilitate community development for application of open hardware and 3-D printing in aquaculture and aquatic sciences, a workshop will be held at 2023 Aquaculture America to provide user interactions with research devices (A) and outreach kits (B).

RISKIER THAN YOU THINK? UNCERTAINTY IN SHRIMP AQUACULTURE

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Aquaculture has been the world's fastest-growing sector for food production and contributes significantly to rural development and food security in many countries. Despite its proliferation, the uncertainties in aquaculture have not been well addressed. In this paper, we developed a stochastic simulation model to explore the effects of risky economic and biological variables on the profitability of shrimp aquaculture. The profitability is measured with the probability distribution of net present value of the aquaculture production. In the base scenario, we examined the effects of risks in disease, random shocks, and biological growth on the profitability. In the second fold, we modelled the different levels of vertical integration by adding input and output price uncertainties to the simulations. In the last scenario, we were interested in how different social and institutional risks will impact the success of vertical integration. Specifically, we investigated how uncertainties in land rights and international trade interact with various vertical integration profiles. Our results contribute to further understanding of the risk profiles of shrimp aquaculture and how social and institutional issues constrain the industry and rural development.

EXTENSION AND OUTREACH ACTIVITIES FOR SOFT SHELL BLUE CRAB *Callinectes sapidus* AQUACULTURE

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Soft shell blue crab (*Callinectes sapidus*) aquaculture is one of the oldest domestic aquaculture industries along the East and Gulf Coasts of the United States. Historically, soft shell aquaculture in the United States produced over 1 million pounds valued at $5.5 million. However, the industry has been in decline over the last 30 years. Hurricanes, flooding events, and changes in salinity and water quality have attributed to the decline in the industry. In an effort to increase soft shell crab production, we have developed several extension tools (factsheets and videos) and conducted several workshops to increase participation in the industry, and make the industry more resilient to change. Topics covered include: components of recirculated systems, biological filtration basics in recirculating crab systems, crab mortality during summer months, system maintenance, water sources, start-up tips, seasonal costs for recirculated shedding systems, harvesting good peelers or busters, nitrifying bacterial products to acclimate systems, start up and seasonal costs, and virus transmission in shedding systems. Resources developed by our efforts can be found at https://www.laseagrant.org/outreach/projects/soft-shell-crab/.

LITTLE DAUPHIN BAY OYSTER RESTORATION: REEF ENHANCEMENT OF *Crassostrea virginica* THROUGH AQUACULTURE TECHNIQUES

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Oyster reef restoration projects often focus on planting cultch and occasionally seeding of hatchery-reared spat on shell requiring large-scale logistical efforts. Traditional methods frequently include seeding of fresh set spat to restoration locations. Consequently, high mortality has been observed due to predation and sedimentation. The Auburn University Shellfish Lab (AUSL) is conducting a three-year oyster reef restoration in Little Dauphin Bay (LDB), AL utilizing aquaculture techniques to improve survival and remove logistical concerns by using smaller setting material.

Six paired plots (750m^2) were cultched with oyster shell in LDB followed by seeding of spat clusters on one plot per pair (shell height=25mm). Spat clusters are produced by setting pediveliger oyster larvae on small shell at the AUSL hatchery and then moved to an AUSL oyster farm. At the farm, aquaculture gear is used to accelerate growth and protect spat from predation prior to planting seed on plots. Three sets of paired plots are trapped for oyster drills to analyze predator mitigation. Plots are assessed semi-annually for differences in oyster populations between seeded and non-seeded plots and predator mitigation effects.

At the project midpoint (October 2022), 897,600 oysters were planted with deployments continuing through Fall 2023. Sampling indicates a bi-modal distribution of oysters suggesting two recruitment classes, but no significant population difference is currently observed between seeded and non-seeded plots. Oyster drills appeared on reefs in March 2022 with trapping efforts showing little effect on oyster populations. Sampling of plots through Spring 2024 will determine overall interaction of seeding and trapping in enhancement of restored oyster populations.

FITNESS OF AN EMERGENT VIRUS IN RAINBOW TROUT AQUACULTURE

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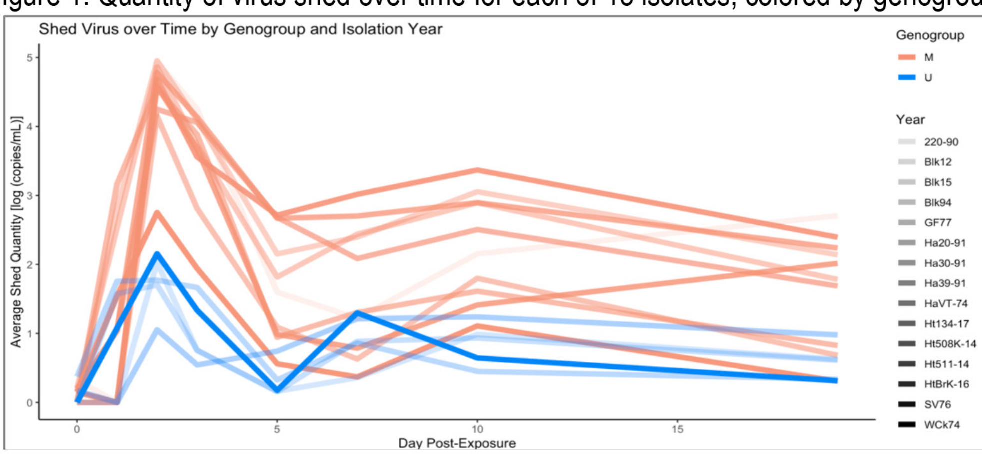
The emergent disease infectious hematopoietic necrosis virus (IHNV) is the most significant disease threat to rainbow trout (Oncorhynchus mykiss) aquaculture. A lethal disease endemic to the Pacific Northwest region of North America, IHNV has expanded its geographic range and genetic diversity alongside trout aquaculture. Aquaculture settings are theorized to constitute novel selective pressures and may drive viral diseases towards more virulent phenotypes. Past work in the system has elucidated IHNV phylogenetics, but less empirical data is available on the phenotypic differences between IHNV genotypes. Viral shedding is the only way to non-invasively measure viral replication. Shedding rate is also used as a proxy for transmission rate. Current research seeks to understand how viral traits of virulence, shedding, and transmission interact and shape viral evolution in aquaculture. It is widely accepted that a diversity of virulence phenotypes exist for IHNV, but it is unknown how viral shedding is linked with virulence. Quantifying post-exposure shedding through time is the best way to approximate viral fitness since shed virions indicate both viral replication as well as transmission potential. Quantifying shedding profiles of different viral genotypes and comparing them to virulence profiles will allow comparison of evolutionary trajectories between different lineages through time, and enable building epidemiological models to better manage fish health.

In this study, we quantified the shedding profiles of 15 IHNV genotypes in rainbow trout. The genotypes represent two major genogroups of virus, pre- and post-host jump to rainbow trout, as well as five decades of evolution. Groups of 15-20 fish were exposed to IHNV via bath immersion. Following exposure, fish were randomly distributed to individual tanks. Daily water samples taken during the first week to record peak shedding, then periodically for one month to measure shedding duration. Water samples were analyzed RT-qPCR for virus quantification. For all viruses, shed load peaked on day 2, but magnitude of M versus U genogroup varied (Figure 1). This is the first work examining the shedding kinetics of this breadth of viruses through time and across IHNV lineages. Results indicate viruses are host species specific and correlated with virulence, which suggests virulence and fitness are linked across host jumps.

Figure 1. Quantity of virus shed over time for each of 15 isolates, colored by genogroup.



SUB-CHILLING OF FARMED ATLANTIC HALIBUT (*Hippoglossus hippoglossus*) IN REFRIGERATED SALT WATER EXTENDS SHELF LIFE COMPARED TO TRADITIONAL ICED STORAGE

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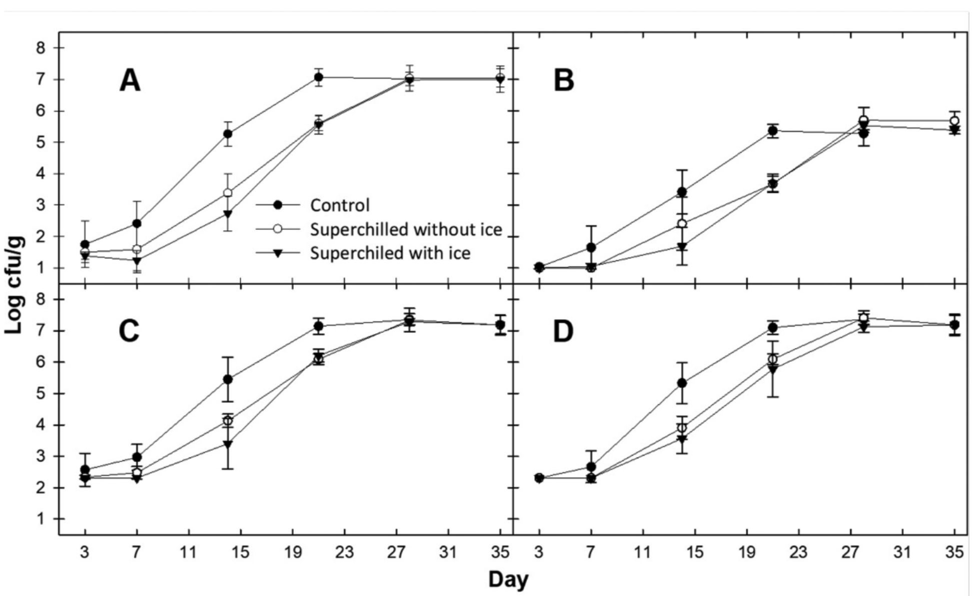
The aim of the study was to assess the shelf life of farmed Atlantic halibut (*Hippoglossus hippoglossus* L.) stored in sub-chilled refrigerated salt water (RSW) versus traditional storage on ice.

Twenty-one farmed halibut were obtained from a farming locality at the west coast of Norway. These were sub-chilled by placing them in a vessel with 3.5% brine and brine ice. The fish were chilled for 24 hours, until the core temperature reached -1.2 / -1.4 °C. The fish were then divided into two groups; the fish in one group (SI) were packaged in boxes with ice and the other group (S) was packaged in boxes without ice. The boxes with fish were placed in a cold room at 4 °C for 24 hours. Ten halibut from the same locality that were slaughtered at a commercial slaughterhouse on the same day, gutted and stored in boxes on ice, served as controls (C). All fish were then placed in a cold room at 0 °C for further storage. Five fish from each of the three groups were used for microbiological analyses including a microbiological shelf-life study according to Nordic Committee on Food Analysis (NCFA) protocol no. 180, and the remaining 5 fish from each group used for quality index method (QIM) analysis.

The microbiological shelf-life was extended by 5-6 days in groups SI and I compared to C. For the quality parameters, group C was the one with the highest values, and thus the lowest sensory quality. SI and I had the same score on most quality parameters, but SI had significantly better sensory score on gill smell and gill colour.

The results indicate that RSW-storage may minimize the need for ice and extend the shelf-life of Atlantic halibut. The novel storage technology offers significant savings in energy and water
compared to traditional storage on ice.

The growth of aerobe mesophile bacteria (A), H_2S-producing bacteria (B), and psychro-tolerant bacteria
at 15 °C (C) and 8 °C (D) during storage at 0 °C.



The growth of aerobe mesophile bacteria (A), H_2S-producing bacteria (B), and psychro-tolerant bacteria at 15 °C (C) and 8 °C (D) during storage at 0 °C.

AFFORDABILITY INFLUENCES NUTRITIONAL QUALITY OF SEAFOOD CONSUMPTION AMONG INCOME AND RACE/ETHNICITY GROUPS IN THE UNITED STATES

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The 2020 U.S. Dietary Guidelines for Americans recommend that the U.S. population consume more seafood. Most analyses of seafood consumption ignore heterogeneity in consumption patterns by species, nutritional content, production methods, and price, which have implications for applying recommendations. We assessed seafood intake among adults (>=20y) by socio-economic and demographic groups using NHANES 2011-2018 dietary data (n=17,559), as well as the cost of seafood at retail using Nielsen 2017-2019 retail sales data to identify affordable and nutritious options. Low-income groups consume slightly less seafood than high-income groups [low income: mean 120.2 (95% CI: 103.5, 137.2) g/wk; high income: 141.8 (119.1, 164.1) g/wk] but substantially less seafood that is high in long-chain n–3 (ω-3) PUFAs [lower income: 21.3 (17.3, 25.5) g/wk; higher income: 46.8 (35.4, 57.8) g/wk]. Intake rates, species, and production method choices varied by race/ethnicity groups and within race/ethnicity groups by income. Retail seafood as a whole costs more than other protein foods (e.g., meat, poultry, eggs, beans), and fresh seafood high in n–3 PUFAs costs more ($P < 0.002$) than fresh seafood low in n–3 PUFAs. Retail seafood is available in a wide range of price points and product forms, and some lower-cost fish and shellfish were high in n–3 PUFAs, calcium, iron, selenium, and vitamins B-12 and D.

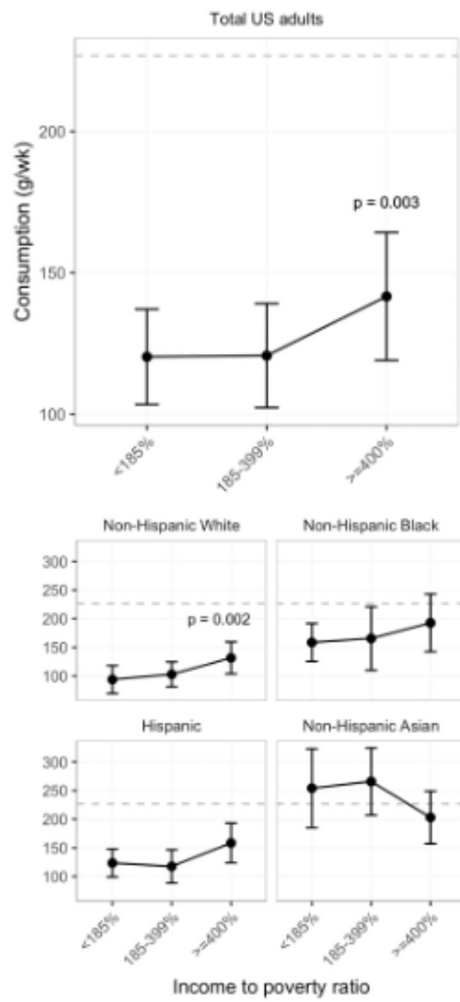

Figure 1. Per capita seafood consumption of United States race/ethnicity and income groups, 2011-2018 NHANES.

CHARACTERIZATION OF MATERNAL IMMUNITY FOLLOWING VACCINATION OF BROODSTOCK AGAINST *IHNV* OR *Flavobacterium psychrophilum* IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Infectious hematopoietic necrosis (IHN) is a significant viral disease affecting salmonids, whereas *Flavobacterium psychrophilum* (Fp), the causative agent of bacterial coldwater disease (BCWD), remains one of the most significant bacterial pathogens of salmonids. We explored maternal immunity in the context of IHN and BCWD management in rainbow trout aquaculture. Two experimental trials were conducted where different groups of female broodstock were immunized prior to spawning with an IHNV DNA vaccine or a live attenuated *F. psychrophilum* (Fp B.17-ILM) vaccine alone, or in combination. Progeny were challenged with either a low or high dose of IHNV at 13 days post hatch (dph) and 32 dph or challenged with *F. psychrophilum* at 13 dph. Mortality following a low-dose IHNV challenge at 13 dph was significantly lower in progeny from vaccinated broodstock vs. unvaccinated broodstock, but no significant differences were observed at 32 dph. Mortality due to BCWD was also significantly reduced in 13 dph fry that originated from broodstock immunized with the Fp B.17-ILM vaccine. After vaccination broodstock developed specific or neutralizing antibodies respectively to *F. psychrophilum* and IHNV; however, antibody titers in eggs and fry were undetectable. In the eggs and fry mRNA transcripts of the complement components C3 and C5 were detected at much higher levels in progeny from vaccinated broodstock and showed a significantly increased and rapid responses post-challenge compared with unvaccinated broodstock. After challenges pro-inflammatory cytokine expression was immediately and considerably elevated in the fry from vaccinated broodstock vs. unvaccinated broodstock, whereas adaptive immune genes were elevated to a lesser degree. Results suggest that maternal transfer of innate and adaptive factors at the transcript level occurred since development of lymphomyeloid organs is not complete in such young fry. In addition to documenting maternally derived immunity in teleosts, this study demonstrates that broodstock vaccination can confer some degree of protection to progeny against viral and bacterial pathogens.

EFFORTS TO IMPROVE SPAWNING CAPACITY AND JUVENILE QUALITY IN CALIFORNIA HALIBUT *Paralichthys californicus*

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Broodstock spawning capacity and juvenile fish quality are among the most critical aspects of any aquaculture operation, whether for stock enhancement or commercial production. California halibut (*Paralichthys californicus*), an emerging candidate species for both applications, is the subject of ongoing research at Hubbs-SeaWorld Research Institute. The spawning characteristics of a broodstock population held under an experimental thermal regime have been monitored since 2019. Volitional spawning occurs during seasons that last up to 10 months (more frequently and for longer than separate broodstock populations exposed to ambient water temperature conditions); this reliable source of high-quality eggs has facilitated sustained research on key areas of juvenile quality, specifically sex determination, pigmentation, and malformations.

Supported by evidence of temperature-dependent sex determination in related paralichthids, California halibut were reared at three distinct water temperatures (15, 19, and 23°C) through the presumed critical development window. Visual sex identification of fish >100 mm TL indicated that complete masculinization occurred at the highest temperature, with females apparent at the lower temperatures. Ongoing development of molecular biomarkers, to identify phenotypic sex as early as possible, may facilitate more complex (and shorter duration) experimental designs. Exceptionally high rates of pseudoalbinism (>95%) and ambicoloration (100%) have been observed in prior production cohorts of California halibut. A stepwise series of replicated rearing experiments have been undertaken involving manipulations of rotifer enrichment, dietary timing, and water temperature. These resulted in incremental reductions of malpigmentation (up to 46% normally-pigmented juveniles), with additional modifications of key nutritional and environmental parameters planned to reduce this further. Lastly, preliminary work has also begun to apply an external malformation surveillance protocol (originally developed for a white seabass stocking program) to California halibut. Cultured and wild California halibut have been used to refine existing malformation categories and establish a baseline reference for this species.

This research has been undertaken on intensively cultured California halibut primarily in the context of a pilot stock enhancement program. Thus, the focus has been on achieving morphologically, physiologically and behaviorally wild-like fish. As the program develops, we are continuing to explore the synergistic development of commercial production and the optimization of quality attributes more suited to that purpose (e.g., faster-growing all-female cohorts).

QUANTIFYING THE STRUCTURAL REQUIREMENTS OF AN OPEN OCEAN KELP CULTIVATION FARM FOR CARBON DIOXIDE REMOVAL

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The United Nations Intergovernmental Panel on Climate Change (IPCC) advises that by mid-century approximately 10 Gt CO_2eq must be removed annually to prevent global surface temperatures warming by 1.5°C by 2100. Among the suite of carbon removal strategies is the farming of macroalgae and sinking of the harvested crop in deep water sites.

A robust bio-techno-economic model (BTEM) developed by Coleman et al. (2022) finds that a 1,000-acre sugar kelp (*S. latissimi)* farm in the Gulf of Maine could achieve a levelized cost of carbon of \$1,257 CO_2eq^{-1} when fully optimized. This represents a 14-fold decrease when compared with baseline assumptions.

This BTEM includes a structural analysis of a 100-acre kelp farm. The farm is composed of flexible members – ropes, chain, and kelp – which renders static and quasi-static analysis methods unsuitable. Therefore, a time-domain Hydro-Structural Finite Element Analysis (HS-DFEA) of the farm was developed to compute minimum capacities of structural members. The HS-DFEA informed selection of farm components such as ropes and anchors, which were used to estimate farm costs and the emissions associated with manufacturing, installation, and maintenance.

TABLE 1: Farm cost per biomass yield for the three farm dimensions considered

Option	Aspect Ratio	Grow-line Length	\$/kg Biomass
A	1.6	10,740 m	\$1.95
B	2.5	14,742 m	\$1.69
C	10	35,914 m	\$1.31

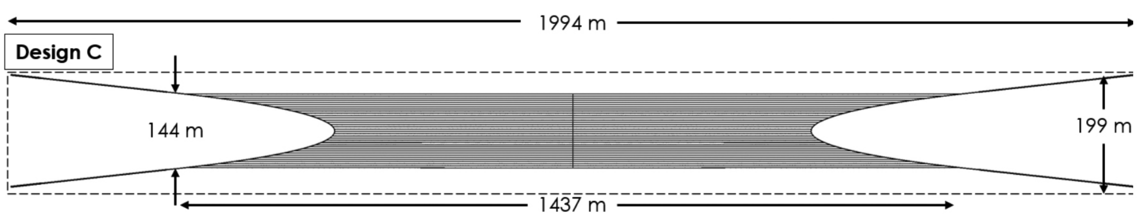

FIGURE 1: Top-down schematic and dimensions of Option C.

A NOVEL APPROACH FOR RECIRCULATING AQUACULTURE SYSTEM (RAS) BASED ON PHISICOCHEMICAL WATER TREATMENT PROCESS

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Aquaculture is the fastest-growing sector in animal protein production projected at 100 million tons by 2030. But with the limited availability of land and water, the only viable solution is intensification – to produce more fish per unit of area and water. This shifted the focus towards longevity and sustainability of aquaculture, now driven by innovative, highly sustainable and cost-effective solutions. One such solution, Recirculating Aquaculture Systems (RAS), are based on the treatment and reuse of water via the application of mechanical filtration, followed by biofiltration, disinfection, and oxygenation. Available RAS technologies suffer from several limiting factors restricting their wide application: (1) Difficulty in meeting desired environmental standards, namely related to inefficient removal of nitrogen and phosphorus compounds; (2) bio-filter limitations, such as long start-up time, temperature dependency, possible amplification of pathogens within the biofilter (3) generation of fish off flavor agents such as MIB & Geosmin generating a muddy taste of the fish causing tremendous lost for the farmer. These factors result in increased production costs due to environmental-related expenses, fish health issues affecting both growth performance and survival rates, and high capital costs particularly apparent in RAS focusing on cold-water fish, which require large bio-filter surface areas.

A new operational approach for RAS, is based on physicochemical water treatment techniques. Within this concept the fish are grown at high TAN concentration and around neutral pH that is calculated to maintain the toxic NH_3 concentration lower than a predetermined threshold. The inherently high Cl^- concentration in seawater enables efficient electro-generation of $Cl_{2(aq)}$ which consequently oxidizes ammonia directly into innocuous $N_{2(g)}$. The system's water passes in a semi-batch mode through the water treatment unit and then returns to the fish tanks, supplying disinfected water with zero TAN and off-flavor agents, and most of the acidity required for maintaining the alkalinity mass balance in the RAS. A powered controller with a highly-intuitive graphic UI monitors and controls the water's pH, temperature, O_2, ORP, Cl_2 and NH_4^+ levels for maintaining optimal water quality for the fish. The BioFishency ELX enables real-time data collection and management via an intuitive dashboard through a cloud-based solution. Remote monitoring and operation are facilitated by an easy-to-use mobile app, accessible from any location, at any time, via any mobile device or tablet. Intelligent process adaptation using Machine Learning technologies, are planned for future versions.

The presentation will include a technical description of the Biofishency ELX technology and describe it value proposition to both investors and fish farmers in three major market segments: Off-flavor purging under regular feeding regime, Full grow-out period in RAS, and Zero discharge transportations of live fish in welboats.

FIND MY OYSTER – A SOFTWARE TOOL FOR IMPROVED BOTTOM CULTURE SHELLFISH FARMING

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The success of a bottom culture shellfish farm depends on many factors, including water quality, temperature, and food supply. But monitoring the health of shellfish and predicting yield can be challenging due to the lack of data. In many cases, farmers rely on manual record-keeping and subjective estimations. Moreover, unexpected events, such as sudden die-off, can severely impact yields and make planning difficult. Here we present FindMyOyster, a software tool to help manage and monitor a bottom culture shellfish farm. The tool is composed of a web based dashboard and a mobile app for collecting data in the field. This allows farmers to easily track their operations and metrics directly from their mobile devices. The mobile app includes features for mapping and navigation, making it easy for farmers to locate specific areas on the farm and record data accurately. The collected data is automatically synced with the dashboard, giving farmers access to real-time information to make informed decisions.

The tool is based on FarmOS, an open-source application for farm management, planning, and record keeping. FarmOS provides many capabilities that are generally useful for any type of farm operation. We leverage many of those features and extend them to help specifically with bottom culture shellfish farming. We extend the functionality of FarmOS with a custom module to introduce concepts needed for shellfish farming, such as *water lease* and *oyster species*. With the mobile app we add a custom workflow that would not work with the web application alone. The app makes it easy to capture precise location data e.g., mark observations with a GPS coordinate or record the path of the boat when seeding and harvesting. The app can help navigate the boat within a farm plot to cover the bottom evenly on repeat visits.

FindMyOyster aims to help shellfish farmers by giving them additional data and insights about their farm, with minimal disruption to the workflow they are already used to. Our hope is that the tool can help farmers make informed decisions and that over time it can be used to make accurate predictions.

AQUACULTURE AND FISHERIES R & D: ROLE OF BFRI IN TECHNOLOGY INNOVATION

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Aquaculture and fisheries have been recognized for their remarkable contribution in nutritional food security, livelihood development, export earnings and economic development of the country. In 2020-21 FY, the contribution of the fisheries sector was 3.57% to the total GDP of the country and approximately 26.50% to agricultural GDP. The Bangladesh Fisheries Research Institute (BFRI) since its inception has been conducting research reflecting the national policy and needs of the sector. Keeping this in view, BFRI has so far developed 75 improved aquaculture and management technologies many of which have been disseminated in the field. As a result, fish production has increased from 2.70 million MT to 4.621 million MT during last 12 years. In global context, Bangladesh ranked 3rd place in inland fish production and 5th in aquaculture production (FAO 2020-21).

Freshwater fishes of Bangladesh comprise 260 species of which 64 were endangered due to habitat degradation and over exploitation. The institute has been conducting comprehensive research to conserve these Small Indigenous Species (SIS) and already developed the artificial breeding and culture technologies of the 37 endangered fish species. As a result, the production and the availability of these fish species have significantly been increased. The production Hilsa has been increased by 85% in the last 12 years and the availability of large size Hilsa (800-1000 gm) has increased by 25%. The Maximum Sustainable Yield (MSY) of Hilsa fish has been uplifted to 7.02 lakh MT in 2020-21 FY and ranked 1st in the global production.

The institute is intensively working to conserve the genetic resources and non-conventional (Crabs, Shellfishes, Seaweeds, Oysters, Snails etc) fishery items through the implementation of the compatible research projects. Bangladesh fisheries Research Institute (BFRI) has made commendable initiatives in fisheries R&D activities on the development of the marine fisheries resources which have great potential to enhance Blue Economy. In the meantime, the institute has successfully produced a number of seaweed- based food products and has given special emphasis to explore this thrust research area for the development of the existing resources.

THE COMPLETE GENOME SEQUENCE OF *Penaeus vannamei* SINGLY ENVELOPED NUCLEAR POLYHEDROSIS VIUS (PvSNPV) REVEALS MORE SIMILARITY TO NUDIVIRUSES THAN BACULOVIRUSES

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Penaeus vannamei singly enveloped nuclear polyhedrosis virus (PvSNPV) (also known as Baculovirus Penaei (BP)) is the first viral pathogen of penaeid shrimp described in 1974. Although PvSNPV was discovered more than 45 years ago, the complete genome of BP is elucidated in this study for the first time. Until few years ago, BP was listed in the World Organization for Animal Health (Paris, France) list of crustaceans diseases. Development of management strategies enabled to reduce the negative impact of the virus in recent years, but it still remains prevalent in the hatchery

We detected the virus in a quarantine stock of *P. vannamei* by light microscopy of fecal samples and by PCR screening of broodstock. Subsequently, next generation sequencing (NGS) was deployed to determine the complete genome sequence of PvSNPV. The PvSNPV genome is a circular, double-stranded DNA molecule of 119,883 bp in length encoding 100 open reading frames (ORFs). The deduced amino acid sequences from 25 ORFs were homologous to 28 core proteins from all identified nudiviruses. Phylogenetic analyses based on deduced amino acid sequences of the core genes and orthologous genes revealed that PvSNPV clusters with *Penaeus monodon* nudivirus (PmNV). Therefore, we propose to rename BP/ PvSNPV as *Penaeus vannamei* nudivirus (PvNV) and re-assign the virus to the family *Nudiviridae* instead of *Baculoviridae*.

Availability of the BP genome sequence will be useful in studying host-virus interactions at a molecular level and developing strategies to mitigate the negative impact in the captive breeding program to develop Specific Pathogen Free (SPF) lines of shrimp.

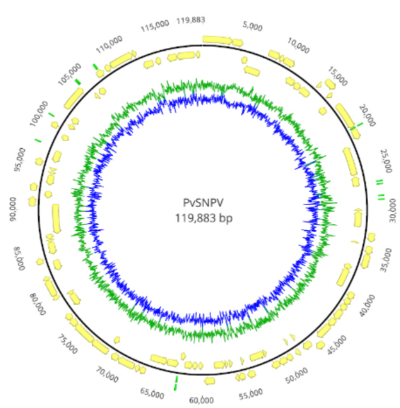

Figure 1. Genome organization of Baculovirus Penaei (BP), also called *Penaeus vannamei* singly enveloped nuclear polyhedrosis virus (PvSNPV).

MINIMIZING SALTWATER DISCHARGES FROM MARINE RAS SUPERNATANTS FROM BIOCLARIFIER BACKWASH WATERS AND SLUDGE MINERALIZATION

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The discharge of saltwater complicates placement and increases the operational costs of both coastal and inland marine RAS. In salt tolerant coastal areas, operations are increasingly challenged with nutrient discharge limitations, a logical consequence to highly visible coastal eutrophication issues. Inland areas, particularly in the western US, are sensitive to both salt and nitrate because of their adverse impact on heavily taxed groundwaters.

The quantity of water physically lost is ultimately limited by the sludge that must be removed from the system when the solids capture device is backwashed. The development of pneumatically washed bead filters that inherently recycle backwash waters allows the sludge discharges to be reduced, so water (salt) reuse can approach several months. Coupling a pneumatically washed floating bead filter with a localized sludge digestor can facilitate nitrate control and further reduce sludge mass by about 50 percent. Secondary consolidation coupled with recycling of supernatants off the surface of the mineralization basin can result in another twofold reduction in sludge discharge volumes potentially extending water reuse beyond a year.

Mineralization of sludge is a straight-forward operation as the degradation process is spontaneous. If the only objective is reduction of sludge discharge volumes, then a continually mixed, aerated basin is the most efficient. AST uses a sizing criteria of 83 liters/kg fed-day (10 gals/lb fed daily) @20^{o}C to reduce the sludge mass by about 50 percent. Discharge concentrations of 4-6% solids are readily obtained with good reactor designs. Nitrate removal requires anaerobic digestion. With some intermittent mixing, a sludge sizing of 170-250 liters/kg fed-day (20-30 gals/lb fed daily) can approach a mass reduction of about 40% while substantially reducing nitrate accumulations. Carbon addition can be used to enhance the denitrification that benefits from high levels of readily digestible organic carbon.

With cumulative feed burdens (CFBs) more than 100,000 mg/L, closed RAS for some marine species may warrant further treatment or supplements to adjust for mineral drifts or dissolved organic accumulations. An extended water (salt) reuse strategy provides an avenue for RAS expansion without the environmental conflict that is plaguing more open approaches.

CHARACTERIZATION OF LEPTIN RECEPTOR DEFICIENT RAINBOW TROUT *Oncorhynchus mykiss*

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Leptin is known for regulating appetite and metabolism and it has been implicated in many other facets of vertebrate physiology. Teleost leptins often exert anorexigenic effects although responses can vary between species. 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, plasma leptin, and leptin transcripts (*lepa1*) were elevated in LepR mutant fish. 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*. All detectable fatty acids (FA) were higher in muscle of fed fish mutant fish compared to WT, albeit not significant. However, fasted mutants exhibited significantly lower muscle FA for virtually all FAs, suggesting an increase in FA mobilization during fasting in LepR mutants. These data demonstrate a key role for leptin signaling in lipid and energy mobilization in a teleost fish. Leptin clearly plays a key role in food intake in rainbow trout, however, additional studies are needed on adipose- and glucoregulatory pathways to help better understand the phenotype of the LepR deficiency.

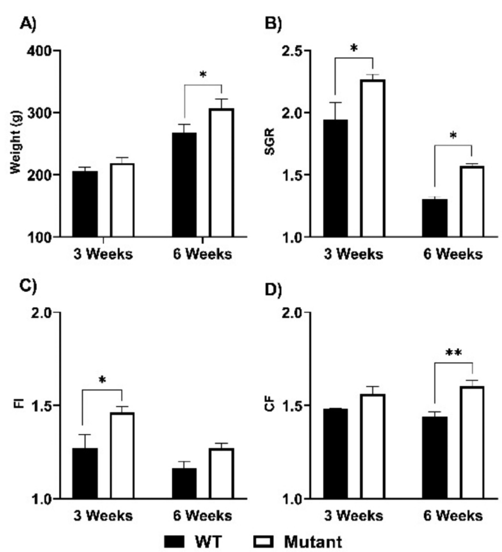

Figure 1. LepR CRISPR/Cas9 edited rainbow trout fed to satiation. WT and LepR mutant fish were sampled at 3 and 6 weeks for: **A**) body weight (g), **B**) specific growth rate (SGR), **C**) feed intake (FI), and **D**) condition factor (CF).

EFFECTS OF ENVIRONMENTAL MANIPULATIONS ON SURVIVAL, GROWTH, AND FEEDING INCIDENCE OF LARVAL *Dascyllus auripinnis*

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The golden domino damselfish (*Dascyllus auripinnis*) is a popular fish in the aquarium trade and is sought after by ornamental collectors. The commercialization of other damselfish species and the demand from the public is driving the development of production techniques for this species. Significant mortality from starvation is observed during the “first feeding” period, where larvae transition from endogenous to exogenous feeding. Environmental conditions can also influence the identification and capture of prey items, which can contribute to increased mortality in the initial stages of development. Ideal conditions for growth are species-specific and improvements in culture protocols would help to facilitate the commercialization of this species.

To assess the effects of various rearing conditions on the culture of damselfish, manipulations of algae density, copepod species, copepod density, prey type, and photoperiod were evaluated at 0 – 5 days post hatch (DPH), with the duration of the experiment dependent on the parameter. Results from the algal density experiment indicated the greatest survival in the microalgal density 150,000 cells mL^{-1} of *Tisochrysis lutea* and in the control, with no significant differences in feeding incidences and growth among treatments. Feeding larvae *Parvocalanus crassirostris* resulted in significantly higher feeding incidence over *Oithona calcarva* but had no effect on larval survival of the larvae. Using these results, *P. crassirostris* was fed at densities ranging from 2.5 - 10.0 nauplii/mL for the copepod density experiment. Feeding incidence and larval growth were not affected by copepod density, but survival was significantly greater in the 5 copepods mL^{-1} and 10 copepods mL^{-1} treatments (Figure 1). The prey type experiment tested the inclusion of rotifers at first feeding and resulted in a significantly higher survival in larvae that were fed a 50/50 mix of rotifers and copepods, as well as an equally high survival in the diet of exclusively copepods. The inclusion of rotifers had no effect on initial feeding incidence and growth. A photoperiod of 24-hour light had a significantly higher larval survival and initial feeding incidence. These results contribute to the development of commercial production techniques for *D. auripinnis* and can provide important insights into larval feeding behavior.

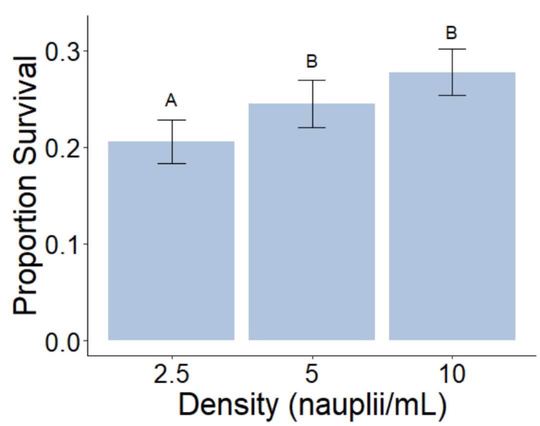

Figure 1. Mean survival (±SE, n = 6) of *D. auripinnis* larvae at 5 DPH in response to densities of *P. crassirostris* nauplii. Density is represented by number of prey items mL^{-1} per day. Letters above denote significance between treatments ($P \leq 0.05$).

MANIPULATING ENVIRONMENTAL PARAMETERS TO IMPROVE SURVIVAL, GROWTH, AND FEEDING INCIDENCE OF LARVAL *Neocirrhitus armatus*

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The flame hawkfish (*Neocirrhitus armatus*) is a marine ornamental fish endemic to the Indo-Pacific and is popular in the aquarium trade due to its bright coloration, small size, and charisma. Due to their popularity, captive rearing of hawkfish is of great interest, but is limited by difficulties in broodstock spawning and a lack of larval rearing data. Natural spawning of *N. armatus* began in June 2022 and several attempts have been made to rear the larvae to the juvenile stage. The larvae take approximately 24 hours to hatch at 27°C and are 2 mm in length (TL). At hatching, larvae lack functional eyes, mouth, and digestive tract, thus are dependent on endogenous yolk reserves. The stage of "first-feeding", where endogenous reserves are exhausted and the larvae must switch to exogenous feeding, is critical for development and survival. Mass larval mortality is common during this period from lack of appropriate nutrition. *N. armatus* larvae reach this critical period at 3 days post hatch (DPH) at a length of approximately 3 mm (TL). Attempts to rear these larvae have been successful until 12 DPH, with significant mortality at 10 DPH. Mortality at this developmental stage could be due to other developmental bottlenecks, such as swim bladder inflation, which occurs at approximately 7 DPH, or the beginning of notochord flexion, which occurs at approximately 9 DPH.

There is no current information on the larval rearing of *N. armatus* and initial experiments will test the effects of environmental conditions on survival, growth, and feeding incidence from 0 – 5 DPH. Experiments evaluating the effects of algal density are currently underway using densities of 0 – 600,000 cells $mL^{-1}$of *Tisochrysis lutea*. Other environmental conditions of interest include algae species, copepod species, copepod density, and prey type. These experiments will all focus on improving survival at the first feeding bottleneck, and later experiments may focus on bottlenecks in production at 10 DPH. The development of early larviculture protocols for *N. armatus* will provide critical information necessary for commercialization of this species.

COLORIMETRIC BIOSENSOR BASED ON THE CATALYTIC ACTIVITY OF SPIONS FOR BACTERIA DETECTION IN BIVALVES – PRELIMINARY RESULTS

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Contamination of food by bacteria is very common and seafood, particularly bivalves, may be affected by several infectious diseases having a direct impact in human health. The transmission of pathogens among bivalves is direct and the areas of high production are often affected by faecal contamination. Thus, the accumulation of pathogenic bacteria in these filtering organisms may favor the increase of the bacterial contamination, bringing serious issues for consumer's health. Colorimetric biosensing, based on color conversion for the detection of target analytes, emerged as a very promising possibility in biosensing development. In this field, the enzyme-like activity of some NPs has gained significant interest since the ability of these NPs to catalyze the oxidation of certain substrates, generating a color change, is a very promising route for colorimetric biosensors development.

In this work, a colorimetric nanosensor of silica-based coated superparamagnetic iron oxide nanoparticles (SPIONs) was developed.

SPIONs were synthesized through a co-precipitation method run in air at room temperature. The amino-silica coating was achieved following the Stöber protocol. Several types of nanostructures were developed, and the catalytic activity of the synthesized nanostructures was evaluated. Also, their performance as biosensors for bacteria detection was analyzed by testing different parameters through colorimetric *in vitro* assays.

The obtained preliminary results are promising. The catalytic activity of the NPs was verified by an increase on the color intensity observed at naked eye and confirmed through the measured absorbance values. When in presence of bacteria, a decrease on the color intensity also corroborated with the absorbance values, was observed. Thus, these preliminary results allow us to conclude that this colorimetric nano biosensor can be a valuable tool for a low-cost, easy and rapid monitoring of the presence of bacteria in bivalves.

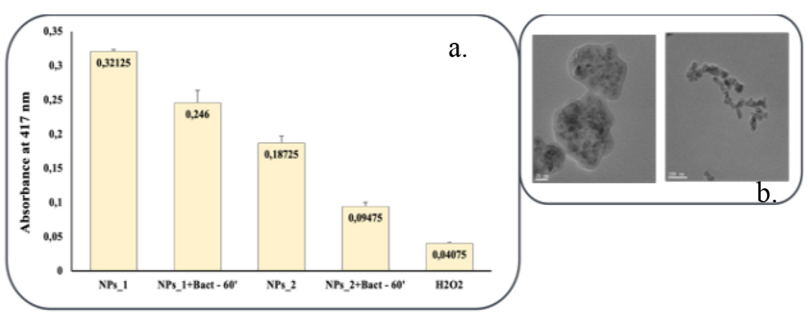

Figure 1: Colorimetric biosensor for bacteria detection: a. colorimetric assays – absorbance values; b. TEM images of the developed NPs.

HYPOXIA EXPOSURE OF PACIFIC OYSTER SEED INCREASES SURVIVAL TO SUBSEQUENT ANOXIA STRESS

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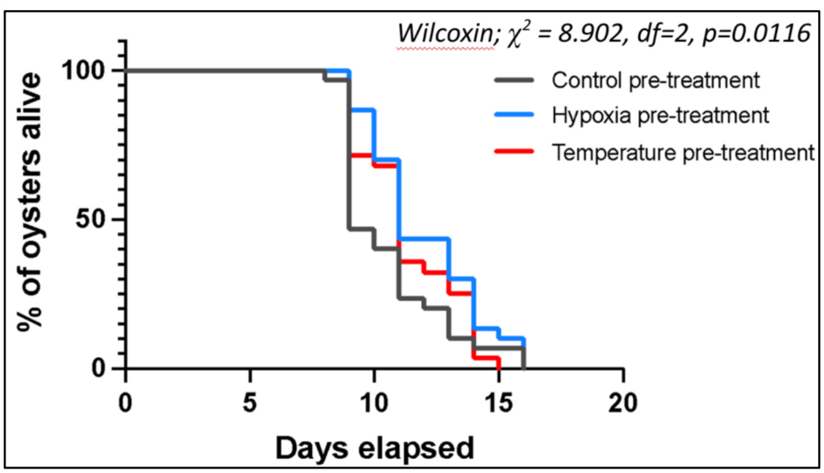
Oyster aquaculture operations are intermittently plagued by mass summer mortality of both juvenile and adult Pacific oysters (*Crassostrea gigas*). These catastrophic mortality events can be marked by the loss of >50% of oysters, with some of the highest losses in juvenile oysters grown in 'on-bottom' cultures. Mass mortality events have become so common that they are now known collectively as Pacific Oyster Mortality Syndrome (POMS) events. POMS have been variably associated with elevated temperatures, eutrophication, low dissolved oxygen, and pathogen loads. This project explored the potential benefits of 'priming' oyster seed to acute, sublethal environmental stress – specifically, hypoxia and temperature – with the hope that this conditioning will enhance oyster survival during the subsequent commercial grow-out phase. Following conditioning oyster seed was allowed to recover for 2 months before repeat exposure to anoxic conditions. Our physiological results provide evidence that prior exposure to hypoxic conditions significantly increased survival of oyster seed to subsequent anoxia exposure. In addition, we have maintained individuals from these treatments on a commercial oyster farm in Morro Bay, California where we have monitored the long-term impact of these exposures on growth and survival across a summer growing season. Together our results may provide a new opportunity to condition Pacific oyster seed prior to out planting that may increase survival during hypoxic conditions.



MONO-SPECIFIC ALGAL DIETS SHAPE MICROBIAL NETWORKING IN THE GUT OF THE SEA URCHIN *Tripneustes gratilla elatensis*

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Algivorous sea urchins can obtain energy from a diet of a single algal species, which may result in consequent changes in their gut microbe assemblies and association networks. To ascertain whether such changes are led by specific microbes or limited to a specific region in the gut, we compared the microbial assembly in the three major gut regions of the sea urchin *Tripneustes gratilla elatensis* when fed a mono-specific algal diet of either *Ulva fasciata* or *Gracilaria conferta*, or an algal-free diet. DNA extracts from 5 to 7 individuals from each diet treatment were used for Illumina MiSeq-based 16S rRNA gene sequencing (V3–V4 region). The niche breadth of each microbe in the assembly was calculated to identify core, generalist, specialist, or unique microbes. Network analyzers were used to measure the connectivity of the entire assembly and of each of the microbes within it and whether it altered with a given diet or gut region. Lastly, the predicted metabolic functions of key microbes in the gut were analyzed to evaluate their potential contribution to the decomposition of dietary algal polysaccharides. Sea urchins fed with *U. fasciata* grew faster and their gut microbiome network was rich in bacterial associations (edges) and networking clusters. *Bacteroidetes* was the keystone microbe phylum in the gut, with core, generalist, and specialist representatives. A few microbes of this phylum were central hub nodes that maintained community connectivity, while others were driver microbes that led the rewiring of the assembly network based on diet type through changes in their associations and centrality. Niche breadth agreed with microbes' richness in genes for carbohydrate-active enzymes and correlated *Bacteroidetes* specialists to the decomposition of specific polysaccharides in the algal diets. The dense and well-connected microbial network in the gut of Ulva-fed sea urchins, together with the animal's rapid growth, may suggest that this alga was the most nutritious among the experimental diets. Our findings expand the knowledge on the gut microbial assembly in *T. gratilla elatensis* and strengthen the correlation between microbes' generalism or specialism in terms of occurrence in different niches and their metabolic arsenal which may aid host nutrition.

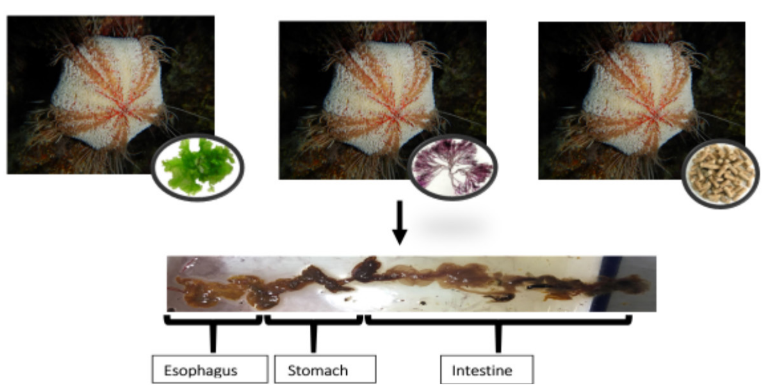

Figure 1. The dissection process of sea urchin *T. gratilla elatensis* for sampling of the three major gut parts.

PRODUCTION OF FISH PROTEIN FROM CONTINUOUSA RECYCLING OF NUTRIENT-RICH WATER: AQUAPONICS

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For many different reasons, some parts of the world are experiencing a decline in resources, one being food. While increasing human population is still an issue, the resources from which we get our food are being depleted. Agriculture, alone, cannot meet the demands that our current population needs. Both plant and animal agriculture require copious amounts of land for the yield to be worthwhile to the farmer and to the food sector. As the human population continues to increase the amount of arable land for farming decreases, the amount of space needed to raise animals also decreases. While many forms of aquaculture also require a large amount of space to produce a high yield of product, there is one variety that may provide a solution to Earth's space dilemma- Aquaponics. Aquaponics combines hydroponic and aquaculture practices so plant and animal products can be produced at the same time and within the same space. When you have a farm that grows both animals and plants, they each need their own space, this is not the case with aquaponics. If properly maintained, aquaponics systems use less water than other agricultural methods and exposes the environment to fewer pollutants. The objective of this experiment is to determine how much product a laboratory scale aquaponics system can produce. This experiment will gather weight and length data of Nile tilapia and the yield of various herbs from various projects that have utilized the aquaponic system at Purdue University Fort Wayne.

FUNCTIONAL FEED FORMULATION: COMPARATIVE GROWTH, INNATE IMMUNITY, DIGESTIVE ENZYME INDICES AND BODY COMPOSITION IN NILE TILAPIA (*Oreochromis niloticus)* UNDER 75% FISH MEAL REPLACEMENT WITH SOYBEAN MEAL

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In aquaculture, functional feed formulation is an optimal solution of reducing high feed cost in an environmental friendly way; by replacing animal origin ingredients with plant based sources and functional additives. In the present study, effects of functional feed having 75% fish meal replacement with fermented or non-fermented soybean meal (supplemented with lysine & methionine) with two probiotic levels (2% & 4%) were investigated with reference to growth response, innate immunity, digestive enzyme activity and body composition of Nile tilapia for 16 weeks in laboratory conditions. Nile tilapia received 35% CP feed as T_0 (containing 100% fishmeal), T_1 (75% fermented soybean meal (FSBM) replacement and 2 % probiotics), T_2 (75% non-fermented soybean meal (SBM) replacement and 2% probiotics), T_3 (75% FSBM replacement and 4 % probiotics), T_4 (75% SBM replacement and 4 % probiotics). The observed weight gain was 14.1 g in T_0, 8.7g in T_1, 8.4 g in T_2, 9.38 g in T_3 and 9.96 g in T_4. The better value of FCR (1.88) was observed in T_0 group and the maximum value of SGR (1.9) was also observed in T_0. The WBCs count and IGM were highly reduced in non-fermented soybean groups. However, FSBM showed significant increase in WBCs count and IgM. Highest level of WBCs counts and IGM was observed in FSBM+ 4% probiotics. IgM level of *O. niloticus* was 10.13 mg/ml in T_0, 9.76 mg/ml for T_1, 9.30 mg/ml in T_2, 11.36 mg/ml in T_3 and 10.43 mg/ml in T_4. The results of the digestive enzyme activity with non-fermented SBM had negative effect on digestive enzyme activity. Fermented soybean groups showed an improvement in digestive enzyme activity in comparison to non-fermented groups but not comparable to control group. Lipase activity of *O. niloticus* was 196.6 U/mg for T_0, 190.30 U/mg for T_1, 186.30 U/mg for T_2, 190.21 U/mg for T_3 and 186.2 U/mg for T_4. Amylase activity was 4.46 U/mg for T_0, 4.40 U/mg for T_1, 4.56 U/mg for T_2, 4.26 U/mg for T_3 and 4.50 U/mg for T_4. Protease activity was 71.04 U/mg for T_0, 68.51 U/mg for T_1, 64.42 U/mg for T_2, 68.54 U/mg for T_3 and 64.54 U/mg for T_4. The results of present study indicated that FSBM had no significant effect on the proximate body composition with respect to moisture content, protein, fat and ash content, however crude protein and fat level were significantly decreased in non-fermented SBM groups. The levels of crude protein of *O. niloticus* were 16.05% for T_0, 16.05% for T_1, 13.54% for T_2, 16.02% for T_3 and 13.616% for T_4 and total fats were 3.66% for T_0, 3.65% for T_1, 2.24% for T_2, 3.64% for T_3 and 2.25% for T_4. The moisture contents were 78.26% for T_0, 78.16%, for T_1, 80.20% for T_2, 78.16% for T_3 and 80.55% for T_4 and total ash contents were 3.19% for T_0, 3.21%, for T_1, 4.57% for T_2, 3.21% for T_3 and 4.48% for T_4. From the results of present study it is evident that high level fish meal replacement with soybean meal did not provide better growth, innate immunity, digestive enzyme activity or proximate body composition in Nile tilapia, however if we pretreat the soybean meal through fermentation then a comparable performance may be achieved.

Suaeda linearis AN EMERGING SUPERFOOD SEA VEGETABLE

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FAU Harbor Branch Oceanographic Institute (HBOI) has successfully cultured sea vegetables for human consumption in a variety of studies since 2014. The plants utilize dissolved nutrients from the production of fish and shrimp in the HBOI Integrated Multi-Trophic Aquaculture (IMTA) system. Currently HBOI cultivates four species that are native to Florida: *Batis maritima* (saltwort*) Sesuvium portulacastrum* (sea purslane), *Salicornia bigelovii* (sea asparagus) and most recently, *Suaeda linea*ris (sea blite). *Suaeda linearis* is an emerging superfood sea vegetable with a fast growth rate, robust mineral content, and high physiological plasticity that allows it to thrive in harsh coastal environments. The nutritive content and biomass production of the genera *Suaeda* has been documented in several studies. However, the commercial production of this genera is still in its infancy, and it remains unclear how variation in harvesting methods may affect nutritive content and harvestable product. This study investigated nutrition, phenology, growth rate, and total harvestable biomass using four different harvesting treatments during a 10-week study period. Under the first treatment (T1) plants received no intermittent harvests, the second (T2) received harvests every five weeks, the third (T3) received harvests every 3.3 weeks, and the fourth (T4) received harvests every 2.5 weeks. The goal of this study was to elucidate the ideal *Suaeda* harvesting method that maximizes total nutritional and harvestable yield across a growing period.

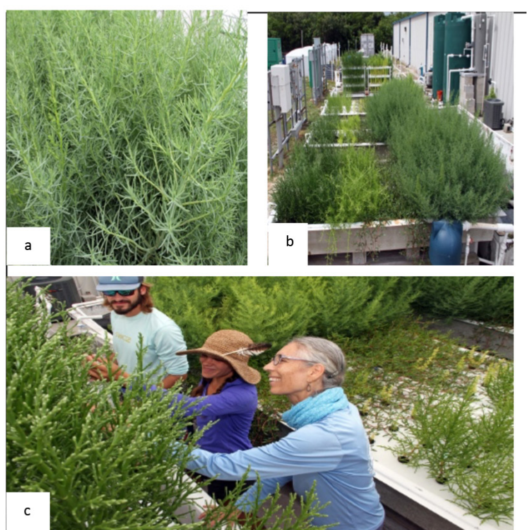

Figure 1. Sea vegetables growing in FAU Integrated Multi-Trophic Aquaculture system: a. *Suaeda linearis,* b. overview of system, c. harvesting sea vegetables

CONSUMER ACCEPTANCE AND SENSORY PROPERTIES OF BURBOT, *Lota lota*, COMPARED TO OTHER AQUACULTURE PRODUCTS

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Burbot are the only cod-like freshwater species with a great potential for diversification of freshwater aquaculture. They have low-temperature requirements, high fecundity, and offer high market value as a food fish, and their liver, and skin byproducts have high value. However, regardless of the numerous advantages, this fish species has limited availability in the US market. For this reason, two studies were conducted to evaluate the market potential and sensory characteristics of burbot fillet.

A preliminary trial (survey) was carried out at Snake River Grill restaurant in Hagerman, Idaho from March through July 2017 for initial evaluation of consumer acceptance and market potential of burbot *Lota lota*. Burbot grown to approximately 1lb in size at one of the commercial trout facilities in Southern Idaho were filleted and prepared with different flavors each week, and customers who ordered burbot were asked a series of questions. Of over 150 customers who participated in the survey, 96% liked the texture of burbot, 92% liked the flavor of burbot, and 96% would try burbot again. Survey results showed that burbot are one of the most promising candidates for commercialization as a foodfish.

Based on these results, a follow-up study (sensory analysis) was conducted in April 2019 to investigate consumer preferences and sensory properties (firmness, smell, flavor, and overall preference) of burbot *Lota lota* fed a trout commercial diet over a 42-d period. After the feeding period, a sensory taste panel was carried out during which burbot fillets were compared to two other prominent aquaculture species (trout and tilapia). Panelists were presented with pairs of fish samples (burbot (Control) and trout, burbot (Control) and tilapia) (Figure 1) in separate flights, and in a random order. Out of a group of 84 untrained panelists, 87% and 81% preferred burbot (Control) to tilapia and trout, respectively (Table 1). The directional Paired Comparison Test showed that burbot significantly had more aroma, fishier flavor, and oilier flavor as compared to trout or tilapia. However, for firmness, consumers found tilapia and trout to be significantly firmer as compared to burbot (Table 1). Overall, the findings for this study demonstrate that burbot would be a highly marketable species and would add value to U.S. domestic market by offering a white fillet option to salmonids and diversifying production for producers entering the market.

Table 1. Consumer scores indicating which sample had more aroma, was more fishy, oilier, firmer and which fish sample was preferred (n=84).

Attributes	Percent composition (%) for sensory attributes			
	Trout	Burbot	Tilapia	Burbot
Aroma Intensity	12	88*	19	81*
Fishy flavor	30	70*	23	73*
Oily flavor	20	80*	13	87*
Firmness	79*	21	71*	29
Overall preference	19	81*	13	87*

Figure 1: Fish samples before being served to consumers.

DEVELOPING THE MAINE AQUACULTURSIT – AN ONLINE KNOWLEDGE PORTAL

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In October 2020, The Gulf of Maine Research Institute (GMRI), in partnership with Maine Aquaculture Association (MAA), and sponsored by FocusMaine launched The Maine Aquaculturist (www.themaineaquaculturist.org) a business knowledge portal. Our aim was to help Maine aquaculture businesses fully utilize the incredible suite of business-relevant support resources provided by various organizations. The site was created with one customer in mind –Maine's aquaculture industry. Our process was driven by extensive user testing with a variety of aquaculture business including new and veteran growers. While we had our own ideas going into the development process, the team checked their assumptions at the door and listened; and learned a lot along the way. The resulting architecture, design, and features of The Maine Aquaculturist were created based on direct industry feedback during the user testing process.

After launch, anecdotal industry feedback was gathered, the site's usage was tracked using Google Analytics and additional user testing sessions were conducted. As a result, it was decided to refine the first version of the site and The Maine Aquaculturist 2.0 was launched in September 2021. These updates included the addition of new pages, minor changes to the visual design, and the optimization of some advanced features. The portal currently includes a Getting Started Guide which walks you through starting your own aquaculture business, a Resource Database that houses over 250 resources, a Job Board, a Regulation/ Legislation Updates page, and an Industry Perspectives page that contains insight for industry veterans. GMRI had continued to update content and gather feedback from the industry which has been positive and constructive.

ESTABLISHING A COMPREHENSIVE WORKFORCE TRAINING SYSTEM FOR MAINE'S AQUACULTURE SECTOR

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Workforce shortage in Maine's aquaculture industry has been an obstacle to growth for many businesses and could stunt the industry's tremendous growth potential. To address this, the Gulf of Maine Research Institute (GMRI), The Maine Aquaculture Association (MAA), and Educate Maine partnered on The Maine Aquaculture Workforce Development Strategy- a forward thinking strategic roadmap for Maine to achieve a cohesive and comprehensive workforce training pipeline that meets the needs of today's industry and anticipates future workforce needs as the industry evolves. To ensure objectivity and learn from countries with developed aquaculture industries that have already gone through 'aquaculture workforce growing pains,' we hired a team of Scottish consultants who brought global aquaculture expertise in business development, workforce development analytics, and workforce training at both higher education and vocational levels. Direct input from Maine's commercial aquaculture businesses – including established and prospective land-based operations, marine producers, service providers, and supply chain companies - formed the base of the analysis. Over the course of the work, we met with 15 businesses and 62 businesses were formally interviewed or surveyed. In addition, we met with representatives of 17 of Maine's education or training institutions and surveyed another 33. GMRI convened a steering committee of 30 representatives from various Maine education and training institutions who met four times to provide feedback throughout the project.

Considerable progress has been made towards implementing the Strategy since it was published in 2020. The first-generation Maine Aquaculture Occupational Standards, which specify the current workforce skills and training needs of Maine's aquaculture sector, were developed collaboratively by MAA, GMRI, and Educate Maine; and were published October 2021. Four standards have been released, focusing on occupations related to (1) Marine Shellfish and Sea Vegetables; (2) Marine Finfish; (3) Land-Based RAS; and (4) Land-Based Shellfish Hatcheries. A supplementary document, the Maine Shellfish Aquaculture Career Pathways Map was also designed to provide an overview of the most common job types, entry points, and career pathway opportunities in Maine's shellfish aquaculture sector today. Currently GMRI, MAA, Educate Maine, and Southern Maine Community College are working together to develop and pilot a Maine Department of Labor Aquaculture Apprenticeship Program where participants will gain valuable experience, receive mentoring, and get trained and tested on defined occupational competencies. We continue to coordinate program development and delivery between the Community College system, Career Technical Education schools, Department of Labor, and University of Maine to establish matriculation pathways and dual-credit programs that enable fast-tracked degree completion.

THE GULF OF MAINE RESEARCH INSTITUTE'S WORK TO ADVANCE THE SUSTAINABLE GROWTH OF AQUACULTURE

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In October 2020, GMRI and MAA launched a free-to use aquaculture business knowledge portal, called The Maine Aquaculturist. The site was created with one customer in mind –Maine's aquaculture industry. Our aim was to help Maine aquaculture businesses fully utilize the incredible suite of business-relevant support resources provided by various organizations. The creation process was driven by extensive user testing with a variety of aquaculture businesses therefore, the resulting architecture, design, and features of The Maine Aquaculturist were created based on direct industry feedback. After launch, anecdotal industry feedback was gathered, the site's usage was tracked using Google Analytics and additional user testing sessions were conducted. As a result, it was decided to refine the first version of the site and The Maine Aquaculturist 2.0 was launched in September 2021. These updates included the addition of new pages, minor changes to the visual design, and the optimization of some advanced features. The portal currently includes a Getting Started Guide, a Resource Database that houses over 250 resources, a Job Board, a Regulation/Legislation Updates page, and an Industry Perspectives page. GMRI had continued to update content and gather feedback from the industry which has been positive and constructive.

BALANCING NATURAL AND ARTIFICIAL SELECTION IN CAPTIVE REARING PROGRAMS

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Traits adapting animals to their natural local environments need to be considered during the development of multi-generational captive rearing programs. In artificial rearing environments unintentional selection may shift naturally balanced polymorphisms in directions that lower fitness in nature. Reproductive traits of concern include age of maturation, return time, spawn time, nest site location, substrate preference, nest construction, nest defense, egg size and fecundity. Salmonid culture tools with the potential to counter these reproductive concerns include spawning channels and sourcing each generation as eyed eggs, parr, or smolts. Survival traits of concern include emergence timing, habitat selection, habitat use, freshwater habitat migration, anadromous migration, foraging behavior, digestive physiology and physiological tolerances (temperature, salinity, dissolved oxygen, etc.). Culture technology using locally adapted temperature profiles, seawater rearing during ocean life history phases, and timing life stage events to match natural profiles may help maintain the natural balance of alternative traits in the cultured population. It is anticipated the use of artificial culture for developing self-sustaining natural populations will be directly tied to its ability to maintain the natural balance of polymorphic traits that adapt the released population to their local environment.

EVALUATION OF GROWTH RATE EFFECTS IN NILE TILAPIA *Oreochromis niloticus* AND BUTTER LETTUCE *Lactuca sativa l* FROM MEALWORM SUBSTITUTION IN FEED IN RAS

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Extensive negative impacts to the seafood industry and marine food webs have occurred due to increased forage fish catch. Forage fish are smaller species of fish (e.g., anchovies and sardines) collected and fed to domesticated fish and shrimp. Forage fish is the main ingredient in fishmeal (FM) and fish oil (FO), and the single highest cost in farming aquatic animals, for fish feed used in the aquaculture industry. Overfishing FM and FO has decreased their availability, inflating costs of fishmeal for commercial fish feeds worldwide. Many seafood farmers in developing countries have also realized that, in the long run, they will be unable to afford FM as a significant protein source in aquafeed (Abdel-Fattah et al., 2020). Incorporating insects as a protein source alongside, or in place of FM or FO, has the potential to increase food security in developing countries and for the aquaculture industry in the future.

A 12-week (84 days) greenhouse feeding trial was conducted with 6 separate recirculating aquaponics systems (RAS) with Nile tilapia (*Oreochromis niloticus*) and butter lettuce (*Lactuca sativa l*). Each system contained 25 fish in 1 m^3 fiberglass tanks, a 200 L biofilter, and an 800 L trough with 35 butter lettuce each, supported in floating rafts. Water flowed from the tanks to biofilters, into the floating raft grow beds, and was pumped back to the tanks from the grow beds. Temperature, pH, dissolved oxygen, and conductivity were determined daily for fish tanks. Ammonia, nitrite, and nitrate levels were taken every other day for tanks and biofilters. The objective of the study was to determine the impact of insect meal substitution in fish feed on growth rates of butter lettuce and tilapia. Formulation of the feeds with a 15% and 25% insect meal (IM) substitution included commercial feed, mealworm meal, vegetable oil, and gelatin.

To conclude, plant growth increased with a modified diet of 15% IM treatment and the results were statistically significant (Table 1). The 15% IM treatment also had a higher fish biomass average increase (Table 1).

Table 1: Sample Statistics, ANOVA table, and Tukey's Post hoc test for Shoot Biomass and Fish Biomass Gain

	ANOVA: Shoot Biomass				
	DF	SS	MS	F-statistic	P-value
Treatment	2	47053	23526.5	3.7105	0.03399
Residuals	37	234599	6340.5		

	Tukey's Test: Shoot Biomass			
	Difference	Lower CI	Upper CI	P-value
Treatment_15-Control	40.28462	-28.97602	109.545253	0.341182
Treatment_25-Control	-52.61538	-143.75569	38.524923	0.346501
Treatment_25-Treatment_15	-92.9	-178.27566	-7.524344	0.030405

	Shoot Biomass		Fish Biomass Gain	
	Mean	SD	Mean	SD
Control	278.6153846	72.02492446	2833.5	386.7874093
Treatment_15	318.9	91.27511911	3261.5	973.6860377
Treatment_25	226	48.40110192	1885	789.1311678

GENETIC PARAMETERS FOR HEAT AND SALINITY STRESS TOLERANCE IN NORTHERN GULF OF MEXICO EASTERN OYSTER, *Crassostrea virginica*

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Eastern oyster production in the Gulf of Mexico is increasingly relying on aquaculture. However, Gulf farms have experienced losses in recent years during summer mortality events. The causes of summer mortalities are not fully understood yet but are hypothesized to be related to excessively high temperature during the late summer months, and also to low salinity events affecting oyster production sites during heavy rain events. In this study, the feasibility of improving tolerance to high temperature and low salinity stresses by selection was evaluated using challenge experiments applied to a mixture of 160 families (10 non-overlapping 4 males x 4 females mini factorial crosses) raised in a common garden. Thermal tolerance was evaluated at the seed (2 months post set) and adult stages (14 months post set) to determine if breeding values for tolerance to high temperature stress in market-size oysters can be predicted during the first year of life. Challenges simulated a heat wave where temperature was maintained between 38-39 °C until all oysters expired. Similarly, tolerance to an acute low salinity stress was tested in a challenge where salinity was reduced from 14.5 psu to 2.5 psu over a period of 4 days. This challenge was applied to juveniles (9 months) and adults (15 months). Survival duration during both challenges was recorded for all individual oysters and tissue samples were taken for genotyping and parentage assignment. The condition index was also recorded for juvenile oysters to characterize the level of stress under challenge.

Oyster seed mortalities peaked after 11 days at 38 °C and complete mortality was recorded after 22 days. Adult oyster mortalities under the same challenge conditions peaked after 5 days at 38 °C and all tested oysters expired after 7 days of challenge. Juvenile oysters tested in the low salinity challenge experienced minimal mortality (2.3% over a period of 40 days). Their level of tolerance will be characterized based on the condition index. In contrast, a peak in mortalities was observed after 14 days at low salinity in the adult challenge followed by complete mortality at 25 days. During both challenges conducted on adults, oysters spawned during the acclimation period, suggesting that their reduced ability to deal with the stressors was related in part to spawning activity.

Challenged oysters will be assigned to parents using a 192 SNP array. Heritability of tolerance to the two challenge stressors and genetic correlations between the two traits will be estimated using general linear model and proportional hazards models.

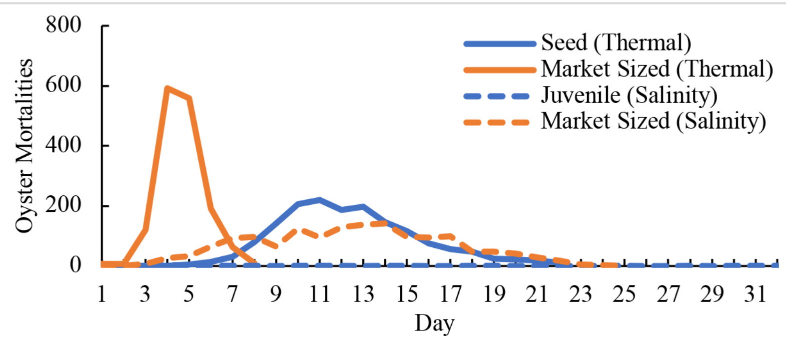


Figure 1. Daily mortality observed during thermal and salinity stress challenges.

BIOSOLIDS WASTE MANAGEMENT THROUGH INNOVATIVE ROTARY PRESS; LESSONS LEARNED AND PERSPECTIVE FOR THE FUTURE

Scott McKay, M.Sc. SMK Consulting

Jean-Félix Trudel, CPI, MBA. Fournier Industries Inc.

Closed-loop RAS allows for efficient solid waste capture and collection. However, the intensive nature of the process also results in the production of a concentrated waste stream. With the increasing use of large-scale RAS, more waste is being produced in land-based systems and agricultural use of liquid waste becomes non-viable in many locations. Waste treatment and disposal leads to additional costs for farmers. A biosolids waste management strategy is required to ensure a sustainable expansion of the industry. The aim of this paper is to offer a concrete contribution to the development of an economically and ecologically sustainable biosolids waste strategy for the RAS industry.

This involves the use of solids/liquid separation technologies that are efficient in human resource (operations and maintenance) and consumables (water, energy, conditioning). An innovative technology has been developed by Fournier Industries to achieve such a goal. The Fournier Rotary Press™ has been recently introduced in the RAS industry both in North America and in Norway. We use empirical data drawn from Fournier's experience in North America and in Scandinavian countries to outline lessons learned. Process data (dryness, capture rates, water, energy and chemical conditioning consumption) collected from Rotary Press operations are presented.

We also examine the contribution of this technological innovation to other land farming waste management operations, such as dairy and swine manure used in renewable natural gas (RNG) generation, to open perspectives for the future of RAS.

From this operational experience, we draw conclusions on technical and commercial strategies that will be key for the successful transitioning of the RAS community to a more circular and low-carbon economy.

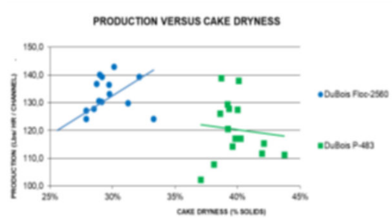
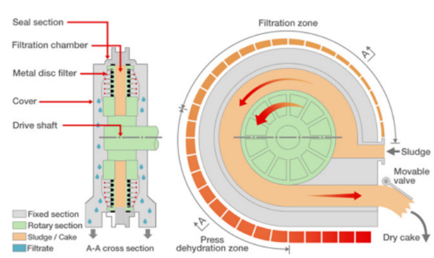
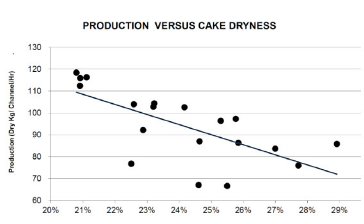


Figure 1





Graphs

PRELIMINARY OBSERVATIONS ON GROWTH OF MALE AND FEMALE LARGEMOUTH BASS IN PONDS AND FLOATING RACEWAYS

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Largemouth bass (*Micropterus nigricans*) were stocked into two floating raceways (41 m^3) and three ponds (0.04 ha) for a second season of growth. Fish were split into ponds and raceways from a common source. Mean weight of pond fish at stocking was 81 g and 111 grams in the raceways, 10 days apart. A Passive Integrated Transponder (PIT) tag was inserted into the abdominal cavity of 50 fish in each pond and 100 fish in each raceway at stocking. Fish in the ponds and the raceways were fed slow sink pellets (45% protein 20% fat) daily to apparent satiation. Water quality was monitored to maintain conditions for growth throughout the experiment with temperature and dissolved oxygen measured daily and total ammonia nitrogen, nitrite, pH, and alkalinity measured every other week. At the end of the growing season, fish were harvested to determine average weight, length, condition factor, survival, and yield. For each tagged fish recovered, weight, length, and sex was determined following euthanization and examination of the gonads. Samples from each sex and each production system were taken for proximate analysis.

Ponds were harvested after 161 days and raceways were harvested after 158 days. At harvest, mean weight for all fish in ponds was 356 g and 287g for all fish in raceways. Mean recovery of PIT tagged fish was 84.7% for ponds and 85% for raceways. Average weight gain among tagged fish in ponds was 270 g for males and 242 g for females. Average weight gain among tagged fish in raceways was 167 for males and 162 g for females. Preliminary analysis by sex reveals significantly greater weight gain in male fish grown in the ponds, and no difference in weight gain by sex for fish grown in the raceways. Condition factor for tagged pond fish at harvest was significantly greater than tagged raceway fish. No difference in condition factor was observed between tagged male and female fish in either system. Proximate body composition results will be reported in the presentation.

FIGHTING THE HEADWINDS: CHANGING THE NARRATIVE ON AQUACULTURE IN THE PACIFIC NORTHWEST

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American industrialist and inventor Henry Ford once wrote, “When everything seems to be going against you, remember that the airplane takes off against the wind, not with it.”

In November 2022, Washington state Commissioner of Public Lands, Hilary Franz, stunned the aquaculture industry by issuing an Executive Order banning commercial net pen fish farming in state-owned aquatic lands.

Several days later, Commissioner Franz held a press conference where, set against the backdrop of net pens in Washington’s Rich Passage, she proceeded to make unsubstantiated claims about the “evils” of commercial net pen fish farming.

As could be expected, the Seattle and state media continued to carry the Commissioner’s message blaming fish farming for the demise of the region’s iconic “wild” salmon and Orcas, despite 40+ years of science to the contrary. The piling-on, however, continued, with stories from the Times being picked up not just around the state but around the country.

As an advocacy organization, the Northwest Aquaculture Alliance (NWAA) knew we had to take swift action to push back against this seemingly overwhelming anti-aquaculture sentiment by changing the narrative and making our collective voices heard—despite the strong headwinds.

In our presentation in the AQUACULTURE COMMUNICATIONS session, we will outline not just our overall strategy in dealing with this Franz-caused crisis, but also the actions and tactics that we used to get our message out and reframe the story. We will talk about building a coalition of scientists, veterinarians, and aquaculture trade associations and using this coalition to issue a demand to the Commissioner to produce the science upon which she issued her Order.

We will also talk about how we used traditional and social media, helped prepare spokespeople, and how we worked collaboratively with other communications professionals from member companies to expand and amplify our message along with influencers from other trade groups.

It is important to state here that the leadership of NWAA was 100 percent behind our advocacy plan.

EVALUATION OF KRILL MEAL AS A FEED PALATANT FOR ATLANTIC SALMON *Salmo salar* L. 1758 FED FISHMEAL AND FISH OIL-FREE DIETS

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Feed is the most important variable cost for Atlantic salmon culture, representing around 47% in major salmon-producing countries. Traditionally, salmon feed has relied on fishmeal (FM) and fish oil (FO) as major ingredients. However, there is pressure to replace these increasingly expensive and finite resources with cheaper alternatives. One problem encountered when switching from FM/FO-based feeds, however, has been reductions in feed intake and subsequent growth penalties. Accordingly, the impact of replacing FM and FO from salmon feed, using blends of animal (A; poultry meal, blood meal) and plant (V; soy and corn concentrate) proteins, combined with FO replacement using canola and algal oil, was evaluated over a 63-day timeframe. The influence of krill meal (+; 2.5%) as an alleged palatant was also investigated with diets being compared against a FM/FO-based control feed. Diets were randomly assigned to one of 20 flow-through tanks (n = 4 tanks per diet) initially stocked with 60 fish (148.4±12.9 g; 23.3±0.8 cm; K = 1.16±0.08), reduced to 45 at day 42.

At trial termination differences in group weights ($P < 0.05$) were observed between salmon fed the control feed and those fed diets without krill meal (Table 1). Fish fed feeds containing krill had similar weights to those fish fed the control feed. Length gain was greatest ($P < 0.05$) in fish fed the control feed with all other groups exhibiting identical length growth. This resulted in measurable differences ($P < 0.05$) in final condition factor K (Table 1). Proximate analyses of whole animals at study end revealed no differences between treatments ($P > 0.05$).

Table 1. Comparisons of morphological characteristics between control and experimental groups of post-smolt salmon fed at trial start and 9 weeks later at trial termination. N = 180/data point. Different superscripts in a row indicate significant differences at the P < 0.05 level.

	C	A-	A+	V-	V+
Initial weight (g)	149.0±12.5	147.6±13.1	148.6±13.2	148.7±13.0	148.7±12.7
Final weight	542.1±88.1^{a}	520.5±82.9^{b}	553.2±87.2^{a}	496.8±70.9^{b}	527.7±81.5^{a}
Initial length (cm)	23.32±0.79	23.28±0.78	23.37±0.78	23.40±0.80	23.43±0.87
Final length	34.38±1.75^{a}	33.63±1.76^{b}	33.78±1.72^{b}	33.31±1.46^{b}	33.54±1.69^{b}
Initial *K*	1.18±0.08	1.17±0.08	1.16±0.07	1.16±0.07	1.16±0.08
Final *K*	1.33±0.10^{a}	1.36±0.08$^{b, c}$	1.37±0.10$^{b, d}$	1.34±0.09$^{a, c}$	1.39±0.09^{d}

LOUISIANA'S ALTERNATIVE OYSTER CULTURE GRANTS PROGRAM: DEVELOPMENT, IMPLEMENTATION, AND PRESENT STATUS

Earl Melancon*, Wood Ogelsby, Anne Dugas, Leslie Davis, Albert (Rusty) Gaudet, Brian Callam, James Wilkins, Julie Lively, Evelyn Watts, Thomas Hymel.

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The Louisiana Department of Wildlife and Fisheries (LDWF) developed the *Louisiana Oyster Management and Rehabilitation Strategic Plan* at the request of the state legislature and the Governor's Office of Coastal Protection and Restoration (CPRA). The fourth initiative (of 12) within the plan is *Expansion of Alternative Oyster Aquaculture (AOC)*. In Louisiana with its historical and highly productive traditional wild-harvest oyster fishery, hatchery-spawned cage-cultured oyster farming is known as AOC. AOC is not a replacement for the traditional fishery, but a supplement for those fishers who have an interest in developing this "new" state fishery.

CPRA and LDWF awarded Louisiana Sea Grant (LSG) a $3M, three-year grant, to help enhance existing AOC farmers within the state and to expand the fishery. Specifically, LSG addressed the program's implementation through $1.8M of competitive grant awards to Louisiana fishers and companies to enhance and develop: (1) aquaculture parks with multiple farms located within each ($100K each), (2) seed nursery farms ($15K each), (3) grow-out farms for market oysters ($45K each), and (4) private in-state hatcheries ($225K each); see Figure 1. To facilitate this effort, LSG has developed workshops, instructional materials, and other forms of outreach to support the grant awardees and other individuals interested in AOC.

We are at the end of our second (2nd) year of the three-year program. We discuss how grant awardees were selected through an competitive process that combines environmental metric scoring with interviews; discuss how the AOC grant program was accepted by the traditional Louisiana oyster industry; discuss state-focused AOC legal and regulatory hurdles; discuss the challenges to lessen bureaucratic grant awards implementation by contracting with the Iberia Development Foundation (IDF); and, the challenges to adequately educate new farmers about best management practices, preparing for hurricanes, and challenges associated with the ever-present need for an adequate and timely supply of seed oysters. There is the possibility of additional funding beyond the three years, and we also discuss options for the next phase moving forward if those funds do become available.

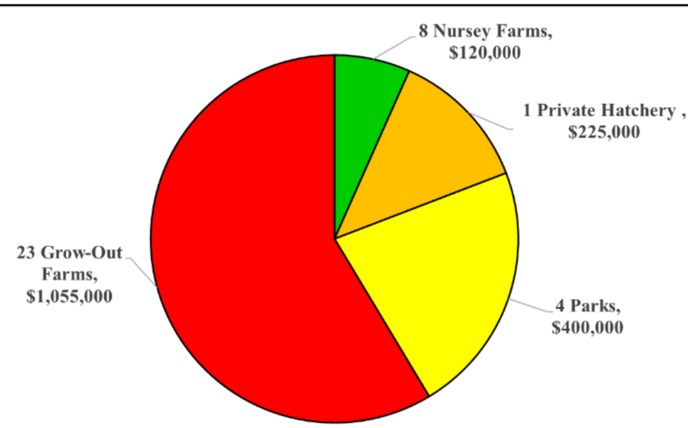

Figure 1. Allocated Funds = $1.8M

PRE-SMOLT ATLANTIC SALMON MATURITY IN FRESH WATER: HOW TO TACKLE THE ISSUE

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Introduction:

It's known that there are environmental factors as photoperiod and temperature that influence the onset of puberty in critical seasonal periods, however at commercial scale it is not so clear how this factors works to explain the high rates of early maturity observed. According to gonadal ultrasound monitoring performed frequently in Chilean salmon production, it has been identified a series of hatcheries with a high prevalence of minijacks before the seawater transfer. In the present study, we examine if manipulation of light intensity and temperature variations can trigger sexual maturation in male Atlantic salmon during freshwater phase and the specific weight of each of those factors in the stimulation of sexual maturity in pre-smolt Atlantic salmon

Materials and methods:

The study was carried out in flow trough commercial facility in La Araucanía region, Chile. 2 experimental groups were formed, each group in duplicate, with different regimes of light as described in the scheme of **Fig 1**.

Results:

There was a significant reduction of maturation observed in the results of regime 2 at high temperatures **Fig 2.**

The L allele and the homozygous LL genotype are fixed in all samples in the 2 groups, which should not predispose to greater maturity any treatments. LL genotype represent a low genetic disposition to early maturation. **Table 1.**

Conclusions:

In this field trial we observe how is possible reduced the maturation effect of temperature using an adapted photoperiod regime. Gonad ultrasound is a very useful tool to predict and control the maturation risk particularly as in this study with high temperature water. This may be of particular importance for FW salmon producers in flow through systems in warm regions such as northern salmon producing regions in Chile and Tasmania, also for production in RAS closed-containment where high temperatures are frequently observed.

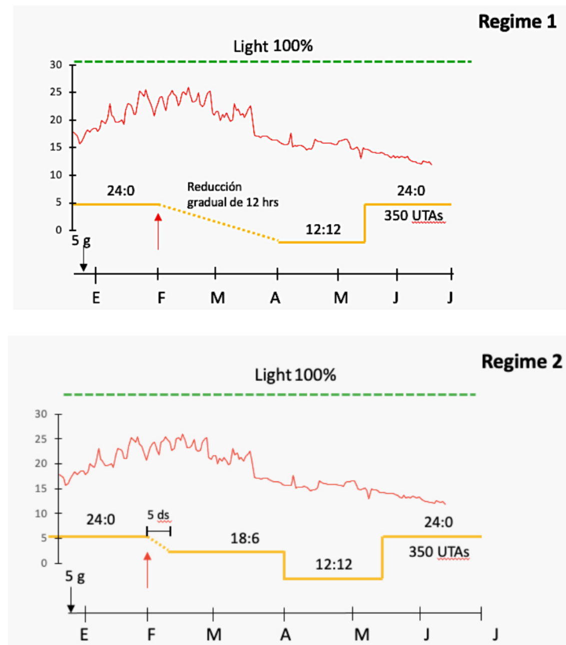

Fig. N° 1. Experimental design scheme describing different light regimes .

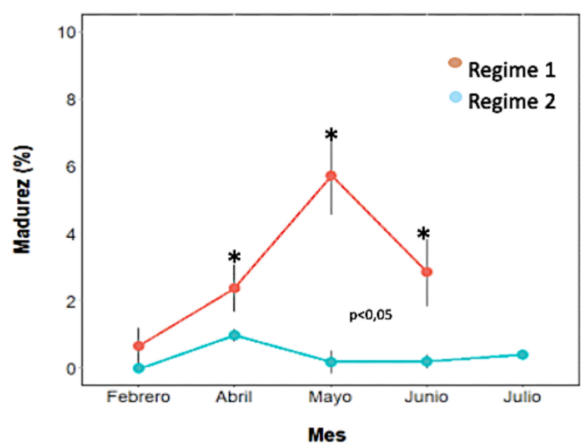

Fig. N° 2. Ultrasound index performed in monthly sampling,. of male of male salmon from 50 to 200 g. during the progression of the spermatogenesis in the different sampling points. Were 6 represent an advanced degree of gonadal development or maturation.

Table 1 Absolut and Relatives frequencies for the genotypes early early (EE), early late (EL) y late late (LL) in each treatment

Genotipos	Frecuencia absoluta	Frecuencia relativa
EE (TT)	0	0
EL (TC/CT)	0	0
LL	382	1
Total	382	1

LIGHT SPECTRUM DECOMPOSITION INDUCED BY TURBIDITY IN SALMON FARMED CONDITIONS

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Introduction:
The application of artificial photoperiod has helped the salmon industry reduce the early maturation, enhance smoltification and growth. Artificial lighting seek to stimulate fish photoreceptors as pineal gland and retina. Regarding light perception, the intensity (quantity) and the spectral composition (quality) of light determine the physiological response of salmon (Bromage et al. 2001; Migaud et al. 2007; Vera et al. 2010). Changes in water quality can have profound effect on the transmission of light. In RAS experimental conditions NOFIMA researchers had observed differences in the absorbance of different wavelengths in RAS. The present study was conducted to determined if turbidity in RAS and sea cages farmed conditions there are changes in the spectral composition of the artificial lighting used in the photoperiod regimes on farmed Atlantic salmon.

The study was carried out in two commercial smolt RAS facilities with two different levels of turbidity (NTU) and in a sea site in two different days with two level of transparency measured by sechhi disc in the X region in Chile. In all the measurements the light source was a 650W multispectral (white spectrum with a blue and green pick) LED underwater luminaire. The underwater spectrum was register at different depths (Fig.1) using a hyperspectral radiometer (RAMSES G2, TriOS, Germany)

The results of this study demonstrated the light spectrum decomposition generated by the water turbidity, generating a selective penetration of the different wavelengths, particularly important in the blue end of the spectrum at the bottom of the culture units suggesting that there might be an effect of the fish photoreception. So, green lights might be the most suitable for RAS, even though we are still working on proving this in field conditions.

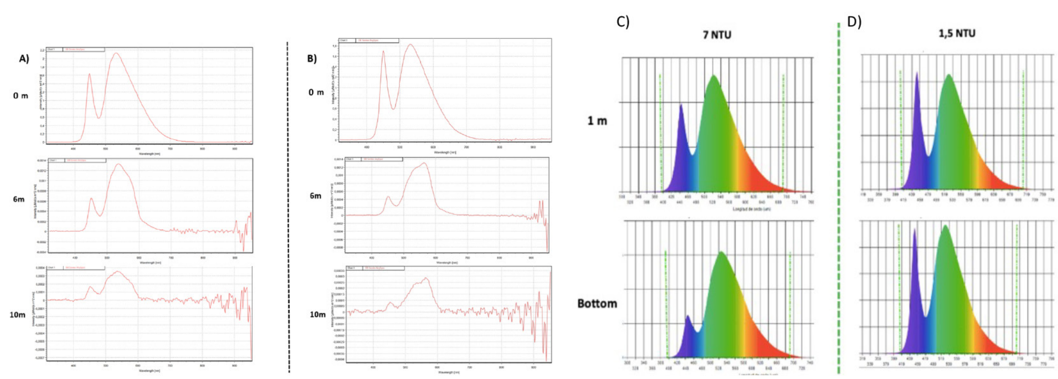

Fig. N° 1. Differences in the wavelengths penetration at two depths in a commercial sea farm at a) 10 m and b) 3 m of transparency. RAS differential wavelenghts penetration at c) 7 NTU (turbid water) and d) 1,5 NTU (clear water)

MICHIGAN DNR MODERNIZES HATCHERY ALARMING SYSTEMS FROM PC BASED SYSTEM TO A CONTROLLER SYSTEM, EMBRASING CELLULAR TECHNOLOGY

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To reduce cost and risk, Michigan DNR is taking advantage of available technology by modernizing hatchery emergency alarming systems, moving from PC based Opto-22 systems to controller based Opto-Epic systems. This presentation will discuss the process of an emergency callback, benefits system modernization, and some other capabilities of the system.

To reduce risk, Michigan's hatchery alarm systems use more than one method for alerting and calling in staff to emergencies. 800 MHz radios are used to instantly communicate any alarm detected by Opto-Epic, taking advantage of existing State of Michigan's statewide emergency communication equipment to send a signal from local towers. After a set time delay, Opto-Epic sends a text message followed by a phone call to the person who is on-call. Both methods send the message to staff that they need to return to work to acknowledge the alarm and address an emergency.

Benefits of Opto-Epic include reducing frequency and costs associates with needed routine PC replacements; keeping equipment that is compatible with State of Michigan IT policies; adding capabilities for staff to be able to adjust and monitor the systems remotely and in real time; plus using cellular capabilities to alert staff, even from locations with poor cellular signal.

Other capabilities include the ability to trend parameters associated with production water, such as temperate and flow. It also allows for easy remote control of feeder systems, plus increased our ability to easily modify the system to meet future needs.

SEQUENCING ALL THE FISHES IN THE DEPP BLUE SEA: IMPACTS OF SAMPLE STORAGE TIME, TEMPERATURE, AND BUFFER TYPE ON DNA QUALITY OF FISHES VALIDATED THROUGH LONG READ SEQUENCING AND ASSEMBLY

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There are over 1.6 million species of animals and 435 thousand species of plants on Earth. Long-read sequencing technology is enabling biologists to digitally archive the genomes of these organisms to understand fundamental basic questions in evolution, boolster conservation strategies in the midst of massive extinctions, and improve food production through crop and livestock genomics. One of the primary challenges however, is obtaining samples from field sites and preserving them in a way that ensures high quality DNA for long read sequencing. Here we demonstrate the impacts of storage time (0 days, 2 days, 7 days, 21 days, and 42 days), storage temperature (4 Celcius, 22 Celcius), and storage buffers (95% EtOH, RNAlater, EDTA) on the preservation of fish blood across multiple species of fish. We extracted DNA using the Monarch HMW kit. DNA yield was consistently high between 10-100 ug and pure (260/280, 260/230) across most time points for the RNAlater and 95% EtOH buffers while most EDTA only samples failed after a week. Fragment lengths based on TapeStation and FEMTO pulse were highest for 95% EtOH stored at 4C even out to 6 weeks whereas for room temperature, RNAlater faired better up to 3 weeks. We show that high quality DNA as measured by yield, purity, and fragment size can be obtained from samples stored at 95% EtOH at 4 Celcius for up to 6 weeks and RNAlater at room temperature for up to 3 weeks. We compare these storage impacts on sequencing read length, genome assembly quality, and methylation variation. For jack mackerel, our sequencing read length N50 on the Promethion was the following: gold standard control 'snap frozen' (37 kb, 33.2; LSK110, LSK112), 2 days at 4C in 95% EtOH (31kb; LSK110), 3 weeks at 4C in 95% EtOH (38.9 kb, 24.4 kb; LSK110, LSK112), 6 weeks at 4C in 95% EtOH (30 kb, 25 kb; LSK110, LSK112), and 3 weeks at 22C in RNAlater (18.4 kb, 11 kb; LSK110, LSK112). The LSK112 kit read lengths were 30% (mean, SD 11.5%) lower than LSK110 but with an increase in quality. Assembly results were also comparable across time treatments (for 95% EtOH at 4C) when controlling for coverage. The overall read lengths were high for all of the 95% EtOH samples regardless of storage time suggesting this as an appropriate storage mechanism for field sampling. For room temperature, RNAlater also did modestly well for up to 3 weeks. We compare our sequencing results to 10 other species of fishes sequenced using the gold standard 'snap frozen' method and demonstrate the feasibility of using this new storage method.

THE EFFECT OF ROTIFER LEVEL FED TO LARVAL RED DRUM *Sciaenops ocellatus* ON DIGESTIVE HEALTH

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Rotifers are the first live zooplankton fed to larval red drum when cultured in controlled conditions within tanks. An investigation was conducted to identify a rotifer level that sustains growth and survival without overfeeding or negatively affecting health. The liver and gut, widely accepted organs to assess effects of diet on the health of finfish, were assessed histologically to determine the effect of rotifer level on fish health. Red drum larvae were cultured in 520 L tanks from 2 to 28 dph with rotifers fed continuously during a 12-hour photoperiod to maintain targeted rotifer levels from 2 to 12 dph. Vacuolization of the liver cells (hepatocytes), and intestinal enterocyte and microvilli heights were measured with digital image analysis (DIA) as indicators of pathology. There was an interaction between rotifer level and age of larvae wherein hepatocyte vacuolization was higher at the higher rotifer levels during the rotifer feeding phase, but vacuolization increased to similar measurements at all rotifer levels after the rotifer feeding phase. Enterocyte and microvilli height tended to increase with age and with rotifer feeding level. Although there was no effect of rotifer level on digestive pathology after the rotifer feeding phase, we did not test for long- term effects. The data suggests that feeding rotifers at 0.5-1.5/mL continuously to red drum during the first several days after hatch may provide suitable food for normal growth and survival and minimize short-term digestive pathology.

SHELLFISH AND SEAWEED AQUACULTURE ECOSYSTEM SERVICES: INFORMING FUTURE RESEARCH AND MANAGEMENT NEEDS

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Shellfish and seaweed aquaculture are increasingly recognized for their role in ecosystem services production, and a growing body of peer-reviewed science documents their connections to all categories of ecosystem services: cultural, provisioning, regulating, and supporting. This project aims to assess the state of the science and utilize the knowledge of experts throughout the United States to identify key next steps for advancing the field of shellfish and seaweed aquaculture ecosystems services research. Framed as a horizon scan, this project seeks to identify not only research gaps and needs, but potentially areas where policy or management has yet to operationalize well-documented aquaculture-associated benefits and services.

In early 2023, research scientists, industry leaders, resource managers, extension personnel, and others well-versed in aquaculture ecosystem services will be invited to contribute to this aquaculture ecosystem service horizon scan. Drawing upon the collective knowledge and responses shared, findings will be synthesized and presented to introduce a preliminary prioritization and discussion of related research and management needs. In the spring of 2023, project participants will be asked to provide feedback on this synthesis. Similarly, session attendees are encouraged to share their thoughts to advance the discussion, implementation, and prioritization of aquaculture ecosystem services research.

WWF CREATING ACCOUNTABILITY IN GLOBAL FEED SUPPLY CHAINS

WWF's progress towards removing habitat degradation from the global food system is hampered by the lack of accountability in the animal protein and feed sector which drives deforestation and conversion. We seek to gain this accountability through the development of a feed ingredient decision support tool (DST) that is fueled by a global hub of information to evaluate environmental, social and governance (ESG) risks of ingredients as well as those that supply them.

Animal protein production is the largest driver of habitat change. Habitat degradation and the over-exploitation of natural resources also are linked to human rights abuse and worker mistreatment. Thus, the production of animal protein and food creates enormous stress on both people and the planet. WWF is currently in the early stages of a global movement to eliminate habitat degradation from food supply chains. While there is progress on the slowing of habitat conversion for the rearing of farmed animals (cows, chickens, fish and swine), the habitat conversion for animal feed ingredients continues to grow because of a lack of transparency, traceability and accountability in supply chains.

The challenge of producing more food with fewer resources will require intensification of the animal protein production sector. Intensification creates a greater reliance on formulated feeds and those feeds, and the ingredients used to make them, are not under the control of producers, yet producers are held responsible for liabilities and risks that may affect their customers (retail/food service), their investors or consumers. Retailers are increasing their attention on feed related issues (see Costco and Tesco letters of support), but investors are as well (see FAIRR letter of support) because their investments in food companies may become "stranded assets" if the risk in these supply chains is not transparent. Thus, it is not only WWF that is concerned about the sustainability of feed ingredients, forward thinking animal protein producers are spotting the trends that have landed major retail chains in court.

For animal protein producers to increase production in a responsible manner, they must be equipped with the knowledge and information that allows them to identify risks – in real time - in feed ingredient supply chains as well as changing market preferences and demands. The purpose of this project is to shine the light on where, how and who produces the ingredients used in feeds for animal protein production beginning with salmon aquaculture in order to begin the transformation of the animal feed sector. We are starting with salmon because the sector has worked together to achieve common goals already. This goal is to create an ESG risk-based DST that is powered by an AI-enhanced global information hub on current impacts of ingredient production such that the most accurate and current knowledge of the current science as well as environmental, social and governance impacts of ingredient production can be shared publicly and catalyze ingredient decisions that are based on current science and values aided by transparency which disrupt the status quo. The DST will allow animal protein producers to avoid ingredients and ingredient suppliers that create the greatest impact and pose the greatest reputational and financial risks to downstream supply chain actors.

Once released, the tool will have direct application to avoiding ingredients produced by the clearing of natural habitat (legal or illegal) for row crops (Brazil, Russia, USA, etc.). The tool will also provide information on the stock status of fisheries to avoid species that are over-exploited (Chile, Norway, USA, Peru, Europe (north Atlantic fisheries). Additionally, the safeguards that are desired for workers will be on display through the transparency of policies around labor and working conditions that occur in specific countries as well as specific locales. Utilizing the human risks component of the tool will raise greater awareness on how production of ingredients can occur while providing the necessities for human well-being of workers. Fundamentally, we are seeking to have global application of the tool and create a race to the top in terms of performance such that the right actors are being rewarded for the specific attributes we expect from those who were underperformers in the past.

This project builds on existing research on protein demand changes in the developing and least developed regions of the world, as well as the patterns of climate and habitat change that will shift supply chains to different regions of the world. WWF will also merge our current efforts to decouple habitat degradation from commodity supply chains with this project to provide greater consensus around the real and perceived impacts of feed and feed ingredient production.

The work to create greater transparency and accountability in the animal feed sector is paramount for the sustainability of food systems. Without this accountability, feed ingredients will remain in the shadows and continue to consume a tremendous amount of the planet's resources. To our knowledge, this is the first effort to truly confront these issues with a lens to promote greater stewardship in resources used through the production of animal feed. The sustainability of animal feed is one of the greatest challenges for our food system with direct implications for the emissions that contribute to climate change, the conversion of our natural ecosystems that buffer humans from climate change and zoonotic diseases and the protection of the biodiversity in habitats threatened by conversion.

DEVELOPING TECHNOLOGY TO INDUCE TETRAPLOIDY IN SAUGEYE (*Sander vitreus* x *Sander canadensis*) AS A MEANS TO ESTABLISH SAUGEYE AQUACULTURE IN THE U.S.

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Saugeye, a hybrid of walleye (female) and sauger (male), is a highly popular sport fish, produced and stocked for recreation in 12 U.S. states. Because of its popularity, market potential, and traits well-suited for aquaculture, Saugeye has been identified as a priority species for aquaculture development in North-Central U.S. (NCRAC, USDA). Barriers that have prevented Saugeye aquaculture establishment include reliance on wild broodstock as source of gametes and potential of escapees to contaminate wild gene-pools of wild stocks of parental species. As part of the overall goal of establishing Saugeye as a species for aquaculture, we aimed to conduct the first investigation of Saugeye domestication (production and evaluation of second-generation hybrids) and development of conventional polyploidy methods and innovative genetic manipulation techniques (stem cell transplantation) to produce tetraploid (4n) Saugeye, which can be crossed with normal diploid Saugeye to produce sterile triploid progenies with low costs, and high yields.

We first attempted to develop optimal physical shocking conditions to produce the first 4n Saugeye. In spring 2022, five different shock types were tested: 1) Pressure shocks at 3 different intensities, each applied at 2 different times post fertilization; 2) 2 heat shocks of different intensities applied at the same time post fertilization; 3) a long (120 min) cold shock applied 4 minutes post fertilization (mpf); 4) double shock consisting of a long (120 min) cold shock applied 4 mpf followed by pressure shock applied at 2 different times post fertilization; and 5) double shock consisting of the long initial cold shock followed by a second cold shock. Control, non-shocked, diploid siblings were produced alongside. All shock treatments produced viable hatched larvae except a pressure shock of 9000 PSI applied at 260 mpf. Flow cytometry was used to determine ploidy of first-hatched larvae. Of the 14 shock protocols tested, 3 produced tetraploid individuals, though induction rate was low (7.7-20%). Triploidy was induced in 6 of the 14 protocols, and mosaic individuals (2n and 4n) were observed in 3 treatment groups. Surviving larvae from each treatment group were stocked to separate 37L aquaria within a recirculation system for first-feeding and grow-out to early juvenile stage. Once fish reached 7 months of age, they were transferred to 60L flow-through tanks for further grow-out. Survival and growth were monitored throughout and will be reported.

Next steps will include further refinement of shocking protocols to induce tetraploidy, production of second-generation hybrids in spring 2023, and evaluation of their survival, growth, and overall quality compared to that of traditionally produced, first generation Saugeye hybrids.

SEQUENCING ALL THE FISHES IN THE DEPP BLUE SEA: IMPACTS OF SAMPLE STORAGE TIME, TEMPERATURE, AND BUFFER TYPE ON DNA QUALITY OF FISHES VALIDATED THROUGH LONG READ SEQUENCING AND ASSEMBLY

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FISH MICROBIOTA 101: HOST TRAITS, ECOLOGY, AND ENVIRONMENTAL FACTORS INFLUENCE MICROBIAL DIVERSITY AND BIOMASS ASSOCIATED WITH MARINE FISHES

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Fish are the most diverse and widely distributed vertebrates, yet little is known about the microbial ecology of fishes nor the biological and environmental factors that influence fish microbiota. The microbiota from 101 species of Southern California marine fishes, spanning 22 orders, 55 families, and 83 genera representing ~25% of local marine fish diversity, was analyzed to identify factors that explain microbial diversity patterns in a geographical subset of marine fish biodiversity. We compared fish microbiota from (gill tissue, skin mucus, midgut digesta, and hindgut digesta) using alpha, beta, and gamma diversity while establishing a novel method to estimate microbial biomass associated with these host surfaces (Qiime2 plugin katharoseq). Body site (anatomy) was the strongest driver of microbial diversity but microbial biomass did not differ across body sites. Midgut had the highest diversity and was most influenced by environment (e.g. habitat), but not host phylogeny. Larger, pelagic fishes had lower microbial biomass and diversity in the gill. Patterns of phylosymbiosis were observed across the gill, skin, and hindgut. The majority of microbes from all fish body sites were of unknown origin but overall sea water generally contributes more microbes to fish microbiota compared to marine sediment. In a meta-analysis of vertebrate hindguts (569 species), mammals had the highest gamma diversity when controlling for host species number while fishes had the highest percent of unique microbial taxa (92%). In fishes, the midgut, gill, and skin contain the majority of microbial diversity which collectively can be 5.5 times higher than the hindgut. The composite dataset will be useful to vertebrate microbiota researchers and fish biologists interested in microbial ecology with applications in aquaculture and fisheries management. In future studies, I am now investigating how the host genome influences or restricts the host-microbiome. By sequencing each of the four major tribes of Scombridae along with a control group of pelagic fishes from a different order (Carangiformes), we can compare the host genomes to identify if immune regulation is enhanced in pelagic fishes.

ROTENONE HAS LITTLE EFFECT ON WATER QUALITY, PHYTOPLANKTON, ZOOPLANKTON, OR MACROINVERTEBRATES IN AQUACULTURE NURSERY PONDS

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Rotenone application has been reported to cause significant declines in zooplankton populations with cladocerans and copepods being the most susceptible and possibly taking months to recover. Because copepods and cladocerans are preferred by catfish fry, rotenone application could have significant effects on nursery pond production. Effects of rotenone on zooplankton and time required for recovery has not been studied in eutrophic aquaculture ponds typically drained and refilled yearly.

We quantified rotenone application effects on water quality, phytoplankton, zooplankton, and aquatic macroinvertebrates in either mostly drained ponds or full ponds (0.04 ha) in northwest Mississippi. The study was conducted during May (23 °C)/June (30 °C) when most catfish fry ponds are stocked. For Study 1, nine ponds were drained to about 15 cm water. Six ponds were treated with 4 μL/L rotenone; three treated ponds were then treated with 4 mg/L potassium permanganate ($KMnO_4$). The three additional ponds were drained but untreated. All ponds were then filled and fertilized with urea. In Study 2, six full ponds were treated with 4 μL/L rotenone. Three of those ponds were then treated the next day with 4 mg/L $KMnO_4$. Three ponds were left as untreated controls.

Applying rotenone to these experimental ponds with 15 cm or less water had no effect on water quality, phytoplankton, or zooplankton. Neutralization with $KMnO_4$ did not affect any measured variables. Desirable zooplankton numbers for catfish culture reached 100 organisms/L 11-14 d after treatment. In Study 2, when a whole pond was treated with rotenone, desirable zooplankton numbers reached 100 organisms/L 7 d after treatment if neutralized with $KMnO_4$ and about 11 d after treatment without neutralization. Rotenone treatment did not reduce predatory macroinvertebrate risk, and this should be addressed using additional management strategies.

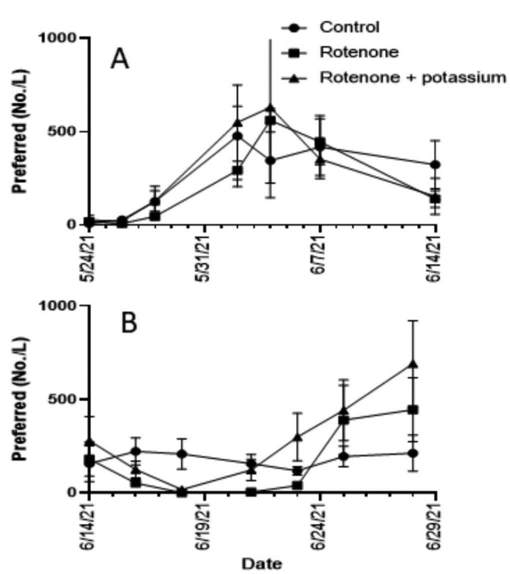

Figure 1. Average (± SEM) number/L of preferred zooplankton in empty ponds (A) or full ponds (B) treated with rotenone, rotenone followed by $KMnO_4$, or untreated controls.

EFFECTS OF MULTIPLE, LOW DOSE COPPER SULFATE TREATMENTS ON THE MARSH RAMS-HORN SNAIL, *Planorbella trivolvis,* AND THE GHOST RAMS-HORN SNAIL, *Biomphalaria havanensis*

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Two snail species, *Planorbella trivolvis* and *Biomphalaria havanensis* are common inhabitants of commercial catfish ponds and known to transmit the trematode *Bolbophorus damnificus*. Copper sulfate application is a promising management tool to control snails and thereby reduce trematode infestations.

We determined 24, 48 and 72-hr acute copper (Cu) toxicity to both species. Additionally, sequential low-dose treatments ranging from 0.1 to 0.8 mg/L Cu were evaluated on eggs, juveniles, and adults.

LC_{50} values were similar for both species of adult snails. Exposure times and LC_{50} values were: 24-h = 1.7 mg/L Cu; 48-h = 1.1 mg/L Cu; 72-h = 0.2 mg/L Cu. For multiple doses, four treatments of 0.4 mg/L Cu were enough to kill all snails of both species. Four doses of 0.1 mg/L Cu prevented all eggs from hatching in both species.

From this data, repeated doses of at least 0.1 mg/L Cu would be required for a strategy targeting eggs and juveniles. However, for strategies aiming to eliminate adult snails, four repeated doses of 0.2 mg/L Cu may be effective. Taken together, these data indicate repeated low-dose copper treatments can be effective for snail control in commercial catfish ponds.

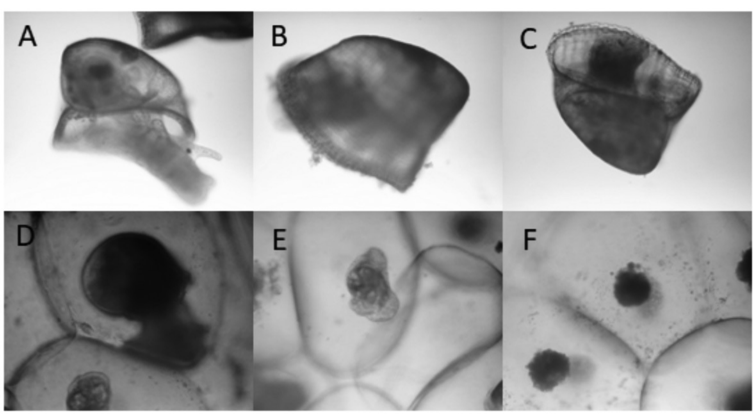

Figure 1. Planorbella trivolvis egg development 10 d post-spawn. Snail egg masses were treated with Cu on day 0 and day 7 post-spawn. Snails from representative Cu treatments are as follows: A = control (normal, hatched snail), B = 0.01 mg/L Cu (normal, hatched snail), C = 0.02 mg/L Cu (normal, hatched snail), D = 0.05 mg/L Cu (alive, unhatched snail), E = 0.10 mg/L Cu (alive, undeveloped embryo), and F = 0.19 mg/L Cu (dead, decaying embryo).

THE CHANGING ROLE AND DEFINITIONS OF AQUACULTURE FOR ENVIRONMENTAL PURPOSES

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Aquaculture is the fastest growing form of food production across the globe. The expansion of the industry has brought about a diversity of approaches to mitigate social and ecological impacts associated with aquaculture production systems. At the same time, there is a growing interest in utilizing aquaculture for conservation purposes including species recovery, habitat restoration and offsetting the impacts of wild capture on vulnerable harvested species. The diversification of the aquaculture sector and the overlapping use of terminology to describe alternative aquaculture approaches can create challenges for policy makers, managers and industry practitioners. Clear distinction between alternative aquaculture approaches and intent may improve regulatory, permitting, monitoring and consumer awareness outcomes.

We examined the use of four primary aquaculture approaches in the scientific literature: 'commercial aquaculture', 'conservation aquaculture', 'restorative aquaculture' and 'regenerative aquaculture' to elucidate the similarities and differences and improve understanding of the approaches. Proposed definitions for the terms were based on empirical analysis of related words used in scientific texts and fitness into a particular initiative. In addition, we discussed the use of those terms within the context of benefits to 'people and nature', namely activities that include economic, social and environmental outcomes and the variability therein. Clear definition of terms and related activities in a burgeoning field can minimize semantic confusion while improving opportunities to craft robust policy guidelines and improve stakeholder understanding and practice of aquaculture activities.

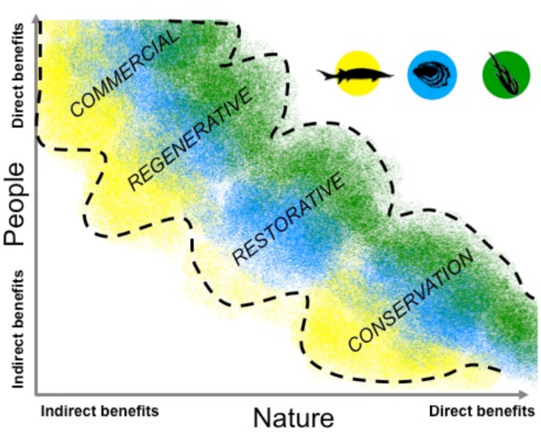

Figure 1: Conceptual graph of aquaculture types by taxonomic group and expected probability of benefits for people versus nature for a given aquaculture system. Outcomes (people and nature benefits) of initiatives are expected to differ and vary based on the chosen approach and species taxa, and can overlap among forms as well, therefore aquaculture approaches are represented by merging 'clouds' to show variability and overlap spread.

CO-CULTURE OF SEA URCHINS AND OYSTERS: A NATURE-BASED SOLUTION APPROACH FOR BIOFOULING CONTROL

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Oyster culture is a longstanding traditional business in the Chesapeake Bay where environmental conditions support the production of shellfish that can be marketed under different grades (size, shape), and taste profiles, including from sweet to briny, with an increasing emphasis on aspects of quality. The control of biofouling, which is exacerbated in high salinity areas, is still an issue for farming on the Eastern Shore, especially in traditional and conflict-reducing bottom cages. Physical methods for biofouling control are time and staff-consuming and can be a considerable expense for the farmer, while types of anti-fouling paints are either prohibited or under development (natural base). Recognizing these limitations, growers have sought alternatives with an interest in more sustainable forms of aquaculture (conservation, restorative, regenerative), seeking natural and most sustainable solutions is the best strategy.

Within the Nature-based Solutions (NbS) framework from the United Nation to which aquaculture can potentially abide to, the farming industry should take advantage of ideas that are both environmentally friendly and provide the best social-economic outcomes. One such approach is the co-culture of different species with a focus on positive ecological interactions, higher revenue for the farmer, and possibly additional marketability.

Within that scope, this work explores if the co-culture of suspension feeding Eastern oysters (*Crassostrea virginica*) and native grazers, Atlantic Purple Sea urchins (*Arbacia punctulata*), represents an effective (or not) natural solution to control biofouling on farming bags (Figure 1) without compromising – and instead potentially improving - main product. Two different urchin sizes and urchin stocking densities are being tested for comparing potential effects on the cleanliness of cages and species conditions, while maintaining available space inside the farming gear and species survival, which will be assessed for both species. Broadly, we expect the advantage of deploying urchins with oysters to be two-fold depending on the target urchin species: in locations where the urchin species have become a biological nuisance, the species can be harvested from nature for grow-out in aquaculture devices; in locations where a past fishery has led to low species stocks, (future) demand for hatchery seed would support another farmed product for the market without the need to further alter natural stocks and possibly contribute to restoration efforts.

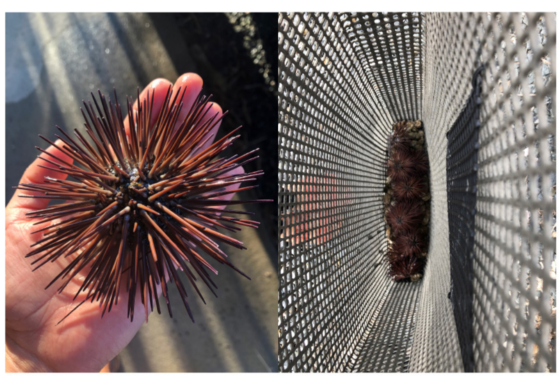

Figure 1: Purple urchins *(detail left)* deployed in farming bags (right) with Eastern oysters in a coculture design.

PLEOMORPHISM IN THE OPPORTUNISTIC FISH PATHOGEN *Aeromonas hydrophila* FOLLOWING OSMOTIC CHANGES IN GROWTH CONDITIONS

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Aeromonas hydrophila, a freshwater, Gram-negative, motile, rod-shaped bacterium ubiquitous in the aquatic environments throughout the world, is the causative agent of motile *Aeromonas* septicemia (MAS) in fish. Virulent *A. hydrophila* has severely impacted catfish farming in the southeastern United States for over a decade. Despite the extensive economic losses that this pathogen inflicts on the aquaculture industry, its ecological attributes are poorly understood. Analysis of farm-level risk factors associated with MAS outbreaks in farmed catfish in Alabama indicated that the use of salt (sodium chloride, NaCl) significantly lowers the odds of *A. hydrophila* infections. Exposure of bacteria to stressful environmental situations such as high salinity may lead to modification of physiological and phenotypical characteristics. Sodium chloride can inhibit bacterial growth and/or attachment and have beneficial effects on the fish host health.

The objective of this study was to describe the adaptive morphological and structural changes that *A. hydrophila* cells undergo in response to different salinity conditions. Multiple strains of this pathogen were grown in tryptic soy broth medium with varying salt concentrations (5, 15, and 45 g/L) at 28 °C and monitored for 14 days. Specimens of bacterial cultures were processed for light and scanning electron microscopy at different times during osmotic stress. Analysis of stressed *A. hydrophila* cells revealed the presence of filamentous morphotypes under the highest (45 g/L) salt concentration. The length of some elongated cells exceeded 15 µm. However, normal short rod cells ranging from 1.0 to 3.0 µm in length prevailed in cultures with 5 and 15 g/L NaCl. This study suggests that exposure of *A. hydrophila* to osmolarity stressful conditions leads to generation of an elongated morphotype, which allow bacterial cells to cope with the adverse conditions, prolong survival, and repopulate in post-stress favorable environments. Moreover, viability, culturability, biofilm formation, and the virulence potential of *A. hydrophila* cells were assessed throughout the study and the results will be presented. The findings of this study would help understand the mechanism of *A. hydrophila* survival in catfish ponds and facilitate research on prevention and control of the recurring MAS outbreaks.

NEW APPROACH TO DEVELOPMENT OF TAILOR-MADE FEED FOR FISH LARVAE USING ZEBRAFISH *Danio rerio* AS A MODEL

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This study proposed a practical controlled hydrolysis method that 1) utilizes all the endogenous enzymes contained within the fish body to "auto-hydrolyze" its tissue proteins and 2) provides a meal that is tailored to meet nutritional requirements and absorptive capacity of larval fish.

The two main objectives for this experiment were to determine: 1) the effect of the proposed hydrolysis method on tissue protein breakdown level; and 2) the effect of dietary inclusion of obtained hydrolysate on fish growth performance in its larval stages.

Adult Zebrafish (ZB) carcasses were utilized in the hydrolysis process. Four different ZB-meal products were produced for this study, an intact (non-digested) product (control), and three digested products that underwent hydrolysis for 1, 2, and 3 hrs, respectively. The hydrolysate solution was kept at 27^{o}C with a pH of 7-9 during the entire process. The efficacy of the hydrolysis was analyzed using SDS-PAGE. At three days post-hatch, larval ZB were randomly distributed in 15 (3 L) tanks, with 100 larvae per tank. Fish larvae received five dietary treatments with three replicate tanks each; live feed or formulated dry commercial diet (reference groups), intact ZB-meal-based diet (Intact); 50% intact and 50% ZB hydrolysate-based diet (50% Hydro); and 100% ZB hydrolysate-based diet (100% Hydro). The hydrolysate-mix utilized in this study contained equal parts of the three digested products. All fish were fed to satiation from first feeding until the larvae fully metamorphosed to a juvenile stage.

The results from the SDS-PAGE showed that the proposed hydrolysis method was able to efficiently hydrolyze the protein within ZB body. The results from the feeding trial found no significant differences in final weight, total length, or condition factor between the Intact, 50% Hydro, and 100% Hydro groups. However, total length was significantly lower in the experimental groups compared to both reference groups while body weight was lower in both hydrolysate groups compared to the reference groups. The gene expression of Pept1 intestinal transporter showed a significant increase in 50% Hydro group compared to the Intact group but similar expression compared to the remaining groups in 24-hours starved fish. However, 2-hours after feeding the gene expression of Pept1 showed no significant differences between 50% Hydro and the remaining groups. These results found that although the hydrolysis method effectively produced hydrolyzed ZB-meal, the inclusion of the hydrolysates generated the same growth of the larval ZB compared to the intact ZB-meal. The growth results paired with Pept1 gene expression potentially indicate ZB larvae to be highly adapted to dry feeds at first feeding and able to utilize dietary protein in different molecular forms efficiently for growth. Overall, the proposed hydrolysis method provides a practical and cost-effective approach to producing species-specific fishmeal hydrolysates. Further research is necessary to determine whether the produced hydrolysates can improve the growth of larval fish in other fish models.

THE EFFECT OF DIETARY ESSENTIAL AMINO ACID DEFICIENCY ON FEEDING RESPONSE IN STOMACHLESS FISH

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Fish have been shown to present an opposite trend to mammals with an increase in feed intake of diets that are imbalanced in essential amino acids (EAA). However, fish response to individual dietary EAA deficiencies remains unclear. The objective of this study was to assess the effect of EAA deficiency on feed intake, growth performance, muscle post-prandial free amino acid (FAA) levels, and the expression of genes associated with amino acid sensing in the intestinal tract using Zebrafish *Danio rerio* as a model species.

Six semi-purified diets were formulated for this study. The first diet, CG, contained casein and gelatin as its only protein sources. The remaining five diets had 50% of its protein supplied through casein and gelatin, and the other 50% supplied in the form of free amino acids (FAA50). The FAA50 diet was formulated to contain the same level of amino acids as the CG diet. The (-) Lys, (-) Met, and (-) Thr diets were formulated to match the FAA50 diet, but were deficient in lysine, methionine, or threonine, respectively. Finally, the Deficient diet was not supplemented with any of the three EAA. At 21 days-post-hatch, Zebrafish were randomly distributed in 18 (3 L) tanks, with 30 fish per tank in three replicates per dietary treatment. The fish were fed three times a day to satiation, and the study lasted until 50 days-post-hatch.

At the conclusion of the study, the feed intake of the CG group was significantly higher than all other groups. Neither the (-) Lys, (-) Met, or (-) Thr groups had a feed intake that was significantly different from that of the FAA50 group. However, the feed intake of the Deficient group was significantly higher than the feed intake of the FAA50 group. The feed efficiency (FE) of the (-) Lys and Deficient groups was significantly lower compared to the CG group. The FE of the FAA50, (-) Met, and (-) Thr groups were not significantly different from any of the other groups. The final weight and weight gain (%) of the CG group was significantly higher than all other groups and there were no significant differences in growth between the FAA50 group and any of the groups with EAA deficiencies. Additionally, survival did not significantly vary between any of the groups. Overall, the results from this study show that Zebrafish do not significantly adjust their feed intake in response to individual deficiencies of lysine, methionine, or threonine, but the apparent additive effect of a combined deficiency of the three EAA triggered a significant increase in feed intake as a response. The results on muscle post-prandial FAA levels and gene expression will be included in the oral presentation.

EFFECTS OF PURIFIED BREWERS' YEAST (*Saccharomyces cerevisiae*) ADDITIVES ON THE MUCOSAL HEALTH OF ATLANTIC SALMON PARR

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Cell wall components of *Saccharomyces cerevisiae* (rich in β-1,3 and -1,6-glucans and mannan oligosaccharides) in isolated or whole forms have been shown to confer immunomodulatory effects in fish. These benefits are at least partially induced by improvements of intestinal health. Despite the reported benefits, many knowledge gaps exist with regards to the optimal form and dosage. An experiment was conducted to investigate the efficacy of highly purified β-glucans (PβG) and whole yeast cell walls (WYCW) to enhance the mucosal health of Atlantic Salmon (*Salmo salar*) parr. A total of 120 parr (ca. 21g) were randomly assigned into six experimental units (20 per tank) and fed either 1] Control (no yeast additives), 2] PβG (0.02% Leiber® Beta-S) or 3] WYCW (0.2% Biolex® MB40) treatments for 4 weeks. All treatments were fed to the same % of biomass (between 1.5% and 2% per day). At the end of the experiment, there were no significant differences in zootechnical performance (weight gain, SGR and FCR) between fish fed the different diets.

Histological appraisal revealed that fish fed the WYCW treatment had a 39% increase (P = 0.0422) in goblet cell abundance in the distal intestine and that the PβG treatment-fed fish had a 49% increase (P = 0.0459) in goblet cell abundance in the skin when compared to the control group.

In addition, transmission electron microscopy (TEM) analysis of the distal intestine revealed significantly different microvilli morphometrics. Fish fed the PβG treatment had significantly longer (P < 0.0001) and more densely packed (P = 0.0001) microvilli than the other treatment groups. Fish fed the WYCW treatment had significantly denser microvilli arrangement (P = 0.0056) than the control group.

Ongoing analysis includes gene expression profiling of immunomodulatory and barrier function genes.

In conclusion, both dietary products demonstrated the potential to enhance the epithelial barriers studied.

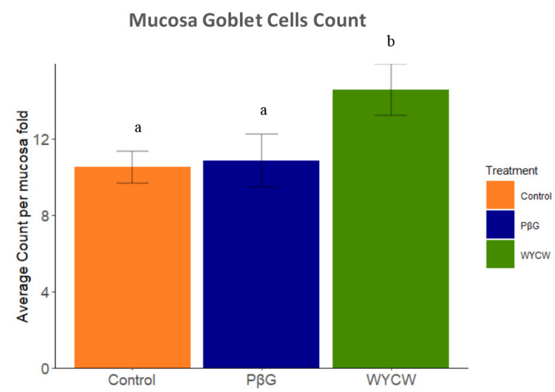

Figure 1: Goblet cells levels of the distal mucosal folds of Atlantic salmon parr after 4 weeks feeding on experimental diets. Treatments with different letters are significantly different (P < 0.05).

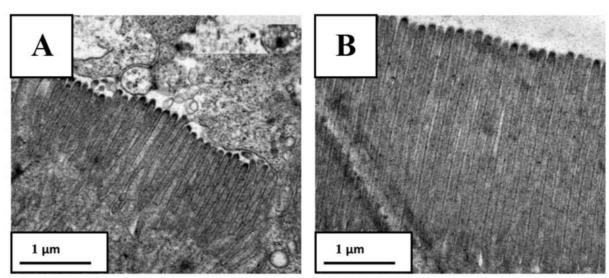

Figure 2: TEM electron micrographs of the microvilli from the distal intestine of Atlantic salmon parr subjected to (A) Control (B) PβG treatments. Scale bars = 1 µm.

OUTCOMES OF IN VITRO FERTILIZATION WITH FROZEN-THAWED BLUE CATFISH SPERM AND IMPLICATIONS FOR GENE BANKING

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Catfish farming constitutes about 75% of total U.S. finfish aquaculture production, where the channel catfish, *Ictalurus punctatus* female × blue catfish, *I. furcatus* male hybrid accounts for >50% of the harvest. Current hatchery technology to produce hybrids is labor intensive and requires the sacrifice of males for in vitro fertilization. This is expensive and time-consuming, as blue catfish males take 4 to 7 years to mature. Sperm are then often of low quality/quantity and do not necessarily give high fertility and hatch. Therefore, paternal gametes should be used sparingly, using the minimum quantity of sperm for fertilization, while still maximizing offspring production. This is particularly the case when using cryopreserved cells, as each male's sperm, whether frozen or in living gene banks, is in limited supply. The objectives were to (i) compare sperm swimming kinematics and health metrics before and after cryopreservation; (ii) determine minimum quantity of frozen-thawed sperm required to maximize hatching success; and (iii) decipher how early offspring development is affected when eggs are sired with fresh and frozen-thawed sperm. Sperm kinematics were evaluated by computer assisted sperm analysis, while health metrics (i.e., viability, oxidative stress, DNA fragmentation) were assessed using fluorescent imaging and flow cytometry. Over 2 spawning seasons, eggs from 19 females were collected and fertilized using cryopreserved sperm from 38 males at 10 sperm to egg ratios (range from 1.0×10^4 to 9.0×10^5 sperm per egg). Embryos were then incubated under common environmental conditions and growth performance documented over 40 days post-hatch.

Generally, sperm kinematic traits and health metrics decreased after cryopreservation (Fig 1A). Despite this, hatching success using cryopreserved sperm was high, but dependent on sperm to egg ratio. For instance, at ratios of 1.0×10^4 to 5.0×10^4 sperm per egg, hatch increased from 18.0% ± 8.5 to 43.9% ± 8.2 (Fig. 1B). Adding greater than 5.0×10^4 sperm per egg had no significant effect on hatching success. Preliminary analyses suggest cryopreservation will not impact offspring performance. These data improve understanding of frozen-thawed sperm quality and paternal effects for blue catfish to improve reproductive sustainability and reduce production costs.

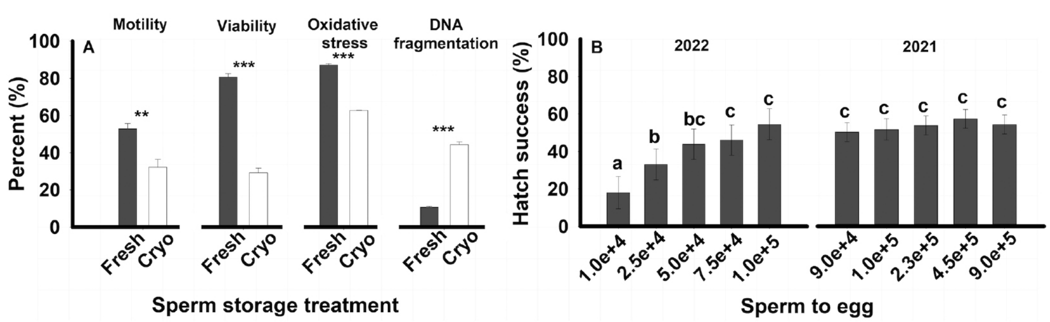

Fig. 1. Sperm swimming kinematics and health metrics before and after cryopreservation (A) and minimum quantity of frozen-thawed sperm required to maximize hatching success(B).

THE EFFECT OF POLIDORIDS AND CO_2-CARBONATE SYSTEM ON GENE EXPRESSION OF PACIFIC OYSTER *Magallana gigas* BROODSTOCK CONDITIONED IN A RAS

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The Pacific oyster (*Magallana gigas*) is the most cultured bivalve in the world. In Mexico, many mollusk hatcheries from Baja California, produce oyster seeds to supply producers. Broodstock conditioning is necessary to obtain gametes and produce seeds. However, most of the conditioning is performed in natural conditions. Consequently, the maturation of broodstock can be variable. Recently, in our laboratory, Recirculating Aquaculture System (RAS) with control of the CO_2-carbonate system has been designed to enhance oyster broodstock maturation. However, we observed that oysters did not mature and had been infested by Polidorids (shell-boring polychaete parasites). Thus, this work aimed to evaluate the effect of Polidorids and the CO_2-carbonate system on the relative gene expression of Pacific oyster broodstock conditioned in RAS.

The experiment was performed in triplicate using two types of systems. Control systems consisted of RAS without control of the CO_2-carbonate system (RAS-C) and the experimental with control of the CO_2-carbonate system (RAS-R). Oysters were conditioned at 12°C for 45 days. Then, the water temperature increased to 18°C (60 days). After that, a second period continues for 70 days in which water temperature changes from 18°C to 20, then to 22, ending at 24°C. Gonad and mantle edge tissue was obtained at 18°C and 24°C. The parasite prevalence was determined by visualizing mud blisters in the inner oyster shell. The relative gene expression was determined by qRT-PCR; reproduction genes in the gonad (ATPhe, Gprot, SP1b); innate immune (MAPK, PRPL1, TLR2), and biomineralization genes (Tyr, VpATP) in the mantle.

Our results showed that at 18°C, oysters kept in RAS-C with Polidorids repressed reproduction effort, evidenced by less gene expression of signal and proliferation of germ cells (ATPhe, SP1b) and receptor of GnRH (Gprot) (Fig. 1A). Also, Polidorids promote change in the energy balance from reproduction to immune and shell protection, because of increased gene expression of innate immune wide recognition (TLR2), inflammatory response (MAPK), production of the shell matrix protein Tyrosinase and active transport H^+ (VpATP) in the mantle. At 24°C, oysters in RAS-C had higher expression of signal and proliferation of germ cells than RAS-R, regardless of the presence or absence of Polidorids (Fig. 1B). In contrast, oysters in RAS-R reduced the proliferation of germ cells and increased innate immune wide protection. In conclusion, Polidorids affected the reproduction performance of oysters conditioned in RAS. Nevertheless, oysters with Polidorids in RAS-C changed the energy balance at 18°C allowing them to reach the same reproduction performance as those without Polidorids at 24°C.

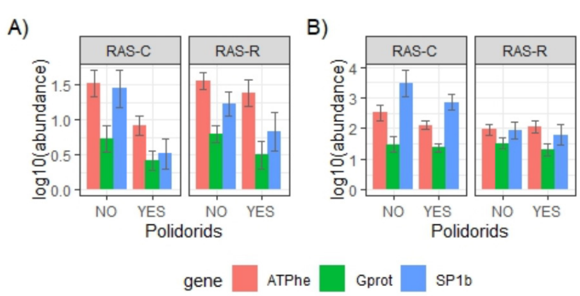

Figure 1. Relative gene expression in gonad tissue at 18°C (A) and 24°C (B).

LARVAL SEA LICE (*Lepeophtheirus salmonis*, Krøyer) 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.

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DEEPCHILLING: MAXIMIZING YIELD AND FRESHNESS WITH A SUBZERO COLD CHAIN

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Seeing as how seafood is a highly sensitive and perishable product, it presents major challenges in handling and maintenance of optimal conditions during harvesting, processing and distribution. The current cold chain, which utilizes the melting of flake or crushed ice, does not effectively meet the requirements of today, due to the fact that the chilling of seafood in ice does not lead to great results. Whereas, Deepchilling (rapid cooling to, and the ability to maintain, subzero temperatures without freezing the product) seafood can optimize the cold chain, offering increased shelf life, higher quality and bigger yields compared to traditional methods such as Refrigerated Sea Water (RSW) or flake ice.

Studies have been conducted at various stages of the cold chain (harvesting, processing to distribution) using different species of fish such as farmed salmon, trout, cobia and yellowtail. The tests analyzed cooling rates, bacterial growth, texture after maturing and freshness and evaluated the impact on the yield, shelf life, and quality of the fish.

When salmon and yellowtail are harvested before being gutted, bacteria are quickly developing inside of the fish. Deepchilling fish immediately upon harvest minimizes bacteria growth and maintains freshness from the beginning, so fish are kept safe (as in, below 0 degrees C) with an extended shelf-life. Salmon, once gutted, are typically stored overnight or additional days before filleting and pin-boning. This is a critical period where bacteria growth needs to be controlled, and firm texture must be maintained to prevent the meat from gaping. Also, to go through the filleting machine efficiently, a deepchilling process can be arranged, where fish are kept at subzero temperatures. This reduces fat accumulation on the knife, allowing better operation with cleaner cuts and less gaping. As a result, the quality of fillets is demonstrably higher and a greater filleting yield can be achieved.

Yellowtail are very sensitive fish, requiring a fast processing time to maintain the highest quality. Often shipped overseas, yellowtail requires extreme quality control. Deepchilling maintains yellowtail's prime condition even during filleting, packaging and overseas shipping. With the extremely high cooling rate and ability to consistently maintain subzero temperature, Deepchilling enables sashimi quality fish and allows distribution from as disparate locations. Similarly, it has been observed that the deepchilling process provides an efficient method for the rapid chilling of farmed cobia from a typical tropical temperature (25-30C) to 0°C in a relatively short time period, and reducing the rate of bacteria growth which affects spoilage.

So, deepchilling has been shown to significantly improve quality, shelf life and yield as the food is kept relatively colder, fresher for longer than using other methods like flake ice. Deepchilling supports a sub-zero cold chain and is a proven, demonstrable method for keeping fish at optimal temperatures, controlling bacterial growth, maintaining freshness and extending shelf-life.

INSIGHTS FROM A PILOT SCALE DEPLOYMENT OF A NOVEL KELP CULTIVATION SYSTEM FOR EXPOSED AND OFFSHORE ENVIRONMENTS

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We report the performance of an advanced kelp cultivation system for offshore and exposed ocean environments. The field test was completed in the Gulf of Maine in 2022. The project is funded by the US Department of Energy's ARPA-E program to develop technologies that can enable large-scale macroalgae cultivation in deep water. Multiple new technologies were deployed in the field test, including a unique mooring layout, unconventional materials, novel anchors, and a robotic anchor installation tool. With two seasons of field tests completed, project team members are using collected data to evaluate the cultivation structure and component technology performance, to improve upon those designs, and to better inform the concept's economic feasibility.

The desired scales of production, harsh offshore environment, permitting restrictions related to marine mammals and low-cost requirements for offshore macroalgae aquaculture present a challenging engineering design problem. To approach economic feasibility our system integrates: shared use of infrastructural components, scaling efficiencies, structural resilience in overlapping modularity, and strategic agronomic processes. Our system sets the typical hectare scale longline farm within the context of massive (square kilometers scale) arrays of semi-independent farm "modules" supported by a lattice of novel multi-line moorings capable of increasing structural efficiency and resilience through distributed accommodation of hydrodynamic loads. ROVs designed to install the anchors enable low-cost deep-water deployments. Wave actuated tethered hydrofoil upwellers integrated into the mooring system harness ambient renewable wave energy to elevate deep cold nutrient rich seawater enabling conditions capable of extending temperate macroalgae growth seasons (tested separately from pilot field trials due to permitting restrictions). Fiberglass rods replace conventional ropes as mooring lines and growth substrate to reduce the risk of marine mammal entanglement.

This presentation will focus on characterizing and evaluating the performance of our proposed kelp cultivation system and component technologies through the lens of collected data and observations from our recent pilot scale field trials.

REARING TEMPERATURE AND LIVE FEEDS INFLUENCE THE DEVELOPMENT OF WHITE MUSCLE IN GREATER AMBERJACK

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Greater amberjack *(Seriola dumerili)* is a very promising candidate for the diversification of aquaculture, yet high growth dispersion at hatchery stages and unsynchronized development is a major drawback in intensifying juvenile production. In this study, we investigated the influence of different rearing temperatures and different live feeds, on the process and variation of myogenesis at early development in greater amberjack.

Rotifers (R) and copepods (C) were the live feed alternatives studied in a 2X2 rearing experiment combined with two different rearing temperatures (20 °C vs 24 °C). Larvae samples were collected at notochord flexion (FL), end of larva rearing (ELR) and middle metamorphosis (MM) and the total cross-sectional area (TCSA) of white muscle was measured along with the expression of *mylpfa* (myosin light chain 2a, hypertrophy), *mylpfb* (myosin light chain 2b, hyperplasia), *myog* (myogenin, coordination of myogenesis).

Total length increase was almost linear after hatching and the combined effect of temperature X live feed significantly affected larval growth (p=0.037), whereas TCSA was strongly affected by the type of live feed (p= 0.039). The expression levels of *mylpfa* gene were affected neither from the temperature nor from the type of live feed. Significant differences were identified in the expression of *mylpfb* and *myog* genes between the four groups; at FL, expression of *myog* that drives muscle cell differentiation and of *mylpfb* that signifies hyperplasia, was higher in larvae reared at 24°C. By ELR, group 24R exhibited the highest *myog* and *mylpfb* expression. The shift from larvae to juveniles is dependent on the proper deployment of hyperplastic and hypertrophic processes during the early phases of muscle development. Based on the *mylpfa*/*mylpfb* expression levels, it appears that hyperplasia dominates the white muscle development up to metamorphosis and it is strongly affected by rearing temperature.

The combined results from histological and gene expression analyses indicate that temperature is a major driver of white muscle development, with 24°C favoring a higher pace in comparison with 20°C. Within 24°C, the type of live feed resulted in different phenotypes at MM with rotifers supporting longer larvae with smaller TCSA as compared with copepods.

Acknowledgement: This research was Co-financed by Greece and the European Union, European Maritime and Fisheries Fund in the context of the implementation of the Greek Operational Programme for Fisheries, Priority Axis “Innovation in Aquaculture”, Project title “ Investigation of size variability in reared juveniles of greater amberjack towards improved production and husbandry practices” MIS 5010923.

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USER CONFLICT MITIGATION IN NORTH CAROLINA'S AQUACULTURE INDUSTRY

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The NC Division of Marine Fisheries (DMF) 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.

Navigation, recreational and commercial fishing, and conflicts with riparian landowners remain the largest challenges associated shellfish leases in North Carolina. The Marine Fisheries Commission recently adopted new rules that can help mitigate some of these user conflicts, but strategies beyond the scope of regulation are often necessary to maintain public license. I will use several case studies discussing contentious shellfish lease applications to highlight the following strategies and their utility.

DMF staff identify and engage with specific (often localized) stakeholder groups to discuss specific concerns with proposed shellfish leases, but also to gather broader feedback that can be used to inform rulemaking and DMF policy related to aquaculture. Some groups feel excluded from the shellfish leasing process, and incorporating their feedback helps DMF improve public license for shellfish aquaculture and allows them to be a part of the process. DMF also uses these meetings as opportunities to educate the public about shellfish aquaculture regulation to address preconceived notions and misconceptions that often conflate concerns.

DMF has developed a set of strategies that can be applied to specific shellfish lease application conflicts. Growers are encouraged to network with their 'neighbors' (riparian property owners and adjacent leaseholders) prior to selecting a location for a new lease. This can identify unique local concerns that can be addressed prior to submitting a formal shellfish lease application. Additionally, grower-driven outreach to build community support for proposed leases among stakeholders can play an important role in the public hearing progress.

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.

LAKE STURGEON *Acipenser fulvescens* RESTORATION: A COOPERATIVE EFFORT IN SOUTHEASTERN UNITED STATES

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In 1995, the Tennessee River Lake Sturgeon Working Group was created to reintroduce lake sturgeon into their historic range in the Tennessee River. The original working group consisted of a multi-agency partnership between the Tennessee Wildlife Resources Agency (TWRA), Tennessee Valley Authority, US Fish and Wildlife Service (USFWS), Tennessee Aquarium Conservation Institute, Tennessee Cooperative Fishery Research Unit, University of Tennessee, Wisconsin Department of Natural Resources, US Geological Survey, Tennessee Technological University, World Wildlife Fund, and the Tennessee Clean Water Network. In 2006, the Cumberland River was added, and since then, other agencies and groups have joined from states such as North Carolina, Kentucky, and Georgia. The group was re-named the Southeastern Lake Sturgeon Working Group. Over the years, geneticists from the USFWS and TN Aquarium have evaluated the genetic diversity of the lake sturgeon in the program. In addition, fish health biologists from USFWS Warm Springs Fish Health Center have examined lake sturgeon from hatcheries and the Tennessee River and have not found any health concerns.

Members of the working group spawn lake sturgeon at Shawano Dam on the Wolf River in Wisconsin. The fertilized eggs are transported to Warm Springs National Fish Hatchery for early rearing. After approximately thirty days, the fish are divided among grow out facilities. Edenton National Fish Hatchery is one of over ten hatcheries that rear lake sturgeon as part of this restoration program.

Lake sturgeon feeding regimes vary at different restoration facilities but these fish are typically fed a combination live brine shrimp (*Artemia sp)* nauplii, frozen bloodworms (chironomids), frozen krill (varying species), and/or a commercially prepared diets. Prior to release, the lake sturgeon are marked by removal of specific scutes, which denotes their year class if these fish are recaptured. Sturgeon are reared for approximately five months at the facilities, and are released in different locations in the Tennessee and Cumberland rivers or their tributaries.

Since 2000, over 300,000 lake sturgeon have been stocked. From 2011 through 2021, biologists have captured and released approximately 700 lake sturgeon during monitoring efforts on the Tennessee and Cumberland rivers. TWRA has enlisted the help of anglers to photograph, and report catch information on lake sturgeon in these rivers in exchange for a certificate. Since 2006, TWRA has issued 873 certificates to anglers for reporting the catch and release of lake sturgeon in Tennessee. The goal of the restoration program is for the lake sturgeon to reach healthy, self-sustaining populations and support a well-managed sport fishery.

EXAMINING THE DIGESTIVE PHYSIOLOGY OF LARVAL *Betta splendens* TO IMPROVE LARVAL CULTURE PROTOCOLS

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Betta splendens, also known as the Siamese fighting fish, is an anabantoid native to Southeast Asia and is among the top ten freshwater ornamental fish species imported into the USA for the aquarium trade. This fish is popular in the aquarium trade due to its ornate appearance, variety of color morphs, and ability to withstand suboptimal water quality conditions. Although *B. splendens* is a staple in the ornamental aquaculture industry, little peer-reviewed research has been conducted on larval digestive physiology and weaning. These data could be used to minimize the reliance on costly live feeds during the larval production process by indicating when larvae can transition to an inert microdiet (MD). Using the timing of the development of a functional stomach to guide MD introduction can help reduce live feed use while maintaining survival and growth. A 30-day trial was conducted to examine the digestive enzyme ontogeny, developmental milestones, and digestive morphology of *B. splendens* to determine the timing of the maturation of the digestive tract. At 1-day post-hatch (DPH), larvae measured 3.11 ± 0.06 mm (TL) with a large yolk sac, unopened mouth, and non-functional eyes. Mouth opening and first feeding occurred at 2 DPH, with subsequent swim bladder inflation occurring at 3 DPH. Flexion began at 6 DPH and completed at 13 DPH. Larvae were fully developed by 40 DPH, where the swim bladder extended to the caudal peduncle and fin coloration was present. Trypsin and lipase (Fig. 1) activities were quantified using standard microplate assays from 1-30 DPH. Both trypsin and lipase were detectable from 1 DPH and increased steadily throughout the larval period. Acid protease activity from 1-30 DPH was also quantified, but the results from this assay did not reveal a clear trend. Results from ongoing histological investigations will confirm the timing of gastric gland development and stomach functionality. Together, these data will be used to design a weaning trial to determine the earliest timepoint at which *B. splendens* larvae can be weaned from live feeds to MD without affecting larval growth or survival.

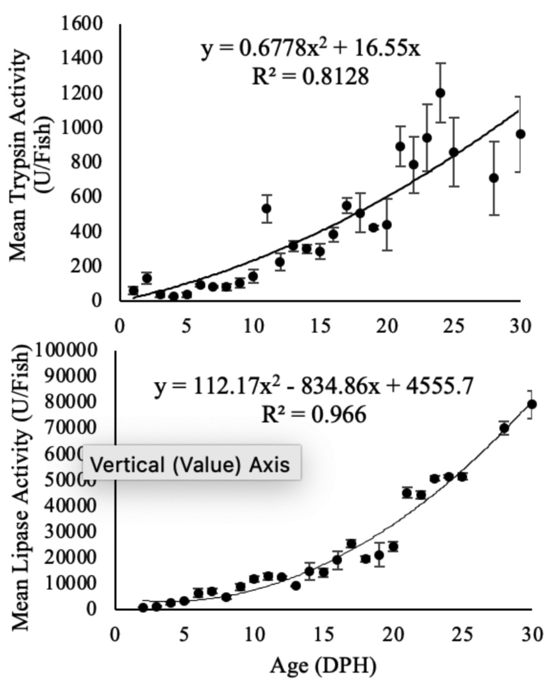

Figure 1. Mean trypsin (top) and lipase (bottom) activity (±SE) of larval *B. splendens* from 1-30 DPH (n=3).

CHARACTERIZING THE GASTROINTESTINAL DEVELOPMENT AND DIGESTIVE ENZYME ONTOGENY OF LARVAL *Amphiprion ocellaris*

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Clownfish (*Amphiprion ocellaris*) are the most recognizable and popular marine aquarium fish and are a key commodity for marine ornamental fish producers. Although these fish have been raised in captivity for decades, producers are still experiencing issues regarding larval survival, reliance on live feeds, and post-larval deformities. To improve the culture process and reduce reliance on live feeds, the ontogeny of larval development and digestive capacity, namely stomach development, was examined. The timing of stomach functionality indicates when larvae have the capacity to better digest and utilize inert microdiets (MDs) that can be used to replace costly live feeds during larval rearing. The morphological development, digestive enzyme ontogeny, and digestive tract development of *A. ocellaris* was examined in a 16-day trial. Standard microplate assays were conducted to quantify the activity of trypsin, lipase, and acid protease enzymes from 24 hours before hatching to 15 days post hatch (DPH). Histology was used to visualize morphological changes in the digestive tract throughout the larval period. At hatching, *A. ocellaris* has functional eyes, an open mouth, and a coiled, differentiated digestive tract. Gastric gland formation was observed at 6 DPH, with stomach functionality occurring between 7 – 9 DPH, indicated by gastric gland proliferation and an increase in acid protease activity (Fig. 1-A). Lipase (Fig. 1-B) and trypsin (Fig. 1-C) activities were detectable before hatching and increased significantly after 6 DPH. The timing of stomach development from this study will be used to design a weaning trial to transition larvae from live feeds to MD without affecting larval growth or survival. Overall, understanding larval digestive ontogeny can aid in streamlining larval rearing and reducing reliance on costly live feeds.

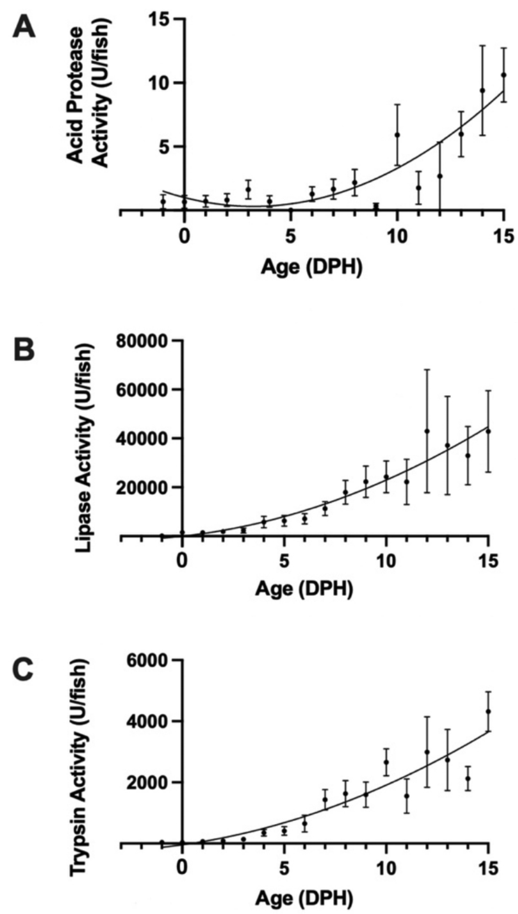

Figure 4. Mean digestive enzyme activity (A – Acid Protease, B – Lipase, C – Trypsin; ± SE) of larval *A. ocellaris* from 24 hours before hatching to 15 DPH (n = 4 from 0 - 10 DPH; n = 3 from 11 - 15 DPH).

PHYSIOLOGICAL AND IMMUNOLOGICAL EFFECTS OF L-THEANINE ON STRESS IN NILE TILAPIA (*Oreochromis niloticus*) IN A CLOSED LOOP AQUAPONICS SYSTEM

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Nile Tilapia are rapidly growing as one of the most popular commercially farmed fish in the world due to their relatively quick growth cycles and easier production than most fish. However, farmed raised fish often get labeled as a lower quality of food than wild caught fish because it is a widely accepted belief that meat from stressed animals is of lower quality in texture, taste, and nutritional value. By the year 2050, the global population is estimated to surpass the 10 billion mark, and the need for a sustainable source of food will be even more prevalent in 2050 than the struggles seen in the COVID-19 era (2020-2022). The aim of this investigation is to measure the impact of acute and chronic effects of L-Theanine on the stress and immune response of Nile Tilapia (*Oreochromis niloticus*) raised in a closed loop aquaponics system where the only variable altered is the feed consumed by the tilapia. The tilapia will be divided into five groups - Control (Cortisol feed), Negative Control (normal feed), Treated Negative Control (normal feed and L-Theanine), Preventative (normal feed and L-Theanine with cortisol feed), and Treatment (Cortisol feed and L-Theanine). In Nile Tilapia, stress levels can be gauged by various physiological parameters such as blood glucose levels, packed cell volume percentage (PCV%), phagocytic activity, and spleen size. Several physiological and immunological parameters will be compared with the control. Our results have not yet been collected. Detailed information will be presented at the conference during the presentation.

ANTIBACTERIAL ACTIVITY OF SEA URCHINS (ARBACIA PUNCTULATE AND STRONGYLOCENTROTUS DROEBACHIENSIS) BODY WALL EXTRACTS AGAINST GRAM POSITIVE AND GRAM NEGATIVE PATHOGENIC BACTERIA

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Nowadays antibiotic resistance is one of the major concerns all over the world. Discovery of anti-bacterial agent from natural source is creating a new dimension to overcome antibiotic resistance problem. Sea urchin, a marine invertebrates of the Phylum Echinodermata, have lots of therapeutic activities. This study aims to determine the antibacterial activity of ethanol and ethyl acetate body wall extract of *Arbacia punctulate* and *Strongylocentrotus droebachiensis*. The body wall extracts of these two species of sea urchins were prepared by following a standard protocol. Then crude concentrated extracts were used against five gram-positive and five gram-negative pathogenic bacteria by disk diffusion method to observe the zone of inhibitions. Our study revealed that both ethanolic and ethyl acetate extracts of *Arbacia punctulate* and *Strongylocentrotus droebachiensis* have potential antibacterial activitis against pathogenic bacteria. Moreover ethyl acetate body wall extract of both sea urchins species showed significant inhibitory activity against all the bacterial species (five gram-positive and five gram-negative) used for this experiment. Detailed observation and statistical significance will be presented at the conference.

AQUACULTURE: IT IS TIME TO FOCUS ON THE QUALITY

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The world's population is also rising and the subsequent demand for high protein sources of food is rising with it. Hence, aquaculture is rising in prevalence and importance in today's society with the increase in fish consumption and the limitations imposed by normal fishing methods. A push for healthier sources of protein is also increasing demand. Other sources of protein such as cattle and other livestock are not adequate to meet the demand due to a lack of grazing area, climate limitations, long time spans to harvest, and other considerations. Wild fish populations are not large enough to meet this demand and many populations are showing signs of overharvesting. Aquaculture offers a solution to these issues by providing a high protein food that requires less space to farm, a faster harvest time, lower cost to produce, and is less detrimental to the environment. However, current aquaculture techniques involve the use of antibiotics and other chemicals in order to reduce disease and mortality within the crops. Disease and mortality present a significant problem in that farmed fish are in crowded conditions leading to certain results. They are susceptible to contagious diseases that can spread rapidly in close quarters as well as their stress levels are increased in these unnatural conditions. This increase in stress over time leads to a reduction in immune response and therefore an increase in susceptibility. In order to provide solutions to the problems of farming and the needs of the people without the use of potentially harmful substances many researchers are looking in to the use of nutraceuticals (feed additives) in feed in order to decrease stress responses, increase immune responses, increase growth, and increase the nutritional value of farmed aquatic animals – both fish and shellfish. In this presentation, I will highlight all these issues and suggest solutions.

OPTIMIZING THE SOYBEAN MEAL INCLUSION LEVEL IN DIFFERENT LIFE STAGES OF ATLANTIC SALMON (*Salmo salar*) DIET

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Soybean meal (SBM) is one of the most commonly used alternative plant-based ingredient 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, dietary utilization of SBM in carnivorous fish feeds has been limited by the presence of several anti-nutritional factors (ANFs) that may affect the digestion or absorption of nutrients, resulting in decreased growth performance and feed efficiency in finfish. When Atlantic salmon is fed on SBM-based diets, the morphology of the distal intestine is disturbed in terms of 'non-infectious sub-acute enteritis'. The changes in the distal intestinal mucosa are described as a deep shortening of the mucosal folds (MF), a decreasing number of supranuclear vacuoles (SNV) in absorptive cells, a widening of the central stroma with a correspondingly high amount of connective tissue and an increased infiltration of inflammatory cells in the lamina propria (LP), an increased number of goblet cells (GC), and a shortening of the microvilli (Mv).

The overall aim of this study was to increase the usage of SBM in feeds for Atlantic salmon. The specific objectives of this study were to optimize the dietary SBM inclusion level in Atlantic salmon at different life stages. Five experimental diets were formulated. Diet 1: FM based diets (30% FM and 0% SBM); Diet 2: 10% SBM, Diet 3: 20% SBM; Diet 4: 30% SBM and Diet 5: 40% SBM. Diets 2 – 5 contains 10% FM along with SBM. A total of 525 fish (juvenile) were stocked in triplicates in 15 tanks of recirculating aquaculture system. The fish were fed by hand to apparent satiation two times per day, six days per week for 22 weeks. Feed intake was measured every day. Fish were weighed and sampled (distal intestine and liver) on 4th, 8th, 12th and 22nd week.

The results showed growth performance, feed intake and feed utilization parameters were significantly affected by dietary treatments. Feed conversion ratio was significantly highest in 10% SBM diet fed group up to 8 weeks whereas growth rate was highest in 40% SBM fed group. Growth performance and feed utilization data were measured for 22 weeks. Results for gut histology and immune related genes will be presented.

THE EVOLUTION AND INNOVATION OF NET PEN CULTURE IN THE GREAT LAKES

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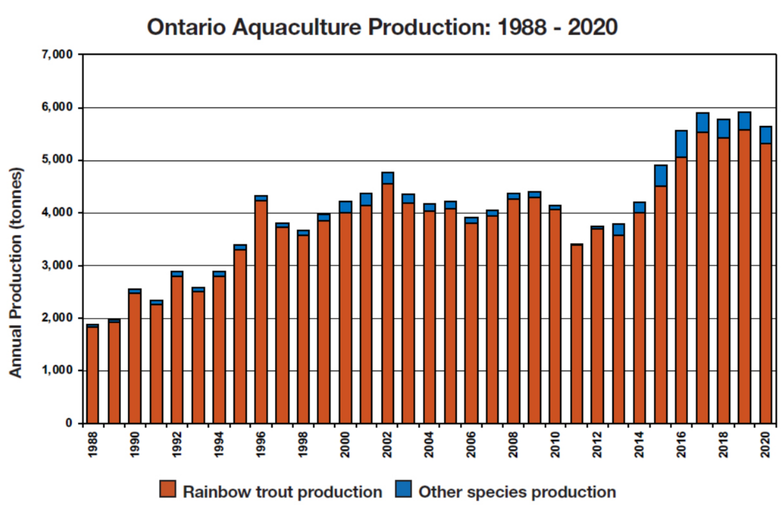
Fish Farming in the waters of Lake Huron started in the early 1980s with small proof-of- concept wooden cages that demonstrated a suitable rearing environment for rainbow trout. The sector has grown over the past four decades to produce over 5,000 tonnes of farmed aquaculture product for the Ontario, Quebec and Michigan markets. In 2022, the first net pen farm began operations in Lake Superior and has demonstrated considerable success.

Many obstacles needed to be overcome; from a lack of science, ice movement, increasing summer water temperatures, social license and invasive species. Farmers have shown great resiliency to the many challenges and developed new technologies and some very innovative approaches to deal with the challenges.

The industry has adopted certification protocols that similarly support the economic and ecological sustainability of the sector. The development and regulation of freshwater net pen aquaculture has been based on an investment in science to support best culture practices to minimize environmental impacts.

Over the past 30 years, there has been considerable involvement of Indigenous groups in the culture of Rainbow trout and, recently, Lake whitefish. Farms within First Nations territories now represent over 75% of the production on Lake Huron and all the emerging production from Lake Superior.

We will review the evolution of net pen culture in the Great Lakes and demonstrate the innovations that are taking place to ensure a future of responsible net pen farming in the face of a warming aquatic environment.



USE OF HIGH PROTEIN DISTILLER'S DRIED GRAIN WITH YEAST IN PRACTICAL DIETS FOR THE TILAPIA, *Oreochromis niloticus*

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A novel technologically advanced high protein distiller dried grains with yeast in it (HP50Y & HP40Y) was evaluated for juvenile tilapia. This experiment assessing the nutritional potential of using high protein (40 and 50%) distillers dried grain to replace corn protein concentrate (CPC) in formulation of tilapia diet for more sustainable development of aquaculture. A 10-weeks growth trial was conducted, using nine diets formulated to contain 32% protein and 6% lipid. Each protein was included at levels (0, 5, 10, 15 and 20%) replacing CPC on protein basis. Juvenile tilapia (mean initial weight 5.23 ±0.20g) were evenly distributed in thirty-six, 75-L aquaria working as a recirculating system and fed twice daily to apparent satiation throughout trial. Tilapia exhibited no significant ($p>0.05$) differences in growth, FCR, survival, whole-body proximate composition, mineral composition, and hematological parameters when fed HP50Y and HP40Y supplemented diets compared to the control diet. Digestibility coefficients for the test ingredients were determined in tilapia for dry matter, energy, crude protein, individual and total amino acids using 1% titanium oxide as the inert marker with 70:30 replacement strategies. All the values were found in acceptable range for the distiller grains when compared to literature. Results from this study revealed that HP50Y and HP40Y both are good alternative protein sources and can be used up to 20% inclusion level in the diets of tilapia.

Table 4: Response of juvenile Tilapia (mean initial weight 5.23 ±0.20g) fed diets containing different levels of HP50Y and HP40Y over a 10-weeks experimental period. Values represented the mean of four replicates.

Diets	HP50Y level (%)	Final Biomass (g)	Final weight (g)	Weight Gain (g)	Weight Gain (%)	FCR[a]	Survival (%)	NPR[b] (%)
Basal	0	783.25	54.93	49.90	992.37	1.23	95.0	45.90
50Y-5	5	760.92	56.34	51.08	972.98	1.24	90.0	45.80
50Y-10	10	787.25	56.10	50.75	948.53	1.25	93.33	42.70
50Y-15	15	772.27	55.16	49.85	939.01	1.23	93.33	42.80
50Y-20	20	773.47	59.63	54.46	1055.66	1.22	86.66	44.90
P-value		0.99	0.45	0.42	0.15	0.93	0.77	0.07
PSE		102.21	3.81	3.72	66.20	0.05	10.0	1.91
40Y-5	5	779.67	56.02	50.93	1001.33	1.24	93.33	45.50
40Y-10	10	809.07	57.71	52.44	994.47	1.23	93.33	40.80
40Y-15	15	811.17	57.87	52.65	1006.45	1.19	93.33	43.10
40Y-20	20	795.52	58.75	53.47	1013.35	1.16	90.0	42.50
P-value		0.97	0.74	0.78	0.99	0.61	0.90	0.20
PSE		88.97	4.43	4.40	84.0	0.08	7.25	3.25

[a]FCR=Feed conversion ratio = feed offered/ (final weight-initial weight)
[b]NPR= Net protein retention

EVALUATION OF FERTILITY OF TRIPLOID ZEBRAFISH (*Danio rerio*)

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Triploidy is the result of producing an organism with three sets of chromosomes (3n). Induction of triploidy requires the application of physical shocks (temperature, pressure and chemical) during meiotic cell division leading to the retention of the second polar body (3n chromosomes). Triploid offspring can also be obtained by the combination of gametes from a diploid and tetraploid parent. Previous studies suggested that triploid Zebrafish are male-biased and sterile due to abnormal gonad development. We aimed to evaluate the fertility of triploid Zebrafish by crossing triploid individuals with another triploid (3n x 3n) and with diploids (3n x 2n), to address the contradictory information about triploid fertility. Cold shock (4°C) was applied at 3 minutes post fertilization for 20 minutes to produce triploid Zebrafish. Ninety percent of offspring were confirmed triploid at six days post fertilization (dpf) by flow cytometry analysis. Fish were grown out to sexual maturity and sex ratio was determined. At 90 dpf, 12% of triploid offspring were determined to be females. Among those triploid females (n=6), two produced offspring by sibling-crossing (3n*3n) and back-crossing (3n*2n) in six trials.

Triploid females produced eggs when spawned with diploid males in all trials except one (Table 1). All larvae were found to be aneuploid (C-value = 2.24 ± 0.07 pg) by flow cytometry analysis at 6 dpf and no larvae survived more than 10 dpf. Sibling-crossing of triploid male and triploid female produced a comparatively smaller number of eggs, and very few were fertilized (Table 1).

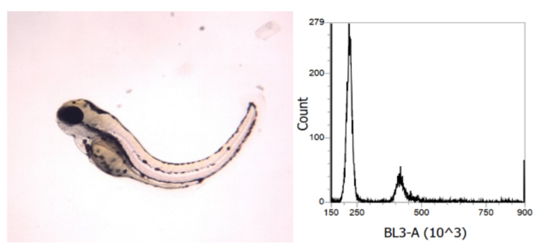

Fig: Hatched larvae from triploid female and diploid male cross and flow cytometry analysis (C value= 2.12 pg)

Table1: Fertilization rate, larval survival, and hatching rate of sibling-cross and back cross triploids

Cross Female*male	Number of eggs	Fertilization rate (%)	Larval survival (%)	Swim-up survival (%)
3n* 2n	172	94	65	15
3n*2n	80	77	80	12
3n*2n	618	93	74	8
3n*2n	386	95	49	14
3n*2n	49	91	10	0
3n*2n	141	88	55	7
3n*3n	5	0	0	0
3n*3n	3	0	0	0
3n*3n	252	2	0	0

INNOVATIONS IN FISH HEALTH EDUCATION: REGIONAL AND HYBRID APPROACHES

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The Great Lakes Aquaculture Collaborative was established in 2019 by Great Lakes regional Sea Grant programs. The collaborative aims to enhance and coordinate aquaculture extension and research work in the region by gathering input from state industry advisory committees, helping to coordinate and fund research and by providing direct education programing to aquaculture related individuals.

In 2022 the collaborative heard from industry that a top priority was fish health education for farmers as well as regulators and future fish health professionals. Due to the limited availability of fish health experts in the region, as well as the relatively small number of potential participants for the workshops, a regional and hybrid approach to a fish health workshop was enacted. This approach was designed to maximize the impact of the workshop by providing a comprehensive overview of fish health with hands on training, while minimizing the number of speakers needed to host the workshops. The format for the workshop included:

1) DAY 1: An all virtual 3 hour series of presentations
2) DAY 2a: A morning of virtual presentations streamed into 7 in person meeting locations
3) Day 2b: An afternoon of in person fish necropsies and fish health farm planning

The first day took place as a typical virtual series of presentations related to fish health and specifically focused on preventative measures. The second day had in person meeting locations in NY, OH, IL, IN, WI, MI and MN. The day started with two zoom presentations focused on reactive measures in fish health. The presentations were viewed live by in-person participants in each 7 locations with Q&A sessions. Then each state transitioned to separate in person fish necropsies, meeting time with local fish health professionals and regulators, and time to work on daily maintenance and best practices plans for attendees' farms/labs.

Results from post-evaluations showed successful learning outcomes as well as appreciation for the wide variety of content provided through virtual presentations paired with hands on in person training. Continued post-evaluations will be taking place over the next few months to assess if the learning resulted in actionable changes from attendees.

EFFECTS OF ECO-FRIENDLY AND CLEANLINESS MESSAGES ON CONSUMER ACCEPTANCE OF AQUAPONIC TOMATOES (*Solanum lycopersicum*)

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Aquaponic-grown produce offers several environmental benefits compared with other production methods, including the remediation of aquaculture waste streams and the reduction of water, fertilizer, and energy inputs. However, for the environmental benefits of aquaponics to be fully realized, consumers must be willing to consume and pay for aquaponic products. Although environmental benefits are the main advantage of aquaponic produce, highlighting sustainability benefits alone has a minimal impact on consumer willingness to pay. The failure of environmental messaging to improve aquaponic produce acceptance may be due to the higher priority consumers place on attributes such as taste, health, and price or concerns regarding aquaponic cleanliness and safety. Therefore, highlighting other benefits of aquaponics – such as the absence of pesticides and herbicides due to the nature of raising multiple species in a water-circulating system – may be a worthwhile strategy to improve consumer acceptance.

A taste test was conducted with participants (n = 204) randomly assigned to one of the four conditions of a 2 (message type: eco-friendly vs. clean and safe) × 2 (product type: aquaponic-grown vs. soil-grown) experimental design. The subjects first read a brief product description. The information about the aquaponic-grown sample was framed as either eco-friendly or clean and safe. The information for the soil-grown sample was consistent across conditions. After reading the information, participants tasted both aquaponic-grown and soil-grown tomatoes one at a time in a counter-balanced order. After evaluating each sample, participants responded to two 9-point items (1 = dislike extremely; 9 = like extremely) measuring tastiness, a 7-point item measuring purchase intention (1 = very low likelihood; 7 = very high likelihood) and indicated their willingness-to-pay in dollars. Participants also answered questions to assess their concern for health and concern for the environment.

Our preliminary results show that aquaponic-grown tomatoes were liked as much as soil-grown tomatoes in both conditions. However, the purchase intention of aquaponic-grown tomatoes was lower than that of soil-grown tomatoes in the “clean and safe” condition but not in the “eco-friendly” condition. Participants’ concerns for health and the environment moderate the effect of the messages, as participants with high health concern and low environmental concern had significantly higher willingness-to-pay in the “clean and safe” condition than in the “eco-friendly” condition. These findings demonstrate that consumer acceptance of aquaponic produce may be increased by tailoring aquaponic produce messaging to distinct health- and environment-concern consumer segments.

EFFECTS OF DIFFERENT SOYBEAN PROTEIN SOURCES ON GROWTH PERFORMANCE, FEED UTILIZATION EFFICIENCY, INTESTINAL HISTOLOGY, AND PHYSIOLOGICAL GENE EXPRESSION OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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This study aimed to evaluate the impacts of different soybean meal protein sources, which were the products of fermented, fractionated, and expeller-extruded processes, on the growth performance, feed utilization efficiency, intestinal histology, and physiological gene expression in white shrimp. The trial was conducted in a green water recirculation system with the stocking density at 30 shrimp/ tank, at an initial weight of 0.42 ± 0.01 g (Mean ± SEM) over an 8-week period. A total of nine experimental diets were evaluated. This included a diet containing animal-based primarily animal-based proteins (17.7% fishmeal and 17.7% poultry meal) and no soybean meal along with a basal diet containing 48% soybean meal and 6% fishmeal. The SBM was then replaced (50% and 100%) on an isonitrogenous basis with BrightDay, Soycomil PE, Hamlet HP 300. Additionally, 1 diet contained 100% replacement using expeller-extruded soybean meal. All growth metrics in the trial, with the exception of survival rate ($p>0.05$), showed significant differences among treatments ($p<0.001$). Furthermore, we observed trends concerning feeding utilization efficiency with fermented BrightDay products having significantly higher phosphorus retention ($p<0.001$). Protein retention, however, showed no discernible differences other than for the fractionated Soycomil PE product with 100% replacement. The histology and gene expression analysis are under investigation for enteritis and physiological gene expression. Results indicate that high inclusion levels do not guarantee a good development performance for shrimp, despite the fact that fractionated and expeller-extruded can be used as a protein source in shrimp diets. At the same time, fermented soybean meal, especially at 50% replacement, is a viable protein source and can be considered a feasible animal-based diet alternative. Therefore, additional studies on various plant-based protein sources are required to improve the dietary matrix and diversify the source of the components for improved animal development performance.

Table 1. Growth parameters of Pacific white shrimp cultured in green water recirculation system for 8 weeks fed different protein sources, stocked at 30 shrimps/ tank with an initial weight at 0.42 ± 0.01 g (Mean ± SEM)

	Basal[1]	**BrightDay 50%**	**BrightDay 100%**	**Soycomil PE 50%**	**Soycomi PE 100%**[2]	**Hamlet300 50%**	**Hamlet300 100%**	**Expeller-extruded 100%**	**Animal**	**PSE**[3]	**p-value**
FW[4] **(g)**	20.88^{a}	20.95^{a}	20.26^{abc}	20.53^{ab}	18.36^{de}	19.43^{bcd}	19.24^{cd}	18.06^{e}	19.11^{cde}	0.4841	<0.001
WG[5] **(%)**	4,773.07^{ab}	4,840.64^{a}	4,671.38^{ab}	4,752.31^{ab}	4,180.12^{cd}	4,499.91^{abc}	4,419.55^{bcd}	4,124.63^{d}	4,414.67^{bcd}	144.3	<0.001
FCR[6]	1.10^{bcd}	1.05^{d}	1.05^{d}	1.07^{cd}	1.24^{a}	1.09^{cd}	1.15^{abc}	1.18^{ab}	1.16^{abc}	0.0361	<0.001
ANPR[7] **(%)**	56.80^{a}	56.30^{a}	54.18^{ab}	55.30^{ab}	45.81^{c}	53.54^{ab}	52.56^{ab}	51.90^{ab}	50.27^{bc}	1.120	<0.001
PR[8] **(%)**	31.84^{cd}	38.37^{a}	45.78^{a}	32.10^{cd}	28.32^{de}	28.93^{cde}	26.63^{e}	28.08^{de}	33.22^{c}	0.967	<0.001

[1]n=3, [2]n=3, [3]Pooled Standard Error, [4]Final Weight, [5]Weight Gain, [6]Feed Conversion Ratio, [7]Apparent Net Protein Retention, [8]Phosphorus Retention

PROTEIN SOLUTION AND APPROACHES FOR FISHMEAL REPLACE MENT IN DIETS OF THE SHRIMP & MARINE FISH SPECIES

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The livestock and aqua feed industries continue to grow year-on-year, which has resulted in higher demand for protein ingredients, especially the fishmeal. Although, the supply of fishmeal reaches 6.0 to 6.5 million metric tons each year (thefishsite.com), the supply of fishmeal is stagnant and isn't expected to grow due to environmental pollution and over-catching capability that results in a limited fishmeal supply. In addition, the trends of requiring sustainable growth and environment protection to many industries, including livestock and aqua feeds that requires urgent innovation in developing the novel protein products (microbial biomass/single cell proteins, insect meals, high-quality vegetable protein concentrates, etc.) to address for fishmeal replacement in diets of aquaculture and livestock species.

Fish meal has been a major protein in diets of the shrimp and marine fish species as it has high energy and protein levels, excellent essential amino acid (EAAs) profile and digestibility, good sources of cholesterol and fatty acids as well as minerals (Ca/P), absence of anti-nutritional factors (ANFs), and for its availability in quantity, also. Besides, shrimp and marine fish species require attractants and palatability in such high-quality and clean fishmeal that makes several challenges for the novel and innovated protein products to be successful in completely fishmeal replacement in shrimp and marine fish species (F3 – Fish Free Feed).

Therefore, in this presentation, some key factors on nutritional aspects required for fishmeal alternatives will be discussed and the available novel protein ingredients will be evaluated for fishmeal replacement as well as the approaches how to replace fishmeal in diets of the shrimp and marine fish species will also be suggested in more practical applications.

BIVALVES AND BETA TESTS: NOVEL TECHNOLOGY TO STREAMLINE SHELLFISH OPERATIONS AND COMPLIANCE

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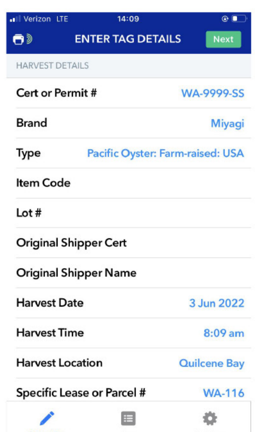
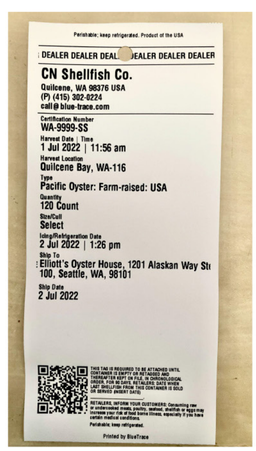
The shellfish industry has some of the strictest harvest and traceability requirements of any food category. And in a context of changing environmental and market conditions, this burden on businesses and agencies will only increase. How do we continue to safeguard public health while furnishing time for operators and regulators alike to do their jobs?

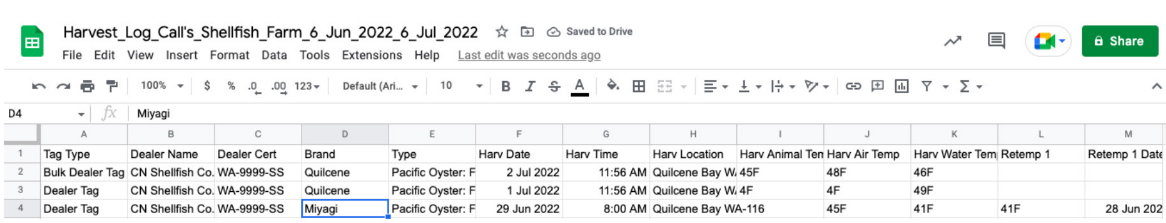
This is the time and place for technology in shellfish. By adapting everyday innovations like mobile devices and QR codes to the shellfish industry, we help businesses accomplish some of their most cumbersome and important — but least glamorous and enjoyable — tasks.

We've built two mobile apps: one for harvesters and one for dealers. Users create a digital harvest or receiving record, complete with all required datapoints (time, temp, location, cert #, etc.). Users can then seamlessly generate tags, logs, and shipping labels with no additional data entry, all automatically formatted to comply with their authority's specific requirements. Save time, reduce errors, and get back to farming shellfish rather than paper.

Our program continues to evolve with industry and agency input. In 2022, we added an automated recall feature to enable more efficient tracking and communication throughout the supply chain. We envision a future in which there is a digital record of every lot of shellfish harvested and distributed in this country. This would let operators focus on their business, give regulators better tools to track product and enforce rules, and improve consumer safety and confidence in shellfish. It may seem like a moonshot, but the technology exists, and with over 300 clients to date, we're well on our way!







	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Tag Type	Dealer Name	Dealer Cert	Brand	Type	Harv Date	Harv Time	Harv Location	Harv Animal Ten	Harv Air Temp	Harv Water Tem	Retemp 1	Retemp 1 Date
2	Bulk Dealer Tag	CN Shellfish Co.	WA-9999-SS	Quilcene	Pacific Oyster: F	2 Jul 2022	11:56 AM	Quilcene Bay W	45F	48F	46F		
3	Dealer Tag	CN Shellfish Co.	WA-9999-SS	Quilcene	Pacific Oyster: F	1 Jul 2022	11:56 AM	Quilcene Bay W	4F	4F	49F		
4	Dealer Tag	CN Shellfish Co.	WA-9999-SS	Miyagi	Pacific Oyster: F	29 Jun 2022	8:00 AM	Quilcene Bay WA-116		45F	41F	41F	28 Jun 202

AQUACULTURE WELFARE ASSESSMENT - A BOTTOM-UP APPROACH TO AQUACULTURE IMPROVEMENT

Oistein Thorsen*, Marius Nicolini, Murilo Henrique Quintiliano, Ralf Onken

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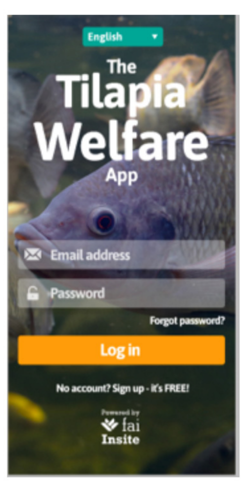
The standard model of implementing welfare standards for aquatic animal production is top down. Certifications bodies and NGO answers to rising citizen awareness about poor farming condition. They create and implement ethical production standards to create seafood products for consumers that understand and acknowledge animal suffering. This model has its limitation because it relies on a niche of educated and sympathetic consumers. Little, if no effort, has been made to understand, support and encourage farmers own intrinsic motivation to do better. FAI believe farmers are the most important actor that drive welfare improvements in aquaculture. We believe they are an untapped force for good who's power can be unleashed through practical farm assessments that align business and welfare objectives.

Farm animal assessment

Working with scientists and farmers in Brazil, Thailand and China, FAI has developed a new approach based on bottom-up farm animal assessments. The assessments help farmers understand what good welfare looks like and show them that it naturally lead to better fulfilment of animal nutritional, health, environmental and behavioural needs. Assessments kickstart a positive spiral of improvements, tapping into farmers innate need to be better and do better. If a problem is identified by the assessor, actions are likely to be taken to fix or improve it, resulting in better welfare. The assessment tool developed by FAI can be used by farmers or processors. Those wishing to use the tool are guided through a series of simple questions that will help them to monitor and drive progress regarding welfare and best practices.

What is FAI offering the industry?

- Protocols that use scientific and operational indicators for the diagnosis of tilapia, carp and white leg shrimp welfare.
- Support for other teams of scientists and practitioners who want to use our rigorous methodology to develop welfare indicators on their own for other species.
- A free application for farmers to perform self assessment and help them monitor and improve welfare and production outcome. It monitors progress, identifies improvement gaps, and provides immediate feedback to the user.
- Free online training series aiming to guide those involved in the sector to integrate the well-established knowledge of animal welfare sciences in their daily routines.



SALTY TALKS PODCAST: USING PODCASTING AS A COMMUNICATION TOOL FOR INFORMATION DISSEMINATION

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Aquaculture has sustained many advancements of the past decade to become more environmentally sustainable, with focus on coastal communities and economic benefit as well. However, public perception and understanding of these developments is not always widely distributed to the general public and therefore not well understood. The aquaculture industry and others must engage in science communication to disseminate information on current and future aquaculture research and development, and community benefits. A content library of podcasts episodes about Aquaculture in Maine will be used as a strategy to disseminate aquaculture topics to the general public. This podcast will be community topic driven and podcast reach will be assessed using social media analytics and analytics provided through Captivate, and survey evaluations. Statistical tests run in R will be conducted quarterly to measure progres once the podcast has been out for 6 months.

Currently there is no podcast about the aquaculture sector as a whole in Maine. Other industries including the photovoltaic industry and healthcare industry have used podcasts as a form of science communication. Additionally, social scientists have demonstrated this form of media is effective in raising awareness and creating social impact, and even potentially changing perceptions on certain topics.

Episode topics are inspired by a study done on diverse perspectives on aquaculture in development in Maine, along with the author's previous research experience to understand perceptions of fin fish aquaculture to guide the focus and think about cross sector and multidisciplinary interactions, as well as the importance of community engagement and social license to operate.

The podcast is on Spotify, Google, and Apple. Episodes consist of various structures including interviews, story telling, and conversations between different stakeholder/industry members. Topics will vary and will include (but not limited to); finfish, shellfish, seaweed, feed, husbandry, disease, indigenous knowledge, regulatory hurdles, history in Maine, and the economy.

SUPPORT IT! PLAN IT! JUST DO IT! LEAD THE EFFORT TO INCREASE DIVERSITY AND INCLUSION AT YOUR WORK PLACE

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I am a Belizean Mestizo – a descendant of Yucatec Maya and Spaniards. I live in Kentucky, but I grew up the first 26 years of my life within a diverse multicultural society in Belize. My childhood, adolescent, and young adult friends and family were from various ethnic groups that include the Yucatec Maya, Mopan Maya, Q'eqchi' Maya, Creole, Hindu, Taiwanese, Garifuna, East Indian, and Chinese. I am proud of my upbringing, and my cultural heritage. I celebrate diversity. One of my career goals is to inspire and achieve diversity and inclusion in what I say and what I do. I actively seek to include women, diverse minorities, and other underrepresented groups because they contribute invaluable and unique insights and strengths to the development of my goals and program of work as an Assistant Professor of Aquaculture Research and Extension at the School of Aquaculture and Aquatic Sciences, Kentucky State University.

Although my laboratory group is small, we actively plan for and engage in inclusion of diverse minorities and women in what we do. Jasmine Iniguez, graduate student for whom I am the major advisor, received support (mentorship and funding) to attend the '2022 Women of the Water Conference' where she presented a poster on 'The Women and Diverse Minorities of the Aquaculture Program at Kentucky State University' (Figure 1). We currently employ a young woman (as a Farm Technician) from the Stewart Home & School, a residential school in Franklin County, Kentucky that serves persons with intellectual or developmental disabilities. I have engaged in various workshops and panel discussions related to diversity and inclusion, and I served as a speaker and panelist as a person of color at the Professionals Panel Discussion hosted by the *Échale Ganas* Program, California Polytechnic University, Humboldt. One main goal of the *Échale Ganas* Program is to increase the participation of Latinx and other underrepresented minority students in natural resource sciences.

As we go about our daily lives and work, remember – if we want to see change, we need to create it. We need to lead the way! Plan for it! And, just do it!

Figure 1. Jasmine Iniguez's poster presentaton at the 2022 Women of the Water Conference, Sarasota, FL.

GROWTH PERFORMANCE AND INTESTINAL HEALTH OF NILE TILAPIA *Oreochromis niloticus* FED AUTOLYSED BREWER'S YEAST

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The Nile tilapia (*Oreochromis niloticus*) is one of the most common aquacultural species in Nigeria and several African countries. Despite the remarkable growth of global aquaculture, the sector still faces serious health challenges in Nigeria and other sub-Saharan African countries, which undermines sustainability in those countries. With this, there is a constant need to improve existing knowledge and to develop new diets using sustainable additives such as yeast derivates. Brewer's yeast is rich in nutrients and bioactive substances such as β-glucans, mannan-oligosaccharides, and nucleotides which have been demonstrated to improve growth performance, health, and immune response of farmed fish. The present study was conducted to evaluate the potential of dietary supplementation of autolysed brewer's yeast (ABY) on Nile tilapia growth performance, feed utilisation and intestinal health.

A 5-week feeding trial was conducted in a RAS with Nile tilapia fry (0.45 g). Four diets were formulated to meet the known nutrient requirements of Nile tilapia (Table 1). The control diet had no brewer's yeast while the other 3 diets were supplemented with ABY (CeFi® Pro, Leiber GmbH) at 1 g/kg (ABY1), 2 g/kg (ABY2), or 4 g/kg (ABY4). All diets were isonitrogenous and isocaloric. The fish (40 fish/15L tank) were fed one of the four diets (n = 3 tanks) at 5% of body weight per day. At the end of the feeding trial, intestinal samples were taken for gene expression and histological analyses (data not shown).

The growth data showed that the final body weight, specific growth rate, and FCR of the fish fed ABY1 was significantly better ($P < 0.05$) than fish fed the control diet (Table 2). Histological analysis of the mucosal fold height did not reveal any significant differences ($P < 0.05$) among the treatments.

Ongoing analysis on other histological parameters and gene expression is being undertaken to assess intestinal morphometrics and regulation of immunoregulatory genes. Results obtained so far indicate that the ABY investigated had a positive response on tilapia feed utilisation and growth performance during early life stage.

Table 1: Experimental diets (g/100g).

Ingredients (g/kg)	**Control**	**ABY1**	**ABY2**	**ABY4**
Soybean Meal 48	40	40	40	40
Sunflower meal	24.67	24.57	24.47	24.27
Fishmeal	15	15	15	15
SPC60	9.98	9.98	9.98	9.98
Sunflower oil	8.67	8.67	8.67	8.67
DL methionine	0.17	0.17	0.17	0.17
Lysine	0.5	0.5	0.5	0.5
Fish premix	0.5	0.5	0.5	0.5
CMC	0.5	0.5	0.5	0.5
CeFi Pro®	-	0.1	0.2	0.4
Proximate Composition (%)				
Protein	46.21	47.39	47.43	46.44
Lipid	11.14	10.85	10.73	11.32
Moisture	4.47	4.2	3.76	3.6
Ash	6.63	6.85	6.89	6.92

Table 2: Growth performance (mean ± SD) of Nile tilapia fed the experimental diets. Values in the same row with different superscripts are significantly different ($P < 0.05$).

	Control	**ABY1**	**ABY2**	**ABY4**
Initial weight (g)	0.46±0.01	0.45±0.02	0.46±0.02	0.45±0.01
Final weight (g)	1.13 ± 0.07^a	1.38 ± 0.21^b	1.21 ± 0.12^{ab}	1.12 ± 0.02^a
Weight gain (g)	0.67 ± 0.06^a	0.93 ± 0.20^b	0.75 ± 0.11^a	0.68 ± 0.01^a
SGR	2.64 ± 0.12^a	3.28 ± 0.36^b	2.86 ± 0.23^a	2.71 ± 0.04^a
FCR	1.99 ± 0.09^a	1.55 ± 0.23^b	1.78 ± 0.16^{ab}	1.91 ± 0.06^a

PROVIDING BETTER ACCESS TO MEDICATED FEED FOR KENTUCKY AQUACULTURISTS AND A SUBSEQUENT SURVEY OF THEIR OPINIONS AND PERCEPTIONS OF THIS SERVICE

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Many fish farmers in Kentucky are limited-resource and are often faced with challenges when trying to obtain medicated feed to treat internal bacterial infections of their fish. The lack of infrastructure in Kentucky (feed mills, supply sources, etc.) cause fish farmers to choose treatment options other than medicated feed due to difficulties in obtaining it, and ordering medicated feed can be expensive due to ordering small quantities with high shipping costs. According to previous survey studies, the majority of fish farmers in limited-resource states mentioned that it takes approximately 11-15 days for the medicated feed to arrive, which is after the peak of the disease mortalities, and high losses have occurred and/or the fish may no longer be eating. It is against this backdrop that our team, through the collaborative efforts with antibiotics manufacturers – Merck Animal Health, Phibro, AquaTactics and the University of Kentucky's Division of Regulatory Services - is conducting this study. This project focuses on registering Kentucky State University Aquaculture Research Center (KSU ARC) as a veterinary feed directive (VFD) distributor. Its priority will be to assist limited resource farmers in Kentucky gain more access to medicated feed (containing either Aquaflor, Romet or Terramycin) in an expeditious manner after they have obtained a VFD from their veterinarians.

This research would provide survey feedback from stakeholders on the effectiveness of fish health services via medicated feed treatment options to limited resource fish farmers in small-scale aquaculture-producing states, like Kentucky. The outcome of these research findings may assist in reducing economic losses currently witnessed by Kentucky farmers and foster the growth and sustainability of fish farming in the state.

INFLUENCE OF WATER TEMPERATURE ON GROWTH AND PROXIMATE BODY COMPOSITION OF FINGERLING LARGEMOUTH BASS *Micropterus nigricans*

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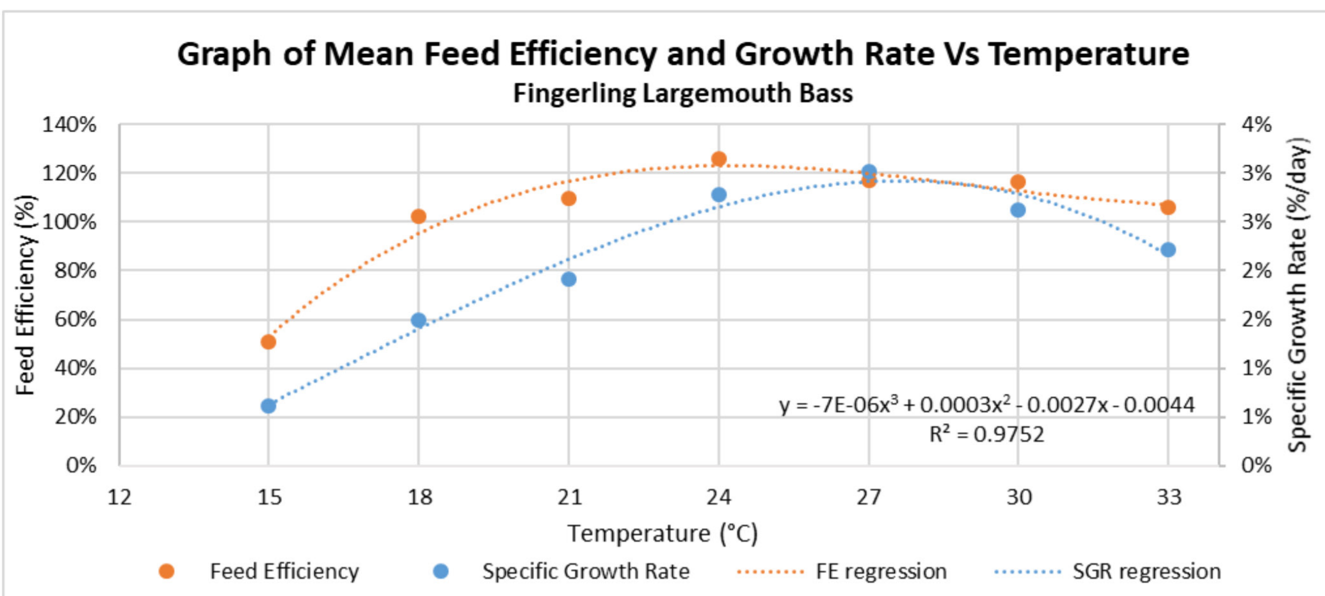
This 6-week study examined the influence of water temperature on growth, condition factor, feed consumption, feed efficiency, and proximate body composition for young of the year feed trained largemouth bass (LMB) fingerlings.

Seven individual recirculating systems with four replicate aquarium tanks per system were randomly assigned a temperature treatment (15, 18, 21, 24, 27, 30, and 33°C). Each tank was stocked with 25 feed-trained fingerling LMB (6.5g ± 0.40/ fish) and fed twice daily by hand to apparent satiation with slow-sinking commercial trout feed (45% protein, 20% fat). Water quality was monitored to maintain conditions suitable for growth. Weight and length for each fish was recorded at the end of the study. Fish in each aquarium were frozen and subsequently processed for proximate body composition. Significant differences were determined with One-Way ANOVA, and relationships described with regression analysis.

Feed consumption and growth among treatments increased to a maximum and then decreased with increasing temperature. Specific growth rate (SGR), final length and weight, feed consumption, and body weight gain (%) were significantly higher at 27°C ($P<0.05$). Regression equation maximum for SGR was 28.45°C with an R^2 of 0.97. Feed efficiency was significantly higher at 24°C ($P<0.05$). Regression equation maximum for feed efficiency was 24.91°C with an R^2 of 0.85. There was no significant difference in survival among treatments. Proximate body composition results will be reported in the presentation.



EVALUATION OF DIETARY ADDITIVES ON GROWTH AND IMMUNE RESPONSE IN CHANNEL CATFISH *Ictalurus punctatus*

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Many bacterial pathogens impact the U.S. catfish industry, and disease control can be challenging for producers. Columnaris disease in channel catfish is primarily caused by *Flavobacterium covae* (formerly *F. columnare*). Using antibiotics and other chemotherapeutics as treatment risks developing resistant pathogens, accumulating antibiotic residues, and potential environmental impacts. In the catfish industry, there is a strong need to evaluate dietary ingredients to enhance catfish health. Immunostimulants may enhance non-specific immune responses and offer an alternative to antibiotic treatments in catfish ponds. Further, dietary protein sources are also essential to fish health and nutrition. Evaluating protein source inclusions, both animal and plant-based, may enhance the overall fish performance in pond culture.

The current project evaluated two immunostimulants: a protease complex (AG175; Jefo Nutrition) and an organic acid substance derived from reed-sedge peat (MFG 50; Kent Nutrition Group). A 60-day trial was conducted to examine the effects of supplementary protein level, dietary formulation, and immunostimulant addition on the growth performance, immune response, and resistance to experimental *Flavobacterium covae* infection in channel catfish. Five diets were tested: 1) a high-quality fishmeal diet (32%; HQFM); 2 a high-protein soy-based diet (32%; CHP); 3) a low-protein soy-based diet (28%; CHL; predominately used in industry); 4) a low-protein soy diet supplemented with AG175 at 1.75 g/ton; and 5) MFG 50 in a low-protein diet at 5 lb/ton. Following feeding for 60 d, juvenile channel catfish were sampled for growth performance and baseline health indicators (n=3 per tank; body mucus, blood for sera, kidney, and spleen). A subset of fish was then subjected to an immersion-based *in vivo* challenge trial with *F.covae* (ALG-00-530; 10^6 CFU per mL exposure).

At 60d post-initiation, there were no dietary differences in percent weight gain (*P*=0.064) or specific growth rate (*P*=0.063), but the 32% diets appeared generally perform best. The cumulative percent mortality (CPM) was found to be different across dietary treatments (*P*=0.003). The mortality in the CHP group was found to be higher than the AG175 (*P*=0.006) and MFG 50 diets (*P*=0.005). These challenge data suggest that the immunostimulant additions may be beneficial in providing protection against *F. covae* when compared to low-protein channel catfish diets.

OPTIMAZATION OF TRIPLOID INDUCTION PARAMETERS AND ANALYSIS OF TRIPLOID STERILITY IN BURBOT *Lota lota*

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Optimization of triploid induction parameters was investigated for burbot using thermal and hydrostatic shock. Additionally, the sterility of sexually mature male and female triploid burbot was assessed. Timing and duration of triploid induction shocks were measured using degree minutes (°C minutes). Hydrostatic shock experiments investigated duration of shock using 7,500 or 8,500psi at 180°C minutes post-fertilization. Thermal shocks investigated duration of shock and post-fertilization shock timing using a shock of 16°C. Assessment of triploid sterility was conducted by fertilizing triploid eggs with diploid sperm, fertilizing diploid eggs with triploid sperm, and comparing fertilization, and survival to diploid males crossed with diploid females. Furthermore, diploid and triploid sperm was assessed via flow cytometry to determine ploidy and sterility. A hydrostatic shock of 7,500psi at 180°C minutes post-fertilization for 10 or 20°C minutes can induce triploidy at or over 90%, and exhibits survival that it statistically similar, $p = \leq 0.05$, to controls. A hydrostatic shock of 8,500psi for 5 or 10°C minutes at 180°C minutes post-fertilization yields triploid induction of 93 and 100%, respectively, with survival that is statistically similar to controls, $p = \leq 0.05$. Thermal induction experiments indicated that shocks at 16°C, 120°C minutes post-fertilization, for durations between 350 and 450°C minutes has potential to induce triploidy at or over 90% while facilitating survival statistically similar to controls, $p = \leq 0.05$. Assessment of sterility determined that milt from triploid males is aneuploid and eggs from female burbot are non-functional. No surviving larvae were produced from the crosses utilizing triploid fish. Tetraploid larvae were detected in three of the optimization experiments. Results presented here provide information on optimum conditions for induction of triploidy in burbot and indicate that tetraploid burbot can be produced via hydrostatic and thermal shocks. Furthermore, assessment of sterility of 4 year old triploid burbot confirmed they are functionally sterile and would present reduced risk of uncontrolled reproduction within or outside of an aquaculture operation.

THE GREENWAVE OCEAN FARMING HUB: A PLATFORM FOR FARMER TRAINING & COLLABORATION

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In April 2022, GreenWave launched the Regenerative Ocean Farming Hub: an open-source platform designed to train, support, and connect seaweed farmers across North America and around the world. In this free-to-anyone digital space, beginning kelp farmers can find curricula and interactive tools to support them throughout planning, permitting, designing, and deploying their farms; as well as how-to videos and lessons on outplanting, monitoring, harvesting, and selling their commercial crops. The real magic, though, is the broader community of farmers this Hub supports. The Hub is designed to encourage collaboration and knowledge exchange across the seaweed industry. In an online community forum, beginning and advanced farmers alike can ask questions of one another, forge connections, and share innovations and updates coming off their farms. In less than a year, the Hub has attracted a global audience of over 3,000 users.

In this presentation, we'll give a tour of the Hub, share examples of our training curriculum, and show how experienced farmers, scientists, and industry experts have used the platform to directly connect with and support active ocean farmers. Additionally, we'll show how GreenWave's broader training & support programming builds off the Hub curriculum to support new and active farmers. We'll share highlights from our guided 6-week learning journey, How to Start a Kelp Farm, where a cohort of aspiring kelp farmers worked through course material, assignments, and attended weekly live sessions with guest experts. Lastly, we'll introduce GreenWave's Farmer Forum, a monthly series of technical conversations and in-person gatherings on seasonally-relevant topics, designed to facilitate connections and community among active farmers. We'll make the case that collaboration — more than competition — will propel the industry forward, and invite members of the broader aquaculture community to join in the movement.

THE USE OF FISH HATCHERIES AND RELATED PROGRAMS IN LOUISIANA TO EDUCATE LOUISIANA STUDENTS AND THE PUBLIC ABOUT FISHERIES MANAGEMENT AND STEWARDSHIP AND PROVIDE FISHING OPPORTUNITIES

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The Louisiana Department of Wildlife and Fisheries (LDWF) manages four freshwater fish hatcheries throughout Louisiana. The hatcheries primarily enhance sport fish populations, increase recreational fishing opportunities, and on occasion, restore desirable sportfish populations in newly renovated waterbodies. The four fish hatcheries include Booker Fowler Fish Hatchery (Forest Hill, LA), Beechwood Fish Hatchery (Forest Hill, LA), Monroe Fish Hatchery (Monroe, LA), and Huey P. Long Fish Hatchery (Lacombe, LA). The hatcheries spawn, hatch, and raise a number of important native freshwater fish species including Black and White Crappie; Hybrid Striped Bass, Blue, Channel, and Flathead Catfish; Bluegill; Redear Sunfish; Threadfin Shad; and Paddlefish. LDWF also raises Florida Largemouth Bass rather than the Northern Largemouth Bass (Louisiana's native bass) since they grow larger, to provide anglers with the potential to catch a trophy-sized fish. The Northern Largemouth Bass, however, has been known to strike a lure better than the Florida Largemouth Bass, so the goal is to create a hybrid of the two subspecies offering anglers the opportunity to catch bass that are both robust and willing to bite. The LDWF Fisheries Outreach section is a crucial component to bridge LDWF fisheries management programs and their mission with public knowledge and perception about Louisiana's natural resources.

The Booker Fowler Fish Hatchery participates in a program called Native Fish in the Classroom (NFC). LDWF collaborated with Louisiana Sea Grant (LSG) in 2002 to develop NFC and provide students in grades 6-12 a hands-on approach to learning about stewardship, Louisiana's aquatic natural resources, and fisheries management. Paddlefish were chosen because their life cycle coincides with the school spring semester, they grow rapidly, and they are a unique fish species. The objectives for this program are: (a) assist students in developing an attitude of stewardship towards the state's natural resources, (b) maintain a classroom-based nursery aquarium and grow Louisiana native paddlefish from eggs to fingerlings, and (c) provide students with information about fisheries management, fish biology, water quality science, species conservation, and aquatic natural resources. During late February/early March students attend a field trip at the Hatchery to assist with Paddlefish spawning. After spawning, students raise the eggs in their classrooms to fingerling size and then release the fish at locations approved by LDWF. As of 2022, NFC has 24 teachers and approximately 2,200 students participating from 22 schools, across 19 cities in 18 parishes. LDWF Fisheries Outreach continues to educate students and the public about conservation, stewardship, and fisheries management using fish hatcheries and programs such as Native Fish in the Classroom funded by the Sport Fish Restoration Program.

GENETIC IMPROVEMENT AND MOLECULAR CHARACTERISATION OF TWO STRAINS OF OREOCHROMIS NILOTICUS

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Complete diallel hybridization and molecular characterization of two strains of *Oreochromis spp* (Makurdi and Swansea strains) was carried out to determine genetic distances, phylogeny of pure strains and their hybrids. Four crosses, namely ♀exotic *O. niloticus* × ♂exotic *O. niloticus* (Treatment 1), ♀local *O. niloticus* × ♂local *O. niloticus* (Treatment 2), ♀exotic *O. niloticus* × ♂local *O. niloticus* (Treatment 3) and ♀local *O. niloticus* × ♂exotic *O. niloticus*. (Treatment 4) were made. Eight 1×1×1m^3 hapa was constructed and placed in a concrete tank of 5×5×1.2m^3 dimension. Broodstocks of the different crosses were sexed and stocked in ratio 1 male: 2 females in the hapas to spawn. Twenty (20) fry were collected from each cross and their weight and length were taken. The fry was reared for 14 weeks in the indoor hatchery of University of Agriculture, Makurdi, situated at the north core in eight bowls of 55 litres each. Fry were fed *ad libitum*. Fecundity, Hatchability, Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI) of pure lines were determined. Physicochemical parameters (Dissolved Oxygen, Temperature and pH) of water were measured fortnightly using multi-channel water parameters checker. DNA was extracted from pectoral fin of F1 generation of the crosses. Forward and reverse primers (VF

(Continued on next page)

Table 1: Population Diversity indices for crosses between local and exotic tilapia

Indices	d	S.E.
Mean diversity in entire population	0.0493	0.0070
Mean inter-population diversity	0.0450	0.0067
Mean diversity within sub-population	0.0043	0.0019
Coefficient of differentiation	0.9125	0.0382
Net between population mean distances	0.0675	0.0101

d=Diversity; S.E.= Standard Error

Table 2: Pairwise Kimura-2 parameter (k2p) distances of crosses between exotic and local tilapia.

Treatment	♀E × ♂E	♀L × ♂L	♀E × ♂L	♀L × ♂E
♀E × ♂E		*0.0106*	*0.0026*	*0.0107*
♀L × ♂L	0.0717		*0.0108*	*0.0020*
♀E× ♂L	0.0057	0.0735		*0.0106*
♀L × ♂E	0.0716	0.0029	0.0702	

♀E = Female Exotic, ♂E= Male Exotic, ♀L = Female Local, ♂L= Male Local

Table 3: Growth Parameters of Pure, hybrid and heterosis for growth and survival of hybrids of *Oreochromis niloticus* reared in plastic bowls.

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Initial Weight (g)	0.30± 0.00^c	0.28±0.00^b	0.30±0.00^c	0.27±0.00^a
Initial Total length	1.30± 0.05	1.30± 0.00	1.30± 0.00	1.30± 0.04
Initial Standard length	1.09± 0.03^c	1.07± 0.00^a	1.10± 0.00^d	1.08± 0.00^b
Final weight	4.76± 2.31^b	1.94±0.73^a	4.89±1.52^b	3.59± 0.97^b
Final standard length	6.50±1.49^b	4.76±0.73^a	6.86±0.52^b	6.14±0.57^b
Weight gain	4.5± 2.00^b	1.7± 0.72^a	4.6± 1.50^b	3.3 ± 0.95^b
Survival	81.3± 2.31^a	80± 4.00^a	82.7± 2.31^b	80± 4.00^a
H for Growth (%)	-	-	45.97	2.54
H for Survival (%)	-	-	2.54	-0.81

Mean in the same column with different superscript differs significantly (p<0.05).***H*= *H*eterosis, ♀E = Female Exotic, ♂E= Male Exotic, ♀L = Female Local, ♂L= Male Local**

5'-GTAAAACGACGGCCAGTCAACCACAAAGACATTGGCA-3'; R 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3') were synthesized to target and amplified the COI gene. The gene sequences were analyzed independently using Maximum Likelihood (ML) and Pairwise distance methods. Kimura-2 Parameter and phylogenetic tree was constructed using Neighborhood Joining of MEGA 7.0.2 at 1000 bootstrap. Genomic DNA was successfully extracted from all the treatments and COI sequenced with the primers designed. The gene sequence was aligned and edited using BioEdit software. Treatments 1 and 3 of the same maternal origin had the highest DNA weight of 710 bp. The DNA sequences for the four treatments were deposited in GenBank at National Center for Biotechnology Information (NCBI) with Accession numbers MK130700, MK130701, MK130702 and MK130703 for treatments 1, 2, 3, and 4 respectively using the bankit format. When Basic Local Alignment Search Tool (BLAST) was used on the gene sequences of the four treatments, treatments 2 and 4 had 99% identity with *O. aureus* inferring they have maternal origin linked to *O. aureus* rather than the originally thought *O. niloticus*. The phylogenetic tree revealed monophyly with two sub-clades at 95% bootstrap, treatments 1 and 3 of the same maternal origin of exotic strain clustered, while treatments 2 and 4 of same maternal origin (local strain) clustered at a separate sub-clade(Table 1). Treatment 3 had the best performance in terms of growth (4.6g), heterosis (45.97) and survival (82.7%). The result of statistical analyses shows that there is significant difference in growth and length between the different crosses (P<0.05)(Table 2). Treatment 3 is recommended for culture due to overall best performance. From the results of laboratory experiment, it is concluded that *O. niloticus* populations obtained from university farm were really *O. aureus*. All the crosses in the four treatments bred through, confirming the possibility of hybridization among the species, thus intraspecific hybridization also was found to be advantageous as maternal inheritance from the exotic female confers hybrid vigor on local strain (Table 3).

Growth performance was monitored and F1 hybrid with the best heterosis was recommended for culture.

ASSESSMENT OF WATER UTILIZATION, WATER QUALITY AND NUTRIENTS IN INTEGRATED RICE-FISH FARMING

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Options for farm diversification through integrated aquaculture-agriculture are being gradually embraced as an alternative to traditional agriculture because of resource use efficiency and increase in overall productivity. Despite numerous ecosystems benefits, the practice of integrated rice-fish (IRF) farming in African countries such as Nigeria is limited. Limited understanding of water utilization and quality in the rice - fish ecosystem can pose a challenge adoption of IRF. A better understanding of the water utilization dynamics of IRF could improve overall food production.

This study, therefore, investigated the water utilization, water quality and nutrient dynamics of the IRF system using an MSU/USAID/FAO IRF plot at the University of Ibadan, Nigeria as a case study. The study was carried out at the University of Ibadan, Department of Aquaculture and Fisheries Management plot (22m x 15m). Fourteen-day rice seedlings were transplanted. Each seedling was spaced 20cm apart on a IRF plot platform. Three weeks after the rice transplanting, the platform was filled with water covering lower part of rice stem and stocked with 1500 *Clarias gariepinus* fingerlings (4.0±1.5g). The fish were fed to satiation three times daily using project formulated feed. This trial was repeated three times over three production cycles lasting 16 weeks each. Biweekly water quality and monthly composite soil samples were collected and analyzed. Water productivity, efficiency and use were also monitored.

All parameters examined were within acceptable range for fish or rice culture. The number of plankton ranged from 50 x 10^3/m^3 to 230 x 10^3/m^3 while in abundance ranged from 148 to 208 (cells/L). Both the number and abundance fluctuated depending on when the fish were stocked with the highest values at week 4 of the trial cycles. The result for water use (m^3), water efficiency (kg/ha cm) and water productivity (kg/m^3) were 440±20.62, 0.9±0.02 and 900±80.2, respectively. These preliminary results indicates that wider adoption of integrated rice-fish system could address water use efficiency while also increasing productivity.

Table 1: Biweekly water quality parameters result

DATE	WK 2	WK 4	WK 6	WK 8	WK 10	WK 12	WK 14	WK 16
ALKALINITY (mg/l)	106.8±22.0	89±45.1	106.8	71.2	71.2	89.0	89.0	106.8
pH	8±2.0	7.5±2.0	7.5±1.2	7.5±1.0	7±0.7	7±1.2	7±0.5	7±1.2
NITRATE (mg/l)	0	0	0	5±0.1	0	0	0	5±0.02
NITRITE (mg/l)	0	0	0	0.25±0.01	0	0	0	0.25±0.02
AMMONIA (mg/l)	0.2±0.01	0.5±002	0.5±0.01	0.1±0.01	0.1±0.04	0.2±0.01	0.2±0.01	0.2±0.02
HARDNESS (mg/l)	89±1.4	178±5.3	106.8±3.0	106.8±1.2	106.8±8.0	106.8±3.1	106.8±4.7	178±4.1
D.O (mg/l)	5.2±0.04	5.4±0.2	5.4±0.7	5.3±1.01	5.3±0.8	4.8±0.04	4.9±0.6	4.2±0.3

Table 2: Biweekly soil quality parameters result

SOIL PARAMETERS	MONTH 1	MONTH 2	MONTH 3	MONTH 4
pH	7±0.1	7±0.1	7±0.2	8±0.2
NITROGEN (g/kg)	1.5±0.2	1.3±0.01	1.8±0.2	0.8±0.01
PHOSPHORUS (mg/kg)	11±1.5	13±0.2	8.8±0.1	8.2±0.1
D.O (mg/l)	4.8±0.1	4.2±0.01	5±0.2	5.1±0.1
ORGANIC CARBON (g/kg)	21±2.2	25±3.1	14±2.4	11±2.1
POTASSIUM (cmol/kg)	0.21±0.1	0.17±0.01	0.19±0.03	0.15±0.01

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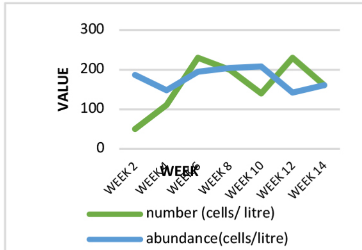

Figure 1: Number and abundance of plankton

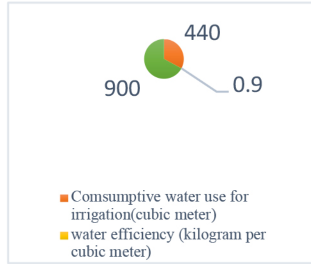

Figure 2: Water productivity, efficiency and consumptive water use result

PARTIAL AND TOTAL REPLACEMENT OF SOYBEAN WITH AFRICAN PALM WEEVIL *Rhynchophorus* ferrugineus LARVA IN THE DIET OF NILE TILAPIA *Oreochromis niloticus*

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The production of quality fish feed is considered a critical factor in aquaculture as it ensures growth efficiency, quality flesh and food utilization. High demand for the conventional feed ingredient by other sectors contributed to expensive and competitive nature of conventional feed ingredients. Current researches in animal nutrition are focused on the use of alternative cheaper energy resources that could replace cereals and supply the required nutrient in adequate amount (Adejumo, 2005). The African palm weevil (Plate 1) is highly nutritious as it is rich in protein, polyunsaturated fatty acids and some minerals (Table 1).

Rhynchophorus ferrugineus larvae meal can partially replace SBM in the diets of Nile tilapia without compromising the growth performance, feed efficiency, and health condition. Therefore it can be used to improve and sustain nutritional composition of fish diet. It was also concluded that *O. niloticus* fed with 100 % inclusion of RF performed best in the growth indices. They had the highest weight gain, feed intake and their fed conversion ratio was the best.

Table 1. Nutritional and mineral compositions of *Rhynchophorus ferrugineus*

Parameter	Composition (%)
Dry matter	94.35
Crude protein	42.46
Crude ether extract	27.54
Ash	5.64
Nitrogen free extract	18.71
Mineral elements	***mg/100g***
Potassium	1015
Phosphorus	650

Table 2: Gross composition (g/kg) of experimental diets for *O. niloticus*

Feed Stuffs (g)	RF1	RF2	RF3	RF4	RF5
Yellow maize	428	428	428	428	428
Rice bran	428	428	428	428	428
Soybean meal	629.2	471.8	314.6	157.4	0
Fishmeal	314.6	314.6	314.6	314.6	314.6
Palm kernel weevil	0	157.4	314.6	471.8	629.2
Fish oil (*g / vol.*)	60	60	60	60	60
Vitamin/mineral premixes	100	100	100	100	100
Starch	40	40	40	40	40

Table 3. Proximate Composition (%) of Experimental Diets

Parameter	RF1 (Control)	RF2	RF3	RF4	RF5
Moisture content	10.84 ± 0.00^{b}	11.03 ± 0.00^{b}	9.85 ± 0.00^{a}	10.18 ± 0.33^{a}	10.88 ± 0.00^{b}
Ash content	8.71 ± 0.00^{b}	8.50 ± 0.00^{b}	8.70 ± 0.00^{b}	8.43 ± 0.26^{b}	7.95 ± 0.00^{a}
Lipid content	11.00 ± 0.00^{b}	10.82 ± 0.00^{ab}	10.71 ± 0.00^{a}	10.83 ± 0.12^{ab}	11.85 ± 0.00^{c}
Crude protein	37.68 ± 0.00^{a}	38.03 ± 0.00^{ab}	38.46 ± 0.00^{b}	38.18 ± 0.28^{b}	38.98 ± 0.00^{c}
Crude fibre	4.72 ± 0.00^{ab}	4.42 ± 0.00^{ab}	4.87 ± 0.00^{b}	4.58 ± 0.29^{ab}	4.30 ± 0.00^{a}
Nitrogen free extract	27.03 ± 0.00^{b}	27.18 ± 0.00^{bc}	27.38 ± 0.00^{bc}	27.77 ± 0.38^{c}	26.00 ± 0.00^{a}

Plate 1: Dorso-lateral view of *Rhynchophorus ferrugineus*

EFFECTS OF HIGH LATITUDE™ OIL INCLUSION ON FISH GROWTH, FILLET n-3 LC-PUFA CONTENT, SURVIVAL, AND HEALTH WHEN FED TO RAINBOW TROUT *Oncorhynchus mykiss* OVER A PRODUCTION CYCLE

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Latitude™ oil is a transgenic canola oil that contains omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Previous research demonstrated that in rainbow trout diets, latitude oil is highly digestible (93%) and a potential candidate as a lipid replacement for diets formulated with fish oil and poultry fat. Given the success of previous research, this study evaluated the effects of high Latitude oil inclusion in trout feeds with the intent to replace fish oil in rainbow trout diets. Three isonitrogenous (50%), isolipidic (16%), and isocaloric (24 MJ/kg) diets that met or exceeded the published minimum requirements for rainbow trout were formulated. Diet 1 (FPO) consisted of a 7.84 % fish oil and 11.53% poultry fat blend, Diet 2 (LPO) contained 16% Latitude oil and 3.26% poultry fat blend, and Diet 3 (LO) consisted of 19.3% Latitude oil. Fish were initially stocked at 17g and were fed their respective diet for 47 weeks.

At the conclusion of the study, there was no significant differences between diets regarding condition factor, survival, feed conversion ratio, hepatosomatic index, visceral fat index and fillet yield. However, final weight, weight gain, and feed intake of fish fed LPO and LO were higher ($P < 0.05$) in comparison to fish fed the control (FPO). Histological analysis of the liver and distal intestine showed no significant morphological differences amongst treatments. Furthermore, LO inclusion reduced hepatic alanine transaminase, suggesting liver health was improved. Analysis of fillet fatty acid content resulted in no significant differences in total EPA + DHA (% FAME) as well as n-3 LC-PUFA (% FAME) amongst diets suggesting dietary docosapentaenoic acid (DPA) and EPA was converted into DHA and deposited into fillet. Overall, Latitude oil improved fish growth and fish health supporting its use as a potential omega-3 lipid source for rainbow trout feeds.

EXAMINING THE GROWTH OF BULL KELP *Nereocystis luetkeana* FROM HATCHERY TO LONG LINE CULTIVATION IN HUMBOLDT BAY, CA

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The cultivation, restoration, and conservation of macroalgae are emerging mariculture practices in the United States. In spring of 2019, Cal Poly Humboldt partnered with GreenWave to establish one of the first small-scale commercial seaweed farm, ProvidenSea, in California. Since then, the farm has been home to several research projects that focus on regenerative and related initiatives in Humboldt Bay. The purpose of this proposal is to evaluate the cultivation of bull kelp (*Nereocystis luetkeana)* in a hatchery at the Cal Poly marine lab in Trinidad, CA, and successfully integrate them to grow out in open water in Humboldt Bay, CA.

This project will consist of 8 aquaria replicates containing spools (pvc wrapped with speeding string). We will evaluate the growth and morphology of bull kelp using a variety of seeding strings commonly used in different regions for open water cultivation of macroalgae.Once the spools reach the optimal development stage and length (juvenile sporophyte at 3 mm in length), they will be transported to Cal Poly Humboldt ProvidenSea seaweed farm, where they will be out planted in the longline for further assessment of growth.

Nutrients in the seaweed's tissues and surrounding water will be analyzed to determine the extractive properties of macroalgae grown in the bay.

Presently, this is one of the first attempts to grow bull kelp from a hatchery to an ocean farm setting on the northern Pacific coast of California. Results from this study will help in expanding the under-developed research in bull kelp cultivation practices, early gametophyte settlement and provide a foundation for future farmers in regenerative seaweed farming practices within the California northern pacific coast.

Fig 1. Remote sensing imaging of Trinidad Bay wild bull kelp canopies where sori are collected. Image captured and processed by Hannah Joss

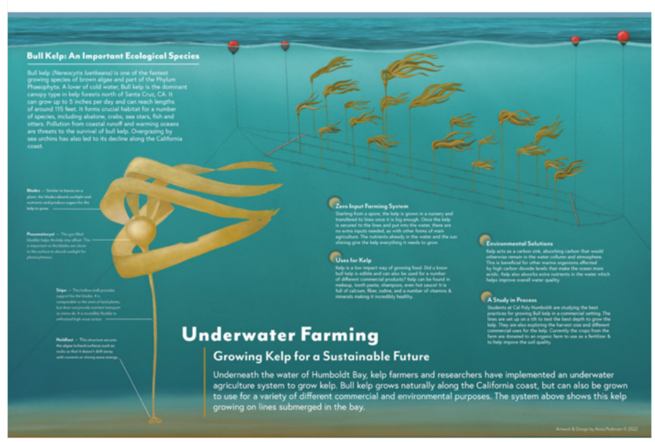

Fig 2. ProvidenSea multi-line system array. Illustration designed by Anna Pederson.

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

VITRIFICATION AS AN ALTERNATIVE TO CONTROLLED-RATE FREEZING OF *Aplysia californica* LARVAE

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The California sea hare (*Aplysia californica*) is an important biomedical model for studies of neurobiology, electrophysiology, learning, and memory due to its well-mapped large neurons and learning capabilities. The National Resource Center for *Aplysia* (NRA, University of Miami) maintains large stocks of live animals and relies on wild sources to maintain genetic diversity. This is risky, cost intensive, and requires labor. One solution to these problems is cryopreservation, which is the process of preserving genetic material, typically sperm, at cryogenic temperatures to be thawed and used at a later time. However, *Aplysia californica* presents additional challenges. For instance, the sperm cannot be extracted efficiently. Instead, early life stages are being used as an alternative substitute. In addition, the early life stages are packaged in egg capsules that are encased within an egg strand. The multiple layers of the egg strand, capsules, and larvae can make the transfer of cryoprotectants, heat, extraction of water, and prevention of ice crystal formation difficult. Vitrification is a specific type of cryopreservation in which freezing occurs so rapidly that ice crystals do not have time to form, resulting in an amorphous glass, minimizing damage to the cell within a sample. This method may be more effective than controlled-rate freezing when cryopreserving the early life stages of *Aplysia californica*. In preliminary experiments, egg strands with veliger-stage larvae were exposed to one of six types of vitrification solutions, loaded in an inoculation loop, plunged and stored in liquid nitrogen, and thawed in a 40°C artificial seawater bath. Several treatments contained larvae with moving cilia, suggesting survival. However, these larvae were damaged with broken shells, were irregularly shaped, or had ruptured velums suggesting they had experienced some ice crystal damage. The method used in this experiment is still preliminary, but the presence of larvae with moving cilia suggests that vitrification may prove to be a suitable method for cryopreserving the early life stages of *Aplysia californica*.

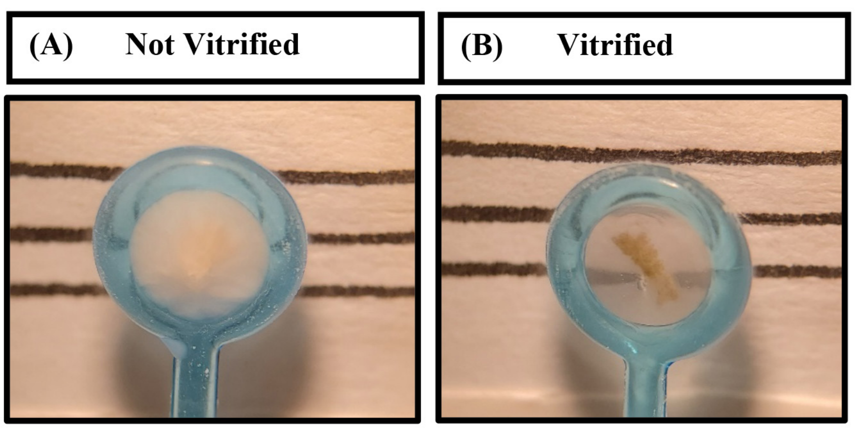

Figure 1: *Aplysia* egg strands in an inoculation loop with 32 ppt artificial saltwater **(A)** and 50% dimethyl sulfoxide **(B).**

MICROBIOME EVALUTIONS IN LARGE SCALE RAINBOW TROUT PRODUCTION FACILITIES

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Understanding host, microbiome and environment interactions is key to optimizing an animals' overall health and feed utilization. Unlike terrestrial animals, fish live in a three-dimensional milieu of microbes and therefore understanding these interactions is of upmost importance for aquaculture. In this project replicant samples were taken from three different rainbow trout production facilities which included environmental- (diets, water, raceway biofilms) and and host-associated (gill, skin mucosa, intestinal mucosa and digesta of fish). The facilities varied in configuration, and number of water uses (either 4 or 8 passes), but the set of samples obtained included triplicate raceway samples from each rearing unit. Using 16SrRNA gene sequencing, samples were characterized for relative microbiome richness and diversity (Fig 1). Within this large and complicated data set the goal is to determine the variance within water usage across replicate raceways within and between facilities and analyze for significant changes between water usages, across disparate sample types. Furthermore, our study highlights relative pathogen levels across sample-types and rearing units and will ultimately be used to evaluate interactions with on-farm water quality. An example of this is demonstrated in Figure 2 where the relative abundance of the bacterial pathogens *Aeromonas* and *Flavobacterium* are presented for the water and fish skin in 3rd-use water. Further in-depth analyses from this large-scale dataset will be presented. This is study serves as an initial study to determine on-farm microbiome compositions in production-scale systems and how they vary within and between farms. As nearly all aquaculture microbiome results presented in the literature being from laboratory studies, this is among the first microbiome studies conducted in production conditions, providing valuable insight for future studies.

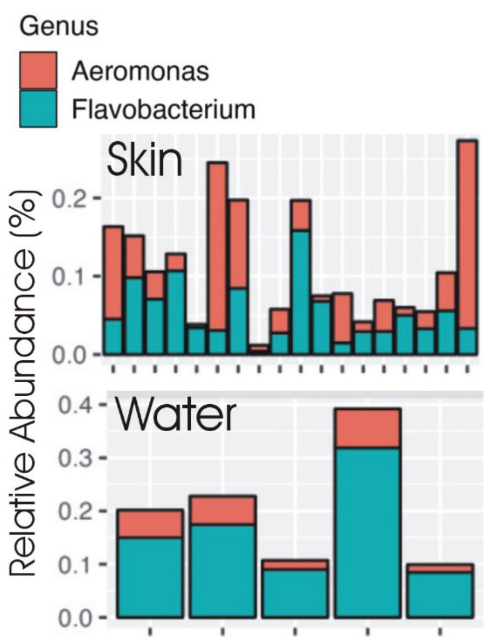

Figure 1. Alpha Diversity of Raceway Microbiome samples by sample type.

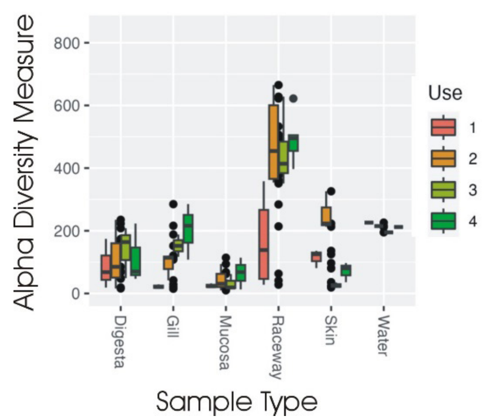

Figure 2. Relative abundance of the two types of pathogenic bacteria, Aeromonas and Flavobacterium found in the water and on the skin of rainbow trout in 3rd use production water.

ESTABLISHING A PRIMARY CELL LINE FROM THE EASTERN OYSTER *Crassostrea virginica,* FOR DEVELOPING CULTIVATED OYSTER

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The world population is rapidly growing and is predicted to grow to 10 billion by 2050, as estimated by the food and Agriculture Organization (FAO), requiring a 70% rise in total food production and 100% increase in meat production to satisfy nutritional requirements and global demand. Thus, developing novel technologies to produce food products is needed.

This study aimed to optimize primary cell line development from adult Eastern Oyster, Crassostrea virginica, as a model bivalve by applying different tissue decontamination, cell dissociation, and culture conditions. Oyster adductor (OAD) cells were obtained via tissue explant, mechanical and enzymatic digestion. Culture vessels were coated with surface proteins such as fibronectin, laminin, Matrigel, and poly-d-lysine to promote cell attachment. The tissue decontamination with Penicillin-Streptomycin (100 μg/mL), Amphotericin B (0.25 μg/ml), and algaecide solution (0.03%) were effective in controlling contaminations. OAD cells grew best at lower nutrient levels in the one-to-one ratio of Lebovitz L-15 media and artificial seawater. Lower fetal bovine serum levels, 1-5%, provided a high number of cell attachments and consistent growth in combination with 1% adult oyster whole-body or larvae extract. The tissue explant method resulted in optimal cell dissociation compared to other methods, and proceeding cultures had attached cells surviving for up to 10 days. All the plate coatings promoted cell attachment. However, fibronectin provided optimal cell attachment of OAD cells. Fibroblast-like, neuron-like, epithelial-like, and rounded cells were observed. Fluorescence cell staining confirmed the presence of cytoskeleton and nuclei in the OAD cell cultures. These advances in primary cell culture methods of OAD cells may be beneficial for establishing mollusk cell lines for cultivated seafood production.

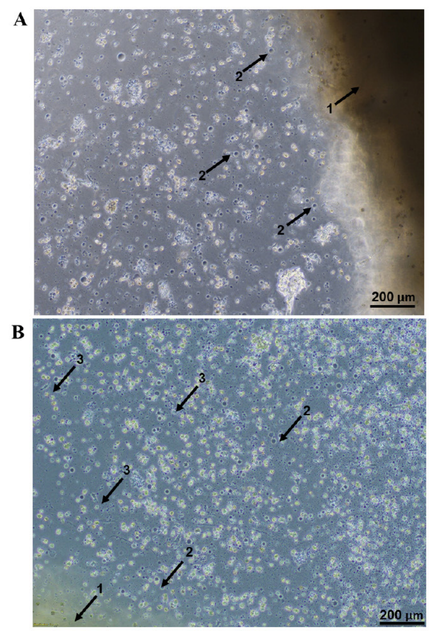

Figure 1. Primary oyster adductor muscle cells dissociate from tissue explant within (A) 24 hours and (B) 48 hours of plating the tissue samples. (1) Tissue pieces (2) Rounded cells dissociating out (3) Dissociated cells starting to elongate and differentiate.

CULTIVATED SEAFOOD: FROM CELL TO FORK

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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).

Future Foods Lab and Cellular Agriculture Initiative program at Virginia Tech. is developing novel cultivated seafood products from Farm to Fork, by focusing on cell line development, media optimization, scaffold development, and addressing food safety challenges.

EVALUATION OF THE EFFECTS OF PROBIOTICS ON THE GROWTH PERFORMANCE OF NILE TILAPIA *(Oreochromis niloticus)* IN A HIGH DENSITY BIOFLOC SYSTEM

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Biofloc technology (BFT) is an aquaculture system that has gained popularity with tilapia producers. Probiotics provide benefits for the host and/or the environment. The use of probiotics has been reported in some cases to enhance growth performance, improve water quality, and prevent infections. One of the administration routes for probiotics is to apply them through the feed. When a probiotic is combined with a biofloc system, the production yield may be improved through better fish growth, disease resistance or enhanced survival. The objective of this research was to evaluate the growth performance of Nile tilapia *Oreochromis niloticus* while offered commercial feed top coated with 2 different probiotics. Nine independent 1000-gallons tanks were set with heavy aeration and managed as individual biofloc type systems. The tanks were stocked with 120 fish/tank (mean initial weight 71.4 ±4.4g). The fish were offered commercial feed (38% protein floating tilapia feed, Optimal Aquafeed, Omaha, Ne) that was top coated with two probiotics, AP193 (*Bacillus* spp.; provided by Dr Mark Liles, Auburn University), and Biowish® Feedbuilder (Biowish technologies ® Cincinnati, OH, USA). The same commercial feed was used as reference resulting in 3 experimental treatments. The trial was conducted for 109 days, and the fish were fed twice a day. The results of the growth trial indicated no significant differences in weight gain percent ($p < 0.4028$), survival ($p<0.4352$), FCR ($p<0.1742$) and final mean weight ($p<0.1293$) of tilapia offered the three diets. Even though the growth performance results presented no significant differences, the results could differ based on the concentration and the route of the probiotic administration, but most importantly, their impact on the microbial community of the water developed in the Biofloc system. Problems with high total ammonia nitrogen were observed in all treatments, what can affect the fish productivity. The fish health is also being analyzed as a secondary part of the trial.

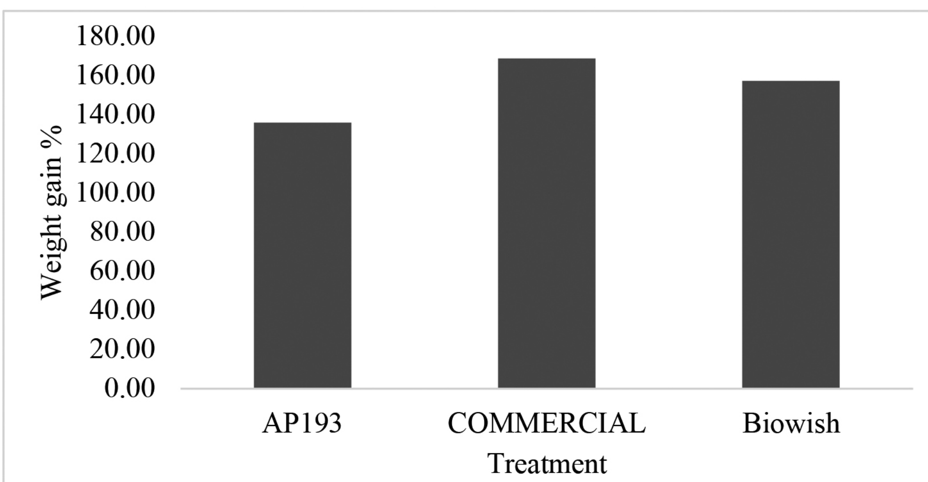

Figure 1: Average of weight gain percent between treatments.

INFLUENCE OF PROBIOTICS ON THE HEALTH OF NILE TILAPIA (*Oreochromis niloticus*) RAISED IN BIOFLOC SYSTEMS

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Biofloc technology is a rearing technique that encompasses water quality by manipulating carbon and nitrogen and their inherent mixture of organic matter and microbes. Biofloc systems have several advantages, which include improved biosecurity, feed conversion, water use efficiency, and increased health and survival of rearing organisms. Beneficial micro-organisms present in biofloc systems contain compounds such as organic acids that may deter the growth of pathogenic microbes. They will also serve as a natural probiotic and increase the immunity and survival of fish and shrimp. This technology could be useful for further integration within many aspects, such as adding probiotics to manipulate the microbial community and its interactions within biofloc systems.

In the present study, two probiotics (AP193 and BiOWiSH FeedBuilder Syn 3) are evaluated for use in Nile tilapia culture in a biofloc system. Nine individual circular tanks (1000 gallons) were stocked with 120 juveniles (71.4 ±4.4 g). Tilapia were randomly assigned 3 diet treatments: a commercial reference diet feed, commercial feed top-coated with AP193, and commercial feed top coated with Syn3. The trial was conducted for 15 weeks, and water quality parameters were kept at their standard limits. At the end of the feed trial, the fish were challenged with *Streptococcus iniae* (ARS-98-60, 6.6 x 10^8 CFU/mL, via intraperitoneal injection) in a common garden setup.

At 10 days post-infection, cumulative percent mortality (CPM) differed across the treatment groups. The CPM of the commercial diet-fed fish was 78±12 %, the AP193-fed fish was 35±8 %, and that of Biowish-fed tilapia was 18±11%. The mortality of fish that did not receive probiotics was significantly higher than AP193-fed fish (p=0.009) and Biowish-fed fish (p=0.003). These values indicate that adding probiotics to Nile Tilapia reared in biofloc systems enhances the disease resistance, and potential immune response against *S.iniae*. Serum lysozyme activity was also analyzed pre- and post- challenge. No differences were found between the dietary groups pre-challenge (p=0.29) and post challenge (p=0.21). To further discern the tilapia immune responses pre- and -post challenge, gene expression of pro inflammatory cytokines is still ongoing. Furthermore, the microbial community of the water was evaluated, and results will be presented. Based on these findings, the probiotic-supplemented diets appeared to enhance tilapia survival after long-term rearing in biofloc culture systems.

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 in 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 up to 100 fish per pond using an electrofishing boat with dip nets. Study ponds will be sampled by researchers 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 the total length (mm), weight (kg), and sex of fish will be recorded. 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. Additionally, looking at factors like seining frequency and pond characteristics will contribute to solutions for the big fish issue. This study is currently in the data collection phase.

DETERMINING THE FACTORS AFFECTING CONSUMER WILLINGNESS TO PAY FOR SEA FOOD SAFETY DECISIONS IN TEXAS, UNITED STATES

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Food safety and consumer satisfaction have become one of the critical issues being discussed across the globe. The process of producing the food is changing and the demand for safe food is also increasing. These demands are challenging the food system to emphasize more on their needs and to meet their demands on healthy, safe, and environmentally sound food products. A study was conducted to evaluate the consumer perceptions on food safety issues and the factors affecting consumers' willingness to pay when the information on food safety changes in Texas. Texas was considered as the site of study due to its diversity and influx of population in recent years. To accomplish the objectives, focus group discussions were conducted to find out the baseline information. Using that baseline information, a survey was designed and implemented to collect the data.

The major portion of the consumers were either neutral or disagreeing with the food safety issues which includes voluntary recalls after encountering food safety issues, same standards of farmers market and supermarket, protection from food borne illness, and same standard of farm fish and wild caught fish demonstrating a knowledge gap providing the spaces for policy recommendation. Multinominal logistic regression was conducted to identify the factors affecting consumers' willingness to pay under company recalled, United States Department for Agriculture (USDA) recalled, Hazard Analysis Critical Control Point (HACCP) controlled and blockchain technology controlled seafoods. It identified the education level and perception have positive impact consistently. Further consumers were willing to pay extra price for food safety was also found significant. However, the elderly consumers (50 and above) were not willing to pay for food safety decisions. Note: This is the result of preliminary data analysis, and more findings will be presented in the final draft.

Table 1: WTP after 3 months for one pound of USDA recalled fresh farm raised catfish (Base price: $6.00)

Price	Extra price for safety	Age group (50 and above)	Education (Master's degree)	Perception
$6.50	1.55 (.53)***	-2.17 (.69)***	.75 (.67)	.06 (.043)
$7.00	2.33 (.50)***	-2.20 (.64)***	1.01 (.61)*	.078 (.03)**
$7.50	3.50 (.64)***	-2.32 (.78)***	1.37 (.78)*	.08 (.047)*
$8.00	3.76 (.77)***	-3.66 (1.02)***	1.56 (.94)*	.079 (.05)

ANTIBACTERIAL ACTIVITY OF SEA STAR, *Luidia clatharata,* BODY WALL AGAINST SLECETED PATHOGENIC BACTERIA

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As antimicrobial resistance to conventional antibiotics has become prominent, it is crucial to look for effective antimicrobial agents in natural resources. Among all resources, marine environment is one of the potential resources to explore as it is the hub of various natural bioactive compounds. To this end, we performed research in *Luidia clatharata* (slender armed sea star), richly endowed species with bioactive potentials. Specifically, the goal of this research is to investigate the antimicrobial activity of *L. clatharata* against the selected five gram-negative and five gram-positive bacteria. The result obtained from the experimentation demonstrates that the crude extract of the body wall with ethyl acetate exhibits impressive inhibitory ability against the tested pathogens. This diffusion ability signals that *L. clatharata* could serve as an important natural resource for nutraceuticals. For this, a detailed study is required to identify and understand the active compound responsible for this inhibitory effect. The detailed findings will be discussed at the conference.

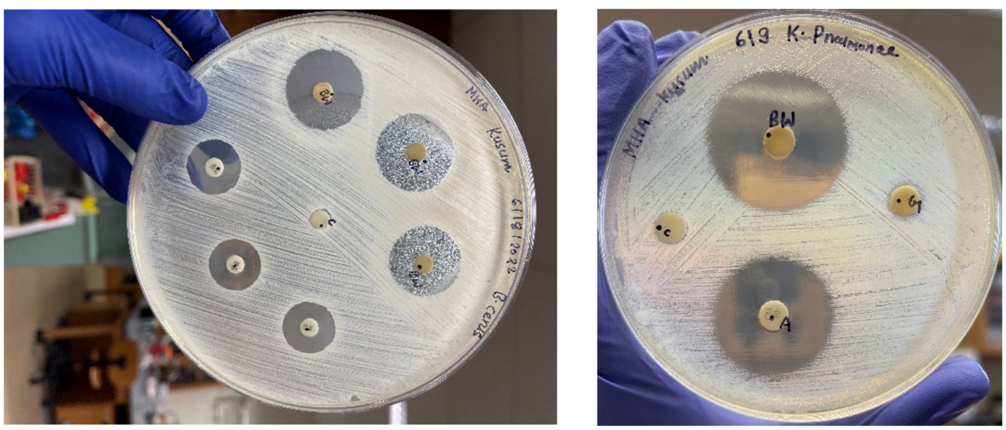

Photo: Anti-bacterial activity exhibited by sea star body wall extract on *Bacillus cereus* (left) and *Klebsiella pneumoniae* (right).

PHYSIOLOGICAL AND BEHAVIORAL STRESS RESPONSES IN SEA STAR (*Luidia clatharata*) INDUCED BY HEAT AND ARM AMPUTATION

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Global warming leading to gradual temperature rise serves as one of the serious stressors affecting the marine community structure. Specifically, keystone predator species like sea stars are vulnerable to temperature stress in various aspects -ecology, behavior, physiology, and immunology. Additionally, these economically important species are subjected to anthropological-induced stress such as invasive fishing activities and heavy metal discharge which can potentially cause arm amputation. Since stress physiology is related to the immune function of the body, a comprehensive study of stress physiology may help understand overall health and survival. As their presence influences community structure, the general well-being and survival of a keystone predator have important ecological implications in the marine environment. Around this scope, first, we are interested in studying the effect of temperature on physiological changes. Shedding off their arm as a defensive mechanism is a very common phenomenon for starfish; however, such phenomena may result in significant energy loss leading to a change in physiological response. Therefore, studying the physiological and behavioral changes resulting from temperature stress (global warming) and arm amputation (natural predation) is important. In addition to the study of individual stress effect, it may be worthwhile to study their synergistic effect. Therefore, we are curious to investigate the effects of temperature and arm amputation in a laboratory setting that mimics the oceanic environment. We are currently observing these effects by leveraging the parameters such as 1. live coelomocyte count, 2. total protein, 3. phagocytic activity 4. righting activity, and 5. antioxidant activity. We will discuss our observations at the conference.

EFFECTS OF DIETARY CARBOHYDRATE SOURCE IN FLORIDA POMPANO GROWTH AND NUTRITIONAL PHYSIOLOGY: *Trachinotus carolinus*

Juan F. Paredes *, Marty Riche, Sahar Mejri, David Bradshaw, Carlie Perricone, Michael Habte-Tsion and Paul S. Wills

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Aquafeed expenses constitute up to 60% of the total operational costs for intensive aquaculture production. Thus, lowering prices without altering feed nutritional balance is a priority for economic sustainability of aquaculture and a target research area.

Carbohydrates are the cheapest dietary energy source. Its adequate dietary inclusion will reduce lipid and protein catabolism for energy; ultimately, reducing feed cost and ammonia release to the environment.Fish utilization of carbohydrates is specie-specific and dependable on carbohydrate source and inclusion levels. Herbivorous and omnivorous freshwater fish will utilize higher carbohydrate levels compared to carnivorous marine fish.Florida pompano reports some nutritional studies, but little is known about carbohydrate metabolism. The objective of this research is to determine the effects of different carbohydrate sources on Florida pompano growth.To this end, five isonitrogenous, isolipidic and isocaloric diets were formulated using different carbohydrate sources. At the end of the 10-week growth trial,fish were assessed:growth performance, feed utilization, body composition, hepatic enzyme activity, gene expression and gut microbiome variations.

The results of this study will reveal for the first time carbohydrate utilization in Florida pompano. The understanding of these findings will allow fish farmers to develop and formulate a more cost-effective diet. Thus, fostering the profitability of culturing Florida pompano.

TIME-SERIES ANALYSIS OF *Lepeophtheirus salmonis* AMONG NEW BRUNSWICK SALMON FARMS (2016 TO 2021)

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The Fish-iTrends database contains weekly counts of *Lepeophtheirus salmonis* and delousing treatments applied to Atlantic salmon aquaculture sites. This longitudinal repeated measures dataset provides the opportunity to estimate the effects of infestation pressures (a measure of the dose of exposure of parasitic stages of sea lice to potential fish hosts) adjusted for delousing treatments in the Bay of Fundy, New Brunswick, Canada, using a time-series model.

The sampling counts for 2016-2021 were aggregated to the site-level at weekly intervals (n = 3,137). These data were used to construct a mixed autoregressive model (AR[2]) for the abundance of adult female (AF) lice. The infestation pressures within and among sites were calculated as the time-lagged weighted averages of the abundance of sea lice. Treatment effects were evaluated by categorizing into short-term (chemical and mechanical) and prolonged treatments.

The infestation pressure of AF within sites was non-significant ($p > 0.5$); its effect was absorbed by the autoregressive function. The infestation pressure among sites (external infestation pressure), however, was shown to increase the abundance of AF by a factor of 1.28 (i.e., a relative increase of 28 % per unit increase of the variable) when other predictors remained constant ($p = 0.002$). For example, the closest sites by seaway distance contributed the most to the external infestation pressure of the selected site (Figure 1). This study provides further evidence that sea lice transmission occurs among the interconnected aquaculture sites in the Bay of Fundy.

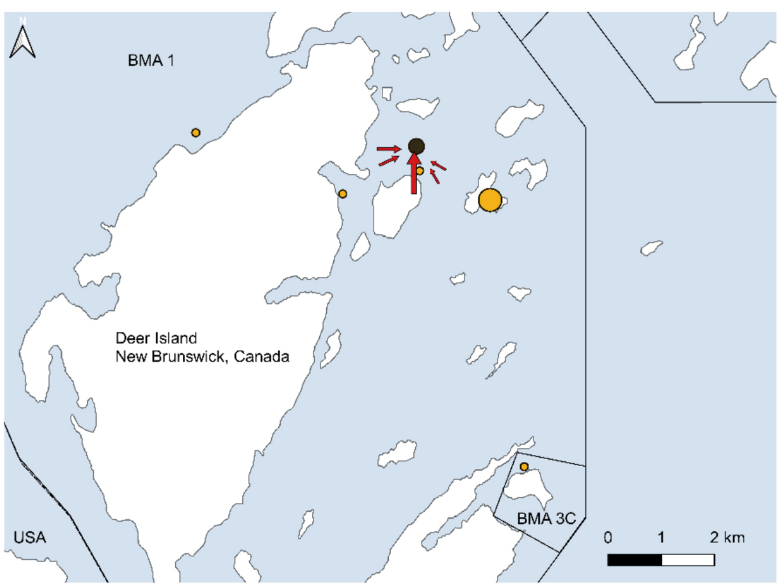

Figure 1. Site contributions (red arrows) from neighbouring sites (orange circles) to the external infestation pressure of a selected site (black circle) in the Bay of Fundy, New Brunswick, Canada. Size of the orange circles and arrows are proportionate to the exposure and contributions, respectively.

UNIVERSITY OF MARYLAND ONLINE ECONOMIC SPREADSHEET TOOL FOR OYSTER AQUACULTURE: A SHELLFISH ODYSSEY

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Come see the recently launched University of Maryland Online Economic Spreadsheet Tool for Oyster Aquaculture (Figure 1). It's not quite HAL 9000, but it's close. Come on a shellfish odyssey as I provide an overview of the tool along with a live demo if WIFI capabilities are available in the session room. Use of the tool allows for more informed financial decisions about starting or expanding a shellfish aquaculture operation. Outputs from the tool may be submitted along with a business plan to support financial projections when applying for loans.

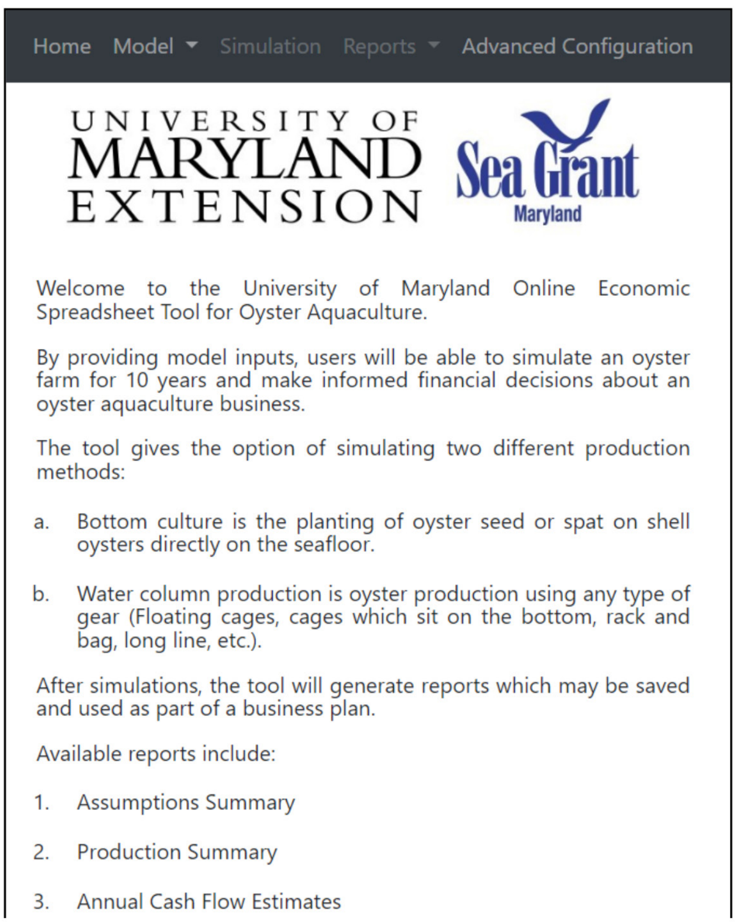

Figure 1. Screenshot of University of Maryland Online Economic Spreadsheet Tool for Oyster Aquaculture

AN ONLINE TOOL FOR BUSINESS ECONOMIC MODELING; TRANSLATING THE TECHNICAL TO UNDERSTANDABLE FOR INDUSTRY

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Often aquaculture economic research data does not easily translate to real world business planning. Providing tools that integrate advanced economic farm modeling with easily understandable interface and outputs is essential for advancing the US aquaculture industry. This presentation presents the University of Maryland Online Economic Spreadsheet Tool for Oyster Aquaculture (Figure 1) which was developed to address stakeholder needs. The tool uses Monte Carlo simulation, based on user inputs, to estimate the risk involved with starting a shellfish aquaculture enterprise. Outputs can be used to make more informed business decisions and apply for start-up or expansion financing.

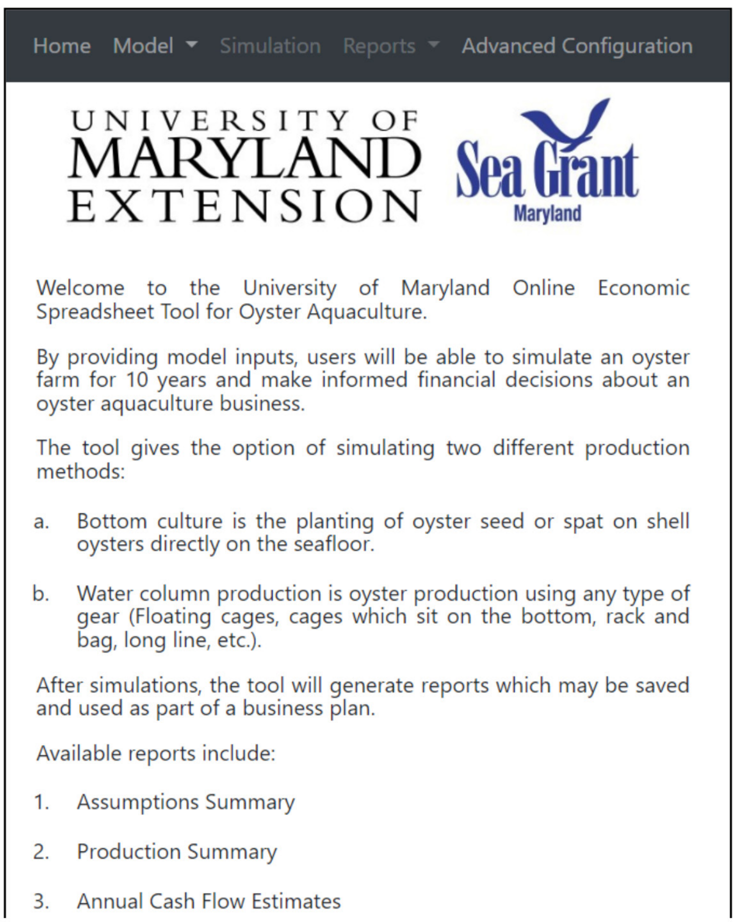

Figure 1. Screenshot of University of Maryland Online Economic Spreadsheet Tool for Oyster Aquaculture

DEVELOPING A RELEVANT TOOL FOR STAKEHOLDERS: STORIES IN FRUSTRATION

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Aquaculture extension specialists strive to be useful for their stakeholders. They also try to identify and develop new tools to help ensure aquaculture businesses are profitable and succeed. Often, we need to rely on others to help develop these tools. This presentation will discuss questions to consider when developing a new tool, handling unanticipated setbacks, and finalizing a tool. A case study for the evolution of the University of Maryland Online Economic Spreadsheet Tool for Oyster Aquaculture (Figure 1) based on stakeholder needs and inputs will be presented so you can learn from my mistakes.

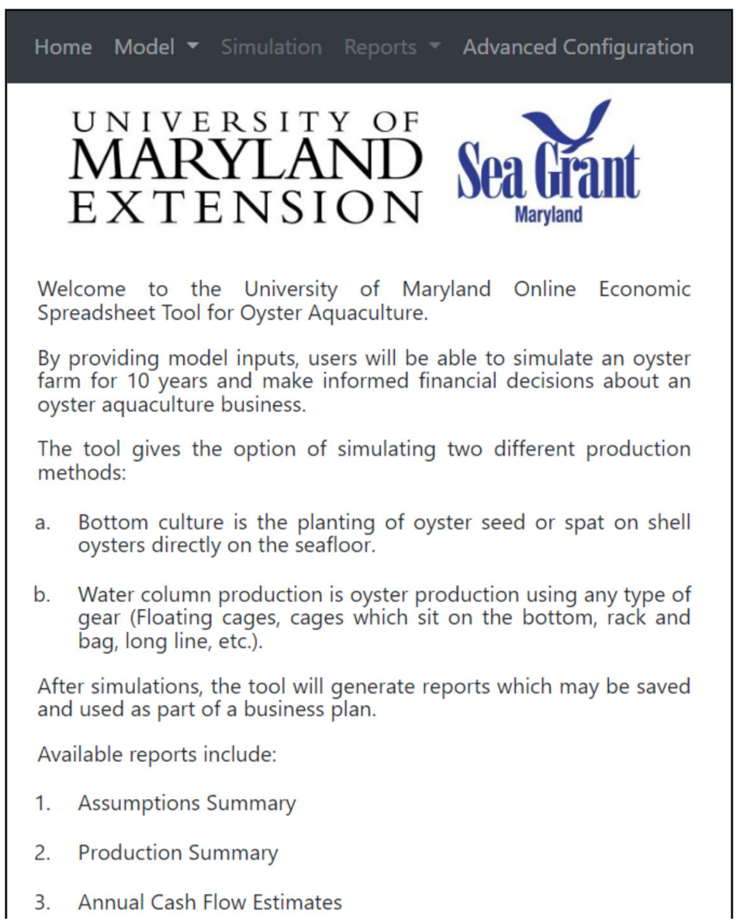

Figure 1. Screenshot of University of Maryland Online Economic Spreadsheet Tool for Oyster Aquaculture

A RAPID AND SENSITIVE COLORIMETRIC PCR-BASED DETECTION METHOD FOR *Vibrio parahaemolyticus* IN SEAFOOD

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Vibrio parahaemolyticus is known as an infectious bacterium that is the lead deadly disease called "Human Acute Gastroenteritis" around the world. *V. parahaemolyticus* commonly has been identified and inhabited at the coastal waters in the United States and Canada, however, it has also been found in seafood products around the world in water and seafood. Detection of the virulent microbial strains like *V. parahaemolyticus* in seafood samples employing traditional culture techniques could be lengthy and requires traditional tools, thus alternative methods for rapid detection of pathogens is of great importance for food safety and disease diagnosis. In this regard, colorimetric detection can be effectively used as a sensitive, rapid, practical, and cost-effective diagnostic method.

The main objective of our study is to introduce and identify *V. parahaemolyticus* using a specific colorimetric detection through DNAzyme as a signaling probe. In our study, colorimetric-PCR based detection method has been used to identify *V. parahaemolyticus* using DNAzyme as a signaling probe. The DNAzyme HRPzyme sequence containing a G quadruplex nucleotide sequence has been used, which mimics peroxidase activity. The HRPzyme would then catalyze the oxidation of ABTS in the presence of Hemin as a catalysis substrate (Figure 1).

We expect the introduced colorimetric-PCR Based assay will be a specific and ultra-sensitive approach for the detection of *V. parahaemolyticus* from a wide verity of environmental samples.

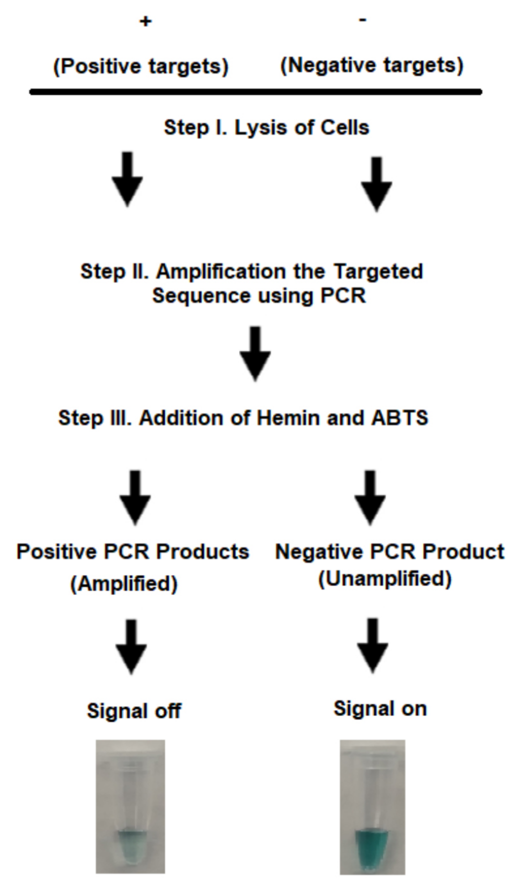

Figure 1. Scheme on the introduced Colorimetric PCR-based detection method using HRPzyme sequence for detection of *Vibrio parahaemolyticus.*

THE ROLE OF SEA URCHIN AQUACULTURE IN A MISSION TO RESTORE SEVEN ICONIC REEFS IN THE FLORIDA KEYS

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Mission: Iconic Reefs is a bold and broad endeavor that has been embarked upon by the National Oceanic and Atmospheric Administration and a suite of partners. The mission goes beyond large-scale outplanting of cultured stony corals to include community stewardship, long-term maintenance and monitoring of restored areas, and incorporation of algae-grazing species. Overgrowth by macroalgae is a primary factor in reduced growth and recruitment of stony corals in the Florida Keys.

Prior to a Caribbean-wide die-off in the early 1980s, the long spined sea urchin *Diadema antillarum* played an important ecological role in consuming large amounts of macroalgae and maintaining Florida Keys reefs in a state favoring stony corals. Other urchin and mobile invertebrate species (e.g. *Maguimithrax spinosissimus* crabs) are also of interest in Mission: Iconic Reefs as algae-grazers. Part of the mission is to develop and implement the capacity to aquaculture algae-grazing species at scale for population augmentation on targeted reefs.

This presentation will detail progress to date with regards to development of scaled aquaculture technology for *Diadema antillarum* and other urchin species of interest. Pilot-scale restocking events and ancillary benefits of aquaculture for these species will also be discussed.

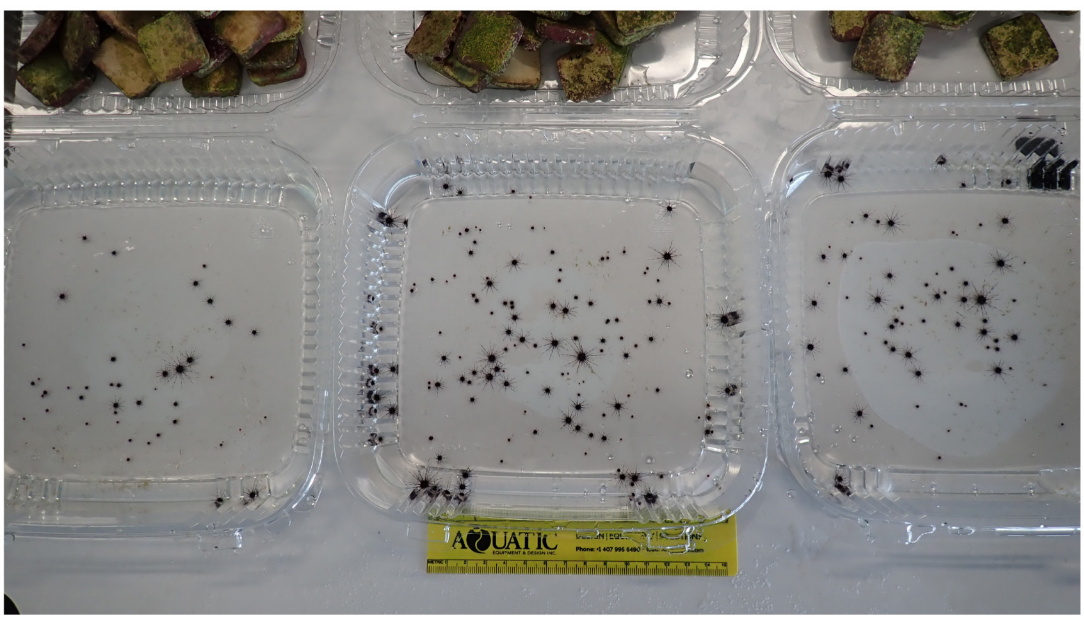

Figure: Hundreds of small aquacultured *Diadema antillarum*

UPDATES FROM THE SMART, SUSTAINABLE SHELLFISH AQUACULTURE MANAGEMENT PROGRAM

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The Smart, Sustainable Shellfish Aquaculture Management (S3AM) program integrates the disciplines of 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 to improve coastal farm productivity and profitability nationwide. 2) Validating S3AM technology through laboratory and farm trials. 3) Modeling and assessing the economic impacts of S3AM technology. 4) Integrating S3AM-generated knowledge into undergraduate and 4H education and outreach activities to enhance the future aquaculture workforce. 5) Building a nationwide extension network to engage stakeholders, disseminate S3AM information, and assess project outcomes.

The S3AM project uses a system-based approach to develop and implement new technologies and management practices that address shellfish aquaculture industry needs, and identify economic barriers/opportunities, to improve 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.

SIMULATION BASED OYSTER DETECTION

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Oyster reefs have significant advantages for the benthic marine ecosystem(s), including boosting species richness and offering habitat, sustenance, and protection for a wide range of marine organisms. Population decline over the 19th century has dramatically impacted oyster standing stocks in the Chesapeake Bay. Massive restoration efforts for oyster habitat are underway throughout the United States and Europe. Effectively tracking the development of oyster reefs is one of the main obstacles to advancing adapting the restoration process. General metrics for oyster reefs include areal dimensions, reef height, oyster density, and oyster size-frequency distribution. These measurements rely on the identification and counting of oysters by skilled human labor. Oyster reefs are manually subsampled with as few as 100 oysters per sampling site. Additionally, similarities between the bottom substrate and the oysters themselves make them difficult to distinguish for people and for computer algorithms. To streamline the process of oyster mapping, we use advancements in robotics and artificial intelligence to gather images from underwater Remotely Operated Vehicles (ROVs) and then automate oyster detection and density calculation. In this study, we provide a mathematical model to generate synthetic oyster image data and employ generative adversarial networks to facilitate the sim-2-real transfer. To the best of our knowledge, this is the first attempt to geometrically model oysters.

In this experiment, we train the convolutional neural network (CNN) using our real dataset (Oreal) and test using OysterNet and another method (DCO). The Intersection over Union (IoU) scores are 18.16% and 18.88% respectively which serves as the baseline for oyster segmentation results for our dataset. Both techniques perform similarly in these cases. Next, we evaluate the model performance using only the synthetic dataset for training (Osyn). We use both techniques to train on Osyn and test on O. The IoU score is lower than our baseline at 7.45% and 6.47%, respectively. Although the network has acquired the ability to detect synthetic oysters, the transfer from the sim to the real world is lacking. For training, we combined a tiny quantity of real data with synthetic data (Osyn_and_real), which yielded better results. In comparison to expert human-labeled ground truth, we achieved a state-of-the-art IoU Score of 24.54%, which is 35.1% better than utilizing only real datasets, and 12.7% better than DCO when trained on synthetic augmented real data.

TABLE I
SEMANTIC SEGMENTATION RESULTS

Method	Train Data	Test Data	IoU Score(%)
DCO	O_{syn}	O	6.47
DCO	O_{real}	O	18.88
DCO	$O_{syn\_and\_real}$	O	21.76
OysterNet (Ours)	O_{syn}	O	7.45
OysterNet (Ours)	O_{real}	O	18.16
OysterNet (Ours)	$O_{syn\_and\_real}$	O	**24.54**

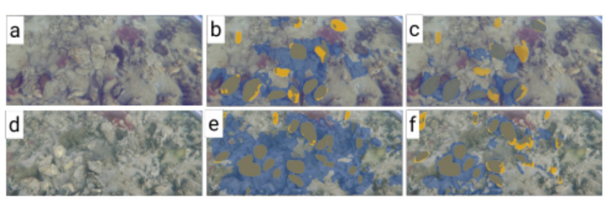

Fig. 1. Each row left to right: Input image, the output of the network when trained using only real data, the output of the network (which we call *OysterNet*) when trained using real data augmented with our synthetic data. Yellow represents the oyster segmentation ground truth and blue is the predicted segmentation result. Notice how the number of false positives and false negatives drop significantly when the training data is augmented with our synthetic data

AUTOMATING OYSTER AQUACULTURE WITH THE SUN

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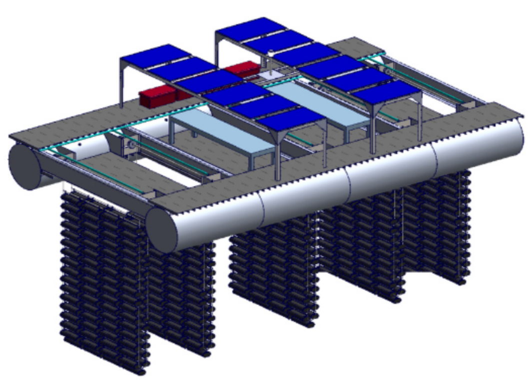
Oyster aquaculture technology has been relatively static in an industry needing to grow to meet global protein demand and advance restoration. Oyster aquaculture is very labor intensive, often growing less than a half million oysters per acre depending on gear types and site location. Floating and off-bottom gear is typically positioned in the near-shore environment or in the upper few feet of the water column, limiting overall growth and increasing conflicts with land-owners and other stakeholders.

The patent-pending Solar Oyster Production System (SOPS) has been developed to take advantage of offshore space that is typically not used by other oyster aquaculture systems. The SOPS supports a series of cages in up to twenty feet of water and utilizes solar power to mechanically rotate the cage array through the water column and above the water, providing desiccation/ air exposure and the opportunity for mechanized washing, which minimizes overall labor inputs. The rotation sequence can be programmed to meet the needs of the grower. The mooring system and robust design of the platform allows location of the SOPs in higher-energy environments, with integrated anti-poaching technology that allows units to be placed further offshore, potentially minimizing stakeholder conflict. SOPS can grow spat-on-shell oysters for restoration or from seed for market oysters. Approximately 200,000 oysters from seed can be grown on one 40’ x 25’ SOPS (see below). A large, 90’ x 67’ SOPS has been designed which would produce over 2,000,000 oysters annually.

In the Chesapeake Bay watershed, nitrogen and phosphorus credits for oysters harvested from aquaculture operations can be traded on the nutrient credit market. Use of the SOPS for 1 acre of high-density oyster aquaculture may remove nutrients equivalent to treating stormwater runoff from over 100 acres of impervious surface.

A SOPS prototype was launched in October 2021 and loaded with spat-on-shell oysters in coordination with the Chesapeake Bay Foundation. Growth was successful and the diploid oysters were deposited on a reef at the entrance to the Baltimore Harbor in early November 2022. Seed oysters are also being grown to gauge the effectiveness of the technology. Additional spat-on-shell will be grown in 2023 for restoration.



EFFECT OF PACKING DENSITY AND TRANSIT TIME ON BIOLOGICAL PERFORMANCE OF JUVENILE LONGSONOUT SEAHORSE *Hippocampus reidi*

Renato Peña*, Eliezer Zúñiga-Villareal, César Cardona-Félix, Luis M. Burciaga-Cifuentes, Carlos McGregor-Bravo, Ana Bodí-Broseta, Melissa Barrera-Flores

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The ornamental fish trade involves transporting live specimens for several days, during which the fish are in starvation conditions and water quality may change in detrimental of the fish. Therefore, packing method, fish density and transit time are important issues affecting the trade activity and representing major cost for traders. We evaluated the effect of transportation time (24 or 48 hours) and fish density (1, 3 or 4 fish/packing unit) on the water quality and biological performance in juvenile long snout seahorse *Hippocampus reidi*.

Seahorses were kept without food 24h before packing. Juvenile seahorses (average 7.6 cm SL and 0.90 gr wet weight) were packed in 1-L plastic bags filled with 400 ml of filtered seawater at the commercial farm “INGENS Cultivos Marinos” in Mazatlán, Mexico. Four threads of raffia were placed inside each bag as a holdfast for the seahorses. A total of 24 bags were used, six bags per fish density (1, 3, 4 fish/bag) and six bags with no fish that were treated in the same way as control. After packing, bags with seahorses and controls were placed inside a closed styrofoam box (12.5” H x 25” L x 15.5” W) and shipped in the morning via air cargo from Mazatlán, México to La Paz, BCS, Mexico. Transit time was 24 hrs. After arrival to the laboratory at La Paz (24H), a total of 12 bags (3 per density and 3 controls) were removed from the box and used for the analysis and the other 12 bags were kept in the box for analysis at the next day (48H). We evaluated the fish survival after 24H of arrival. We also analyzed the change in water quality (temperature, pH, salinity, ammonia, dissolves oxygen, nitrates and nitrites) in each bag. Seahorses were measured and weighted to evaluate the condition factor. Protein concentration was also evaluated. The swimming activity and feeding intensity during 5 mins was evaluated when each seahorse was transferred from the transport bag to a 20L aquarium filled with filtered and UV sterilized seawater at the same temperature of the transportation bag.

Survival at arrival to the lab and after 48H was 100%. Nevertheless, water quality was significantly affected by transit time and fish density, particularly the ammonia concentration, fluctuating from 0.0 to 6.7 mg/L and the dissolved oxygen concentration from 10.2 to 6.0 mg/L. Transit time or packing density had no effect ($p > 0.05$) on the condition factor of the seahorses (average $k = 0.20$). However, the protein concentration of the juveniles was affected, but only significantly ($p < 0.05$) by the transit time (24H = 1.63 mg/ml, 48H = 1.57 mg/ml). The behavior analysis and feeding response showed that a 48H transit time had a significant effect on seahorses’ activity. The feeding intensity was significantly higher ($p < 0.05$) in the seahorses of the 48H treatment (4.6 prey/seahorse) than in the 24H treatment (2.1 prey/seahorse). The packing density had no significant effect on feeding intensity ($p > 0.05$). The results suggest that 3 or 4 seahorses/bag is an adequate packing density and that up to 48H of transit time had no significant effect on survival or welfare of the juveniles.

MACROALGAL SULFATED POLYSACCHARIDES MODULATE IMMUNE RESPONSE AND IMPROVE PATHOGEN RESISTANCE IN TILAPIA

Raquel Tatiane Pereira, María García Suárez, Danièle Marzin, María Ángeles Rodríguez

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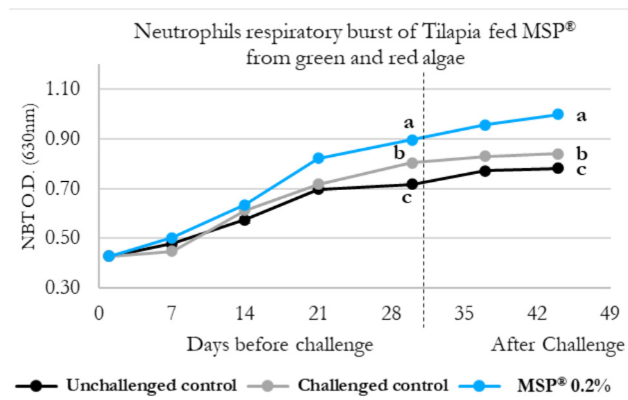
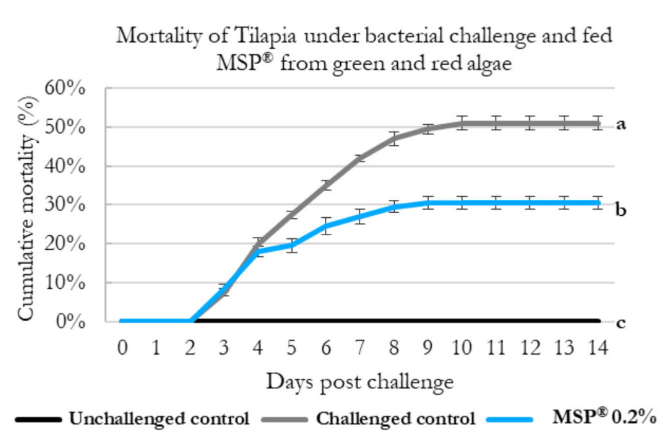
The cell wall of green and red marine algae is mainly composed of water-soluble sulfated polysaccharides with several biological activities, such as immunomodulation and strengthening of the intestinal barrier components respectively. Recent research has highlighted the potential of in-feed marine macroalgal polysaccharides to improve fish immune response, barrier integrity and resistance to pathogens focusing on gill, skin, and gut-associated lymphoid tissues (GIALT, SALT, and GALT). The study aimed to evaluate the effects of macroalgal sulfated polysaccharides on the immune response of tilapia under bacterial challenge. The study was carried out at the Can Tho University in Vietnam at a recirculating system for 44 days. Juveniles of hybrid tilapia (*Oreochromis mossambicus × Oreochromis aureus)*, initial weight 57.3±2.8 g, were assigned to 3 treatments with 4 replicates (100 fish/100L tank), totalizing 1200 fish. Three iso-nutritive diets: Positive Control, Negative Control and MSP® (commercial product based on the combination of green and red algal sulfated polysaccharides), were formulated to differ only on the inclusion of 0.2% of green and red macroalgal polysaccharides at the MSP® group. Fish were fed four times a day at a rate of 5% of the body weight.

At day 31, fish from the Positive Control group and MSP® (n=200/treatment) were intraperitoneally infected with *Streptococcus agalactiae* (0.5 x 10^5 CFU/fish) and mortality was recorded for 14 days. The Negative Control group was not challenged and used as a reference. Blood samples were collected at days 1, 7, 14, 21, 30, 37 and 44 for respiratory burst analysis. Neutrophils oxidative radical production (ROS) was determined in fresh blood (n=12 fish/treatment) incubated with nitrotetrazolium blue chloride (NBT) and absorbance read at 680 nm. Results were subjected to ANOVA and Tukey's test, $p<0.05$). Neutrophils ROS was significantly higher in fish fed MSP® compared to the Positive Control group at day 30 before challenge (up to 11%) and day 44 after challenge (up to 19%). The mortality rate at day 14 was 64% lower in fish fed diets containing MSP® compared to Positive Control group (31% *vs* 51%, $p<0.05$). These results highlight the efficacy of MSP® to improve tilapia's resistance to pathogens.





MACROALGAL SULFATED POLYSACCHARIDES MODULATE IMMUNE RESPONSE AND IMPROVE PATHOGEN RESISTANCE IN OLIVE FLOUNDER

Raquel Tatiane Pereira, María García Suárez, Danièle Marzin, María Ángeles Rodríguez

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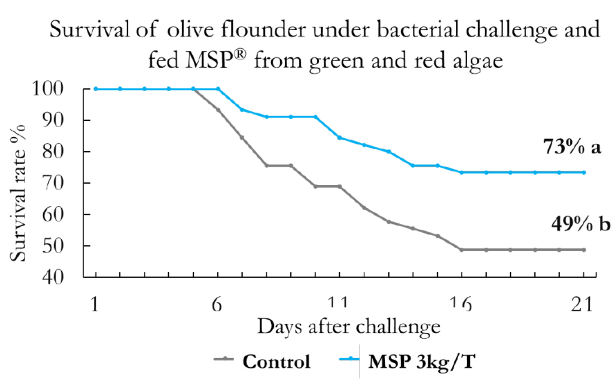
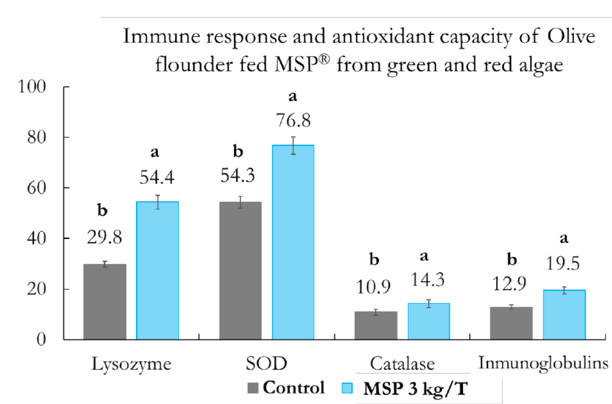
The cell wall of green and red marine algae is mainly composed of water-soluble sulfated polysaccharides with several biological activities, such as immunomodulation and strengthening of the intestinal barrier components respectively. Olmix has been able to isolate, identify and concentrate different macroalgal sulfated polysaccharides which are naturally available on the coast of Brittany (France) being one of the most important sustainable macroalgae resources in the world. Macroalgal extracts are potentially useful in aquaculture to modulate the immune response and to enhance epithelial cells integrity in the gills, skin, and gut which are the most important lymphoid tissues that form the barrier to pathogens in fish (GIALT, SALT, and GALT). The study aimed to evaluate the effects of macroalgal sulfated polysaccharides on the health of olive flounder under bacterial challenge. The study was carried out at Jeju University (South Korea) in a recirculating system for 15 weeks (105 days). Juveniles of *Paralichthys olivaceus*, initial weight 26.51g ±0.02 g, were assigned to 2 treatments with 4 replicates (40 fish/300L tank), totalizing 320 fish. Two iso-nutritive diets, Control and MSP® (commercial product based on the combination of green and red algal extracts) were formulated to differ only on the inclusion of 3 kg/T of green and red macroalgal polysaccharides in the MSP® group. Fish were fed twice a day *ad libitum*. Results were compared using ANOVA and Student test, $p<0.05$). At week 12, the fish (44 fish/treatment) were intraperitoneally infected with *Edwardsiella tarda* (1 x 10^5 CFU/fish) and mortality was recorded for 21 days. Additionally, blood samples were collected from 12 fish/treatment at the 12 before challenging for immune assay and antioxidant capacity. Lysozyme activity (mU.mL^{-1}) was quantified by a turbidimetric method using *Micrococcus lysodeikticus*. Superoxide dismutase (SOD, % inhibition) and catalase activities were assessed by commercial kits, Sigma Aldrich and, Biovision, respectively. Total immunoglobulin (mg.mL^{-1}) was quantified using micro-protein determination method. Lysozyme activity and total immunoglobulins were significantly ($p<0.5$) greater in the MSP® group by 45% and 34%, respectively. Likewise, SOD and catalase activities were increased by 29% and 24% by MSP®. Survival rate was 33% higher in fish fed the MSP® (49% *vs* 73%, $p<0.5$). The use of MSP® improved the immunity, antioxidant capacity, and pathogen resistance in olive flounder.





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

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One of the biggest challenges in commercial marine warmwater finfish production is consistent high-quality eggs, to ensure a steady supply of seedstock. A sufficiently nutritious broodstock diet is needed because a maternal diet would greatly influence the survival and development of the offspring. Florida Pompano (*Trachinotus carolinus*) is a coastal species common to Florida coasts with growing interest for commercial aquaculture production. The objective of this study was to evaluate the impact of different diets of the Florida Pompano broodstock, such as the control diet (cut-bait shrimp), commercial Breed-M and the green seaweed *Ulva*-based diet, on the quality of eggs, specifically the fertilization and hatching rate, the growth rate, the use of endogenous nutrients, and the fatty acid profiles of embryos and larvae during the endogenous and exogenous feeding period. The experimental design includes 40 fish evenly distributed in 4 tanks of 2.5 m in diameter (7.8 m^3) installed in identical Recirculating Aquaculture Systems (RAS). Broodstock in each tank were fed the same diet 4 times a day at 10 % of the fish body weight. At the time of spawning, after each diet treatment, eggs from each tank were incubated in 4 replicates. Eggs were taken for biochemical analysis and frozen at -80°C from each incubator and fresh samples were placed under the microscope for morphological measurements. It is expected that the addition of *Ulva* in the feed would provide sufficient nutrient levels for quality eggs and larvae comparable to the experimental diets and produce a similar or higher yield than the control and Breed-M diets.

THE EFFECTS OF HATCHERY STRESSORS ON GROWTH, AGGRESSION, AND CANNIBALISM OF JUVENILE LUMPFISH

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Due to the high demand for cleanerfish in salmonid ocean farming operations, increasing lumpfish hatchery production and rearing efficiency are of great importance. Juvenile lumpfish are cannibalistic which is controlled, to some extent, though frequent size grading of the fish, however, cannibalism still occurs. Understanding and mitigating for factors that exacerbate aggressive behaviors in juvenile lumpfish, which can lead to cannibalism, would help achieve the goal of increasing juvenile production in the hatchery. We hypothesize that lumpfish cannibalism is linked to a specific ontogenetic period related to fish size and can be exacerbated by various stressors such as stocking density and photoperiod.

To test this hypothesis, we subjected two different size classes of juvenile lumpfish (5g and 11g) to varying densities (40 g/L, 65 g/L, or 90 g/L) under different photoperiod regimes (ambient, constant low light, or constant bright light) for an 8-week duration in winter 2022. Fish growth, survival, and aggression were measured biweekly, and stocking densities adjusted to baseline levels biweekly by removing any necessary fish.

For 5g lumpfish, overall percent growth ranged from 92.60 ± 8.51% to 170.65 ± 24.24%. Both stocking density (two-way ANOVA, $P < 0.01$) and light (two-way ANOVA, $P < 0.001$) significantly affected overall percent growth of 5g fish. Fish subjected to the 65 g/L treatment grew 31.5% faster than fish in the 40 g/L treatment ($P < 0.01$) and 22.9% faster than fish in the 90 g/L treatment ($P < 0.05$). 5g fish exposed to ambient lighting grew 41.8% faster than fish in the constant low light treatment ($P < 0.01$) and 31.3% faster than fish in the constant bright light treatment ($P < 0.05$). Unlike the 5g lumpfish, neither stocking density (two-way ANOVA, $P = 0.53$) nor light (two-way ANOVA, $P = 0.154$) significantly affected the overall percent growth of the 11g fish. For 11g lumpfish, overall percent growth ranged from 31.58 ± 0.73% to 43.92 ± 4.86%. Contrary to our hypothesis, neither variable significantly affected aggressive behaviors in either the 5g (two-way ANOVA, stocking density: $P = 0.460$, light: $P = 0.065$) or 11g fish (two-way ANOVA, stocking density: $P = 0.412$, light: $P = 0.988$). However, a trend towards higher fin nipping in smaller fish was observed (~45% fin nipping occurrence in 5g fish vs ~20% in 11g fish), indicating that cannibalism may be even greater when fish are < 5g but decreases as the fish grow.

Manipulating lighting and stocking density (up to 90 g/L) can be used to suppress or increase growth rates in small lumpfish, depending on a hatchery's desired outcome, without resulting in an increase in fish aggression. However, these variables are less effective tools for controlling growth in larger juveniles. Future studies should focus on how these variables affect lumpfish < 5g as there are indications that aggression is most severe at this size class.

THE VELELLA EPSILON PROJECT: PIONEERING OFFSHORE AQUACULTURE IN THE SOUTHEASTERN GULF OF MEXICO; CHAPTER 5 – FINALIZING PROJECT PERMITTING AND PREPARING FOR DEPLOYMENT: ACT II

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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*) or red drum (redfish; *Sciaenops ocellatus*) 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/Federal laws.

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 5 – Finalizing Project Permitting and Preparing For Deployment: Act II - will walk us through the fifth 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. A review of significant advancements performed with the MAPP development as well as evaluations of potential commercial siting analyses will be discussed.

DEVELOPMENT OF A GULF OF MAINE CAPTIVE REARED LUMPFISH BROODSTOCK PROGRAM

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The USDA National Cold Water Marine Aquaculture Center (NCWMAC) began culturing lumpfish in 2016 with the aim of optimizing husbandry and spawning techniques in the United States. Soon thereafter, the NCWMAC, University of New Hampshire, University of Maine Center for Collaborative Aquaculture Research (CCAR) and Cooke Aquaculture co-founded the US Lumpfish Consortium. A primary goal of the Consortium has been the establishment of a Gulf of Maine (GoM) captive reared brood stock population. Beginning in 2018, staff from CCAR have developed techniques for capturing wild young-of-the-year lumpfish (often less than 1g) during the summer months from coastal Maine waters. Captured fish were then weaned to commercial diets and reared for two-three years until the fish reach sexual maturity. Once sexually mature, fish are transferred to NCWMAC to conduct spawning and rearing of the filial generation. The NCWMAC has developed and optimized techniques for incubating and rearing family batches of eggs and fish. We have also started a health screening program for our captive spawned population. The 2022 spring spawning season resulted in the establishment of 15 unique families at the NCWMAC from captive spawned fish. These families will form the nucleus of a GoM captive reared brood stock program at NCWMAC.

FIRST INVESTIGATIONS OF SETTLEMENT DYNAMICS FOR THE ECOLOGICALLY IMPORTANT LONG-SPINED SEA URCHIN *Diadema antillarum*

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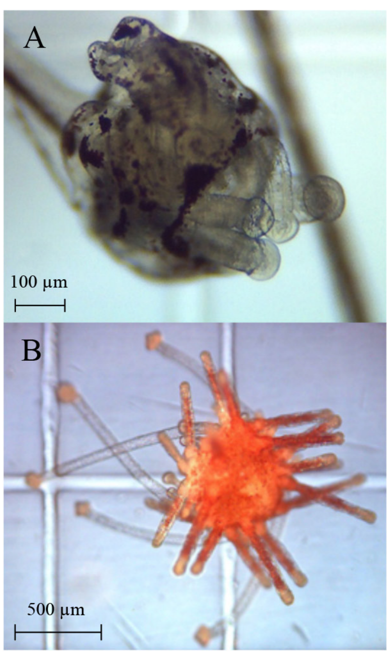
Sea urchin herbivory can have profound effects on coastal marine ecosystems. Understanding early life histories of ecologically relevant species can provide insight into population dynamics and broader ecosystem functioning. Settlement dynamics, defined as the transitional process from planktonic larva to benthic juvenile, are largely unknown for the long-spined sea urchin *Diadema antillarum* despite its importance to Caribbean coral reefs and being the most widely studied tropical sea urchin. Recent advances in larviculture methods for this species have resulted in large numbers of late-stage larvae and the ability to perform replicated settlement research that otherwise is prohibitively difficult to study through field observations. In this study, laboratory culture of late-stage larvae and settlement assays were employed to report first empirical observations of *D. antillarum* settlement response to a suite of cues. Positive settlement to two types of calcareous macroalgae and a biofilmed ceramic tile, along with zero settlement in three other treatments, revealed the need for obligatory cues. An agonistic relationship between tiles and biofilm revealed the importance of both a structural and biochemical cue, and evidence for a tactile response in *D. antillarum* larvae. Results from an assay comparing settlement rates between two larval morphologies further aided in the development of a general description of competence, or the point at which larvae are capable of metamorphosis. Subsequent experimentation revealed positive effects of phytoplankton food availability on competence acquisition of late-stage larvae and on proportional settlement of mixed development larval populations.



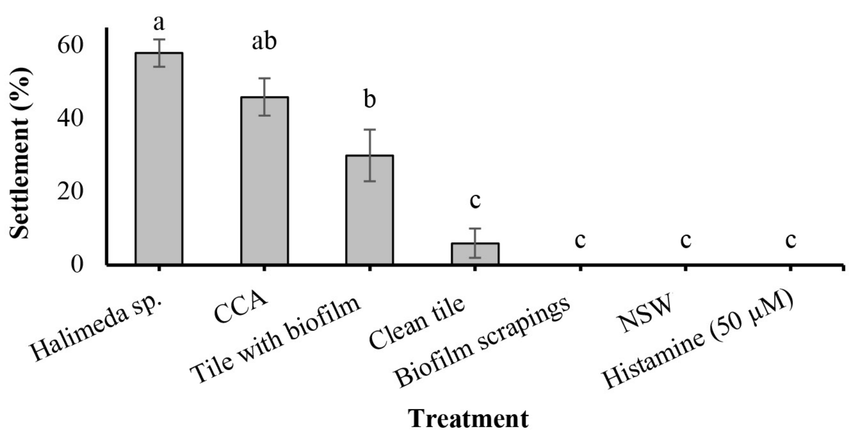


Fig. 1 (left): *Diadema antillarum* (A) displaying metamorphic competence during larval development, (B) post-settlement juvenile.
Fig. 2 (top): Percent settlement of competent *Diadema antillarum* larvae in response to a suite of cues. Letters denote statistical differences in the proportion of settled urchins (PERMANOVA, post-hoc pair-wise test). Data are presented as means ± SE.

NON-INDIGENOUS SPECIES UNINTENTIONALY TRANSPORTED TOGETHER WITH FARMED MUSSELS: DO WE KNOW ALL WE NEED ABOUT THESE HITCHHIKERS?

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Mussels are widely farmed and one of the most traded aquaculture products worldwide. As ecosystems engineers, mussel's clusters constitute hotspots of biodiversity in terms of the associated fauna. Therefore, given the huge and broad mussel international market, usually consisting in imports of live and acclimatized specimens, unintentional introductions of associated or "hitchhiking" species may occur potentially inducing relevant impacts on receiving habitats. Indeed, aquaculture international imports of live organisms have been associated to incidental transport of non-indigenous species (NIS), being particularly relevant whenever the introduced species become invasive. Nevertheless, it has been recognized that the success of invasive species depends more on their particular traits rather than on a random process of selection of species.

The epifauna associated to mussels farmed (*Mytilus galloprovinciallis*) in southern coast of Portugal was identified and quantified in order to assess the species with spreading potential through commercial transport. Overall, the study aimed to: i) characterize the epi- and endofaunal communities associated to mussel clusters from an offshore aquaculture; ii) assess the biogeographic distribution of associated species across mussel transposition areas; iii) identify the associated species with spreading potential in mussel destination areas through a functional/behavioral approach.

Among 105 associated *species*, 44 were not previously reported in at least one of the common mussel export/transposition countries. Among those *taxa*, fouling species such as the anemones *Paractinia striata* and *Urticina felina*, the acorn barnacles *Balanus glandula* and *Balanus trigonus*, or the bryozoans *Bugulina stolonifera* and *Schizoporella errata*, exhibit functional attributes that allow them colonizing and spreading into new geographical areas. In this context, predicting and preventing the spread of NIS is a crucial challenge for the scientific community involved in this research field.

Figure 1 – Farmed mussels colonized by fouling (above) and free-living (below) epibenthic species, including non-indigenous species with invasive potential.

FEEDING MICROENCAPSULATED ALGAE TO BIVALVE MOLLUSCS - A POSSIBLE VECTOR TO IMPROVE FOOD QUALITY

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Marine bivalves frequently require depuration before being commercialized alive for human consumption. This process is vital to the food safety of these food items and aims to reduce microbiological fecal contamination (content of *Escherichia coli*). After depuration, bivalves are packaged and commercialized refrigerated (4 – 6°C). During this food supply chain, bivalves may suffer nutritional loss as they are in starvation. Thus, adding microencapsulated algae-based food during the depuration process can reduce the possible nutritional losses of the organisms, assuring their quality. To evaluate that, clams (*Ruditapes philippinarum)* depuration was performed for 48h in 250L recirculated tanks, with a filtration system composed of a protein skimmer, a 25W UV-c unit, and a temperature control system (15 ± 1°C). During depuration, one of the groups (Diet) was fed with a microencapsulated diet, incorporating *Nannochloropsis* sp. in 40μm diameter pellets, after 22h and 46h. The other group, which served as a control (Ctrl), was not fed during the trial. Samples for microbiological analyses and biochemical biomarkers were collected at 24h and 48h depuration periods. Additionally, clams were sampled for biochemical biomarkers 6 days after depuration to evaluate whether food addition during depuration would promote the energy budget of the animals during the posterior shelf-life.

After 24h depuration, the clams fed presented higher values of protein content and, after 48h, presented higher lipid content. Regarding energy consumption, both groups presented similar results. Depuration process significantly decreased *E. coli* values reducing from 9450 NMP/100g to 490 NMP/100g in the Diet group and 330 NMP/100g in the Ctrl group and, *Salmonella sp*. was not detected in all groups of clams. Further studies are needed, encompassing different types and concentrations of microalgae in feeds. However, preliminary results indicate that adding food during the depuration process may contribute to maintaining bivalves' quality and add value to this natural resource.

This work is part of the project DepurD (MAR-01.03.01-FEAMP-0046), supported by Portugal and the European Union through MAR2020, Portugal2020 and FEAMP

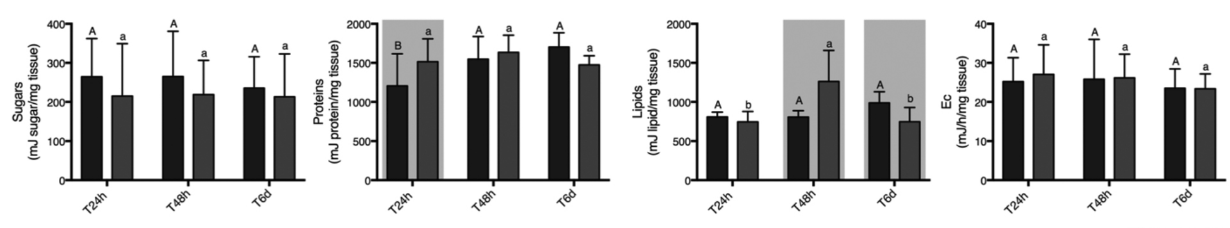

Figure 1 - Mean values of sugars, proteins, lipids and energy consumption (Ec), measured in edible tissue of *R. philippinarum*, n = 10.

MULTIPLEX PCR AND MINION SEQUENCING FOR DIRECT WHOLE GENOME ASSEMBLY OF AQUATIC VIRUSES FROM TISSUE AND ENVIRONMENTAL SAMPLES: A CASE STUDY INVOLVING PRV-1

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Many aquatic viruses such as Piscine orthoreovirus genotype 1 (PRV-1) are not culturable, necessitating genome assembly directly from samples. This approach is typically limited to samples with high viral load to ensure accuracy and adequate coverage during sequencing. Unfortunately, many natural infections and environmental substrates (e.g., seawater) have low viral loads. We adapted a recently developed multiplex PCR tiling method for targeted enrichment of Zika virus (Quick et al. 2017) to target the aquatic salmon virus PRV-1 from tissue and seawater and optimized library preparations for MinION sequencing. Specifically, PRV-1 from British Columbia was used as reference to design 36 sets of overlapping primer pairs using Primal Scheme software. RNA extraction and cDNA synthesis followed standard methods (Polinski et al. 2019) and was used for multiplex tiling PCR. Products were sequenced using MinKnow high-accuracy base calling and aligned using Nanopipe.

We sequenced 24-48 barcode-separated samples per flow cell, obtaining 2-3 million reads per sample. At this sequencing depth, near full-length genomes were obtained in samples with as low as 10 PRV-1 copies per µl with near-perfect agreement (>99.9%) to Illumina-derived assemblies for read depths of ≥ 20 reads per position. Samples which had received improper handling or were stored under suboptimal conditions often had significant loss of amplification across portions of the genome; however, even these typically yielded acceptable sequence coverage for most of the genome. By assembling over 300 PRV-1 genomes from a diverse set of fish and environmental samples, we were able to explore transmission pathways of PRV-1 including those within and between farmed and wild salmon over a large temporal range and spatial scale. We believe these methods are fundamentally suitable for application to a wide range of infectious agents in aquatic environments.

STRIPERHUB AND THE NATIONAL BREEDING PROGRAM FOR THE STRIPED BASS *Morone saxatilis* INDUSTRY

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The expansion of American aquaculture is critical to address the ~$17 billion seafood trade deficit that is caused in part by our importation of over 80% of seafood products. Striped bass (*Morone saxatilis*) is a prime candidate to help expand the United States aquaculture industry as it has been captively bred (domesticated) for decades. This effort was initiated in response to the wild population collapse in the 1980s and now supports the fourth largest aquaculture industry in America, hybrid striped bass, with striped bass gaining traction as a standalone industry. Breeding and domestication have resulted in better dress-out yield, improved growth rate, and greater stress tolerance in these seven-generation domesticated fish compared to earlier generations and their wild counterparts. The reduced time to reach market size is a key outcome that supports economic feasibility for producers, as striped bass can now reach market size (1.36 kg or 3 lbs.) in under two years and the eighth-generation captively bred fish are anticipated to require even less time.

Further, the use of exogenous hormones to induce captive spawning has been eliminated, and instead spawning behavior of domestic fish is prompted by modifying water salinity and temperature to mimic natural conditions. In addition to the qualities that make striped bass a commercial culture-ready candidate, these fish are a popular product among consumers with a slightly sweet flavor and firm texture once cooked. The presentation of these domestication efforts is in part to address challenges stemming from producer and consumer education with the ultimate goal of advancing aquaculture nationally.

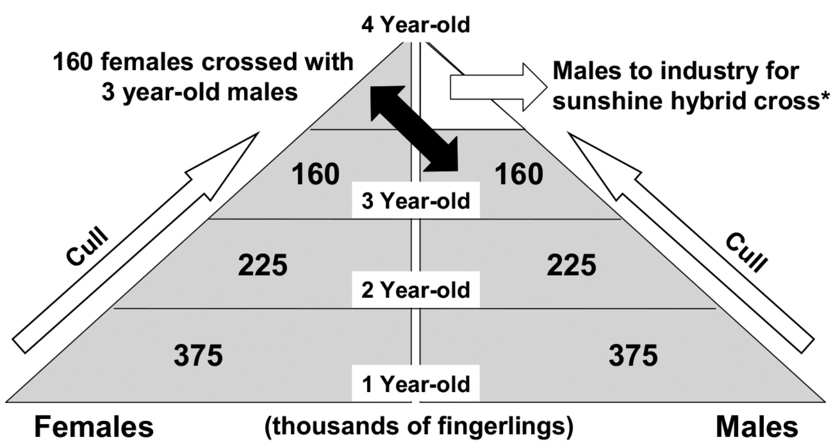

Figure 1. Striped bass (SB) breeding program structure across four class years. A new year class is produced each spring. These four-year classes collectively represent one filial generation (F) of captive breeding, or domestication, as the age at maturity for female SB is four years. Performance data of fish are collected between each of the year classes. Individuals exhibiting superior traits are retained for the next year and the remaining individuals are culled. Age at maturity for male SB is three years and at this age they are crossed with four-year-old females to produce the next generation of domesticated SB.

EFFECTS OF DIETARY SOYBEAN MEAL INCLUSION ON CALCIUM-BINDING PROTEIN EXPRESSION AND INFLAMMATORY GENE MARKERS IN LIVER AND INTESTINE OF ATLANTIC SALMON (*Salmo salar* L.)

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Atlantic Salmon (*Salmo salar* L.) are sensitive to the inclusion of soybean meal in the diet which can result in inflammation in the distal intestine. The complex mechanism and etiology of soy-induced distal enteritis is not fully understood. However, calcium-binding proteins produced from *S100* genes are known to be involved in the facilitation of inflammatory responses. The intestine of Atlantic Salmon is also involved in calcium uptake and calcium-binding protein expression has not been studied in conjunction with plant protein diets containing soybean meal. We examined the expression of *S100* genes in the distal intestine and liver between groups of Atlantic Salmon fed fishmeal (FM) or soybean-meal (PM) diets for 12 weeks. Fish were randomly assigned to FM or PM diet groups and fed daily to satiation. Tissues from each experimental group were sampled every four weeks to assess gene expression. Expression of *S100I2* in the distal intestine was elevated by 8 weeks but was not significantly different between diets. Expression of *S100V2* was significantly elevated in fish fed the PM diet compared to FM diet fed fish. This upregulated expression of *S100V2* but not *S100I2* at 8 and 12 weeks differs from previous studies and suggests Atlantic Salmon raised in freshwater may use calcium-binding S100 proteins differently than Rainbow Trout. Expression *S100I2* and *S100V2* in the liver were not responsive to dietary treatments. However, other inflammatory genes examined showed expression patterns similar to those observed in previous studies. Histological examination showed mild but significant distal enteritis in the PM diet group. Overall, these results provide further understanding in the expression of *S100* genes and the inflammatory processes associated with soy-induced distal enteritis in salmonids.

LOW COVERAGE WHOLE GENOME ANALYSIS OF PARENTAGE STRUCTURE AND SEX MARKERS IN *Paralycthys olivaceus*

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Genomic tools have been applied in agriculturally significant species such as pigs to assess questions such as parentage and inbreeding and to develop selective breeding programs. However, the aquaculture industry is just beginning to apply genomic tools to improve broomstick management and product quality. Olive Flounder, *Paralichthys olivaceus*, is a popular aquaculture species in the Republic of Korea and Japan, with most of the United States supply being imported from those countries. Studies using this species have generally used micro-satellite markers (highly repeated segments of DNA) to analyze brood stock relationships and parentage. Most genomic studies have been genome-wide association studies and transcriptome analyses of disease resistance and developmental pathways, while some questions concerning industry applications remain unanswered.

In this study, low-coverage whole genome sequencing (lcWGS), a type of next-generation sequencing that interrogates the whole genome of an organism and provides many thousands of genetic markers at a lower depth (0.5-2X) and lower cost than Whole Genome Sequencing, will be used to identify parentage and sex markers for *P. olivaceus* from an experimental hatchery. Fin clips were collected from 20 brood stock *P. olivaceus* and 60 fish from 2 groups of offspring from the same cohort, a "Runt" group, and a "Future Brood Stock" group which were isolated due to a faster growth rate post-hatch. The samples were sequenced using a lcWGS approach and single nucleotide polymorphisms (SNPs) will be used to identify parentage relationships between brood stock and offspring. Results will be used to determine the relative contribution of specific brood stock to each offspring group (runt *versus* future broomstick). In addition, an association study will be used to identify SNP markers for sex that will allow for sex identification in a species that has no morphological distinction between males and females. These results will provide valuable broodstock management data and further prove the utility of lcWGS approaches as cost-effective methods of answering questions significant to aquaculture operations.

REPLACING FISHMEAL WITH A SINGLE CELL PROTEIN FEEDSTUFF IN CHANNEL CATFISH *Ictalurus punctatus* DIETS

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There is a continually growing interest in novel ingredients which can serve as alternatives to fisheries-derived proteins in aquaculture. The alternative ingredients that have been receiving more attention recently include insect meals and single cell proteins obtained from bacteria, algae, and yeasts. The inactivated dry yeast *Saccharomyces cerevisiae* product DY-Pro (Meridian Biotech, LLC) is produced using co-products of the dry mill corn ethanol fermentation process and holds great potential as an aquafeed ingredient. Although many dry yeast products have been evaluated as providers of various immunostimulating compounds in several aquaculture species, only a few studies have shown that dry yeast products may replace fishmeal in diets for aquatic organisms. Indeed, in a previous collaboration, graded replacement of 2 to 100% of fishmeal protein by DY-Pro did not significantly affect Nile tilapia's weight gain or gut morphometric parameters, but linearly improved feed and nutrient utilization.

Farm-raised catfish is the largest aquaculture industry in the United States with production valued at approximately $400 million annually. Although fishmeal is typically not a major protein feedstuff in catfish diets, replacing it with alternative protein feedstuffs such as DY-Pro could contribute to reducing diet costs and sparing marine resources. Therefore, this study was conducted to investigate the effect of either partially or totally replacing fishmeal (included at 14.8% by weight in the control diet) by the inactivated dry yeast product DY-Pro. The DY-Pro replaced 0% (DY-PRO0), 5% (DY-PRO5), 25% (DY-PRO25), 50% (DY-PRO50), 75% (DY-PRO75) or 100% (DY-PRO100) of the protein provided by fishmeal in six isonitrogenous and isocaloric experimental diets. Each diet was fed to quadruplicate groups of the experimental fish (initial average body weight 39.4 ± 1.6 g) for 8 weeks.

At the end of the feeding trial, the graded replacement of 5 to 100% of fishmeal protein by the DY-Pro did not have any negative impacts on fish survival, fillet yield, hepatosomatic and viscerosomatic indices. However, as higher levels of DY-Pro were included in the experimental diets, weight gain and feed efficiency were progressively reduced. Broken-line regression analysis of weight gain data indicated that up to 21% of dietary protein contributed by fishmeal could be replaced with DY-Pro without significantly affecting growth performance and condition indices of channel catfish juveniles.

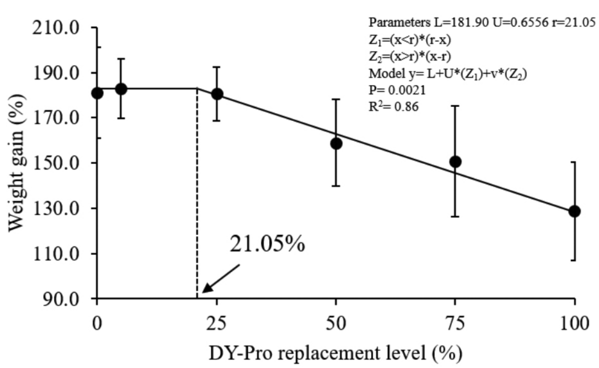

Figure 1. Broken-line regression analysis of catfish weight gain in response to incremental levels of DY-Pro ingredient replacing fishmeal protein.

REMOVAL OF OXYTETRACYCLINE FROM AQUACULTURE WASTEWATER USING A LOW-COST NATURAL MATERIAL

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Oxytetracycline (OTC) is an FDA-approved antibiotic that is commonly used in aquaculture as a prophylactic treatment or to treat fish bacterial diseases. OTC can be administered to fish in 3 ways: medicated feed, bath treatment, and injection. Regardless of the treatment method, 70 to 80% of OTC may not be metabolized by fish and will end up in the water system. Accumulation of OTC in aquaculture systems may lower the nitrification performance of biofiltration as well as increase the risk of antimicrobial resistance in cultured fish. OTC may also pose a persistent contamination issue that can impact the health of other animals and humans when it is released into the environment. Studies have shown that adsorption is the most efficient and cost-effective method to remove OTC. In this study, *Moringa oleifera* (MO) husk, a byproduct from Moringa oil and protein production, was tested as a low-cost biosorbent for the OTC removal.

The biosorbent was prepared by grinding the MO husk to 300 to 500 μm in diameter, followed by chemical and heat treatments. The changes in the surface morphology of the treated MO husk were evaluated by scanning electron microscopy (SEM). The concentration of the OTC was measured by detecting the absorbance at 355 nm using a UV–VIS spectrophotometer. The OTC removal study was done by mixing the appropriate amount of MO biosorbent with 25 mL of 20 mg/L OTC in a 50 mL tube wrapped in aluminum foil for 3 hours. The pH of the solution was adjusted using HCl and NaOH to the targeted value. The effect of the biosorbent dosage (0.4 to 2.0 g/L) and solution pH (5, 7, and 9) on the adsorption was tested by comparing the adsorption capacity under different conditions.

The results showed that the adsorption capacity decreased with the increase dosage while the removal rate increased (Fig.1a). The adsorption was significantly higher at the initial pH of 5 than at pH of 7 and 9 (Fig. 1b). Nonetheless, the equilibrium pH was consistently lower than the initial pH. This indicated that ion exchange might be the primary adsorption mechanism. Based on these results, we plan to investigate the effect of interfering ions commonly found in aquaculture wastewater as well as the kinetics and the isotherms of the adsorption to further understand its mechanism. Lastly, the performance of the biosorbent on the removal of OTC from the actual aquaculture discharge water will be assessed.

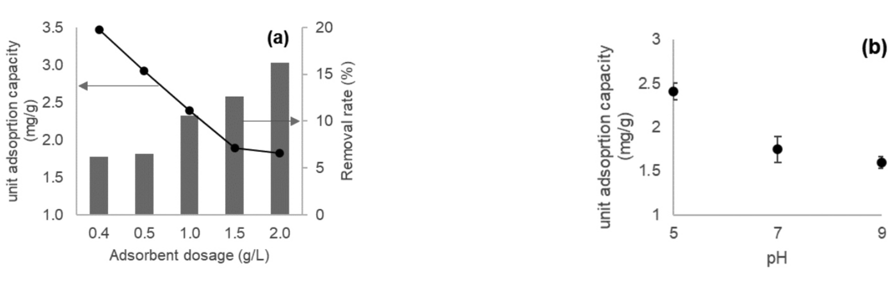

Figure 1. Effects of (a) MO biosorbent dosage and (b) pH on the OTC adsorption

ARE TRADE CREDITS A DRAIN FOR GAIN IN THE AQUACULTURE INDUSTRY OF BANGLADESH?

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Most small-scale aquaculture producers in the developing countries have limited access to formal external finance. More easily accessible alternative source of finance for these producers is trade credit. However, earlier literature argues that trade credit is a drain on one hand and the gain on the other hand for fish producers. Based on farm level cross section data, this study empirically examines the sources of drain and gain of financing through trade credit using Stochastic Meta Frontier and Propensity Score Matching model, whereas determinants of trade credit are identified using Probit regression. The findings imply that users of trade credit are as technically efficient as the non-users and are using improved production technology (Figure 1). In addition, there is positive tradeoff between benefits and costs of using trade credit (Table 1). Furthermore, collateral, documentation, number of suppliers and revenues influence the farmers' decision on use of trade credit as financing tool (Table 2).

TABLE 1: Impact of trade credit on farm performance

	Means Treated (Users of TC)	Means Control (Non-users of TC)
Culture period (days)	221.00	197.00
Yield (Kgs)	21243.00	14359.00
Revenues ($)*	21881	17813
Feed Quantity (Kgs)	33094.00	20875.00
Feed Cost ($)*	16291	10722
FCR	1.68	1.54
Feed cost Per Kg. ($)*	0.50	0.53
Efficiency	0.79	0.79

* 1 USD equivalent to 80 BDT.

FIGURE 1: Differences in technical efficiency and technology gap ratio between users and non users of trade credit

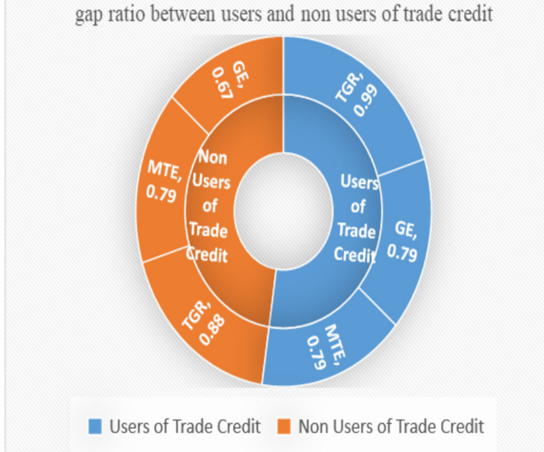

TABLE 2: Determinants of trade credit

	Estimate	Pr(>|t|)	Marginal Effects
Collateral is problem: Yes/No	0.04*	0.08	0.018*
Trade credit is convenient than formal: Yes/No	0.08**	0.017	0.015**
Trade credit int. is lower than formal: Yes/No	0.12***	0	0.026***
Number of suppliers offering Trade credit	0.23***	< 2e-16	0.070***
Education	0.06**	0	-0.001**
Log (Revenues)	-0.08**	0	-0.023**
Log (Feed costs)	0.07**	0	0.027**

ADVANCING AQUATIC ANIMAL DRUG APPROVALS FOR MARINE AQUACULTURE

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Since its inception the U.S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership Program (AADAP) has played a role in the FDA approval of almost all of the currently approved aquaculture drugs. Despite these successes, there are still currently no approved drugs for use on marine finfish. With the significant potential for mariculture expansion in the U.S. and the renewed commitment to the support of marine aquaculture by federal agencies, it is imperative to stock marine aquaculture's medicine chest. In the past few years, AADAP has increased our efforts to help secure the first aquatic animal drug approval in saltwater in the U.S. With the help of our partners, we have made progress on saltwater finfish claims for AQUI-S 20E, Aquaflor, and 35% Perox-Aid. There are currently several challenges with saltwater drug approvals that do not exist for freshwater approvals, since the paradigm of what is required by FDA has not been fully established. This is further complicated by the small scale of the U.S. marine aquaculture industry, which creates a lot of uncertainty in the current and future use patterns of each drug in marine aquaculture. This presentation will summarize the successes, failures, obstacles, challenges, and opportunities for advancing marine aquaculture drug approvals, and outline ways to make the process as efficient as possible by utilizing the lessons learned through decades seeking aquaculture drug approvals in freshwater.

POPULATION DYNAMICS ASSESSMENT AND PREY DETECTION OF THE BLUE CRABS (*Callinectes sapidus*) USING eDNA ANALYSIS AT DELAWARE INLAND BAYS

Juan Ramos*, Tahera Attarwala, Marcus Teat, Zachary Riggi, Kevin Knight, Cassandra Miller, Emily Andrade, Ali Paraseimehr, Ph D., and Gulnihal Ozbay, Ph.D.

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Blue Crab (*Callinectes sapidus*) fisheries is a multi-million-dollar industry, which is extremely important to the Mid-Atlantic States. *C. sapidus* is an important keystone species to the aquatic ecosystem of Delaware Inland Bays. In aquatic ecosystems, *C. sapidus* serves both as predator and prey. The objective of this study focuses on the population dynamic of *C. sapidus* and its relationship with its surroundings such as oyster reefs or oyster aquaculture activities and its roles in biodiversity preservation. The primary research area is selected in Rehoboth Bay, Delaware where most oyster aquaculture and oyster restoration activities are taking place. We have deployed 18 crab traps within 6 study sites (2 aquaculture, 2 artificial reefs, and 2 controlled sites) since June 2022. While collecting water samples to process water quality parameters we include “Bay City” as the 7th site. We deployed the crab traps every two weeks and kept the traps in the water no longer than 24-36 hours. Each site receives 2 commercial-size cages and 1 half-moon recreational-size pot. Each commercial cage is baited with 2 Menhaden *(Brevoortia Tyrannus)*, while the recreational pots get 1 Menhaden. Additionally, with field assessment, the molecular biology approach has been used for monitoring the biodiversity of the prey of *C. sapidus* using Environmental DNA (eDNA). Water quality has been monitored at the sites every week using YSI Multiprobe 5600 and water samples have been tested for Nitrate, Nitrite, Total Alkalinity, and Total Hardness while Total Chlorophyll-a and Turbidity have been monitored using Turner Design Fluorometer. Per harvest totals range from 140-230 blue crabs have been captured. All of the crabs were captured from June 15th through the end of the crabbing season on November 30th. Blue crab size and sex determination have been conducted along with sampling for e-DNA. We monitored higher female crabs throughout all study sites. The overall result is promising and shows a relationship for increased blue crab activities that varies by habitats such as aquaculture, artificial reefs, or controlled in Rehoboth Bay, Delaware.

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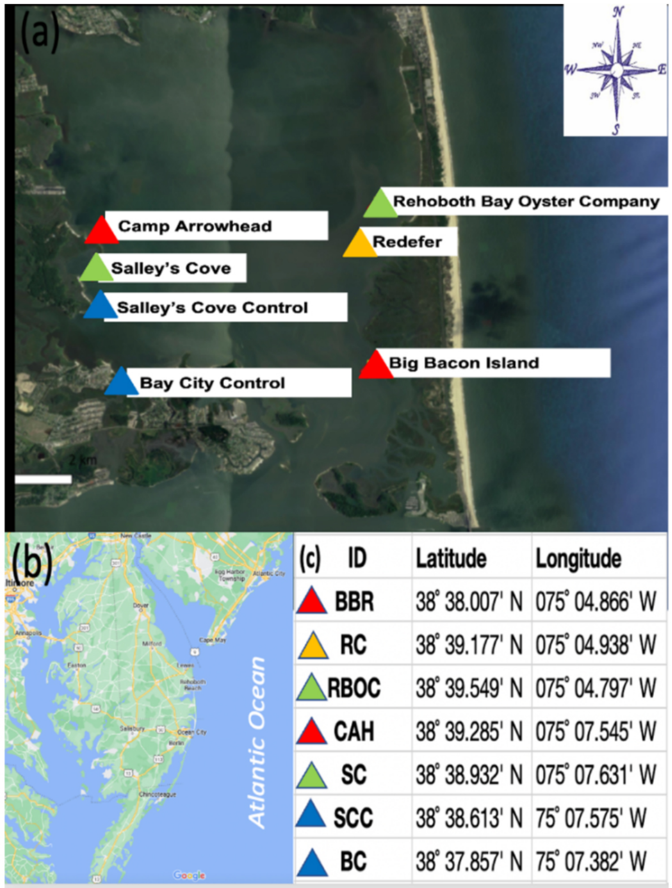

ID	Latitude	Longitude
BBR	38° 38.007' N	075° 04.866' W
RC	38° 39.177' N	075° 04.938' W
RBOC	38° 39.549' N	075° 04.797' W
CAH	38° 39.285' N	075° 07.545' W
SC	38° 38.932' N	075° 07.631' W
SCC	38° 38.613' N	75° 07.575' W
BC	38° 37.857' N	75° 07.382' W

Figure 1 (a-c). Study sites at Rehoboth Bay, Delaware: (a) Names of the sampling locations, (b) Overall geographic location of Rehoboth Bay in Delaware, and (c) The GPS locations of the study sites. Camp Arrowhead and Big Bacon Island sites (in red) represent artificial reefs. Sally's Cove and Rehoboth Bay Oyster Company sites (in green) represent on going aquaculture sites. While Redefer (in yellow) represent a retired aquaculture site. Lastly, controls are located at Sally's Cove and Bay City (in blue) represent natural sites.

POPULATION CHARACTERISTICS ASSESSMENT OF THE BLUE CRABS (Callinectes *sapidus*) LOOKING AT RELATIONSHIPS BETWEEN CARAPACE DIMENSIONS AND SEX ORIENTATION IN DELAWARE INLAND BAYS

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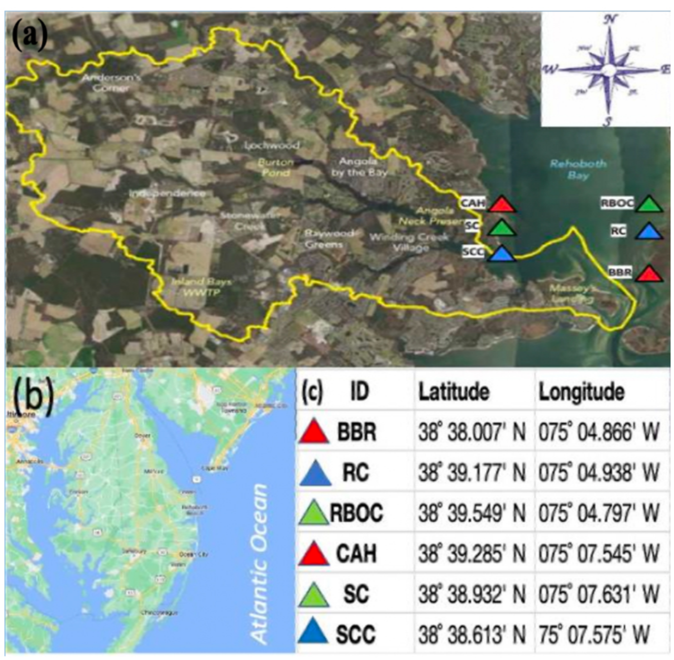
Blue Crab (*Callinectes sapidus*) fisheries is a multi-million-dollar industry that is extremely important to the Mid-Atlantic States. *C. sapidus* is an important keystone species to the aquatic ecosystem of Delaware Inland Bays. In aquatic ecosystems, *C. sapidus* serves both as predator and prey. The objective of this study focuses on the population characteristics of *C. sapidus* and its relationships with its surroundings, such as artificial pilot reefs or oyster aquaculture activities, and its roles in biodiversity preservation. The primary research area is selected in Rehoboth Bay, Delaware, where most oyster aquaculture and oyster restoration activities are taking place. We have deployed 18 crab traps within 6 study sites (2 aquaculture, 2 artificial reefs, and 2 controlled sites) since June 2022. Additionally, water samples were collected to process water quality parameters. We deployed the crab traps every two weeks and kept the traps in the water for no longer than 24-36 hours. Each site receives 2 commercial-size cages and 1 half-moon recreational-size pot. Each commercial cage is baited with 2 Menhaden *(Brevoortia Tyrannus)*, while the recreational pots get 1 Menhaden. Water quality has been monitored at the sites every week using YSI Multiprobe 5600, and water samples have been tested for Nitrate, Nitrite, Total Alkalinity, and Total Hardness, while Total Chlorophyll-a and Turbidity have been monitored using Turner Design Fluorometer. Per harvest, totals range from 140-230 individuals, and the blue crabs were captured and released. All of the crabs were captured from June 15th until October 31st. The overall result is promising and shows a relationship for increased blue crab activities that varies by habitats such as aquaculture, artificial reefs, or controlled (natural) in Rehoboth Bay, Delaware.



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ECOGENOTOXICOLOGICAL EFFECTS OF FWATER POLLUTION ON *Scamberomorus commerson* AND ITS USE FOR EARLY TOXICITY SCREENING AND MONITORING IN THE ARABIA GULF, NEAR DAMMAM, SAUDI ARABIA

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The current research was planned to investigate genotoxicity in fish to screen and monitor the aquatic ecosystem. *Scamberomorus commerson* collected from the contaminated areas of the Arabian Gulf Near Dammam Saudi Arabia were analyzed for genotoxicity. DNA fragmentation was detected by Comet assay and Micronucleus assay. All water quality parameters and concentrations of heavy metals (Pb, Cr, Zn, Mn, Cu, Cd, Sn, and Hg) were extensively higher than the WHO permissible limits. They were more than enough to have adverse effects on fish health. Considering DNA damage as a biomarker of freshwater pollution, the highest DNA fragmentation was observed in *Scamberomorus commerson*, indicating its most heightened sensitivity to pollution. *Scamberomorus commerson* showed comet head diameters 63.33±2.2, 83.59±3.38, and 66.28±2.13px from RS1-RS3, respectively. *Scamberomorus commerson* erythrocytes showed comet tail lengths as 16.66±1.65, 16.20±1.63, and 19.07±1.81px from RS1-RS3. DNA damage was found to be 19.14±1.38, 16.38±1.26, and 19.95±1.33% from RS1-RS3, respectively. The tail moment was found to be 6.46±0.79, 4.72±0.69, and 7.14±1.08, while the olive moment was found to be 5.31±0.51, 5.14±0.52 and 6.01±0.49 respectively from RS1-RS3. *Scamberomorus commerson* collected from the polluted area of the Arabian Gulf exhibited the highest frequency for single micronucleus induction, double micronucleus induction, and nuclear abnormalities as 50.0±6.3‰, 14.4±2.5‰, and 15.0±2.9%, respectively. This study proposes that this fish species, along with these novel DNA damage assays, could be the best tools for toxicity screening and monitoring freshwater bodies.

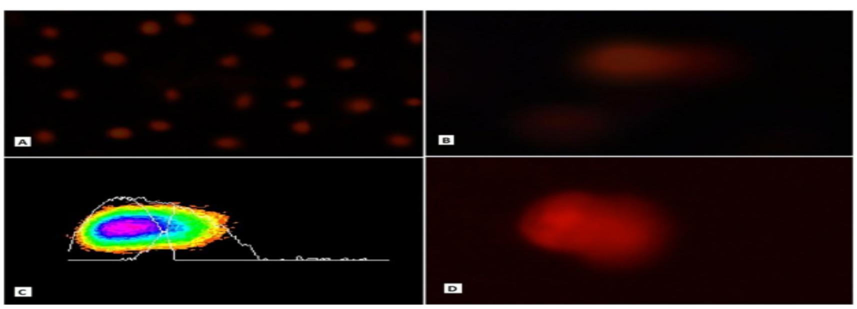

Fig. 1. Comet Assay images of fish *Scamberomorus commerson*. Contol fish were showing normal erythrocytes (A) Fish erythrocytes indication DNA damage and comet tail formation 60x (B). Fish erythrocyte was analyzed through TriTek Comet Score Freeware 1.6.1.13, indicating significant DNA damage (C). Fish erythrocytes indicate migration of DNA from the core head region 100x (D).

THE EFFECTS OF SYSTEM TYPE AND HORIZONTAL SUBSTRATE ADDITION ON PACIFIC WHITE SHRIMP *Litopenaeus vannamei* PRODUCTION AND WATER QUALITY IN RAS

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Shrimp is the most consumed seafood item in the United States, but its production is still inadequate to meet consumer demand and high-quality domestic supplies are lacking. To facilitate intensive farming of brackish water species, recirculating aquaculture systems (RAS) can be adopted. This allows farmers to control inputs and outputs, helps save water and salt costs, and prevents adverse environmental impact. This project focuses on assessing the effects of two different types of RAS (clear water and hybrid), along with horizontal substrate on intensive Pacific white Shrimp production and water quality. Clearwater (CW) RAS is an effective strategy for shrimp production but may have relatively high equipment and operational costs. Simple hybrid systems (HY) have some advantages as external biofilters maintain water quality, but solids filtration is relatively minimal. Artificial horizontal substrate in RAS may help to enhance shrimp production by increasing the surface area for shrimp to graze and facilitating higher shrim

In this study two levels of each experimental factor will be used, and they are system type (CW vs HY) and presence of an artificial substrate (WS) versus absence of substrate (NS). There will be a total of 4 treatments: HY-WS, HY-NS, CW-WS, and CW-NS each of them having four replicates randomly assigned to 1 m^3 circular tanks. Ozone, a strong oxidizing agent, will be used in CW tanks. CW will have a foam fractionator, a settling chamber, and a biofilter. HY will have a settling chamber and a biofilter, and suspended solids will be allowed to accumulate in the tank. Four layers of horizontal cloth net, each layer covering 0.5 m^2 area, 20 cm apart from each other, and square in shape will be installed as substrate in the eight circular tanks with substrate. Shrimp will be stocked at 400 m^{-3} in the experimental tanks and reared for 12 weeks.

Results from this experiment are expected to show significant differences in the average weight, total harvest, FCR, and growth rate of shrimp between system types and level of horizontal substrate. We expect shrimp performance to be enhanced by the extra filtration of CW systems and by the added surface area and grazing opportunity provided in the WS treatments. Turbidity, TAN, nitrite, nitrate, TSS, and VSS concentrations are expected to be significantly higher in HY systems compared to CW, which may create disparities in shrimp production as well. Isotope levels ($\delta\ ^{13}C$ and $\delta\ ^{15}N$) in the tissues of shrimp grown with substrate are expected to indicate that shrimp had access to an additional food source besides commercial feed. Overall, we expect to find a dynamic series of independent and interactive effects of system type and substrate availability. These differences in RAS management may have important implications for intensive shrimp production, especially in locations where low water use, and high product output are critical considerations.

EDIBLE AND MARKETABLE PLANT SPECIES IN BRACKISH-WATER AQUAPONICS

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Brackish-water aquaculture producers often face challenges with nitrate accumulation to the point it becomes toxic to animals. Disposing of high-nitrate water wastes both water and salt, can be toxic to terrestrial crops, and often cannot be discharged into municipal sewer systems. Using aquaponics to reduce waste discharge while producing marketable plants is gaining popularity globally; however, brackish water producers are typically thought to be limited to halophytes, or salt-tolerant plants only. Although halophytes grow well and remove nitrogen in brackish water systems, the plants are often unknown to consumers, or are not palatable. Finding non-halophyte species that are tolerant of salt and better known to consumers may allow producers to utilize aquaponics and reduce water and salt use.

Over 10 different plants have been tested at KSU to determine their suitability for brackish water aquaponics. Each plant is evaluated for salinity tolerance by growing plants in 0, 5, 10, 15, and 20 salinity. Plants that show high survival and growth at elevated salinities are used in further trials that examine plant performance in situations representative of shrimp farming conditions. Variables such as varying nutrient concentrations, plant age, acclimation strategies, decoupled versus coupled aquaponics with shrimp, and the effects of supplemental iron on chloride tolerance have been tested.

Kale has been a standout performer with 100% survival even at 20 salinity. Mustard has also shown high survival while only showing moderate growth reduction at higher salinities. A primary finding is the importance of an acclimation period. This acclimation process involves germinating plants in freshwater, then increasing salinity over a two-week period. Many plants show survival at 5, 10, and even 15 salinity when using this acclimation period. Changes in nutrient uptake and tissue concentrations have been noted, particularly replacement of Ca and K by Na, while Mg seems to be unaffected. Some plants appear to store excess Na in leaf tissue, while other plants (notably kale), appear to have lower amounts of Na in the leaves and can reduce Na uptake at the roots or have a Na transport/evacuation ability. Nitrate uptake rates tended to decrease as salinity increases across all plants, however significant amounts of nitrogen were removed from the water, even at 15 and 20 salinty. Other findings are the increased necessity of dosing certain nutrients at high levels compared to freshwater, particularly iron. This line of research has opened potential opportunities for brackish-water aquaculture producers. Further research will include human sensory profiles of the plants and consumer acceptance studies.

ARTIFICIAL INTELLIGENCE-AIDED REAL-TIME FISH MORTALITY ALERT FOR RECIRCULATING AQUACULTURE SYSTEMS

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Fish mortality is a major production-limiting factor in aquaculture. Real or near real-time mortality tracking can provide valuable inputs to farm managers or automated systems in order to initiate procedures to prevent mass mortality events. Additionally, avoiding excessive mortalities blocking outflow from a tank's bottom drain is critical to maintain normal system operations in recirculating aquaculture systems (RAS). While traditional systems use periodic human operator observation and tracking - often in conjunction with an underwater camera - this study augments this approach with Machine Learning and Internet of Things (IoT) deployed at the Edge Device to provide round-the-clock mortality monitoring and trigger an alarm when mortality thresholds are exceeded. An imaging sensor [Pi camera (model: Pi 4 HQ, Raspberry Pi foundation, Cambridge, UK)] integrated with an edge computing device [model: Pi 4] was customized for underwater application and has been deployed in the tank at 0.6 m above the central drain to collect one image every hour for six weeks. Acquired images will be annotated as live and dead fish in Roboflow (Roboflow, Inc., Des Moines, Iowa, USA) and will be split up into training (70%), validation (20%), and test (10%) dataset to train a custom Yolo V6 mortality model. The accuracy of the model will be evaluated in terms of mean average precision and F1 score. The model predicted daily and cumulative mortality will be compared with the ground truth data, and the reliability of the alarm events will be analyzed and presented.

STRIPERHUB: STRIPED BASS (*Morone saxatilis*) AQUACULTURE

Benjamin J. Reading*, Linnea K. Andersen, Jason Abernathy, David L. Berlinsky, Greg Bolton, Russell J. Borski, David Cerino, Michael Ciaramella, Robert W. Clark, Michael O. Frinsko, S. Adam Fuller, Steve Gabel, Bartholomew W. Green, Steve Hall, Eric Herbst, Michael Hopper, Linas W. Kenter, Frank Lopez, Barry Nash, Matthew Parker, Kwamena Quagrainie, Steve Rawles, and Hanping Wang

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StriperHub is one of the National Sea Grant Aquaculture Hubs coordinated by North Carolina Sea Grant. The StriperHub integrates diverse Sea Grant programs (IL/IN, OH, MD, NC, NY, NH), industry partners, government researchers (NOAA and USDA), policymakers, cooperative extension agents, and academic scientists to consolidate and streamline striped bass (*Morone saxatilis*) aquaculture efforts in the US through demonstration of commercial culture, economics, and marketing. By value, >80% of seafood products consumed in the US are imported, totaling over $17 billion in the annual US trade deficit. The Sea Grant StriperHub centered in North Carolina will help to address this deficit by developing striped bass as a candidate aquaculture species and expanding hybrid striped bass (*M. saxatilis* x *M. chrysops*) aquaculture to strengthen the domestic seafood industry and boost the economies of coastal and rural communities. This collaboration will define striped bass markets and economics of production, develop education and training programs, clarify regulatory permitting and licensing procedures, and promote comprehensive outreach and visibility among likely producers and consumers of this new seafood product, which is now available in markets along the Eastern US Coast. For continued development of the Sea Grant StriperHub, we anticipate acquiring additional partners (academic, government, and private sector) as the success of striped bass aquaculture continues to expand from the East Coast to nationwide. Specific StriperHub program goals include:

1. Continue development of the Sea Grant Aquaculture Hub: A nexus to commercialize striped bass as a major aquaculture industry (The Sea Grant StriperHub);
2. Improve seed stock production, distribution, growout, and better define parameters for production economics of domestic striped bass aquaculture;
3. Continue marketing strategies, market economics, permitting clarity, and business models for domestic striped bass aquaculture; and
4. Expand communication, outreach, extension, and training to support domestic striped bass aquaculture development

Specifically, the StriperHub will continue its efforts in broodstock and seedstock production and distribution to cooperating farmers and collaborators to ensure consistent commercial striped bass production. Additionally, members are performing an evaluation of reduced frequency feeding strategies to limit feed input costs at commercial density; are synchronizing batch spawning to improve seedstock production; and expanding the number of commercial domestic striped bass producers. The StriperHub will produce a revised culture manual for striped bass.

THREATS TO U.S. FOOD AND AGRICULTURAL RESOURCES: SEAFOOD

Benjamin J. Reading* (on behalf of the DHS TFAR Team)

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The Food and Agriculture Sector (FAS) accounts for 20% of the United States (US) economy and has been designated a Critical Infrastructure Sector by the Department of Homeland Security (DHS). This sector consists of an extensive, open, globally interconnected, diverse, and complex array of privately owned "just in time" networks; and encompasses a variety of goods and services including the production and manufacturing of crops, livestock, poultry, and seafood products and by-products. As such, the threats to food and agricultural resources will come from various sources including foreign and domestic events, naturally occurring and/or human-induced, and the interdependencies of FAS with other critical infrastructures. Given the broad scope of these possibilities, the 2021 Threats to Food and Agriculture Resources (TFAR) Teams focused on the myriad of threats that could disrupt or devastate supply chains within the vast FAS of the US; examine shortfalls in US capacity to prevent and mitigate the threats; and recommend best practices, policy, and research priorities that will foster preparedness and resilience of the FAS against all threats. The TFAR discussions centered on terrestrial and aquatic environments in the context of food and agricultural systems, climate change, food adulteration, disruptions in the transportation sector, water shortages, globalization of trade/travel, biosurveillance limitations, social culture, cyberthreats, agro/bioterrorism, and economic coercion. In-depth capability and vulnerability analyses of the FAS identified several key areas for utmost attention by the public and private sectors, with recommendations to prepare for and address the likelihood of emerging threats that could severely impact the food, agriculture, and aquaculture industries, including: Aquatic/seafood safety and biosecurity.

Globalization will remain the determinant factor to the world's economic, technological, and societal progress; with caveats that geopolitical disputes amongst dominant world powers for access to natural resources, including agricultural and aquaculture/seafood products, may change the dynamics of the global food supply chain. This will significantly impact the FAS and its interdependencies with other critical infrastructures. This report can be leveraged to support and address a variety of research requirements embedded within existing US government policy and doctrine. In particular, six key recommendations were provided, one of which was: The US government needs to promote domestic aquaculture for food production. National Security Memorandum-16 (NSM-16) on *Strengthening the Security and Resilience of United States Food and Agriculture* (2022) assigns key roles to the DHS related to overall strategic guidance and enhancing national unity of effort. DHS, in coordination with the Department of Agriculture, the Department of Health and Human Services, the Department of Justice, and other relevant agencies, will continue to integrate food and agriculture sector efforts across the Homeland Security Enterprise to promote the security and resilience of the Nation's critical infrastructures.

DHS TFAR Report: https://foodsafetytech.com/wp-content/uploads/2022/09/Threats-to-Food-and-Agriculture-Coordinated-Research-Agenda-AEP-final-paper-2021.pdf
DHS NSM-16 Bulletin: https://www.dhs.gov/sites/default/files/2022-11/NSM_DHS%20FACT%20SHEET%20on%20NSM%20Food%20and%20Agriculture.pdf

EXCHANGE RATE PASS-THROUGH IN NORWEGIAN AQUACULTURE EXPORTS

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Aquaculture and fisheries industries plays a major role in overall exports from Norway, the world's second largest exporter of seafood. The industry has a global reach as there exist several destination markets and exporters for seafood, indicating a substantial heterogeneity in the industry.

It is widely assumed that a country's export can be stimulated through a depreciation of the domestic currency, but while the actual response of exports to exchange rate movements has been debated for decades, the current evidence is mixed. How trade actually responds to changes in exchange rates varies widely across products, trade orientation and choice of invoicing currency. In this paper we investigate exchange rate pass-through (ERPT) into destination prices for Norwegian Aquaculture exports. Following the literature of international trade, ERPT is assumed to be incomplete when exports are invoiced in a foreign currency. An examination of how exporters of aquaculture products responds to exchange rate variation, and if the response differs between different invoicing currencies, are of interest as the literature considers such products are as homogeneous products and the estimated trade response can then be considered as a direct effect of exchange rate movements.

In this paper we employ Customs data for Norwegian exports of seafood. Each observation is recorded with an anonymous ID for the exporter, the statistical value and weight of the shipment, date of the shipment, destination country and invoicing currency. Data on invoicing currency and destination country makes it possible to measure ERPT for seafood exports at the product level.

The results indicate a strong prevalence of ERPT in Norwegian seafood exports invoiced in producer currency. The effect appears to be different for exporters of aquaculture products than for exporters of products from traditional fisheries. Pass-through for exporters of aquaculture products are sensitive to marked shares and export orientation.

CLOSING THE LOOP IN AQUAPONICS AND RAS THROUGH SUSTAINABLE WASTE MANAGEMENT

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Aquaponics tends to be thought of as one of (if not the most) sustainable food production methods currently being utilized. One issue that is often overlooked or dismissed with this type of system is solids management. In a typical recirculating aquaculture system (RAS), solid waste is simply removed from the equation. Whether it is discarded to the sewer or dumped on the land as irrigation, minimal thought goes into its management. In aquaponics, we often mineralize some of our solids to increase nutrient content to plants, but solids often accumulate in the system and must eventually be dealt with. In small-scale systems, solids are often emptied into gardens or to feed vegetation, but this proves problematic in commercial systems. We have shown that the aerobic mineralization process increases concentrations of plant-available forms of various nutrient, while eventually leaving behind a stabilized biosolid. This study examines the nutrient content of these biosolids and discusses how they have been and can be successfully used in agricultural applications. The implications of this study are wide reaching and can potentially offer economic solutions for large scale aquaponics systems and RAS when determining a way to sustainably deal with their solid waste accumulation.

DEVELOPMENT OF SEAWEED FARMING SYSTEMS FOR NEW NORI AND DULSE AQUACULTURE CROPS

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The potential of US seaweed aquaculture is immense, but the industry remains stalled at low value brown kelp crops. Springtide Seaweed, a leading aquaculture innovator, is developing red seaweed cultivation solutions that leverage key partnerships, refined nursery and farm technologies, and significant market opportunities that will enable new high-value seaweed crops for sustainable economic opportunities and aquaculture diversification.

Phase I successfully supported critical nursery work for production of dulse and nori seed, laying the groundwork for commercial nursery production of seed. Phase II is working to refine nursery and farm seaweed cultivation systems for commercialization, optimizing three dimensional designs and advanced growing substrates targeted to red seaweed crops. Research and development objectives include: 1) Refine nursery technologies and designs for efficient scalable seed production; 2) Develop and test cultivation substrates for successful seed production and farm cultivation; 3) Design and test four cultivation system designs on the farm for dulse and nori crops; and 4) Develop sustainable business strategies for all phases of innovation development.

Commercialization of cultivation systems will take a three-phase approach: (1) growth of new crops for Springtide; (2) seeded substrates and systems for other farms; and (3) full seaweed farm cultivation system solutions commercialized for the global aquaculture industry. New turnkey systems, adaptable to red and brown seaweed cultivation, will increase sustainability and efficiency in the industry, reduce gear and crop failure, and enable high-value red seaweeds to be easily integrated into existing aquaculture industries, including shellfish, finfish, and seaweed farms worldwide.

IMMUNOLOGICAL RESPONSES TO COLUMNARIS

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Arkansas is home to the baitfish, sportfish fingerlings, hybrid striped bass fingerlings, largemouth bass, triploid grass carp, and feeder goldfish. Arkansas baitfish and sportfish farms, and state fish hatcheries routinely combat Columnaris disease, caused by *Flavobacterium covae*. Columnaris outbreaks are especially prevalent during feed training and when holding fish in vats. Understanding the immunological responses of fish to columnaris, would be useful to understanding how fish respond to columnaris outbreaks. Sixty channel catfish were stocked into separate 190 L tanks, which were part of a recirculation system. Thirty fish were injected with lipopolysaccharide (LPS) and 30 with physiological saline (PBS). Five fish were sampled from each group at 6, 24, and 72 hours. Blood, spleen, gills and liver were sampled from each of the five fish. Cortisol and gene expression were examined. Data analysis is currently ongoing, and results will be analyzed and presented.

ECONOMIC UPGRADING OF BRAZILIAN TILAPIA VALUE CHAIN: A REGIONAL ANALYSIS

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Fish farming corresponds to approximately 90% of the Brazilian aquaculture production, which in 2020 reached 643,279 t. Tilapia production alone accounts for approximately 50% of this production and 40% of the commercialized value (IBGE, 2020). Despite being one of the largest aquaculture producers in the world (FAO, 2020), export rates of the country's production are still very small. In the case of tilapia, historically (2013 to 2020), Brazil exported less than 1.1% of its total production (CIAQUI, 2022).

Another characteristic of the tilapia value chain in Brazil is the existence of eleven productive zones distributed in almost all regions of the country. According to 2019/20 data a little more 30% of the national production of tilapia was concentrated in five of these eleven zones (CIAQUI, 2022). These poles have different socioeconomic and infrastructure contexts (i.e Human Development Index-HDI, Per capita GDP and Km of highway/1.000 km^2 territory), value chain structures (i.e level of production, number of fish processing plants and fish farmers) and economic performances (i.e cost of production, sell price and gross margin).

In this context, the objective of this research is to compare tilapia poles in Brazil, using economic upgrading indicators. This study collected data from tilapia production units (TPUs) distributed in four selected and categorized production zones (TPZs). The Global Value Chain (GVC) structure was the background for building the economic update indices estimated by a fuzzy inference system.

The results indicate that more integrated governance structures point to better upgrading trajectories, better management of the risk associated with the activity in general and a higher probability of compliance with international eco-certifications.

The research also highlights that, in productive configurations where these integrated forms are not fully dominant, active technical assistance and competent suppliers can be an important factor in boosting the economic upgrading of TPUs.

Table 1. Upgrading fuzzy indexes

Group	Tilapia Production Zone	Upgrading Indexes Means
Emerging	Boa Esperança	0.426*
	Serra da Mesa and Cana Brava	0.743*
Consolidated	Submédio Baixo São Francisco	0.092*
	Oeste of Paraná	0.813*

*Indicates statistical significance at 1% between zones of the same group

INTERAGENCY COORDINATION FOR OFFSHORE AQUACULTURE IN THE U.S. GULF OF MEXICO: A REGIONAL MODEL FOR ESTABLISHING EFFICIENCIES IN THE PERMITTING PROCESS FOR AQUACULTURE

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Often cited as one of the barriers to offshore aquaculture development in U.S. waters, the permitting and environmental review process for marine aquaculture is composed of a series permits, authorizations, consultations and reviews necessary for operations to comply with one of the most comprehensive regulatory environments in the world. Offshore aquaculture projects must navigate through the current regulatory environment influenced by evolving agency priorities, policy directives, emerging science and finite agency resources. Efforts to foster a more transparent and efficient permitting and environmental review process for offshore aquaculture remains a top priority and focus of the federal agencies involved in these processes.

In the Gulf of Mexico region, staff from NOAA Fisheries, the U.S. Environmental Protection, U.S. Army Corps of Engineers and other federal agencies have a longstanding history of close coordination on aquaculture initiatives, dating back to the development of the Fisheries Management Plan for Regulating Offshore Aquaculture in the Gulf of Mexico. NOAA's Gulf-wide planning effort to identify Aquaculture Opportunity Areas in the Gulf of Mexico has helped to improve aquaculture coordination across agency district and region boundaries, strengthened existing partnerships and fostered new ones across the federal agencies. This presentation and panel discussion with regional members of NOAA Fisheries, the U.S. Environmental Protection and U.S. Army Corps of Engineers will discuss the methods, tools and techniques developed to improve interagency coordination, leading to more efficient and effective permitting and environmental review processes for offshore aquaculture projects in Gulf of Mexico.

A SIMPLE, LOW-COST TRAP FOR MONITORING SNAIL POPULATIONS IN CATFISH AQUACULTURE PONDS

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Bolbophorus daminificus is a commercially important trematode parasite within catfish aquaculture. Infection by this parasite can lead to reduced growth due to lack of feeding, as well as mortality. In severe cases, economic losses may also occur as a result of processing rejects due to a heavy parasite burden in surviving fish. The complex life cycle of the parasite consists of two intermediate hosts, snails and fish, and a single final host, piscivorous birds, making control and eradication difficult.

In catfish ponds, two snail species are known to serve as intermediate hosts of the trematode: the Marsh Ramshorn *Planorbella trivolvis* and the Ghost Ramshorn *Biomphalaria havanensis*. However, basic ecological information about these two snail species is still largely unknown, both in commercial aquaculture and in the wild. Information such as snail density, movement patterns, and seasonal behavior may all play important roles in the management of snail populations and their parasites. Thus, monitoring of snail populations in catfish ponds and development of a threshold density could help farmers make more informed management decisions throughout the production season.

This study evaluated the use of a small, cost-effective snail trap that could be easily implemented in catfish ponds and maintained by farm managers and employees. Traps were deployed in each of 8 commercial catfish ponds – 4 fingerling and 4 foodfish. Traps were checked every week from May to September to evaluate the numbers and species of snails present throughout the production season. Foodfish ponds produced significantly more snails than fingerling ponds throughout the season. Our data also show recovery of snail populations in less than one month following copper sulfate treatment. The results of this study show promise for the use of passive traps as tools for monitoring snail populations within catfish ponds. This study also suggests that fingerling and foodfish ponds likely differ in their ecology, and that copper sulfate treatments are a short-term solution that may require multiple applications and/or more targeted approaches.

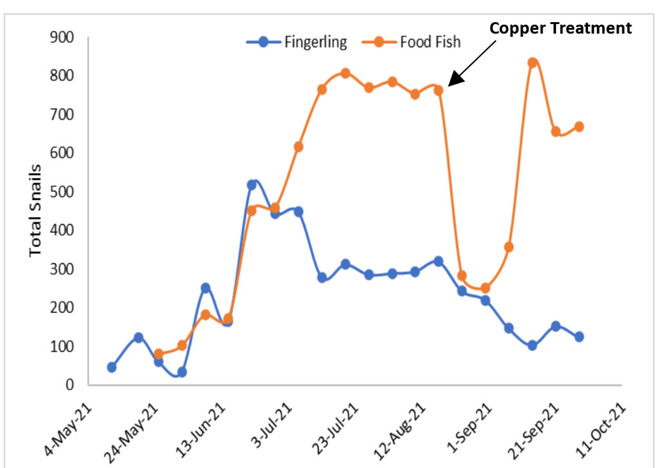

Figure 1. Plot of total snails collected from traps in commercial fingerling and foodfish ponds at each sampling date. Values include all snail species collected.

DIFFERENCES IN EGG PRODUCTION OF MARSH RAMSHORN SNAIL *Planorbella trivovlis* AND GHOST RAMSHORN SNAIL *Biomphalaria havanensis* BASED ON MATE AVAILABILITY

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Bolbophorus damnificus is a digenetic trematode causing economic losses in commercial catfish production. The trematode is transmitted by two different snail species commonly found in commercial catfish ponds, *Planorbella trivolvis* and *Biomphalaria havanensis*. Controlling the snail host is the most common management strategy for reducing trematode infections. However, little is known about the population dynamics of the two species.

We performed a 21-week study to evaluate how mate availability influences snail reproduction rates. Three scenarios were tested to investigate egg production and viability when provided available mates continuously ("continuous"), cyclically ("cyclic"), or at a single timepoint ("loner"). Both snails are hermaphroditic and can self-fertilize, though there is a preference for out-crossing. Snail eggs were collected weekly from each treatment, and the total number of eggs (viable and non-viable) and clutches were counted.

Over 93,000 eggs were collected and counted over 21 weeks. Egg production patterns differed between species. *B. havanensis* produced more eggs per day at the start of the study but declined sharply over the course of the sampling period. Non-viable eggs began to increase near the end of the study, particularly in "loner" treatment, suggesting a depletion in stored sperm. Additionally, *B. havanensis* showed a dramatically higher mortality rate compared to *P. trivolvis*.

The data collected from this study sheds new light on mechanisms that may help snails inhabit and persist in new ponds. This also provides an initial estimate of replacement rates in commercial ponds throughout production. Basic biological information on each snail species can aid in developing more targeted management strategies for farmers.

Table 1. Total number of eggs collected from each species (columns) and treatment group (rows) during the 21-week sampling period. Continuous = continuous mate availability; Cyclic = weekly mate availability; Loners = single timepoint mate availability.

	B. havanensis	*P. trivolvis*	Total
Continuous	9,317	21,542	30,859
Cyclic	9,810	29,859	39,669
Loners	11,541	11,928	23,469
Total	30,668	63,329	93,997

TOWARD INTEGRATED PEST MANAGEMENT OF THE INVASIVE APPLE SNAIL *Pomacea maculata* IN CRAYFISH, RICE, AND CATFISH PRODUCTION

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Invasive apples snails, including *Pomacea maculata*, are considered one of the most destructive invasive species worldwide, and considered pests to rice production in Asia, Europe, Central America, and North America. Louisiana has seen large production impacts in both crayfish and rice production, which are often used in a crop rotation.

Apple snail densities can reach > 20 snails/m^2 and current management strategies are limited. The conspicuous, pink eggs are deposited on emergent vegetation and structures overhanging the water; the eggs must remain dry to hatch. Currently, mechanical tactics for knocking eggs into the water has been used to limit reproduction but the method is laborious and costly. Snails effect production directly and indirectly. Direct predation on rice seedlings leads to crop loss and requires replanting which further increases production costs. In crayfish production, large snails clog traps, reducing catch rates of target species, while smaller individuals intermix with the captured crayfish, requiring each trap to be carefully sorted to remove snails from marketable catch. Still unknown is the influence that apple snails may have on fish production systems. Snails are known to serve as hosts for a wide variety of parasites, many of which are further transmitted to vertebrates, both terrestrial and aquatic. These parasites can have detrimental effects on aquatic production systems, as well as human populations.

This project aims to broaden our understanding of invasive apples snails in the context of developing applicable management strategies. Evaluating apple snail population dynamics is crucial in understanding variables associated with infestation rates and damage assessments. Chemical therapeutics such as copper sulfate have a variety of uses in aquatic production systems, including gastropod treatment. The current study will evaluate the applicability of this and other molluscicides in the control of apple snails. Additionally, an environmental DNA assay will be developed and evaluated as a method of monitoring new infestations and snail densities. Lastly, a survey will be conducted to identify parasites relevant to crayfish and catfish production, as well as human-health.

Figure 1. Conspicuous pink egg masses of the invasive apple snail on emerging vegetation in a Louisiana crayfish pond. Photo credit: B. Wilson

CSRV1 TRANSMISSION AMONG BLUE CRABS IN SOFT SHELL BLUE CRAB *Callinectes sapidus* AQUACULTURE

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Soft shell blue crab (*Callinectes sapidus*) aquaculture is one of the oldest domestic aquaculture industries along the East and Gulf Coasts of the United States. Soft shell blue crabs are produced by culturing pre-molt (peeler) crabs in shallow dockside or land-based shedding systems until they shed their hard shell in order to grow. Soft shell production is most successful with peeler crabs harvested one to three days before molting. However, crabs are often held for 14 to 21 days. High mortality in shedding systems has always been a problem for the industry, and diseases, such as *Callinectes sapidus* reovirus 1 (CsRV1), can play a significant role. The prevalence of CsRV1 in dead peeler crabs ranges from 22 – 75% in blue crab shedding facilities in the Chesapeake Bay region.

To understand more about CsRV1, we conducted experiments examining the fate of naturally infected wild-caught crabs, the mortality rate in virus-injected juvenile (Florida) and adult (Louisiana) crabs, the potential for water-borne virus transmission, and the impact of salinity (1, 5, 15 ppt) on CsRV1 transmission among crabs in shedding systems. Crabs used in threshold experiments were kept in individual tanks until they died. Crabs used in transmission experiments were kept individually in cages in scaled-down recirculating systems. The cages prevented physical contact among crabs. Real-time qPCR was used to detect CsRV1 in crabs prior to the start of all experiments (Time 0) and at death or end of the experiment (Time Final).

Wild caught crabs with natural infections of CsRV1 died on average 13.8 days with viral loading greater than 500,000 copies of CsRV1 per mg of tissue. Injected, juvenile crabs showed significant mortality (50%-66%) when injected with virus loads between 10^6-10^7 copies of CsRV1 per mg of tissue after 18 days. Injected, adult crabs showed significant mortality (40%-100%) when injected with virus loads between 10^5-10^7 copies of CsRV1 per mg of tissue after 25 days. The absence of physical contact among crabs in the transmission study indicated that the transmission of CsRV1 among crabs is waterborne. Virus transmission and mortality was reduced in lower salinities (1 and 5 ppt) compared to higher salinity (15 ppt).

Information gathered from these experiments is being used in extension work with soft shell crab producers in an effort to increase production efficiency. We encourage producers to reduce the amount of time they hold crabs in their systems prior to molting, staying as close as possible to one to three days. We encourage the use of lower salinity water in recirculating systems and the discard of dead crabs into land based facilities to reduce the spread of the virus in their systems.

HOW CAN SEASONALITY CHANGE THE ACCUMULATION OF METALS IN CLAMS PRODUCED IN THREE DISTINCTIVE AREAS OF PORTUGAL?

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Bivalve molluscs are traditionally used in gastronomy, and their harvesting represents a critical socioeconomic activity to coastal communities. However, due to their filter-feeding behavior that facilitates the ingesting of bioavailable contaminants, regular monitoring is needed to ensure food safety. Specifically, these organisms may act as vectors for transferring metals to higher trophic levels, including humans. Arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are listed in the Priority Pollutant List from the Clean Water Act by EPA, as these metals require particular attention due to their high toxicity, persistence and bioaccumulation. Although the recent efforts to reduce anthropogenic emissions worldwide, historical contamination leads to considerable burdens of metals in the sediments of aquatic systems. Therefore, anthropogenic activities, seasonal storms, and tides are expected to resuspend bottom sediments, making metals available to the biota. Therefore, monitoring metals' presence along the Portuguese coast is extremely relevant to environmental and food safety issues. This study aimed to assess As, Cd, Pb and Hg concentrations in clams (*Ruditapes decussatus* and/or *Ruditapes philippinarum*) collected monthly in aquaculture areas of three coastal lagoons (Ria de Aveiro, Ria Formosa and Óbidos Lagoon, April 2019 – January 2020). Metals quantification in the edible tissues of clams was carried out through: i) DMA-80 Evo Direct Mercury Analysis System for Hg quantification; ii) inductively coupled plasma mass spectrometry (ICP-MS), using the Agilent Technologies-7700Series equipment, for As, Cd and Pb. None of the studied metals was found above the legal limits regarding human consumption of bivalves. Clams proved to be good bioindicators of metal presence. Arsenic increased in both clams from Óbidos lagoon in the summer period, and its levels only decreased in *R. philippinarum* in the following months. As expected, clams from Ria Formosa and Ria de Aveiro presented higher levels of Hg than Óbidos lagoon throughout the year (Figure 1). Further fluctuations in As, Cd, Pb, and Hg accumulation due to seasonality will be discussed.

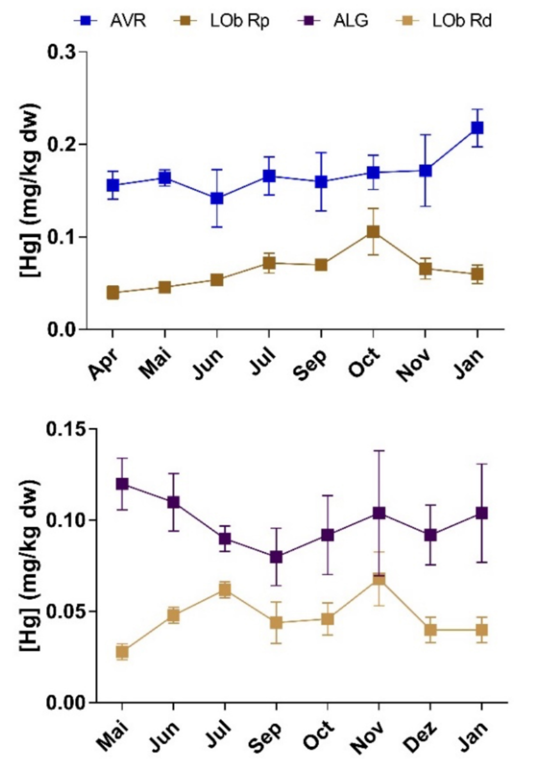

Figure 1. Mercury concentration (mg/kg dry weight) in clams from Ria de Aveiro (AVR), Óbidos lagoon (LOb) and Ria Formosa (ALG).
Data are presented as mean ± SD.

This work is part of the project DepurD (MAR-01.03.01-FEAMP-0046), supported by Portugal and the European Union through MAR2020, Portugal2020 and FEAMP

IMPROVEMENTS TO GROWTH AND INTESTINAL HISTOMORPHOLOGY IN TILAPIA BY DIETARY INCLUSIONS OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE FRASS DEPENDS ON THE TYPE

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Black soldier fly larvae (BSFL) are increasingly being investigated as an ingredient in fish diets, but a by-product is the left over 'frass', which is their excrement rich in minerals and amino acids. There is also interest with dietary inclusions, however, the composition of frass can be influence by the initial substrate. In this study, BSFL frass was made using either spoiled feeds (SF) or vegetables/fruits (FV) that were high and low in protein, respectively. The proximate and amino acid composition of these frass types were analysed to formulate isonitrogenous and isolipidic diets containing 5 or 10% SF or FV frass, while a control diet was made with no frass inclusions. These diets were fed to tilapia juveniles (initial weight = 2.94g) for 2 months in a closed recirculating system.

Results showed that 5 and 10% FV frass diets significantly enhanced growth compared to the control or FV frass diets. Contributors to enhanced growth were increased palatability based on a higher feed intake and a better amino acid profile. Liver histology revealed no inflammation or any other adverse response in any of the treatments, indicating dietary frass at the levels used showed no hepatotoxicity. Moreover, the intestinal villi of tilapia fed the SF frass diets were significantly longer than those in the control (Fig. 1).

Fish muscle phosphorus was significantly higher at FV frass treatment while magnesium was significantly higher at 10% FV frass compared to the 5% SF frass treatment. The remaining biochemical analysis (proximate, amino acid, and fatty acid composition) are being analyzed. Preliminary gene expression analysis indicates that dietary frass inclusions and type greatly influences metabolism and detoxification pathways.

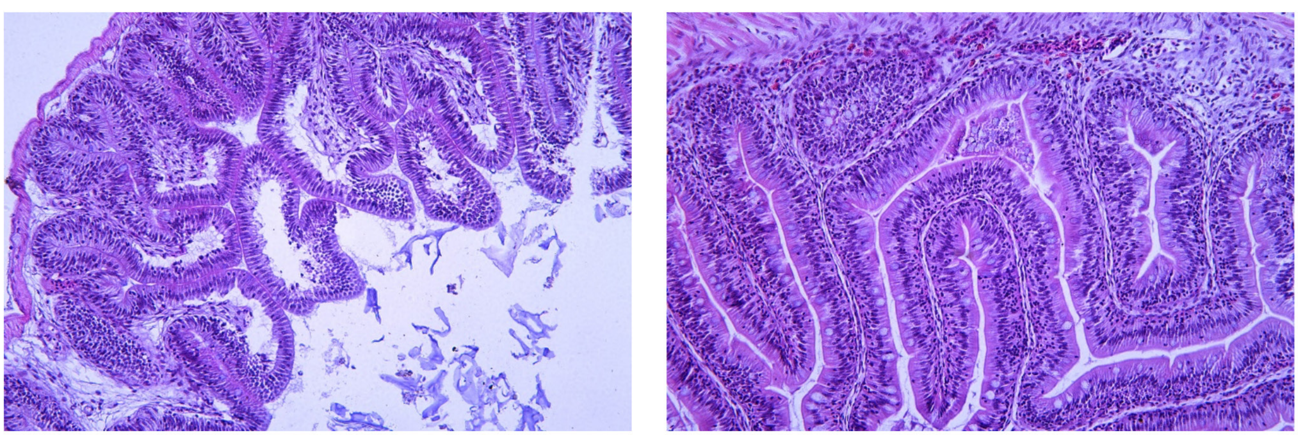

Figure 1: Intestinal histology in tilapia fed the control diet (left) that contained no frass versus those fed 5% SF frass which had longer villi and less inflammation in the lamina propria.

DEVELOPMENT OF EASY-TO-USE OYSTER AQUACULTURE ENTERPRISE BUDGETING TOOLS FOR FLORIDA GROWERS

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Off-bottom oyster aquaculture is a relatively new and growing industry in Florida. The oldest commercial operations are less than a decade old, but the industry is growing rapidly. In 2021 approximately 4.1 million oysters were grown by Florida off-bottom producers, an increase of 37% relative to 2016. Given the newness of the industry and limited availability of commercial production data there are a variety of production approaches, both with respect to equipment (floating bags, floating cages, and suspended longlines) and strategies (number and timing of plantings, biofouling control procedures, etc.), being employed by current growers. Quantifying the financial and economic risks associated with production can allow growers to make better informed decisions regarding their production techniques and practices.

While recent research (Moor et al. 2022) and extension-based risk modeling tools (UF/IFAS Oyster FARM Calculator_Beta Version - https://shellfish.ifas.ufl.edu/news/oyster-farm-financial-and-risk-model-calculator/) have quantified some of the risks of production, they have relied on complicated stochastic risk modeling techniques and have not been widely employed by growers. In an effort to provide growers in Florida's oyster aquaculture industry with tools that can easily be tailored to their current business and production practices we have developed enterprise budgeting tools specific to the most common gear types used in production (floating bag, floating cage, and suspended longline) that allow growers to use enterprise specific information to examine income and cash flows associated with their production decisions. In addition, to providing growers with an easy means to evaluate the potential financial implications of changing production variables such as stocking density, survival rates, output prices, and biofouling control techniques; our tools include built in sensitivity analyses that show how changes to key input and output variables (labor costs, seed costs, survival rate, sales price, etc.) impact profitability. Each of these easy-to-use Excel-based enterprise budgeting tools also includes step-by-step instructional guides on their use. This presentation will cover the design of the enterprise budgeting tools and their functionality.

SYNTHESIZING ECOSYSTEM SERVICES DATA TO SUPPORT SHELLFISH AQUACULTURE MANAGEMENT

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Shellfish aquaculture can provide a variety of ecosystem services beyond food production. Shellfish aquaculture gear creates complex structure that can increase the abundance and diversity of wild fish relative to reference habitats such as mud and sand bottom. A variety of life stages of fish have been observed in association with aquaculture gear, and occurrence of habitat-related behaviors such as foraging, shelter, and reproduction have also been documented on gear. Shellfish can reduce excess nutrients in the nearshore environment by assimilating nutrients into their tissue and shell as they feed and grow and promoting excess nitrogen removal by augmenting denitrification rates in surrounding sediments. Provisioning of this nutrient reduction service has been formally recognized through incorporation of shellfish aquaculture into nutrient management programs in two estuaries in the Northeast United States.

Through a combination of synthesis of the existing literature, and collection of regionally-relevant data, our program seeks to provide shellfish resource managers with an assessment of nutrient and habitat provisioning by shellfish farms in the Northeast region. The assessment is focused on the three most commonly cultivated species in the region: *Crassostrea virginica*, *Mercenaria mercenaria*, and *Mytilus edulis*. Existing literature on nutrient content of all three species indicates low variability in space and time, suggesting that nutrient reduction services are consistently provided region-wide. Existing literature on habitat provisioning suggests that gear used to cultivate all three species can increase the abundance of wild fish relative to reference habitats. Ongoing research programs in New Jersey, Connecticut, and Massachusetts seek to understand how habitat services provided by oyster aquaculture may vary according to factors such as location, season, cultivation practices, and gear types. Results from the synthesis and the regional research programs will be discussed, and data gaps identified. Information on region-scale variation in ecosystem service provisioning associated with shellfish aquaculture can aid resource managers in developing a permitting framework that includes consideration of environmental benefits in addition to potential impacts.

THE QUALITY OF FROZEN-THAWED SALMON FILLETS AS AFFECTED BY SUB-CHILLING PRIOR TO FREEZING

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Aim:
The present study aimed to enhance the quality of thawed Atlantic salmon by implementing sub-chilling prior to freezing.

Methods:
Eighty salmon (4-5 kg) were electrically stunned and bled commercially and divided into two equal-sized groups. The first group (*ice*) was packaged on ice in expanded polystyrene boxes, whereas the second group (*RSW*) was immersed in a premade refrigerated seawater (RSW)-cooling system, created by using a 7% NaCl brine and ice, holding ~-1.0°C. After chilling, the fish was hand-filleted and vacuum-packed (99% vacuum) before the fillets were randomized (20 fillets per group) into a factorial design following the fixed factors "chilling condition" (*ice* versus *RSW*) and "storage condition" (*fresh*, *one*-, and *four-months frozen*, respectively). The "storage condition" *fresh* was used as a control. The control groups (*ice-fresh* and *RSW-fresh*) were, after packaging, directly placed in the cold room (0.6±0.5°C) and evaluated through a 16-day storage experiment. The fillets to be frozen (*ice* and *RSW*, *one-* and *four-months* frozen, respectively) were flash-frozen (30 minutes) with dry ice (-78.5°C) before being stored in a freezer (-28.5±1.4°C). After frozen storage (*one- and four-months*), fillets were thawed in a water-bath (4°C) for 4 hours before being transferred to a cold room (0.6±0.5°C). The salmon fillet quality throughout the storage experiments (*fresh* and after *one- and four-months* of frozen storage) was evaluated by following the fillet drip loss, texture, and colour, as well as protein denaturation, adenosine triphosphate degradation, and microbial parameters on day 1, 5, 12, and 16 post packaging/thawing.

Results:
Sub-chilled fish (*RSW* groups) had a higher drip loss than those ice-chilled, with lower aerobic plate counts and higher concentrations of inosine monophosphate. Moreover, frozen fish showed a higher drip loss than the fresh controls, lower total viable psychotropic counts, surface-breaking force, firmness, chroma, and higher hue. All groups showed a decrease in quality through storage.

Conclusion:
It is concluded that sub-chilling prior to freezing improved the overall quality of fresh and frozen-thawed salmon fillets, whereas no effect of frozen storage time was observed.

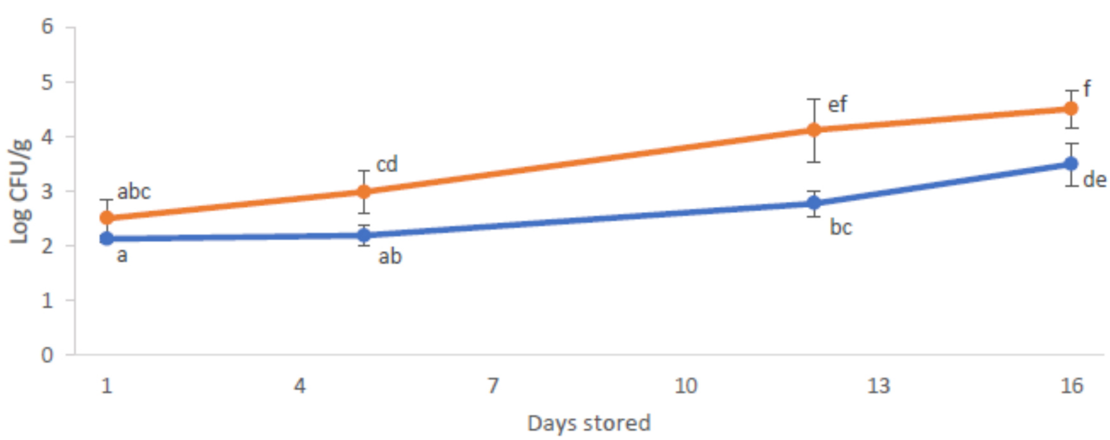

Figure 1: *APC of mesophilic bacteria in RSW chilled and ice-chilled samples throughout storage (significance level p<0.05, GLM). Blue line is sub chilled fish (RSW), and the orange is ices stored fish, as a pre treatment before freezing*

THE PRODUCTION OF COMMERCIAL QUANTITIES OF HAWAI'IAN KANPACHI *Seriola rivoliana* JUVENILES AT THE BLUE OCEAN MARICULTURE HATCHERY; PAST, PRESENT AND FUTURE

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Blue Ocean Mariculture is a vertically integrated commercial fish production company growing Hawai'ian Kanpachi (*Seriola rivoliana)* on and off the coast of Kailua-Kona, Hawai'i. Before the fish can be raised to harvest size, processed, marketed and sold, all of the hundreds of thousands of juveniles transferred to offshore net pens yearly are produced within our single hatchery.

For more than 22-years, Blue Ocean Mariculture and its predecessor Kona Blue Water Farms have worked within a single 2.5 acre lot to write the code on how to efficiently and predictably produce commercial quantities of healthy and whole Hawai'ian Kanpachi juveniles. The vast majority of this work has taken place within a single 2.5 acre lot located inside a one-of-a-kind aquaculture technology park, the Natural Energy Laboratory of Hawaii Authority. During these last 2 decades, hundreds of passionate and tireless biologists, growers, engineers, pathologists and jacks of all trades have been involved in creating the hatchery we now proudly operate.

Beyond the obvious need for a deep institutional understanding of all aspects of culture, our company has learned many important lessons in the challenges of operating a small, and simultaneously high-output facility. The necessity of managing a 365 day/year complex biological process within a high labor cost, remote site has been a catalyst for aggressive and creative management strategies. A prime example, automation, has yielded innovative and high-return on investment solutions to age-old labor-intensive tasks such as siphoning, feeding, greening and counting. Moving forward, using tools like genetics, AI and advanced hatchery equipment, we plan to bring our production to the next level of sophistication and consistency.

WHITE ABALONE (*Haliotis sorenseni*) RESTORATION AQUACULTURE: AN ASSESSMENT OF FORMULATED DIETS AND PROBIOTICS

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White abalone (*Haliotis sorenseni*) are an endangered species found along the California coast and are at historically low densities, nearing extinction. Aquaculture facilities throughout California are currently involved in the captive breeding of the species and growout of juveniles for outplanting to wild habitat. This activity aims to enhance the species recovery by developing self-sustaining populations. However, these efforts are costly because of the species slow growth, high early mortality rate, and reliance on seasonally dependent macroalgae as feed. These limiting factors warrant an assessment of alternative diets and probiotic treatments for the species to shorten the culture time and lower costs before outplanting. Diet administered probiotics have previously shown improved growth rates, feed digestibility, and survivorship in abalone species, while formulated feeds are known to provide adequate nutrition and reduce costs for several cultured species.

This study investigates the effects of a diet administered probiotic (*Bacillus licheniformis*) paired with a commercial formulated abalone feed (ABKelp® by Algamar) on white abalone. Three diet treatments were assessed in a replicated tank trial: untreated formulated diet, probiotic treated formulated diet and fresh macroalgae diet (*Devaleraea mollis* ,*Macrocystis pyrifera*). Live probiotic was cultured and sprayed onto the pellets weekly and stored at 4°C until fed. Feeding rates were measured at every feeding interval, shell length and abalone wet weight were measured individually on a monthly basis. The study was conducted for a total of six months. The fresh macroalgae diet treatment resulted in the greatest growth rate and feed intake (Fig. 1). The formulated diet treated with the probiotic had the lowest feed intake rate, potentially due to a reduced palatability caused by the presence of the probiotic. Despite comparatively inferior growth metrics, the formulated diet treatment still resulted in adequate growth and survivorship in white abalone suggesting it is a viable alternative diet for conservation aquaculture facilities with limited access to fresh marcoalage. The use of a formulated diet is feasible for white abalone restoration aquaculture when considering additional costs associated with fresh macroalgae feeding including, permits, diving, and boat operations for kelp collection as well as culture facilities devoted to macroalgae culture and storage

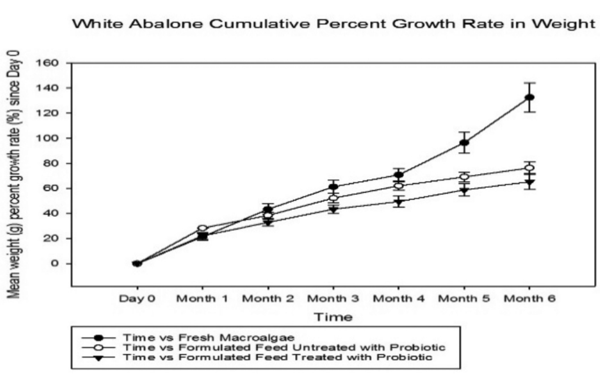

Figure 1. Percent growth rate in wet weight in white abalone by treatment over six months.

MITIGATION OF SOYBEAN MEAL-INDUCED ENTERITIS IN RAINBOW TROUT *Oncorhynchus mykiss* USING VITAMIN D AND GLUTAMINE AS DIETARY SUPPLEMENTS

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Soybean meal (SBM) has become an essential ingredient in fish feed formulations, reducing the aquafeed industry reliance on fishmeal (FM). However, challenges remain when using SBM as the major source of protein, especially for carnivorous species. Reduced growth, and the occurrence of intestinal inflammation, referred to as soybean meal-induced enteritis (SBMIE), are signs of limited tolerance to SBM. Formulation of functional feeds using vitamins or amino acids could help increase SBM inclusion levels in fish diets. Vitamin D (VitD), well known for its role in calcium and phosphorus homeostasis, has more recently been suggested to play a role in intestinal immunity and barrier integrity. Therefore, we hypothesized its potential role as feed additive towards SBMIE attenuation. In addition, possible synergistic effects of VitD and the non-essential amino acid glutamine (Gln), known for its protective action during intestinal inflammation, were also examined in the present study.

A 10-week feeding trial was designed to investigate these hypotheses in rainbow trout (*Oncorhynchus mykiss*). A total of 378 fish initially weighing ~ 9.5 ± 1.0 g, were randomly distributed into 21, 65-L tanks (18 fish/tank). Seven experimental diets (42% isonitrogenous and 20% isolipidic) including a FM diet (control), a SBM30 and SBM40 diet (30 g/Kg and 40 g/Kg inclusion level, respectively); two SBM30 and two SBM40, each supplemented with either VitD or VitD-Gln (5 µg/Kg VitD or 5 µg/Kg VitD + 2 g/Kg Gln) were formulated and fed to apparent satiation.

Feed intake was recorded daily, and fish weight and tissue samples were taken at 5 and 10 weeks. Samples were collected from the distal intestine for histology and gene expression analyses of the inflammatory markers: TNF-α, IL-1β, IL-10; barrier function markers: MLCK, occludin, and claudin-12; as well as the VitD receptor. Vertebra, kidney, and muscle samples were also obtained for mineral analysis and calcium determination.

Fig. 1., shows that by week 10, growth performance was improved in terms of weight gain in the SBM30-VitD-Gln group, which also showed slightly lower FCR ($p < 0.05$). Gene expression analysis showed changes in VitD receptor and MLCK expression at 5 and 10 weeks as well as IL-1β at 10 weeks. Further analysis will help reach conclusions on the use of VitD or VitD-Gln in SBM functional feeds.

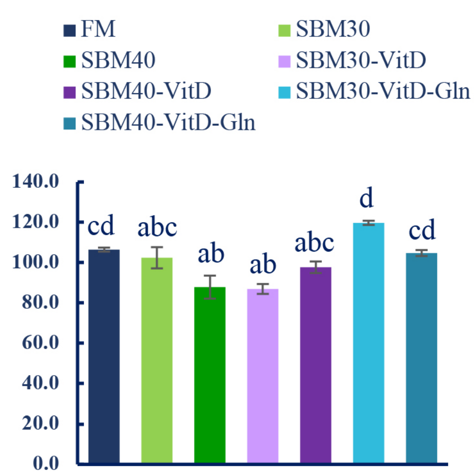

Fig. 1. Weight gain (g) of each dietary group at week 10 of the feeding trial.

CAN WE USE STEPPING STONE TECHNOLOGIES TO GET OCEAN BASED FISH CULTURE MOVING?

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The current industrial model for U.S. marine fish aquaculture requires large investments for high tech cages, automated feeding and monitoring systems, a modern hatchery, and modern extruded feeds. The provision of feed and seed must either be accomplished by vertical development of the grow out company, capacity must already exist or be developed by others in a location. Development of a modern hatchery and/or extruded feed mill each require a certain high level of demand to justify investment, and often the minimum size for profitability is larger than would be needed for a grow-out company. These drivers create a catch-22 among producers of feed, seed and grow-out for marine fish. Especially in small island states and rural areas where shipping in one or more of the inputs to production is impractical, and in political jurisdictions that are hesitant to support a single large company, producing a large amount of seafood that is likely to dominate markets. If local markets are small, a single large farm could dominate them, and may need to target exports to sell the excess. All of this drives up the minimum production size for a grow-out firm to be profitable, and increases the economic, environmental, and social barriers to entry. Where marine aquaculture has developed (finfish in Norway, China, Japan etc.) and is actively developing in the US (seaweed and shellfish in Alaska, Maine and other states) firms started smaller with, and in most cases still use simpler less complex technology and grew organically to their current level of industrialization. I will introduce examples of possible stepping stone technologies for marine fish cages, seed (Floating Hatcheries) and feed production (Wet formulation with compacted or wet extruded pellets) that may help reduce barriers to entry for marine finfish. I will also argue that to provide the level of innovation and development needed to progress from an entry level of technology to higher levels of industrialization needed for a high level of production, it will be necessary for the world to focus on a few species due to the high costs of research and innovation. Conversely, entry level application of low capitol technologies can lead to a smaller level of production but of a more diverse number of species. These paths are not mutually exclusive and can even be complimentary.

SCIENCE FOR ADVICE, AN UNDERDEVELOPED OPPORTUNITY?

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Regulatory inaction has been suggested as a prime cause for slow development of the US's marine aquaculture industry. Many scientists see the solutions to this issue as outside of their skill set. On the contrary, we argue that application of science to inform permitting is underdeveloped and a keystone need for science-informed government decision making. At the heart of the Federal regulatory process for permitting new aquaculture sites is environmental, economic, and social analysis prescribed by the National Environmental Policy Act (NEPA). These analyses are reported in environmental impact statements (EIS) or assessments (EA) and are meant to apply the best available science from a range of scientific disciplines to understand a given project's range of impacts and mitigation options. In many ways, NEPA forces an ecosystem approach to aquaculture permitting by addressing multiple impacts and requirements from numerous applicable federal laws within one document. However, it also sets up a complex process that by its nature requires diverse and specialized scientific expertise. For analyses related to aquaculture, the diversity of issues requires the writers of the NEPA document to consider "best available science" from disciplines as different as economics, engineering, oceanography, ecology, social sciences, genetics, epidemiology, health, spatial planning, nutrition, and many others. It also requires the ability to turn the vast universe of discipline-specific science into a series of *de facto* combinations of risk assessments and best management practices. Typically, these are complex documents and are done by specialists hired by the government (but often using the proponent's money) for the purpose. Given the diversity of issues to be addressed within a NEPA analysis, writers are generalists regarding the various scientific disciplines. In addition, NEPA regulations prescribe limits on document length, necessitating brevity for each topic. In many areas of government decision making (including fisheries management), special issue-specific scientific documents and tools--generically called Science Advice--fill the gap between the universe of science and regulatory decisions. Science advice considers the "best available science" and presents this information in a format useful for decision making. These documents and tools directly provide decision support for environmental, economic and social analyses but do not make the decisions. NOAA has recently published a few science advice products for this purpose (with more in the works), however, the need is still great for targeted advice documents. Recent examples of science advice products are the two Aquaculture Opportunity Area Atlases published in 2022 (Gulf of Mexico and Southern California), however there are a diversity of others covering specific issues. US law applies special requirements to this type of scientific product which has recently been reviewed in an Aquaculture Science Advice Handbook. There is a role for government and non-government scientists to focus their expertise on science advice, and we challenge you to consider applying your science skills to help address regulatory needs.

GENOMICS AND METAGENOMICS PREDICTION OF MUSCLE GROWTH AND FILLET QUALITY TRAITS IN RAINBOW TROUT

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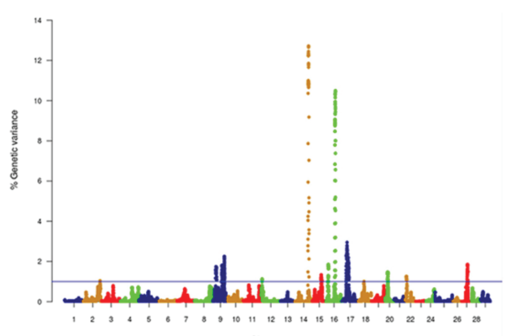
Muscle/fillet is the most valuable product of fish. Increasing fillet yield and reducing fillet downgrading can improve production efficiency and customer satisfaction and have significant economic benefits.

Classical breeding has limited success in improving fillet yield and requires several generations. The aquaculture industry is eager to adopt genomic selection methods to increase the efficiency of genetic improvement, i.e., increasing the accuracy of breeding values and reducing generation intervals, consequently increasing genetic gain.

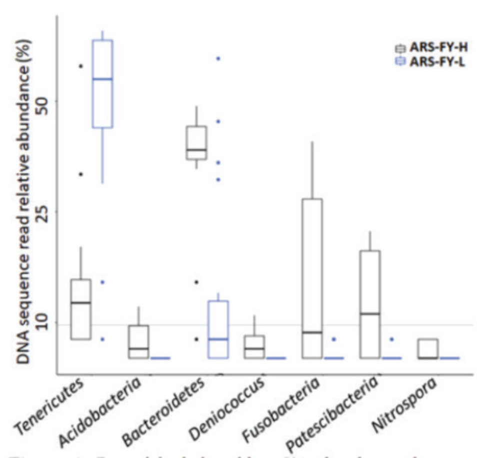
In this project, we demonstrated the utility of genomic selection in fillet yield and quality traits of rainbow trout. We developed a 50K "functional" SNP chip and used it to identify QTL for fillet yield, firmness, color, protein, and fat contents. Our studies showed that the genomic predictions can improve the estimated breeding values by 33.3% for the fillet yield and 53.1% for fillet firmness.

The genomic predictions outperformed the traditional breeding by 35% for fillet yield and 42% for fillet firmness. In addition, we demonstrated that a reduced SNP panel of ~1000 SNPs used in genomic predictions still can provide predictive abilities higher than traditional breeding. These results suggest that genomic selection is a practical strategy to identify and select fish with superior genetic merit within rainbow trout families, even with low-density SNP panels.

Separately, a recent study in our laboratory indicated the presence of heritable fillet yield-associated microbiome components. We are currently assessing the feasibility of improving genetic prediction accuracy when microbiome information is integrated into genomics-metagenomics analyses.



Manhattan plot of GWAS showing association between SNP genomic windows of 50 SNPs and muscle yield. Chromosomes 14 and 16 showed the highest peaks with genomic loci, explaining up to 23.2% of the genetic variance.



Bacterial phyla abundances between the ARS-FY-H (high) and ARS-FY-L (low) fillet yield genetic lines.

STATUS OF THE ASSEMBLY AND FUNCTIONAL ANNOTATION OF RAINBOW TROUT GENOME

Mohamed Salem, Rafet Al-Tobasei, Yniv Palti, Guangtu Gao, Huaijun Zhou

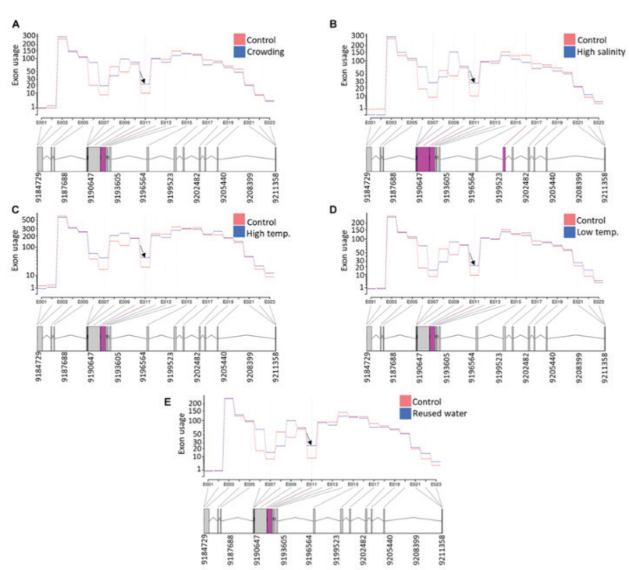
This USDA NIFA-funded project aims to provide a high-quality assembly and well-annotated reference genome of the rainbow trout. The USDA/ARS/NCCCWA lab released significantly improved de-novo genome assemblies for the Arlee clonal line (USDA_OmykA_1.1) and Swanson clonal line (USDA_Omyk_2.0), which can improve the identification of genetic markers and genes related to disease resistance and other physiological traits. Efforts are still ongoing to add a third de-novo assembly from the Whale Rock line toward developing a pan-genome reference for rainbow trout.

A new assembly of the rainbow trout transcriptome (Swanson clonal line) using long reads sequencing has been published, identifying new alternative splicing associated with essential traits, including fish growth, muscle accretion, disease resistance, stress response, and fish migration. The new transcriptome was used for annotation of the USDA_Omyk_2.0 genome assembly.

ATAC-Seq data from four tissues and Chip-Seq data from six tissues were generated to annotate chromatin accessibility of the genome. In addition, Methyl Mini-seq was used for genome-wide profiling of DNA methylation in male and female rainbow trout. The improved assembly and annotation of various genome references will allow comparative functional genomics analyses and identification of genes controlling aquaculture production and animal welfare traits.

Feature	OmykA_1.1 (GCA_013265735.3) (Arlee)	Omyk_1.0 (GCA_002163495.1) (Swanson with Illumina)	Omyk_2.0 (GCA_025558465.1) (Swanson with PacBio)
Number of chromosomes	32	29	29
Number of scaffolds	938	139,799	1,278
Total length	2.3 Gb	2.2 Gb	2.3 Gb
Maximum length	88,429,459 bp		
Minimum length	16,956 bp		
Scaffold N50	39.2 Mb	1.7 Mb	52.4 Mb
Scaffold L50	22	259	16
Contig N50	15,579 Kb	14 Kb	10,668 Kb
Contig L50	41	32,575	57

Summary statistics of 3 genome assemblies available for rainbow trout



An exon in the P4HA2 gene is differentially used under 5 stress conditions [(A) crowding, (B) high salinity, (C) high temperature, (D) low temperature, and (E) reused water].

SETTING THE STAGE: APPLYING EFFECTIVE BROOD DEVELOPMENT TECHNIQUES FOR INCREASING PROBABILITY OF SUCCESS WITH THE REINTRODUCTION OF EXTRIPATED ARCTIC GRAYLING *Thymallus acrticus* IN MICHIGAN

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The goal of the Michigan Arctic Grayling Initiative (MAGI) is to restore self-sustaining populations of extirpated Arctic Grayling *Thymallus acrticus* into some select historic Michigan range river systems. While success will ultimately hinge on habitats, to increase the likelihood of success, several very important factors needed to be considered and addressed far before reintroduction, during the brood development planning process.

This presentation will review considerations taken during the process. This includes considering fundamentals about the founding stock such as availability and reliability to access of the founding stock, ensuring healthy genetic variability, and the existence of a credible founding stock disease history. It also includes how gametes are crossed and are selected, including meeting goals for the number of contributing adults, maintaining 1:1 spawning ratios, and random selection of fertilized eggs.

BIOSECURITY: CONTAINMENT STRATEGY USED DURING MICHINGAN'S ARCTIC GRAYLING *Thymallus acrticus* BROOD DEVELOPMENT

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To ensure no aquatic invasive species were accidentally introduced into the Great Lakes watershed, Michigan DNR upgraded Oden State Fish Hatchery's Isolation building into a containment facility before accepting Artic grayling from Alaska. As originally designed, Isolation was meant to protect the hatchery during brood development using fish from within the upper Great Lakes basin. It however was not designed to protect the waters of Michigan from fish or diseases originating outside of this watershed.

This presentation will review strategies initiated to contain possible invasive species and diseases that might have been accidentally transferred along with the Arctic grayling. Strategies included adding redundant primary filtration and UV sterilization for effluent water; designing the system to not discharge any undisinfected water during emergencies; controlling and limiting incoming water, especially during emergencies; adding additional procedures to reduce risk of accidental biosecurity breach; replacing an outdated generator; plus upgrading the emergency alarming system.

A REVIEW AND CRITIQUE OF NORCAN'S "GATLIN GUN" STYLE FISH FEEDING SYSTEM APPLICATION AT MICHIGAN DNR'S THOMPSON STATE FISH HATCHERY

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In early 2021, a unique fish feeding system was installed at Thompson State Fish Hatchery to feed fingerling and yearling steelhead *Onchorhynchus mykiss*. This system was designed by Norcan Electrical Systems Inc. and uses a computerized centralized blowing system to calculate feed amounts and efficiently distribute precise dry pelleted feed quantities to multiple large raceways in two buildings.

This presentation will show the feeding system in action including the centralized feed distribution area and the unique circular selector wheel (aka "Gatlin Gun"), which directs feed to the correct location. Challenges during the process will also be discussed including coordination with the manufacturer during the pandemic, adjustments needed for proper customization to the hatchery, and the learning curve hatchery staff experienced while learning how to best use it.

Benefits of the system include the ease of feed handling from one centralized location, user-friendly software that automatically calculates and delivers precise amounts of feed, the system having relatively few simple moving parts, plus less electricity required as compared to the hatchery's previous automatic feeding system.

BURULI ULCER: NEW EVIDENCE FOR AN EMERGING ZOONOTIC PATHOGEN OF COASTAL FISHES IN THE NORTHERN GULF OF MEXICO

Michael W. Sandel*, Alex Rakestraw, J. Tyler Newburn, Kayla M. Fast, Yanesha Johnson, Magdalene Dogbe, Heather Jordan, Sophie Picq, Joe Receuver, Alexandra Bauer, Christine Chevillon, Jean-François Guegan, Jennifer L. Pechal, M. Eric Benbow

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Mycobacterium tuberculosis is responsible for Tuberculosis (TB), which is the deadliest infectious disease in human history. Concerted investment in TB research has guided healthcare interventions that have resulted in a 2% annual reduction in global TB incidence and mortality since 1990. In contrast, opportunistic non-tuberculous mycobacteria (NTM) infections are increasing worldwide, even among populations served by the world's most advanced healthcare systems. The simultaneous opposing trends of TB and NTM disease incidence implies a knowledge gap between the evolutionary ecology of transmissible and environmental mycobacterioses. We sought to narrow this gap by investigating an aquatic NTM species recently reported from the Neotropics, including the northern Gulf of Mexico. *Mycobacterium ulcerans* is responsible for Buruli Ulcer (BU), a necrotic infection of skin and soft tissues that is of substantial public health concern in tropical regions of the eastern hemisphere. The same bacterium is also responsible for disease outbreaks in global aquaculture, resulting in economic losses exceeding $1m annually. We surveyed cultured and wild populations of sportfishes, baitfishes, and invertebrates from Louisiana, Mississippi, Alabama, Florida, and Puerto Rico. Using molecular detection methods, we found evidence for diverse and widespread lineages of *M. ulcerans*, including strains known to cause debilitating human disease such as BU. Molecular sequence data was used to infer a new phylogeny of the *M. ulcerans* complex, which reveals evidence for intercontinental translocation of lineages of importance in aquaculture. Though mycobacterial disease in fishes of the Gulf of Mexico appears to be rare, we caution that the presence of this zoonotic pathogen imparts a need for integrative risk assessment among stakeholders from aquaculture and public health organizations.

ADOPTING PRINCIPLES OF FOOD JUSTICE FOR EQUITABLE OYSTER AQUACULTURE INDUSTRY DEVELOPMENT

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The food justice movement is growing, amplifying the need for equity and social justice within the food system. However, the movement has predominantly focused on terrestrial food systems, excluding ocean food systems. At the same time, the Atlantic coastal region is experiencing an expansion of non-industrial aquaculture production, especially oysters, benefitting coastal environments and communities. But, while many Black watermen were critical to the historic development of the wild-caught oyster industry along the Eastern coastal states', today's aquaculture industries in MD, DE, and NJ lack racial diversity, particularly at the business ownership level. This session will discuss research that analyzed barriers to entry in oyster aquaculture in MD, DE, and NJ, the history of Black oystermen in the region, and Food Justice. The research identified 8 major barriers to entry in aquaculture and 5 core Food Justice themes. The resulting framework of recommendations is an opportunity for oyster aquaculture industry managers to engage a more diverse population of entrepreneurs and consumers, promoting economic development, nutrition security, and environmental restoration.

FOOD JUSTICE THEMES AND SUBTHEMES		BARRIERS TO ENTRY		
		Racial / Gender Bias; Exclusiveness	Access to Permits, Capital, & Technical Assistance	Waterfront Access; Exposure; Experience
Creating systemic change throughout the food system.		RECOMMENDATIONS		
		Train all industry staff in: 1) industry history, 2) antiracism and 3) adopting Antiracism, Equity or Food Justice.		
Antiracist and Anti-exploitation Approach	Acknowledging Harm, History, and Trauma	Memorialize Black talent, leadership and labor; acknowledge exploitation	No- and low-interest, forgivable loans / grants	Dock / working waterfront space to watermen of color and descendents
	Reparations		Technical assistance in permitting / business planning / grant applications	Tell the story; Build pride / connection in the region via marketing
Community Determination of a Just Food System	Acknowledge, Invest in Talent and Leadership of Communities of Color	Invest in watermen of color; compensate for participation in decision-making, sharing history, and leadership roles;		
	Community Power and Community-Determination	Invest in: organizations led by people of color, internships, and community engagement to amplify industry exposure		
The Right to the Food System	Right to Food	Facilitate direct-to-consumer sales	Integrate equitable food access goals into management plans	Establish incentive programs for oyster purchases made with Federal Benefits.
	Right to Business Ownershp and Livable Wages	Integrate industry history into creative place-making in urban areas.	Hold info sessions for non-coastal / communities of color Oyster farm-to-institution programs Simplify permitting and provide info and training in multiple languages	Working waterfront space / on-deck equipment Leasing not dependent on landownership Paid internships at state agencies, businsses, universities, and organizations Educational programming (K- College)
	Right to Land and Resources	Engage the public through events and online.	Low cost dock access and "Right to farm" for small scale aquaculture	
Valuing People, Culture, Community, and Environment	The Right to Human Dignity	Multilingual outreach	Promote livable wages via grant / loas Highlight industry role in regional development via marketing Minimize public protests; expand pre-approved Aquaculture Zones	Memorialize the history, celebrate the individuals, and acknowledge injustices. Compensate families of black oystermen for decision-making roles in planning memorials.
	Community / Collaboration	Events that showcase the history	Support businesses via grants, marketing, institutional purchasing Build social capital via education	Community outreach / education; increase interest in industry and increase the buying market
	Environmental Connection and Stewardship	Invest in people of color to lead educ. programming	Priorize oyster purchasing for restoration in state, county, municipal plans.	Invest in environmental organizations for outreach

THE INTERPLAY BETWEEN ACUTE STRESS AND ANTIMICROBIAL PEPTIDE MODULATION IN THE SKIN OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Beta-defensins are one of the most representative antimicrobial peptides throughout vertebrates and the unique class of defensins identified in fish. As in humans, beta-defensins are highly expressed in the skin, where these small cysteine-rich peptides contribute to shape microbiota and the innate immune response to danger signals. Aquaculture poses many different stressors to farmed fish. Acute stress impacts organismal immunity, requiring fast and specific responses toward reestablishing homeostasis. The skin is one of the first tissues that senses and responds to stress and, thus, requires efficient mechanisms of immune regulation in place.

Here, we investigated the effects of acute stress associated with either elevated temperature or transportation on the expression of six beta-defensin (omDB) in Rainbow trout skin. In the temperature stress experiment, we sampled fish kept at 16°C (control) and those exposed to increasing temperatures (19, 22, and 25°C). In the transportation stress experiment, we sampled before (control) and after 5h-transportation. From both experiments, we collect skin and blood plasma.

Our data show that beta-defensins are expressed in a tissue-specific manner in Rainbow trout at the steady state. For instance, omDB-1a, 1b, and 4 genes are highly constitutively expressed in the skin and swim bladder; omDB-2 and 5 in the heart, and omDB-3 in the liver and gut. In both acute stress models, beta-defensin genes were strongly modulated, which occurred at earlier time points than significant changes in cortisol and glucose levels. Specifically, temperature stress caused a down-regulation of omDB-1b, 3, and 5 genes, while up-regulated omDB-1a and 4 genes. On the other hand, transport stress resulted in a down-regulation of omDB-2.

This suggests that different types of stress impact skin immunity differently as well as likely regulated skin microbiota. Our work demonstrates that beta-defensins are associated with the early fish response to acute stress in aquaculture in a stress-specific way, which is evident in the skin, a crucial physical and functional component of the host immune defense.

CRYOGENIC SPERM BANKING OF CATLA *Catla catla*, BIGHEAD CARP *Hypophthalmichthys nobilis* AND GRASS CARP *Ctenopharyngodon idella* AND PRODUCTION OF SEEDS IN COMMERCIAL HATCHERIES

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This study was conducted with the target to establish a cryogenic sperm bank of Catla, Bighead carp and Grass carp for production of quality seeds using cryopreserved sperm in commercial hatcheries which becomes a prime need in this sector. Quality broodstock of Catla was developed by rearing Halda and Padma river-origin fish. Similarly, broodstocks of Bighead carp and Grass carp were developed by rearing recently imported China-origin fingerlings of these species. To standardize the sperm cryopreservation protocols of these species, activation of sperm motility was evaluated in different concentrations of NaCl solution (0.1 to 1.0%). Highest motility and swimming duration of sperm of Catla (95.3±1.5% and 37.6±0.6 min), Bighead carp (96.0±0.6% and 20.6±0.6 min) and Grass carp (96.3±0.3% and 21.9±0.7 min) were observed at 0.4 % NaCl solution. The toxicity of cryoprotectants (DMSO and methanol) to sperm was tested at 5, 10 and 15% concentrations for 5–40 min incubation with two extenders, Alsever's solution and egg-yolk citrate, and better motility was observed at 5% and 10% concentrations during 5 and 10 min exposure. Alsever's solution with 10% DMSO at 1:9 dilution (sperm : diluent) provided best equilibration (Catla 93.3±1.7%, Bighead carp 95.3±1.2% and Grass carp 93.3±1.7%) and post-thaw (Catla 86.7±1.7%, Bighead carp 89.7±1.5% and Grass carp 85.0±2.9%) motility of 10-15 min equilibrated sperm in cryogenic freezing. Breeding was conducted in eight government and private hatcheries for Catla, six for Bighead and five for Grass carp using cryopreserved sperm in different geographic locations and seeds were produced. The average fertilization and hatching were obtained as 38.1±3.2% and 31.0±3.1% in Catla, 41.4±2.0% and 31.8±1.3% in Bighead carp and 28.2±5.8% and 21.4±5.3% in Grass carp with cryopreserved sperm, whereas 77.3±4.2% and 65.2±4.3% in Catla, 76.3±5.4% and 60.9±6.3% in Bighead carp and 69.1±7.6% and 59.4±7.2% in Grass carp with hatchery-origin fresh sperm, respectively. Seeds of both cryopreserved and fresh sperm-origin are stocked in separate nursery ponds in respective hatcheries and being reared with supplementary feeds for comparing growth performances as well as brood production.

Table: Species-wise average fertilization and hatching rate found in breeding trials

Species	Fertilization rate (%)		Hatching rate (%)	
	Cryopreserved sperm	Fresh Sperm	Cryopreserved sperm	Fresh Sperm
Catla	38.1±3.2	77.3±4.2	31.0±3.1	65.2±4.3
Bighead carp	41.4±2.0	76.3±5.4	31.8±1.3	60.9±6.3
Grass carp	28.2±5.8	69.1±7.6	21.4±5.3	59.4±7.2

USER CONFLICTS ASSOCIATED WITH MARINE AQUACULTURE IN THE GULF OF MEXICO

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Marine Aquaculture is relatively new to the U.S. and subject to user conflicts which are only beginning to be understood. The regulatory approval of any marine aquaculture project must consider potential user conflicts through the public review and comment process. NOAA has taken the first steps in understanding potential conflicts through its work on the "Aquaculture Opportunity Area Atlas for the U.S. Gulf of Mexico". In addition, NOAA is conducting a Programmatic EIS for identifying one or more Aquaculture Opportunity Areas (AOAs) in U.S. federal waters of the Gulf of Mexico (GoM) and has held several scoping meetings to collect public comments. To help facilitate the penetration of marine aquaculture in the GoM, the Sea Grant-National Centers for Coastal Ocean Science (NCCOS) GoM Regional Workshop Steering Committee (RWSC) process has been initiated culminating in a workshop in February 2023.

This paper analyzes the ongoing work by NOAA, Sea Grant, environmental non-governmental organizations (eNGOs), and specific aquaculture projects to ascertain the hurdles that an aquaculture venture must address to satisfy potential user conflicts and secure the necessary social license to operate marine aquaculture operation in the GoM. The authors also provide additional stakeholder engagement experience to better anticipate conflicts and hurdles, which may include social acceptance of marine aquaculture, supply chain challenges, government policy, and regulatory obstacles.

THE *XIPHOPHORUS* GENETIC STOCK CENTER: A RESOURCE FOR THE GENUS *XIPHOPHORUS*

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Xiphophorus is a genus consisting of 26 species of small, freshwater, live-bearing fish species. Distinct biological traits make *Xiphophorus* fish useful in a broad array of research topics that include behavioral, evolutionary, physiological, developmental, toxicological, genomics, immunological, and parasitological studies. More specifically to our program, they have been used to study metabolism, sex determination, maturation, chronobiology, cancer, bioinformatics, and optical genetic regulation. The *Xiphophorus* Genetic Stock Center (XGSC; www.xiphophorus.txstate.edu) is a national animal resource center in the US that currently hosts 24 of 26 known *Xiphophorus* species and over 60 different strains within those species maintained for specific genetic traits, as well as a variety of inter-species hybrids. The XGSC has ~10,000 live fish maintained in ~1,400 aquaria. In addition to housing the *Xiphophorus* fish species, the center performs multiple additional duties: analysis of biopsy material, distribution of conserved biological materials, and consultation on husbandry and genetic questions. Further, the center provides resources that are relevant to *Xiphophorus* animal husbandry, histology, genomes, transcriptomics, protocols, and software for research purposes.

FEMINIZATION OF MALE BROWN TROUT *Salmo trutta* BY ESTRADIOL ADDITION TO FEED AT SEVERAL TREATMENT DOSAGES AND DURATIONS

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The Brown Trout is considered one of the 100 worst invasive species worldwide and is difficult to manually suppress making it a good target for possible use of the YY Male eradication approach, the first step of which entails the feminization of juvenile male fish. We exposed Brown Trout fry at first feeding (26DPH) to a range treatment dosages (10-30mg) and durations (60-120d). At 150 DPH, project personnel and COFRH staff collected lengths, weights and genetic fin clips from 75 fish in each replicate tank and PIT tagged them for subsequent growout. On the same day, 10 randomly selected fish per treatment group received a health exam. Health exams involved rankings for fin erosion, anomalies of the head and gills, as well as general body appearance along with weights, length and liver weights. On 29 Nov 2021 project personnel conducted the main sex reversal trial sampling via necropsy. Data at 366 DPH collected (target n = 60 fish per treatment group) included total length, weight, visual phenotype and intersex observations. Genetic fin clips were also taken along with the same suite of fish health data from 10 randomly selected test fish as described above. Genetic sex of all fish was subsequently assessed by the Eagle Fish Genetics Lab (EFGL) using Brown Trout sex markers developed and field tested against five western U.S. populations. Feminization rates (FEM) for genetic males were calculated for each treatment group. Based on sex marker results, the genetic sex of all trial fish combined (n = 1099) closely approximated 50:50 at 50.4% male. There was a small amount of variation in the percent genotypic males across the 10 treatment groups, ranging from 42.9 to 55.0% male for combined replicates. FEM rates for these genetic males ranged from a low of 63.1% for fish exposed to 10 mgE2 for 90 days to a high of 94.9% for fish treated with 20mg/kg for 120 days. Intersex ratios were lowest for both groups treated for 120 days. Based on Hepatosomatic Index (HI) trends, we saw no evidence of long-term (366dDPH) effect of exposure to E2 on liver weight from the treatment regimens evaluated. In terms of the other health factors examined, in the treated groups, there were generally more downward rankings for the two fin rankings than for the body and head/gills/eye variables. Pectoral fins appeared to be more affected by E2 exposure than other fins examined, particularly at 366DPH; however at the same time (366DPH) there were no reductions in head/gill/body rankings with the exception of the 20mg 120 d group which experienced a slight reduction. Based on feminization of genetic males, our results demonstrate that Brown Trout need longer exposure to E2 than Brook Trout to attain high rates of feminization. The two 120 day treatment periods resulted > 94% feminization of genetic males with < 3.4% intersex. The next best feminization and intersex results were observed in the 20mg 90d trial at 81.8% and 7.3%, respectively. However, ascertaining the best of these protocols for possible development of a YY Male broodstock will ultimately depend on growout results. A total of 285 fish from the various treatment tanks remained alive at the cessation of the sex reversal trial. These tagged fish are being reared in a communal raceway and will be examined in the future to ascertain long-term survival, growth and time to maturity. The best treatment protocol will depend, in large part, upon survival and maturity schedules of fish from the above three groups.

INTEGRATING INFORMATION ON BENEFICIAL SERVICES PROVIDED BY SHELLFISH AQUACULTURE INTO THE AQUACULTURE PERMITTING AND REVIEW PROCESS

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Shellfish aquaculture operations can provide a variety of beneficial services beyond food production. Cultured shellfish have been increasingly incorporated into nutrient management strategies due to their ability to assimilate nutrients into their tissue and shell as they feed and grow. Enhancement of denitrification associated with oyster aquaculture has also been documented in the scientific literature. Shellfish aquaculture gear also creates complex structure, and a variety of life stages of recreationally and commercially important fish species have been observed exhibiting habitat-related behaviors such as foraging, shelter seeking, and reproduction in association with aquaculture gear. Other environmental, economic, social and cultural benefits associated with shellfish aquaculture have also been documented to varying degrees in the literature.

Despite the growing body of evidence that aquaculture operations can consistently provide beneficial services, the current aquaculture permitting framework largely focuses on an assessment of possible adverse effects to various environmental and socioeconomic factors. Engagement with resource managers suggests this is likely because adverse effects are often easier to quantify and document than beneficial effects, and variability in aquaculture production practices can limit managers in their ability to make defensible assumptions on the types and extent of beneficial effects a proposed operation may provide. In addition, not all aspects of the current aquaculture review/permitting framework allow for, or easily lend themselves to, a synergistic evaluation of adverse and beneficial effects during the aquaculture review/permitting process.

Through a combination of engagement with resource managers and industry, synthesis of the existing literature and policy, and collection of regionally-relevant data via ongoing research programs in the Northeast region, we have begun to: 1. Identify and describe mechanisms within the current federal and state level aquaculture review/permitting framework that may allow for, or limit, the consideration of beneficial services into aquaculture management and permitting decisions; 2. Understand where current data may already support consideration of beneficial services, and what additional data may be needed to build confidence within the management community; and, 3. Develop information and end products that are accurate and easy for managers and growers to use to facilitate greater inclusion of beneficial effects in the aquaculture review/permitting framework.

We will discuss the results from initial outreach with resource managers to share information on existing regionally-relevant research and literature related to nutrient and habitat provisioning from shellfish aquaculture and data gaps, and the existing regulatory mechanisms and the types of tools/end-products that may support greater consideration of beneficial services associated with shellfish aquaculture in the aquaculture permitting and review process.

INFLUENCE OF FORAGE FISH AS A DIET SUPPLEMENT IN CAPTIVE WALLEYE BROODSTOCK ON THE QUALITY AND PERFORMANCE OF THEIR OFFSPRING

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Advancement of a viable food-fish market for walleye (*Sander vitreus)* requires a domesticated captive broodstock that produces high quality gametes and viable offspring. Furthermore, out-of-season spawning techniques that result in year-round fingerling availability is needed for the walleye market to develop. Both of these industry needs require a better understanding of proper nutrition in walleye broodfish diets. Previous studies in other Percids such as Eurasian perch and pikeperch have shown that dietary lipid and fatty acid composition of broodfish diets play an important role in egg quality and fry survival. These studies have shown that the highest hatching rate and early survival in Percids have been obtained when the broodfish diet included forage fish, either as the sole food source or as a supplement to dry feed. The walleye in this study represent a lower Mississippi River strain from Genoa, Wisconsin. Brood fish were raised under two feeding regimes that included one cohort raised 100% on pellets and one cohort raised on both pellets and forage fish. Results indicate that neither broodstock egg production nor fertilization success was influenced by feed regime. Egg size was significantly smaller for broodstock relative to wild fish but did not differ between pellet fed and minnow fed broodstock. Feed-trained fingerlings from each diet cohort were then co-mingled in an aquaponics system at an average weight of ~20 g. A fin clip was used to distinguish the two cohorts. PIT tags were also used to track the growth of 30 individuals per cohort. Growth has been monitored approximately every 30 days and is ongoing until food-sized fish have been attained with an average weight of approximately 1.0 lb (454 g).

Note: The growth study described above will conclude in January of 2023 to permit final analysis of the growth comparison of the two walleye cohorts to be presented at the 2023 Aquaculture America conference.

REVIEW OF IN VIVO *Enterocytozoon hepatopenaei* (EHP) PROPAGATION FOR USE IN EXPERIMENTAL CHALLENGE STUDIES USING *Penaeus vannamei*

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Enterocytozoon hepatopenaei (EHP), one of the etiologic agents of Hepatopancreatic Microsporidiosis (HPM), continues to inhibit production in shrimp aquaculture. As the disease spreads across the world and affects aquaculture production, farmers, feed producers, and researchers continue to develop genetically resistant lines of shrimp, preventatives, and treatments.

EHP is an obligate parasite and currently there is no *in vitro* culture method to propagate the parasite in a reproducible manner. To aid the aquaculture community in developing treatments to overcome EHP, researchers at the Aquaculture Pathology Laboratory at the University of Arizona conducted challenge studies in which specific pathogen free (SPF) *Penaeus vannamei* were injected into the hepatopancreas with a fresh inoculum prepared from known EHP-infected animals. In less than two weeks following injection, challenged animal display lesions in the hepatopancreas that are pathognomonic of EHP infection, and the parasite load was quantified by real-time PCR. There is currently no in vitro propagation method for EHP and the inoculum is not viable when store at -20 ^{0}C or at -80 ^{0}C, leaving researchers with no option but to propagate EHP in live animals.

This rapid propagation method has been implemented in 11 challenge studies since December 2021 in research conducted by the researchers at the Aquaculture Pathology Laboratory, and its collaborators. The data presented will demonstrate the efficacy of this challenge method, demonstrate its benefits, and present the challenges associated when working with this emerging disease.

ADVANCING THE GREAT LAKES AQUACULTURE COLLABORATIVE (GLAC)

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The Great Lakes Aquaculture Collaborative (GLAC), one of the National Sea Grant supported Aquaculture Hubs, was formed to provide relevant, science-based initiatives that support an environmentally responsible, competitive, and sustainable aquaculture industry in the region. This collaboration has been successful in advancing aquaculture in the Great Lakes region through outcomes such as creating industry advisory groups, providing events and webinars to the community, supporting symposia at national and state aquaculture and fisheries meetings, addressing research questions about aquaculture from the consumer and producer perspective, and generating collaborative projects in the region.

In September 2022, an additional two years of funding was provided by the National Sea Grant Office to continue GLAC with an additional set of objectives. GLAC's primary goal remains the same, and through discussions with our industry advisory groups, we developed five new objectives that will further the understanding and sustainable development of aquaculture in the Great Lakes region. The five objectives for the next iteration of GLAC are:

1. Strengthen GLAC's network leadership by maintaining collaboration among the Great Lakes Sea Grant programs and our state and regional advisory groups. We will use feedback from our advisory groups to pursue funding to develop and coordinate aquaculture research in Great Lakes states.
2. Facilitate collaboration between producers and state aquaculture associations, link these state groups to national and regional aquaculture organizations, and support underrepresented producer, student, and conference speaker involvement at state and national meetings.
3. Develop collaborations among private, state, and tribal organizations including aquaculture producers, commercial fishers, and seafood processors to jointly address food system and supply chain challenges.
4. Deliver to our state agencies, policymakers, and legislators educational materials about aquaculture, the potential for successful aquaculture/aquaponics businesses in both rural and urban underserved areas, and information about how these groups can engage with and support a sustainable aquaculture industry in their state.
5. Synthesize the existing aquaculture laws, regulations, and policies within the Great Lakes states and evaluate how regulatory agencies interpret and implement these rules with the industry.

This presentation will summarize the outcomes from our initial GLAC project and address current progress and plans for achieving our new objectives as we continue to advance GLAC.

Great Lakes AQUACULTURE Collaborative

FOUR STRATEGIES TO ACCELERATE GOLDEN SHINER *Notemigonus crysoleucas* GROWTH IN MINNESOTA AND OTHER NORTHERN CLIMATES

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Over the last twenty years the supply of Golden Shiner in Minnesota has decreased while demand by anglers for use as bait has increased and far exceeds in-state production. Minnesota reported $101,000 in sales of Golden Shiner in the 2018 Aquaculture Census which was about 58% of the $175,000 reported in the 2013 census and 24% of the $425,000 reported in the 2005 census. Recent projections by Minnesota bait dealers estimate a deficit of more than 10,000 gallons of Golden Shiner annually. Consequently, there is pressure from some anglers, bait dealers, and legislators to import Golden Shiner from other states, though this is currently prohibited by law in Minnesota. The primary concerns are that importation can introduce aquatic invasive species, disease, and parasites that may negatively impact native fish communities. Recreational fishing is one of Minnesota's largest industries, with an estimated value of $2.4 billion. Our study explores methods to increase Golden Shiner production as a preferred alternative to importation.

Production of Golden Shiner in Minnesota is limited due to slow growth rates where it takes two years for Golden Shiner to reach market size (8-12 cm) in natural ponds. We are exploring four different strategies to grow Golden Shiner to market size in Minnesota within one growing season (May-October). Our strategies include: 1) intensive indoor rearing of Golden Shiner using a recirculating aquaculture system (RAS), 2) growing Golden Shiner in an indoor recirculating aquaponics systems, 3) stocking outdoor, constructed ponds with newly hatched Golden Shiner sac-fry (0.5-1 cm), and 4) producing feed trained Golden Shiner frylings (2-3 cm) indoors before stocking them into outdoor constructed ponds.

Year one of our project has produced optimistic results and provided additional areas of research to pursue. Results from our project partners in the Minnesota bait industry indicate that Golden Shiner reared in RAS can reach market size in approximately 6-9 months. Sampling indicates many feed trained Golden Shiner frylings stocked into ponds reached market size by early September, however only a small number of sac-fry stocked into ponds have approached market size. Upon harvest in late October, total production and size structure will be determined for each pond and information will be shared in this presentation. Development of the aquaponics strategy was delayed but we expect to initiate this strategy over the next year.

The project has drawn high interest from bait dealers, the Minnesota Department of Natural Resources and the media. If successful and cost effective, these methods could become a new model for production of a variety of minnow species used as bait in Minnesota and other northern climates.

SUPPORTING ECOSYSTEM SERVICES OF HABITAT AND BIODIVERSITY IN TEMPERATE SEAWEED (*Saccharina latissima*) FARMS

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Most kelp (*Saccharina latissima*) farms in the Gulf of Maine reside in coastal, sheltered areas and are ephemeral, being fully deployed and removed every growing season. We assess whether the deployment of gear and growth of biomass at these farm sites provide seasonal habitat used by other organisms. Habitat, and the biodiversity of other species associating with farms, is considered a type of "supporting" ecosystem service. Ecosystem services are the benefits humans receive from healthy ecosystems. Of all ecosystem service categories – supporting, provisioning, regulating, and cultural, – supporting services are the least studied for temperate seaweed aquaculture. Quantifying biodiversity to understand the ecosystem that regenerative aquaculture creates, will promote climate resilience and sustainable industry growth.

We quantified mobile vertebrates (fish, seals) and crustaceans (crabs, lobsters) interacting with kelp farms using GoPro cameras. We also assessed small (< 5mm) invertebrates using mesh settling devices suspended at the same depth as kelp lines (2m). Both fish and invertebrate visual surveys were paired with environmental DNA (eDNA) methods. Alpha and beta diversity results suggest that kelp farm habitat has no impact on the richness, biodiversity, or types of species in this region. However, differences in alpha diversity measurements were seen between seasons, with higher species richness and Shannon Weiner diversity indices during the summer non-growing season when compared to the winter growing season. Differences in the types of species present were also occurring between the two seasons. Specifically, American lobsters were driving this significant difference with a higher abundance of lobsters present during the summer non-growing season. Other studies reveal seaweed farm habitat values vary based on a range of factors including: species cultivated, local environmental characteristics, farm management practices, etc. In the Gulf of Maine, a large percent of the species assemblage migrates offshore during the winter growing-season, resulting in little habitat needed near shore.

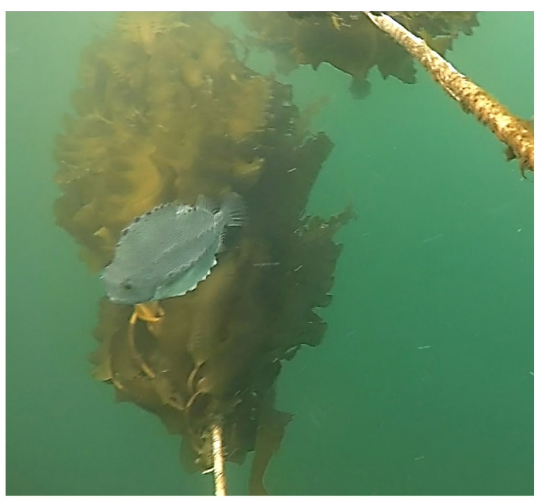

Fig. 1 Lumpfish present on Saco Bay, ME kelp farm

IMPROVING LOW SALINITY TOLERANCE IN LOUISIANA POPULATIONS OF THE EASTERN OYSTER *Crassostrea virginica* USING GENOMIC SELECTION

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Louisiana contributes the majority of eastern oyster (*Crassostrea virginica*) landings in the United States, producing approximately 45% of average national oyster landings annually (NOAA Fisheries Landings, 2011-2021). Like other major fisheries, the eastern oyster fishery has seen major declines in recent years due to changing environmental conditions. As a sessile estuarine animal, oysters are subject to rapid environmental fluctuations, especially salinity, which can decrease rapidly in Louisiana's estuaries due to storm events and freshwater diversions. Salinity in these estuaries often drops below the optimal growth and survival range for oysters (14-28; Lowe et al 2017). Louisiana has seen mass mortality events potentially due to extended periods of low salinity (<5) in the last several years, resulting in reduced *C. virginica* landings.

The Leveraging Opportunities and Strategic Partnerships to Advance Tolerant Oysters for Restoration (LO-SPAT) Project was launched in April 2021 with the goal of selectively breeding low salinity tolerant oysters to restore Louisiana populations in protected and fished reefs. To do this we are building genomic selection (GS) models for low salinity tolerance based on the results of a low salinity challenge (salinity 2, temperature 28°C) using oysters spawned from six Louisiana populations across three regions. Following the 45-day challenge, tissue from all live and dead oysters was sent out for genotyping on a custom *C. virginica* Axiom array (~66k SNPs). We will present the results of a genome wide association study along with the prediction accuracies of various GS models (Bayesian and GBLUP alphabets). Going forward, the best performing model will be used to select candidate broodstock for spawning based on genomic estimated breeding values and produce low salinity tolerant oysters to be planted at sites across Louisiana.

VIRGINIA SEAFOOD AGRICULTURAL RESEARCH AND EXTENSION CENTER - PROGRAMMATIC EXPANSION

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Core research and Extension programs at the Virginia Tech - Virginia Seafood Agricultural Research and Extension Center focus on seafood safety and quality of wild-caught, cultured animals and products, business and marketing support for the commercial and aquaculture industries, engineering, thermal processing, intensive recirculating aquaculture, and education/outreach for industry and consumers. In 2022, the Center moved into a new state-of-the-art 22,000-square-foot facility, with design considerations to enhance coastal resilience. New major program area expansions are currently underway, including Sustainable Food Production Systems (aquaponics, microbiome, RAS, alternative proteins including plants and insects); cellular agriculture; Economics and Marketing (policy and regulatory impacts, farm production economics, economic impact analysis, financial benchmarking, and automation/robotics); coastal resilience; in addition to offshore renewable energy and co-location of synergistic activities.

SITE SELECTION FOR OPEN OCEAN AQUACULTURE

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Site selection is a critical process for every new farm. For open ocean farms, this early planning step is more complex due to the stronger forces encountered in these environments, the high upfront capital costs, and the relative novelty of these environments for farming. The process is further complicated by the trend of siting open ocean farms in data sparce environments with fewer neighbouring farms or other ocean users to supplement data.

However, site selection studies have been conducted in geographies all over the world and for multiple species and offer examples and insights that can be applied to new studies. This presentation will cover Innovasea's process for identifying the most ideal location for a successful farm through remote geospatial analysis, on-location data collection, data analysis, and the decision-making process.

WORKING TO ESTABLISH DEFINITIONS OF THE TERMS "OFFSHORE", "OPEN OCEAN", AND "EXPOSED"

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The terms "offshore" and "open ocean" have been used to describe aquaculture sites that are further from the coast or in higher energy environments. Neither term has been clearly defined in scientific literature or in a legal context and are often used interchangeably. For example, Farmer A is located at an exposed site close to shore with waves up to 6 m, while Farmer B is located in shallower waters but is a further distance off the coast. While Farmer A invests in robust designs and engineering to survive as she fights against the strong forces of waves and currents, Farmer B has more of a focus on better logistics as she has to overcome issues related to accessibility. Both farmers see their concepts as challenges, although these are fundamentally different. Nevertheless, the two farmers both define their undertakings as being part of 'offshore' aquaculture. The terms above, and other related terms (e.g., "exposed", "high-energy"), refer to specific aspects of a site, usually the geographic distance from shore or infrastructure, or the level of exposure to an extended fetch leading to large waves and strong currents.

The main benefits of establishing clear definitions are: 1) promoting common understanding and avoiding misuse for different classifications, which can lead to misinterpretation and confusion among different actors, such as NGOs, licensers, and federal agencies; 2) enabling regulators to identify the characteristics of a marine site; 3) allowing farmers to be able to assess or quantitatively compare sites for development; 4) equipping developers and producers to identify operational parameters in which the equipment and vessels will need to be operating; and 5) providing insurers and investors with better means to assess risk and premiums.

The key differences between these terms are discussed as well as the importance of clearer meanings for various interest groups. The progress of the International Council for the Exploration of the Seas (ICES) Working Group on Open Ocean Aquaculture to develop set of definitions and a rigorous exposure index are presented and additional time for audience engagement and feedback will be provided.

INVOLVEMENT OF THE GH/IGF SYSTEM IN SEXUAL SIZE DIMORPHISM IN TILAPIA

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Tilapia are widely cultured worldwide, partly because they grow well in captivity, tolerate a wide range of environmental conditions and are sexually dimorphic, where males grow larger than females. As in other vertebrates, growth in tilapia is regulated by the growth hormone/ insulin like growth factor (GH/IGF) system. Once in circulation, GH can bind to its receptor (GHR) in target tissues, such as muscle, liver and gonads, resulting in a physiological response that, alone or through the mediation of IGFs, includes cell proliferation and differentiation, and protein synthesis. Moreover, environmental conditions, such as salinity, have been shown to directly modulate growth in tilapia. Less is known, however, on how the GH/IGF system may be differentially regulated between sexes at the tissue mRNA expression level and how salinity may modulate sexually dimorphic growth.

Our laboratory has developed a model for investigating effects of salinity and whole-organism GH by employing the Mozambique tilapia (*Oreochromis mossambicus*), a species that can thrive in either fresh water or seawater. In this model, we surgically remove the pituitary gland (Hypophysectomy - Hx) and replace GH and other pituitary hormones via intraperitoneal injections. With this approach, we examined whether the expression of *ghr* and *igfs* in muscle, liver and gonads were differentially affected between males and females in Hx fish and those injected with GH. We also compared the pituitary mRNA expression of *gh* in male and female Mozambique tilapia (*Oreochromis mossambicus*) acclimated to different salinity regimes.

Our results indicate that male and female fish differ in their sensitivities to GH by differentially expressing *ghr* and *igf*s in muscle, liver and gonads. These results suggest that the sexual size dimorphism in tilapia and its modulation by environmental salinity can at least be partially attributed to sex-specific differential regulation of the GH/IGF system.

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EXAMINATION OF CORN FERMENTED PROTEINS TO REPLACE FISHMEAL IN JUVENILE RAINBOW TROUT DIETS

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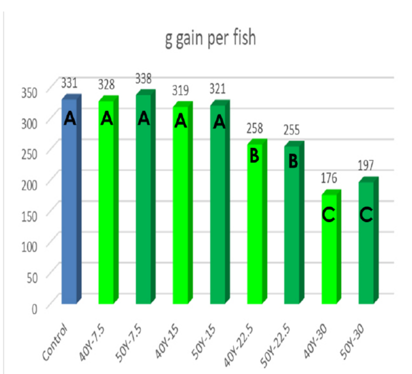
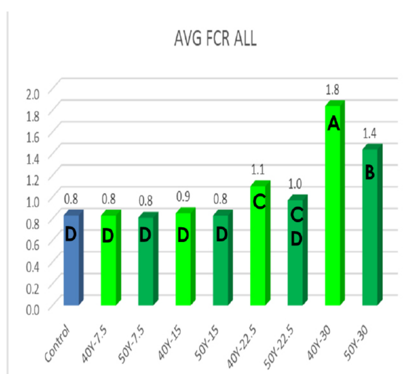
The ability of high value plant protein concentrates to replace fish meal in diets for rainbow trout depends on their available nutrient composition, cost, and consistency. The aim of this study was to assess the effects of two novel corn fermented protein products (ANDVantage™ 40Y and ANDVantage™ 50Y, The Andersons, Inc.) on growth performance of juvenile rainbow trout.

A 2 x 5 factorial design was applied with test products included at 0, 7.5, 15, 22.5 and 30% diet dry weight replacing dietary fish meal and poultry meal on a digestible protein (DP) basis. All diets were formulated to 42% DP and 18% crude lipid, supplemented with Lys, Met and Thr to targets of 3.8, 1.3 and 2.1%, respectively, and manufactured by cooking extrusion. Diets were randomly assigned to triplicate tanks of rainbow trout (*Oncorhynchus mykiss*, Troutlodge Inc., Sumner, WA) with a mean initial weight of 38 ± 0.7 g (mean ± SD). Fish were cultured in poly tanks (320 L) at n = 20 fish per tank in a recirculating system with a flow rate of 4-6 L min^{-1}, temperature at 15 °C, and a 13:11 light:dark cycle, and fed twice daily to apparent satiation six days per week for 12 weeks.

Including ANDVantage products at levels above 22.5% decreased g gain fish (P<0.0001). A significant interaction was observed for feed conversion ratio (FCR; P<0.0001) wherein fish fed ANDVantage™ 40Y had significantly higher FCR than fish fed ANDVantage™ 50Y when fed levels above 22.5%.

Optimized inclusion levels, determined by regression analysis for combined data or for each ingredient when interactive effects occurred, indicate that inclusion levels for ANDVantage 40Y and ANDVantage 50Y in rainbow trout diets range from 13.2 to 19.8% depending on the performance variable assessed.





Maximum inclusion capacity of ANDVantage products in rainbow trout diets. Limits identified for for various performance paramete using regression analysis.

Inclusion capacity[X]	Product	Parameter[X]	P value	R^2	Equation
13.55	Combined	Growth (g gain)	< 0.0001	0.95	$Y = 329.4 + 2.716X - 0.2524X^2$
14.29	40Y	FI (% BW d^{-1})	< 0.0001	0.94	$Y = 1.935 - 0.04284X + 0.002616X^2$
19.75	50Y	FI (% BW d^{-1})	0.0002	0.87	$Y = 1.907 - 0.02141X + 0.001163X^2$
13.23	40Y	FE	< 0.0001	0.99	$Y = 1.251 + 0.01467X - 0.00123X^2$
15.13	50Y	FE	< 0.0001	0.97	$Y = 1.253 + 0.01451X - 0.001019X^2$

[X] % diet dry weight.
[X] Feed intake (FI), Feed efficiency (FE)

PEPTIDOGLYCAN HYDROLASES AS ALTERNATIVES TO ANTIBIOTICS TO TREAT *Streptococcosis* IN FISH

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Infectious diseases are the chief cause of production loss in aquaculture and have severely limited the growth and sustainability of this industry. Gram-positive streptococci are costly pathogens that cause diseases in the aquaculture industry. Specifically, *Streptococcus iniae* is an emerging pathogen of wild and cultured fish, with ~30 species of fish susceptible to this pathogen. The economic impact of streptococcosis on the global aquaculture industry is hundreds of millions of dollars annually. While antibiotics are effective against many bacterial infections of fish, there are fears that their use in aquaculture may cause severe environmental and human health problems. In addition, the use of antibiotics in aquaculture has led to resistance in targeted pathogens, which could reduce their effectiveness. Although antibiotic treatment is effective, multi-drug resistant strains may lead to a potential for farm-to-clinic antibiotic-resistance transfer. These potential complications have led to an intensive effort to develop safer alternatives to traditional antibiotics. These novels (non-antibiotic) antimicrobials should be refractory to resistance development. Phage endolysins are cell wall degrading peptidoglycan hydrolases (PGHs), enzyme antimicrobials that digest peptidoglycan, the major structural component of the bacterial cell wall. Using bioinformatic tools, we identified ten PGHs that can potentially prevent and or eradicate systemic and topical S. iniae from fish. We used the pET21a (+) vector to express PGH-6x His tag in BL21 (DE3) *E. coli*, purified the proteins, and tested against S.iniae strains. Our preliminary data suggests new potential alternatives to antibiotics to treat streptococcosis.

POTENTIAL IMPACT OF HERBICIDE OVERSPRAY ON PHYTOPLANKTON BLOOMS IN ARKANSAS AQUACULTURE AND PRIVATE PONDS

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A common inquiry from aquaculture producers and pond owners is what impact a mistaken aerial herbicide application will have on their fish. These calls frequently follow a fish kill which has occurred after a suspected overspray. With few exceptions, the most used row-crop herbicides will be harmless to fish. What is often then asked is could the herbicides have negatively impacted the phytoplankton bloom, causing it to crash, leading to a fish kill due to low dissolved oxygen.

To investigate the potential for an herbicide overspray to negatively impact an algal bloom, extension crop scientists were consulted for a list of the most common aerially applied herbicide active ingredients (AI). A representative label was selected for each AI to determine the maximum amount of chemical that would potentially be applied to the pond, to the depth of one foot (AI ppm/acre*ft). This value was then compared to the EC50 for a representative green algae *Raphidocelis subcapitata*. These results were then compared with known algicides.

The results of this investigation indicate that some of the most common aerially applied herbicides have the potential to negatively impact planktonic algal blooms. Due to the nature of algae and ponds, while an herbicide overspray might negatively impact algae, this may not necessarily cause a low dissolved oxygen fish kill, but it cannot be categorically dismissed.

Active Ingredient (Label)	Max Application Rate (ppm)	Green Algae EC50 (ppm)[2]	Max Rate: EC50 Ratio
Acetochlor (Breakfree NXT)	0.9651	0.000143	6749
Flumioxazin (Chateau)	0.1406	0.00102	137.8
Thiobencarb (Bolero 8 EC)	1.4706	0.017	86.5
Propanil (Propanil 4EC)	2.2059	0.029	76.07
Metolachlor (Helmet)	0.7169	0.01	71.69
Dimethenamid-p (Outlook)	0.3619	0.014	25.85
Pyroxasulfone (Zidua)	0.0098	0.00038	25.79
Atrazine (AAtrex 4L)	0.7353	0.053	13.9
Paraquat (Gramoxone SL 2.0)	0.3676	0.32	1.15
Fomesafen (Sinister)	0.1385	0.17	0.815

Active Ingredient	Sample Label	Application Rate (ppm)	Green Algae EC50 (ppm)	Max Rate: EC50 Ratio
Copper	Copper sulfate	0.2-0.8 (as elemental copper)	0.0031	258
Hydrogen Peroxide	Pak27 (sodium carbonate peroxyhydrate)	0.3-1.7	0.18	9.44
Diuron	Diuron 80[4]	0.02	0.0024	8.33

DEVELOPING A PROTOCOL TO ESTIMATE DAILY RATION AT A CONSTANT LEVEL BELOW SATIATION

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Intensive production of fish in raceways are conducive to carefully controlled feeding practices and inventory management. Though feeding below satiation will not yield maximum growth, it has been shown to improve feed efficiency. A tool was developed to estimate daily ration and create a production plan based on empirical data describing potential growth across a range of water temperature. Daily ration is based on potential growth, water temperature, size of fish stocked, number stocked, date of stocking, condition factor, and estimated feed conversion. An option to input target mortality distributes loss evenly throughout the culture interval. Each day ration by both weight and volume, total fish weight, average fish weight, feeding rate as %BW/day, are determined creating a day by day production plan. It also estimates the amount and size of feed required for the culture unit during the culture period.

This tool has been in use with rainbow trout and is being developed for use in floating raceways with largemouth bass. Trout (150g/fish) in one set of raceways were fed a commercial diet (44% protein, 20% fat) to apparent satiation. Another set of raceways were fed a daily ration using the tool set at 86% of growth potential. Feeding with the tool yielded a relative harvest of 87% as compared with yield in the satiation treatment. Feed conversion for fish fed to satiation was 1.53 whereas feed conversion was 1.12 for fish fed a ration based on the tool.

As a management device, the tool can run different scenarios to determine what size fish to stock, when to stock, when fish in production can be expected to reach harvest size, or weight of fish over the market interval.

REVIEW OF FLOATING RACEWAYS WITH A PERSPECTIVE FOR THE SMALL FARM

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The concept for a floating raceway emerged from a common need rather than an idea from a single individual. Collamer (1923), Fremont (1972), Fast (1977), and Caillouet (1995) patented early designs. Heard and Martin (1979) described floating horizontal and vertical raceways to culture juvenile salmon in Alaska. Baklien (1989) briefly described production of salmon in a floating raceway in Norway and the influence of exercise on flesh quality. A series of studies evaluating performance of the floating raceway design was conducted at Auburn University in the early 1990s (Masser 2012). Whether floating or fixed to the pond bottom, this approach has become known as the in-pond raceway system (IPRS). Design of IPRS fixed to the pond bottom have been developed further on a commercial scale for production of food fish in projects associated with the US Soybean Exporting Council and Auburn University. This presentation will chronicle use of floating raceways.

Research on floating raceways for the small farm is being conducted at Kentucky State University. The focus is to make use of existing ponds that cannot be drained to provide additional farm production and potential income. Fish grown at high density in floating raceways are readily accessible in modest quantity consistent with niche markets and the labor profile of a small farm. Floating raceways on the small farm can be configured to grow a variety of fingerlings and/or foodfish, for holding fish, or even trapping fish that naturally occur in the pond.

MICROENCAPSULATED ORGANIC ACIDS AND ESSENTIAL OILS (EOOA) AS FEASIBLE FEED ADDITIVE FOR FISH FARMING

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Sustainable nutritional solutions are the focus of modern aquafeed sector. Innovative compounds that support fish growth performance and promote animal health without the use of antibiotics are encouraged to be used in salmonid farming. Being nutrition one of the fundamental pillars of animal health, adequate fish nutrition elevates animal resistance to the stressful effects that are intrinsic to the farm, besides promoting overall fish health without being detrimental to the environment or leaving residue in fish meat or water pond. Advances in the knowledge of fish nutrition and health led to the development of high-performance, natural bioactive compounds to be used as fish feed additives. Aquanat Synergy® (Jefo Nutrition Inc., Quebec, Canada) is a microencapsulated blend of organic acids (inc. sorbic acid, fumaric acid, malic acid, and citric acid) and essential oils (inc. thymol, vanillin, and eugenol). The microencapsulation technology improves the delivery efficiency of the natural bioactive compounds in fish gut. A four-week feeding trial evaluated the dietary inclusion of Aquanat Synergy® (2 inclusion level, seven replicates) on the growth performance and gut health of juvenile – adult Tilapia (O.Mossambicus)

Proximal and distal intestine morphology was assessed by histological analysis.The dietary inclusion of Aquanat Synergy® maintained Tilapia growth, with possitive effects on fish performance. Aquanat Synergy® significantly increased fish proximal intestinal villi length, without causing intestinal edema, inflammation, or apoptosis.

Aquanat Synergy® sustained good performance in both happas and tanks for over 100 days, Aquanat Synergy® is a feasible nutritional phytogenic compound for Tilapia diet, , with a positive effect on fish gut health, and a promising alternative to control intestinal fish pathogens.

RESULTS

<u>Zootechnical Performances</u>:

Table 2. Tilapia zootechnical performances according to treatment after 116 days of supplementation

	Control	Jefo 0,3	Jefo 1,5
Weight Gain (g/fish)	106.12±8.86a	116.53±10.75ab	124.80±7.19b
Specific Growth Rate (%)	1.36±0.06a	1.42±0.06ab	1.47±0.04b
Feed Conversion Ratio	2.21±0.60b	1.94±0.20ab	1.66±0.10a
Survival rate (%)	95.37±2.27a	94.26±3.61a	93.89±2.08a

a, b significantly different when $p<0.05$.

ANALYSIS OF THE GROWTH PERFORMANCE, STRESS, PROFILE OF FATTY ACIDS AND AMINO ACIDS AND CORTISOL IN TILAPIA (*Oreochromis niloticus*), CULTURED AT HIGH STOCKING DENSITY USING IN-POND RACEWAY SYSTEM

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In-pond raceway system technology (IPRS) was introduced in Pakistan in 2019 as solution for sustainable aquaculture approach by effectively increasing production, reducing pollution, and facilitating feed and pond management. Fingerlings of GIFT Tilapia (Oreochromis niloticus) (n= 16,500 in each raceway, initial weight= 32.00±1.26 g) were stocked in June, 2019 in two IPRS raceways (area of each raceway = 220 m3) for 171 days until harvested on November 30, 2019. Fingerlings stocked in traditional earthen ponds (area of each pond = 6,167 m3) were studied as control (n= 3,000 in each pond, initial weight= 32.00±1.26 g). Average harvested biomass from raceways was 57.33 kg/m3 with an average daily weight gain per fish of 4.47 g per day. On the other hand, average harvested biomass from control ponds was 0.38 kg/m3 with an average daily weight gain per fish of 4.60 g per day. Average feed conversion ratio (FCR) in both raceways and control ponds was recorded as 1.25 and 1.24, respectively. Overall survival rate in both raceways and control ponds was above 99 %. No sign of any disease was noted at any stage in either study group. Crude protein and fats contents did not reduce in any raceway despite of high stocking density and sharp seasonal changes. Profile of essential and non-essential amino acids were found to be upto nutritional requirements of adult human. High levels of n-3 and n-6 fatty acids in fish collected from raceways as compared to those in traditional earthen pond show that muscle quality was not compromised due to high stocking density in small area. Return on investment excluding capital cost was 47.05 which implies that IPRS technology can be economically feasible with further modifications.

Table 1 Summary of production and other parameters in traditional earthen pond (control) and IPRS raceways, culturing tilapia (*Oreochromis niloticus*) for 171 days. Area of control pond was 6,167 m^3 while each raceway had water area of 220 m^3.

#	**Location**	**Stocking Density**		**Initial Biomass**			**Morts**	**Survival Rate**	**Harvested Biomass (kg)**			**Biomass Gained (kg)**		**Wt. Gain/ Day/ Fish (g)**	**FCR**
		#	Fish/ m^3	Avg. I. Wt.(g)	Total Biomass (kg)	kg/m^3	#	%	Avg. F. Wt. (g)	Total Biomass (kg)	kg/m^3	Total Gain	Total Gain/ Day		
1	Control-1	3,000	0.49	32.00	96.00	0.015	16	99.45	790.00	2,357.40	0.38	2,261	13.22	4.62	1.24
2	Control-2	3,000	0.49	32.00	96.00	0.015	25	99.17	785.00	2,335.37	0.38	2,239	13.10	4.59	1.24
3	Raceway-1	16,500	75.00	32.00	528.00	2.40	40	99.84	767.00	12,509.6	56.86	11,981	70.07	4.44	1.25
4	Raceway-2	16,500	75.00	32.00	528.00	2.40	26	99.80	772.00	12,718.0	57.81	12,190	71.30	4.51	1.25
Raceways		**33,000**	**-**	**-**	**1,056**	**-**	**66**	**99.82**	**-**	**25,227**	**-**	**24,171**	**-**		**-**

#: Number, Avg. I. Wt.: Average initial weight; Avg. F. Wt.: Average final weight

LOCALLY RAISED FISH AND SHELLFISH IN AMERICA'S SEAFOOD BASKET: UPDATE ON EAT MIDWEST FISH EXTENSION PROGRAM

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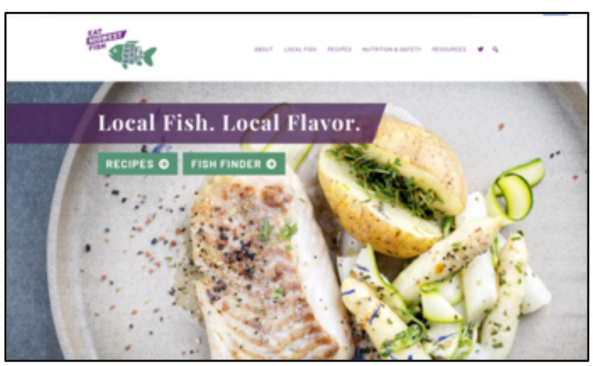
The 2020-2025 Dietary Guidelines for American consumers recommend eating at least 8 oz of seafood per week, for a 2,000-calorie-per-day diet, to support a healthy dietary pattern. Almost 90 percent of Americans fall short of meeting the United States Department of Agriculture (USDA) recommendation for seafood consumption. Still, when looking at consumption rates by region, data confirms that the Midwest is missing the mark by a long shot. The Midwest had the lowest seafood consumption rate (12.9 g/day) in the national, when compared to the Northeast (23.9 g/day), South (17.6 g/day), and West (20 g/day). There is a relatively small but diverse aquaculture industry in the north central region of United States, with a portion of these businesses looking to sell their food fish products locally or regional. Several pieces of information including the average seafood consumption rate; data collected from farmer interviews, focus groups, and consumer preference studies; as well as changes in Choice of Seafood and Emerging Opportunities, all point to a need for consumer facing programming, to come along efforts to grow the aquaculture food fish sector on a regional and national level.

The Eat Midwest Fish project is an extension outreach program that first completed needs assessments, worked with farmers to tell the story of sustainable aquaculture in the Midwest, and developed consumer facing educational resources for the general public. The deliverables include digital products for consumers to explore cooking local seafood at home, and information that raises awareness of regional farmed aquaculture species.

Phase 1 & 2 highlights of the Eat Midwest Fish project:

- Conducted informal and formal interviews: Informal- 5 extension educations (CES) from 4 states and formal – 27 farmers from 10 states.
- Conducted focus groups: 1 in-person and 2 virtual (14 CES from 6 states).
- Developed and launched the online resource hub eatmidwestfish.org. Included a fish finder feature to help consumers find local produced fish and shellfish.
- Pivoted programming to online platforms to reach consumers during the global COVID-19 pandemic.



Home page of the Eat Midwest Fish website and online resource hub

EFFECT OF PROBIOTICS AND IMMUNOSTIMULANT SUPPLEMENTATION ON GROWTH, GUT MICROBIOME AND GENE EXPRESSION OF WHITELEG SHRIMP *Litopenaeus vannamei*

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Due to the increasing demand for shrimp in industrialized nations, shrimp aquaculture is one of the rapidly growing industries producing animal food worldwide. However, the intensification of shrimp culture due to its rising demand is one of the fundamental causes contributing to the spread of infectious diseases. These industries are plagued by diseases, primarily brought on by opportunistic infections, which result in significant economic losses. Probiotics are increasingly frequently used in aquaculture due to the rising demand for environmentally friendly practices. A probiotic is typically described as a live microbial food supplement that balances the intestinal flora of the host animal. Other modern and essential techniques used in aquaculture include immunostimulants. An immunostimulant is a naturally occurring substance that modifies the immune system by enhancing the host's resistance to illnesses mainly brought by microorganisms.

The present study investigated the effect of feeding the white-leg shrimp (*Litopenaeus vannamei*) post-larval (PL) with different levels of probiotics and immunostimulants on their growth, gut microbiome, and gene expression. In this experiment, the 15 days old shrimp PL were kept in 42 different tanks. Three different diets were given, including particle diet, probiotics, and immunostimulants at different levels. The research was carried out for a total of 39 days. Seven different treatments were used in this experiment. Treatment 1, also called the control diet, was only given the particle diet. Treatments 2, 3, and 4 were given the probiotics at the rate of 0.5 ppm, one ppm, and two ppm, respectively. Whereas, for treatments 5, 6, and 7, immunostimulants were given at 0.5 ppm, one ppm, and two ppm, respectively. The best growth performance was shown by treatment 7 (2 ppm immunostimulant) and treatment 3 (1 ppm probiotics). The expression of most immune-related genes was upregulated in shrimp that were fed probiotics and immunostimulants compared to the control group. Alpha diversity indices indicated greater richness and evenness in the treatments that were given probiotics and immunostimulants.

THE VALUE OF CROSS-SECTORAL PARTNERSHIPS IN BUILDING PUBLIC TRUST AND ENHANCING AQUACULTURE LITERACY

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Consumer and community understanding of aquaculture is key to unlocking its full potential to support healthy people, a healthy planet, and a healthy economy. Aquaculture outreach and education strategies that help consumers navigate and take ownership of their decisions can reduce public misperceptions of aquaculture, and paint a more holistic picture of what the sector looks like in their region. However, trust in the messengers of information can matter as much as the information itself. Environmental educators and informal learning institutions provide the necessary platforms through which to build trusting relationships with, and provide credible aquaculture learning opportunities to, the general public.

From 2021-2022 through the eeBLUE aquaculture literacy mini-grants program, NOAA and its partners at the North American Association for Environmental Education (NAAEE) supported ten cross-sectoral partnerships that built place-based learning opportunities for a variety of audience groups. Here, we discuss the impacts of those exciting pilot projects, and the collaborative actions that are taking place to disseminate their best practices and lessons learned. From cooking classes for chefs and K-12 teachers, to new aquarium exhibits and youth-driven career training opportunities, these programs improved community-level awareness of their connections to aquaculture industries, their products, and career opportunities. Join this presentation to learn more about how NOAA plans to continue to support aquaculture literacy initiatives with the help of these mini-grantees.

FROM PLANNING TO PRACTICE: AN UPDATE ON THE EVOLUTION OF NOAA'S AQUACULTURE LITERACY EFFORTS

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Public awareness of seafood farming and perceptions of the intended benefits to industry expansion play an important role in unlocking its full potential to support healthy people, a healthy planet, and a healthy economy. However, there is still limited public understanding regarding environmental impacts, quality, and safety that creates a significant barrier to advancing seafood farming in the United States. At Aquaculture America in 2018, NOAA unveiled an agency initiative to address this public knowledge barrier. Over the course of the past 5 years, the agency implemented a multifaceted approach to engaging and educating the public on seafood farming practices and the science, services, and policies NOAA has to offer in support of the sector.

This presentation will highlight the evolution and impacts of these ongoing efforts, including:

- The Coastal Ecosystem Learning Centers (CELC) Aquaculture Education Initiative that is working to engage millions of aquarium visitors on the environmental, social, and economic benefits of seafood farming,
- Efforts to support and empower cross-sectoral partnerships through a Community of Practice for Aquaculture Literacy (CoPAL),
- NOAA Fisheries, Office of Education, and Sea Grant's partnership with the North American Association for Environmental Education (NAAEE) which funded ten aquaculture literacy mini-grant projects from 2021-2022.
- A look ahead at efforts of NOAA Fisheries to equip trusted messengers of information with the knowledge needed to develop credible, salient, and engaging seafood outreach and communications.

From career exploration to teacher professional development, and from supporting aquarium partners around the country to co-managing funding opportunities with leaders in environmental education, NOAA reaches multiple audiences in a variety of settings. Join us to continue this discussion of seafood farming education, outreach, and communication strategies that improve seafood literacy and help consumers more confidently navigate their decisions about working waterfront industries and their products.

EXPANDING ACCESS TO HIGH-QUALITY SUGAR KELP SEED THROUGH NURSERY FACILITY IMPROVEMENTS

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GreenWave, a 501c-3 nonprofit organization headquartered in south central Connecticut, has been producing sugar kelp seedstring in its New Haven facility since 2016. GreenWave's original seed production facility was based, in part, on the recommendations of the 2013 Kelp Farming Manual produced by Ocean Approved (Flavin, Flavin, and Flahive) and the seminal work of Dr. Charles Yarish and his lab at the University of Connecticut, Stamford. Over the first five years of operation, we realized that our system, which utilized T-12 fluorescent lighting, glass aquaria, and air and water chilling systems, was labor and energy intensive and costly to operate at commercial scale. These system inefficiencies, coupled with the reliance on seasonally sourced wild kelp reproductive tissue, or sorus, represented a significant bottleneck to the availability of affordable, high quality, and timely kelp seed for ocean farmers. In 2022, GreenWave embarked on an effort to revamp its kelp nursery to increase labor and energy efficiencies and improve seed quality while reducing the costs of operation, and to develop a modular model for kelp seed production that could be replicated throughout southern New England and beyond. Using this new system, GreenWave anticipates being able to produce twice the amount of kelp seed in ⅓ of the space and with less than half the labor that was possible in the original facility. This presentation will review the major transformations to GreenWave's kelp nursery, including water treatment systems, lighting, cooling, and standard operating procedures, and will share preliminary findings from the 2022-2023 production season.

OPTIMAL FEEDING RATES FOR JUVENILE WALLEYE *Sander Vitreus* IN TANK-CULTURED SYSTEMS BASED ON GROWTH PERFORMANCE AND SURVIVAL

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Walleye *Sander vitreus* are a highly sought-after northern Midwest fish species known for their nutritional quality and recreational attributes; however, the population has been decreasing with the ever-changing climate of our lakes. The objective of this study is to determine the optimal feeding rates for walleye juveniles and provide baseline information on feed management for walleye aquaculture.

We conducted three two-week trials in indoor systems run with flow through water at 18 to 20°C. Walleye were fed with commercial diets (50-55% protein, and 15% lipid) at 6 different feeding rates (% initial body weight daily) ranging from 5.0 to 17.5 % in Trial 1, and 2.5 to 15% in Trial 2, with 30 fish per tank. In Trial 3, walleye were fed with 5 different feeding rates from 2.0 to 8.0% body weight daily with 15 fish per tank. The initial body weight was 0.95 g, 2.31, and 9.3 g for Trial 1, 2 and 3, respectively. An optimal feeding rate was estimated using polynomial regression method based on the percentage of weight gain during each 2-week feeding. Our results showed that an optimal feeding rate was 22%, 12.5%, and 7.7% for fish ranging from 1-3 g, 2-5 g, and 9-17 g, respectively. The survival was significantly impaired ($P<0.05$) when walleye were fed at 5% body weight daily in Trial 1. Trial 2 and 3 showed high survivals (>97%) from all treatments. Condition factor, expressed as the ratio of body weight and the cube of length, was significantly lower for underfed fish compared to those fed at their optimal feeding rates or higher. The nutritional composition of walleye is pending for analysis and will be available for the presentation. The result of this study will provide preliminary information for developing feed management of walleye culture and designing future studies on nutritional requirement.

	Feed rate					
Trial 1	**5**	**7.5**	**10**	**12.5**	**15**	**17.5**
WG	95.9 ± 8.9^{A}	115.9 ± 9.2^{AB}	130.9 ± 4.3^{BC}	152.9 ± 1.9^{CD}	170.2 ± 8.8^{D}	174.8 ± 5.9^{D}
Trial 2	**2.5**	**5**	**7.5**	**10**	**12.5**	**15**
WG	45.4 ± 0.9^{A}	79.5 ± 7.6^{B}	102.3 ± 2.4^{C}	117.8 ± 3.2^{C}	120.2 ± 2.9^{C}	115.7 ± 1.3^{C}
Trial 3	**2**	**3.5**	**5**	**6.5**	**8**	
WG	33.6 ± 0.6^{A}	53.3 ± 1.0^{B}	57.8 ± 1.1^{B}	78.1 ± 1.7^{C}	71.6 ± 0.2^{D}	

Different superscript letters within the same row indicate significant difference compared by Tukey's HSD as the post hoc test with differences considered significantly at $P<0.05$.

SWIM BLADDER MORPHOGENESIS AND EFFECT OF PHOTOPERIOD ON INITIAL INFLATION IN COMMON SNOOK *Centropomus undecimalis*

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Common snook, *Centropomus undecimalis*, are an economically and ecologically important sport fish species in the southeastern United States. Mote Marine Laboratory, in partnership with the Florida Fish and Wildlife Conservation Commission, are culturing common snook fingerlings for use in stock enhancement research. Advances in aquaculture technologies have led to significantly improved production of common snook. The current research focus is on evaluating environmental parameters to further optimize husbandry protocols. Swim bladder (SB) inflation is an important milestone that often represents a bottleneck in finfish larviculture. This study describes SB morphogenesis in common snook and the impact of photoperiod on SB inflation is evaluated in larvae reared at 28°C and 35 ppt. In the first experiment, larvae (0-10 day post-hatch, DPH) were collected and fixed for histological evaluation of SB ontogeny (Figure 1). As physoclistous fish, the inflation window for common snook began at 3 DPH, or mouth opening. In the second experiment, two photoperiod treatments (24-hour and 18-hour light) were selected (based on previous data) and effects on SB inflation were documented. Freshly anesthetized larvae were assessed for SB inflation via light microscopy on 1, 2, 3, 5, 7, and 10 DPH. SB inflation occurred for both treatments at 3 DPH (18-hour: 3.86 ± 5.60%; 24-hour: 4.52 ± 2.34%). Larvae in the 24-hour light treatment had a significantly higher ($P < 0.01$) SB inflation rate than those reared in the 18-hour light treatment at 5, 7, and 10 DPH. By 10 DPH, inflation rates in the 24-hour light treatment (62.13 ± 6.02%) were over 3 times greater than those found in the 18-hour light treatment (20.14 ± 3.53%). Photoperiod also had a significant effect on larval size at 5 DPH (18-hour: 2.93 ± 0.16 mm; 24-hour: 3.03 ± 0.18 mm; $P < 0.01$) and 7 DPH (18-hour: 3.12 ± 0.18 mm; 24-hour: 3.19 ± 0.24 mm; $P < 0.05$). Ultimately, determining the ontogeny of important physiological milestones in larval development are critical for optimizing larval husbandry protocols.

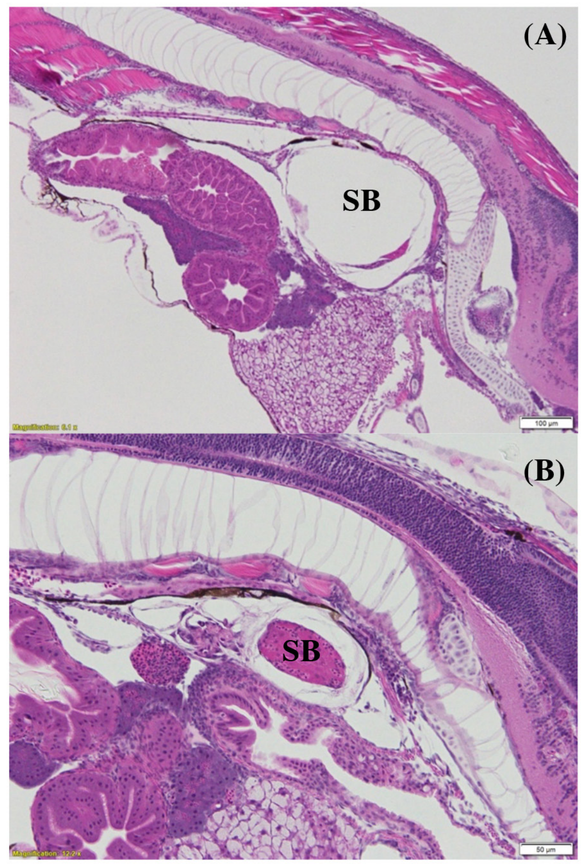

Figure 1. (A) Common snook larvae (8 DPH) exhibiting an inflated swim bladder. (B) Common snook larvae (8 DPH) exhibiting an uninflated swim bladder. SB, Swim Bladder.

STANDARDS FOR POSSIBLE FISH & SILURIFFORMES CONTAMINATION SHOULD BE BASED ON RISJK ASSESSMENT AND NOT APPLIED TO ALL THE SAME

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Escherichia coli is used by many regulators and other agencies as a possible indicator of food fecal contamination and thus possible indicator of bacterial human pathogens in fish. In 2017, USDA-FSIS compliance guidelines for Siluriformes, implemented the testing of generic *Escherichia coli* to ensure product wholesomeness and safety. The BAP standards for processed fish call for rejection of sample if from n= 5 subsamples, 3 or more subsamples exceed 4 MPN per gram or any subsample exceed 40 MPN per gram. The ICMSF has a different standard: n= 5 subsamples, 3 or more subsamples exceed 11 MPN per gram, or any subsample exceed 500 MPN per gram. Others have placed a limit of 1 CFU/g. The USDA-FSIS has stopped testing for salmonella in finished product (fillets) due to the low incidence (~3.3% in farm-raised and wild-caught catfish) and the insignificant risk pathogens have on consumers of this product. *E. coli* and other indicator organisms in catfish, liquid and environment of processing facilities were assessed by season and time of day. Environmental, fish, and liquid samples were collected in 3MTM Swab-Sampler (with 10 ml of Buffered Peptone Water) and Whirl-pak® sterilized stomacher bags respectively from two different catfish processing plants. (site) located in the state of Mississippi, USA. Replications were defined by the number of visits to the processor. *Escherichia coli* counts were similar ($P \leq 0.05$) for all sampling points with slightly higher incidence in the late Summer than the other seasons. Within the types of sampling, liquid and fish part sample had higher counts than ($p \leq 0.05$) environmental samples. It is possible that the presence of indicator organisms evaluated varies according to the sampling season, considering that such microorganisms have been found to have a relationship with pathogens, *E. coli* could be part of the natural microflora of the catfish ponds, thus not a good fecal indicator. Moreover, summer fish still have feed in their gut, and this could contribute to higher incidence of E. coli in fish. It is recommended that this standard be replaced with a risk-based standard depending on region, fish source, fish products prepared (raw vs. RTE) and other applicable parameters.

Date	E coli (log CFU/g)					
	m	c	M	Range	BAP?	ICMSF
25-Aug	40	2	100	0-100	No	No
15-Sep	60	3	100	0-100	No	No
20-Oct	120	2	500	0-500	No	No
17-Nov	ND	0	0	0	Yes	Yes
29-Nov	ND	0	0	0	Yes	Yes
3-Mar	ND	0	0	0	Yes	Yes
3-Mar	<2	1	<10	0-<10	Yes	Yes
25-Oct (10)	ND	0	0	0	Yes	Yes

16S RRNA METAGENOMIC ANALYSIS OF UNKNOWN BELUGA STURGEON (*Huso huso*) POPULATIONS

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An optimal feeding strategy that promotes fish growth at minimal cost and enhanced reproductive performance without adverse effects on fish health is required. Beluga is a valuable fish that is reared for its caviar. But the species is presently critically endangered, which makes researching to improve its production critical toward sustainable fish production. Currently, optimum dietary requirements for developing commercial diets to enhance the performance of Beluga sturgeon during the culturing period of 8 – 10 years before it reaches maturity are unavailable. Most Beluga farmers either use commercially available diets intended for other fishes or use diets formulated based on the dietary requirements of other freshwater fishes. To surmount the challenge associated with culturing of Beluga in the aquaculture industry, the digestive ecosystem of Beluga sturgeon needs to be understood using a next-generation sequencing method. Hence, this study investigated the gastrointestinal tract of three unknown Beluga sturgeon populations.

Hatchery-raised populations of Beluga sturgeon (2012, 2013, and 2018) maintained identical diets and hatchery conditions but differed by age were evaluated. Samples of different sections of the gut samples, blood, and tissues were collected for amplicon sequencing, proximate compositions, fatty acids and amino acids analyses. Distal gut samples were collected for histopathological examination. Data were analyzed for differences among the three populations.

Fish weight was significantly higher in the 2018 population than in fish from the 2012 and 2013 populations ($P<0.05$), but the condition factor was better in the 2013 fish population. Histopathological sections of the fish from 2012, 2013, and 2018 show mild to moderate inflammation. Overall, Firmicutes (78.8%), Proteobacteria (8.2%), and Fusobacteria (8.03 %) phyla were the most predominant bacteria in all three populations. Diversity and differences of microbial population, proximate composition, fatty acids, amino acids data in the three populations will be presented.

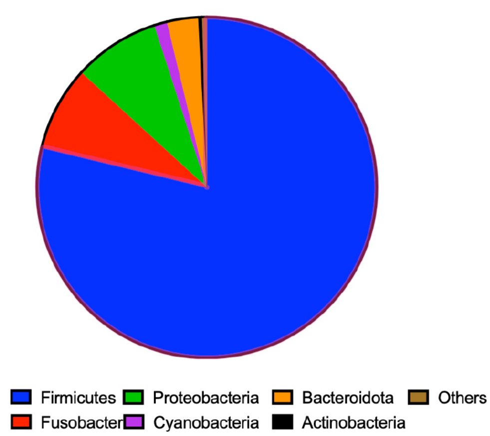

Figure 1: Overall Phylum Compositions

WHAT THEY DON'T KNOW CAN INDEED HURT: WHY OCEAN ERA IS JOINING THE COALITION FOR SUSTAINABLE AQUACULTURE

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The marine conservation community is now increasingly willing to consider marine aquaculture – and particularly offshore aquaculture – more favorably, as an environmentally responsible means of producing animal protein for the planet. Much of this shift has come with the recognition that wild stock fisheries simply cannot sustain the growing demands of 10 billion people, and the realization that aquaculture – when done right – can be minimally impactful. Offshore aquaculture also attracts greater interest now from eNGOs, funds and impact investors, because of the potentially lower externalities, and the abundant ability to scale.

However, eNGOs still seem hesitant to embrace policies in support of offshore aquaculture expansion in the USA. A recent Marine Policy paper (Fujita, et al, 2022) highlights concerns about "ecological risks" and "knowledge gaps". The eNGO-supported SEAfood Act, recently introduced into the US House of Representative, is packed with caveats and precautions, advocating for only incremental advances offshore, such as a few, small "pilot projects".

Offshore aquaculture policy and development is hurt by Fujita, et al.'s perceived knowledge gaps. Uncertainty breeds hesitancy. However, this also now offers us an opportunity to partner with, engage and better educate, the conservation community. There appears to be a genuine eNGO willingness to more fully understand the science of offshore aquaculture, and the realities needed to support responsible industry growth. Industry advocates should ideally, then, work more closely with our eNGO colleagues to ensure that reliable data is available, and understood, and that policies align with the practical needs of commercial operations.

For this reason, Ocean Era, Inc. has joined the Coalition for Sustainable Aquaculture (CSA). This presentation will discuss how industry can quickly and effectively address most of these perceived knowledge gaps, and how we can find areas of commonality and policy agreement amongst CSA members, even where there is incertitude.

What we don't know (or don't share amongst ourselves) can also hurt the industry. We need to improve the way that we exchange information about learned experiences, especially where things go wrong. Siloing knowledge to protect reputations, brand image or proprietary interests can be unhelpful, or downright dangerous. The aviation industry perhaps presents a useful model, where accidents are extensively studied, and lessons applied industry-wide.

We should seek to emulate this safety record, by keeping fish, workers and the environment as safe as airplanes. Either the offshore aquaculture industry should establish its own safety regulatory authority, or perhaps we might ask NOAA to do so. Sharing information with each other, with regulatory authorities, and with the science-driven eNGO community – and learning from our collective experience - is better for us all. But fulsome sharing needs to come from a place of trust, and for this to happen, we need eNGOs and regulatory agencies to recognize the value of such transparency and collaboration.

BASELINE STUDY ON THE QUANTITY AND VARIATION OF MICROPLASTIC POLLUTION IN AQUACULTURED VERSUS WILD EASTERN OYSTER *Crassostrea virginica* IN NEW ENGLAND

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Microplastics (MP), particles <5mm that are either manufactured or occur as breakdown products of larger plastics, are a component of Earth's Plastisphere and are emerging as an important anthropogenic pollutant. They are found in every water system worldwide, including in New England where studies on the distribution and fate of MP are just now beginning. The most common MP found so far in our analyses are Polyethylene and Polypropylene, varying in size and shape. This study is part of a larger study of the body burden of MP in New Hampshire living resources, designed to build a baseline data set and supplement knowledge and understanding of MP that are present in New England. This study sheds light on occurrence of MP in both aquacultured and wild oysters. Oysters were collected throughout the summer field season of 2022. Because oysters are consumed whole, this investigation used whole-body preparations. After removal of tissue from the shell, biogenic material was chemically digested, and the remaining material was stained with Nile Red and analyzed using first confocal microscopy and subsequently LDIR. The results of the study show the incidence of MP pollution in New England oysters from different farm and reef sites, evidence that is helpful in siting oyster aquaculture, selecting oyster depuration locations, and making policies that pertain to estuary pollution and health.

LONG-TERM FEEDING SOY-BASED DIETS SUPPLEMENTED WITH ADDITIVE MIXTURE IMPROVES GROWTH AND FILLET QUALITY OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Plant protein (PP) based feed is desirable to maintain the sustainable growth of aquaculture. However, the presence of anti-nutritional factors, imbalanced nutrient content, and low digestibility limits their utilization. Additionally, PP based feed may deteriorate fish fillet quality. Therefore, using feed additives in PP based feed can potentially improve their utilization in fish and improve the fillet quality. The aim of the present study was to evaluate the effects of dietary supplementation of additive mixture in PP diets (~30% soy) on the growth and fillet quality of rainbow trout.

Two thousand fish (2.22 g) were distributed in four groups with 5 replicates (100 fish/tank) and fed four isonitrogenous (42% CP) and isolipidic (20% lipid) diets: control (30% fish meal, FM), PP, PP+A1 (krill meal, taurine and organic selenium) and PP+A2 (proline, hydroxyproline and vitamin C) for 28 weeks.

Dietary supplementation of additives in PP diets improved the growth performance and feed efficiency of fish compared to PP group but comparable to FM (figure 1). Fish fillet quality was also affected by additives (table 1), hardness, gumminess and chewiness of PP+A2 group was higher than other groups, whereas gumminess and chewiness of FM and PP+A1 groups were higher than those of PP-group. The higher value of chroma and lower value of hue-angle in PP+A1 group indicates improved color of fish fillet. Additionally, muscle fibre requirement pattern using muscle fibre count and genes responsible for myogenesis are being analyzed.

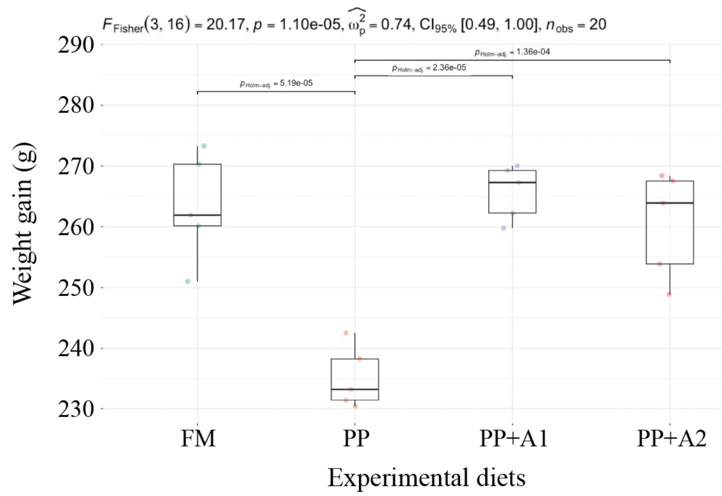

Figure 1: Weight gain (g) of different groups after 28 weeks

Table 1: Texture profile analysis (TPA) of rainbow trout fillet

Diets	Hardness (N)	Springiness (mm)	Gumminess (N)	Chewiness (N.mm^2)
FM	1.72±0.01^a	0.95±0.02	1.29±0.01^b	1.22±0.03^b
PP	1.51±0.04^a	0.92±0.02	1.03±0.03^a	0.95±0.03^a
PP+A1	1.68±0.07^a	0.93±0.03	1.24±0.08^b	1.16±0.06^b
PP+A2	2.15±0.11^b	0.95±0.02	1.57±0.03^c	1.49±0.02^c

Conclusively, PP based diet exhibited negative effects on growth and fillet quality of rainbow trout, which can be alleviated by the supplementation of additive mixture.

DIETARY INTERACTION OF L-ALANINE AND LIPID ON GROWTH AND PHYSIOLOGY OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Dietary role of dispensable amino acids (DAA) in fish nutrition is not well studied. DAAs play important role in growth, cell signaling, gut health. Among DAAs, L-alanine helps in energy-budgeting and acts as a major precursor for gluconeogenesis. Therefore, aim of this study was to determine the use L-alanine to reduce the fish oil inclusion in the diet of rainbow trout.

A factorial design (2 × 6) consisting of twelve isonitrogenous diets (42% crude protein) containing two levels of lipid (L: 14 and 20%) and six incremental levels of L-alanine (A: 0, 0.5, 1.5, 2.5, 3.5, and 4.5%). A total of 576 fish (12.4 g) were stocked in triplicates in 36 tanks of recirculating aquaculture system and fed for nine weeks.

Results revealed that lipid and L-alanine levels have significant effect on growth performance. Dietary supplementation of 2.5% L-alanine in low lipid diet improved the growth compared to control (figure 1). Feed efficiency and protein utilization were improved by supplementation of 1.5 and 2.5% L-alanine (table 1). Interaction on lipid and L-alanine levels was observed for PER, indicates 2.5% L-alanine with lower lipid fed group showed high PER as compared to other groups. Lower lipid fed groups showed significantly lower IPF than high lipid fed groups. Additionally, amino acid metabolism enzymes (GOT and GPT) in serum were significantly affected by dietary lipid and L-alanine levels. Nutritional profile of fish fillet, gene expression pattern for protein and energy metabolism and hepatic metabolomics are being analyzed.

Based on our knowledge this is the first study wherein fish oil inclusion was reduced by using L-alanine as a source of energy in rainbow trout diet without compromising the growth.

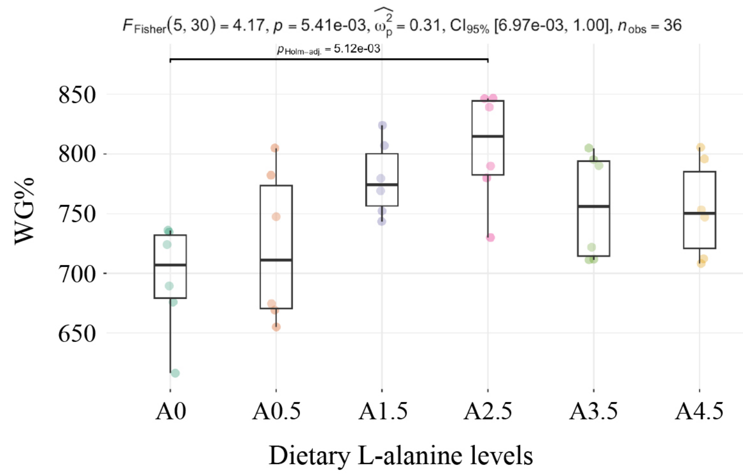

Figure 1: Pairwise comparison of dietary L-alanine levels on percent weight gain (WG%)

Table 1: Two-way ANOVA results (*p value*)

Factors	WG%[1]	FCR[2]	PER[3]	IPF[4]	GPT[5]	GOT[6]
Lipid	**<0.001**	0.755	0.875	**<0.001**	**<0.001**	**0.006**
L-alanine	**<0.001**	**0.005**	**0.003**	0.784	**<0.001**	**0.015**
Interaction	0.118	0.075	**0.048**	0.573	0.488	0.980

[1]WG, weight gain; [2]FCR, feed conversion ratio; [3]PER, protein efficiency ratio; [4]IPF, intraperitoneal fat index; [5]GPT, glutamate pyruvate transaminase; and [6]SOT, glutamate oxaloacetate transaminase

PROTECTIVE EFFECT OF ELEVATED WATER HARDNESS IN CHANNEL CATFISH FOLLOWING CHRONIC CHALLENGE WITH HIGH ENVIRONMENTAL AMMONIA AND SALINITY STRESS

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The aim of this study was to assess whether rearing fish at increased water hardness could assist channel catfish (*Ictalurus punctatus*) juveniles to efficiently cope with later (sub)lethal ammonia exposure or salinity stress. Fish were subjected to 100, 494 (10% of 96 h-LC_{50}) or 1250 mg/L (25% of 96 h-LC_{50}) $CaCO_3$ water hardness levels for two months. Afterwards, fish were challenged with high environmental ammonia (HEA; 12.2 mg/L total ammonia ~ 25% of 96 h-LC_{50} value) or salinity stress (SST;10 ppt) separately for 21 days at their respective water hardness levels.

Results show that after two months, weight gain (%) in 1250 mg/L $CaCO_3$ group was markedly worse than 100 and 494 mg/L $CaCO_3$ groups. This finding was accompanied with a down-regulation of hepatic IGF-I and growth hormone receptors (GHR) mRNA levels. During SST, growth was still significantly worse for those at 1250 mg/L $CaCO_3$ (1250_{SST}). However, exposure to HEA inhibited growth for 100_{HEA} and 494_{HEA} relative to 1250_{HEA}. Ammonia excretion rate (J_{amm}) remained similar at different hardness levels; however, this was strongly inhibited at 100_{HEA}, 494_{HEA} and 100_{SST}. This appeared to align to some degree with high water hardness having a protective effect on the gill histomorphology. In fact, fish at 1250_{HEA} were able to increase J_{amm} efficiently, which was associated with upregulated branchial expression of ammonia transporters (Rhesus glycoproteins 'Rhcg') and Na^+/H^+ exchanger, as well as augmented gill H^+ ATPase activity. These responses prevented branchial Na^+/K^+-ATPase activity indicating this unlikely has a major role in ammonia excretion. a build-up of excess ammonia in plasma. In contrast, both 1250 mg/L $CaCO_3$ and 1250_{HEA} groups displayed inhibited gill Ca^{2+}-ATPase activities reduced at the highest hardness (1250 mg/L $CaCO_3$) and 1250_{HEA} groups, likely to prevent hypercalcemia in plasma. Overall, findings suggest that although elevated water hardness up to 1250 mg/L $CaCO_3$ adversely affects the growth performance of channel catfish, it can ameliorate the inhibitory growth effects of HEA. This was partly attributed to 1250 mg/L $CaCO_3$ protecting the gills as well as mitigating HEA-induced ammonia excretory and ion-regulatory disruption. However, elevated hardness may not be as effective in alleviating salinity stress in catfish

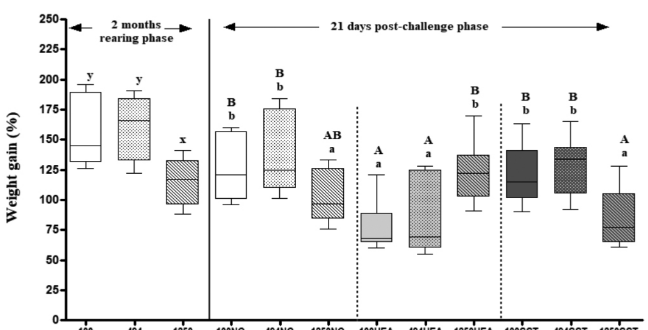

Fig 1. Patterns of weight gain (%). NC: not challenged; HEA: high environmental ammonia; SST: salinity stress.

INSECTS AND TROUT: EVALUATION OF MEALWORM PROTEIN AS A FISHMEAL REPLACEMENT FOR RAINBOW TROUT *Oncorhynchus mykiss*

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The replacement of fishmeal in trout feeds has been a perpetual challenge for the industry since the late 1990's when the global supply of fishmeal fell from its peak, creating greater competition for use and increasing costs. As such, many alternative protein sources have been evaluated over the past 40 years. Leading the way has been soybean meal and soy concentrates, due to their favorable amino acid profile. Even so, dietary inclusion of soybean meal creates digestive challenges for trout and other salmonids. High concentrations of anti-nutritional factors and antigens result in enteritis of the distal intestine, and with it reduced nutrient utilization and fish growth and health. While soy is a staple in fish feeds today, higher prices coupled with soybean meal induced enteritis, has renewed the drive for novel protein ingredients. Furthermore, concerns over rainforest destruction and other sustainability challenges associated with soy have been at the forefront of more recent pushes for sustainable protein alternatives.

As a recent addition to the fish nutrition toolbox, insect meal provides an acceptable macronutrient and amino acid profile. Specifically, defatted, yellow mealworm (AKA Darkling Beetle; *Tenebrio molitor*) meal contains approximately 72% protein and 5% lipid and is only limiting in the amino acids methionine, threonine and lysine. Further, mealworm production is touted as sustainable, with the possibility of 365 harvests/year. Relative to soybean production, mealworm production capacity can achieve 5000x the acre yield of soy with half the power and 2% of the water requirements. As such, the objective of the current study was to evaluate defatted mealworm meal (DMW) fed to rainbow trout as a sustainable fishmeal alternative.

A digestibility and growth study were conducted. Apparent digestibility of DMW fed to trout was evaluated using standard practices, and the apparent digestibilities of dry matter, protein, lipid, energy, and phosphorus were 80, 87, 100, 84, and 90%, respectively. Growth was evaluated in juvenile trout fed over 13 weeks with five graded dietary treatments (0, 10, 20, 30, 40% as fed), substituting 1:1 for sardine fishmeal. No differences in fish performance or feed utilization were detected ($P>0.05$). Differences in body protein, lipid and energy composition were observed in fish fed the highest DMW treatment; however, no differences were observed for fillet composition. Survival, intestinal histology, and blood chemistry were similar among treatments. In total, DMW is highly digestible when fed to rainbow trout and can replace all the fishmeal in the diet, up to 40% as fed, while supporting fish performance, feed efficiency, fillet composition, and health. These results support the use of DMW as a novel and safe ingredient in rainbow trout feeds.

THE NEED FOR SEED: INNOVATIVE METHODS FOR CRYOPRESERVATION AND PROPAGATION OF AQUATIC ANIMALS FOR FISHERIES, CONSERVATION, AND AQUACULTURE

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As many aquatic species vital to ecosystem function and food production continue to decline, techniques to rescue dwindling populations and protect remaining genetic diversity are crucial. Genetic banking via cryopreservation may provide an avenue to catalog and stockpile a multitude of aquatic species. Cryopreservation is the process of cooling biological material (e.g., cells, tissues, ova, sperm, embryos, etc.) for storage in subzero or freezing temperatures. Low temperature storage reduces the biological and chemical reactions in living cells but can also promote damaging intracellular ice formation. Cryopreservation strategies use permeating (i.e., ice reducing) and non-permeating (i.e., dehydrating) cryoprotective agents (CPA) to stem chilling induced damage and reduce ice crystal formation. However, permeating CPA is often toxic and non-permeating CPA can induce damage via osmotic dehydration, making development of successful cryopreservation protocols challenging. Overcoming these challenges is critical for the successful cryopreservation of aquatic embryos or larvae and could help provide long term protection for threatened species and an insurance policy for future generations to restore and reintroduce genetically diverse representatives from critically important populations. Preservation of diploid aquatic embryos or larvae (i.e., aquatic seed - capable of developing into mature organisms) provides advantages over other haploid germplasm (e.g., sperm & oocytes) because it preserves genetic material from both parents. Genetic banking of plant cells, tissues, seeds, and mammalian embryos is common practice in agriculture and farming for selective breeding purposes, in case of disease outbreak or environmental catastrophe. However, genetic banking of aquatic seeds is nearly nonexistent to date with only a small number of successful embryo and larval cryopreservation protocols for invertebrate species such as oysters, clams, urchins, and mussels. The relatively large size (i.e., low surface area: volume ratio), multiple membrane bound compartments (i.e., yolk, blastoderm, perivitelline space, chorion), high yolk content, and chilling sensitivity of aquatic seeds make them extremely difficult to cryopreserve. Recent advancements in rapid cooling for storage in liquid nitrogen (-196°C) and ultra-rapid laser rewarming (>10^7 °C/min) have led to major breakthroughs and new opportunities in cryopreservation for aquatic germplasm. Successful aquatic seed cryopreservation provides an opportunity for 1) immediate culture of embryos or larvae for aquaculture broodstock replenishment, 2) improvement of restocking hatchery systems, 3) reintroduction of locally extirpated wild populations, and 4) alleviating pressures on wild caught populations.

WHAT DO CONSUMERS KNOW ABOUT AQUACULTURE? SURVEY FINDINGS FROM THOSE WHO EDUCATE CONSUMERS

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The Michigan and Minnesota Sea Grant programs and Lake Superior State University administered an aquaculture consumer education survey to people in the Great Lakes region working with aquaculture and to the national aquaculture extension network. The survey was incentivized, open for 30 days during summer 2022, and received 89 participant responses. Data were analyzed both quantitatively and qualitatively. Constructed response questions were coded based on emergent themes. We defined a consumer as a person in the United States who is the end-user of aquaculture products which may include farm-raised fish for food, fee-fishing, bait, stocking, or ornamental purposes. Participants primarily served in extension/outreach roles (42%) and the rest were distributed across research, industry, regulations, and other areas (such as consulting, and government as a non-regulator). Over 45% of respondents spent 20 or more years working in or with aquaculture. Respondents were predominately white (93%) and male (71%). Of those who were female (29%), 83% were between 25-30 years old with aquaculture experience ranging between one and nine years. Of the different types of fish products or topic areas, participants worked primarily with food products (78%), stocking (48%), ornamental (21%), and “other” categories (such as aquaponics or endangered species), bait, and fee-fishing.

Most participants believe consumers are aware of aquaculture, but cannot define it or teach it to someone else. Participants were asked to list 1) the top three facts consumers already know about aquaculture, 2) the top three facts consumers should know about aquaculture and 3) misconceptions (or inaccurate facts) consumers have about aquaculture. The top three facts consumers know about aquaculture were that products are farmed, can be used as food, and aquaculture involves the environment. The top facts that consumers should know about aquaculture were that its products can be used for food, it is sustainable, it affects the environment, and its products are healthy. The top three misconceptions consumers have about aquaculture were that it negatively affects the environment, there are differences between farmed vs wild products, and products have high levels of contaminants. Survey participants indicated there are challenges associated with teaching adults and youth about aquaculture. Specifically, adults already have a set mindset, they tend not to be curious, and they have little to no knowledge about aquaculture. Participants indicated that current resources are not always appropriate to share in order to educate consumers because the type of delivery methods available are not useful (need more visuals, field trips, etc.), information is geared toward producers rather than consumers, and the information is incomplete or outdated. Participants stated that the most effective means of consumer education include: in-person/virtual tours of farms, online websites, and social media.

We will discuss these findings in more detail and explore how these data can help inform ongoing efforts in consumer education and outreach about aquaculture.

USING REAL-TIME DATA MONITORING AND COLLECTION TO ACCESS FISH HEALTH AND CONDUCT SITE SURVEYS, AN INTRODUCTION TO THE BEACON®

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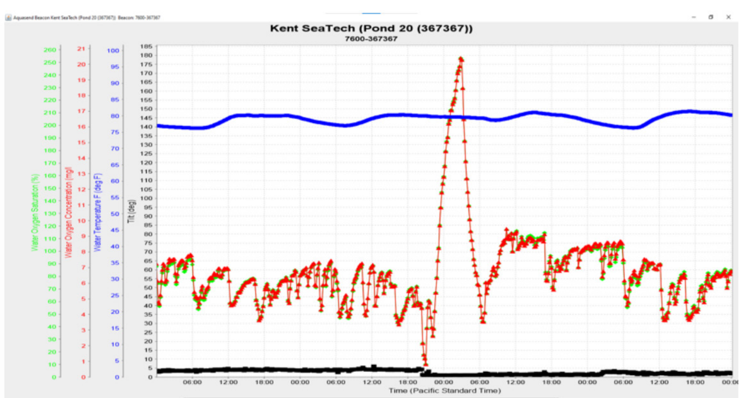
Aquafarm and hatchery managers continually make decisions regarding fish health, how to increase yields and lower overall production costs. Water quality management is an essential tool to any aquaculture operation and is affected by variables that can drastically affect fish health and production procedures. Aquasend®, an enterprise of Precision Measurement Engineering, recently conducted product pilot programs at three aquaculture farms with varying goals to help farmers monitor their water quality and collect data in real-time.

Two farms were in California with a third farm on the island of Oahu, Hawaii. The goals of the deployments varied from measuring dissolved oxygen and temperature to establishing optimal feeding windows, to collecting data to determine if water quality was reaching levels to sustain the reintroduction of aqua farming.

One deployment successfully alerted farmers of a pond's dissolved oxygen crash resulting from a failed aerator. Dissolved oxygen levels hit a life-threatening number of .08mg/L followed by an excessive amount of liquid oxygen pumped into the water causing a sharp increase to 20mg/L. Farmers quickly responded to both drastic changes alerted by the Aquasend Beacon® bringing the water to safe levels preventing fish kills and saving money.

Monitoring the oxygen and temperature levels of tanks, raceways and ponds in real-time can provide essential data to enhance fish health, increase yields and alert managers before losing product, as well as decrease labor costs. Other benefits include determining optimum biomass capacity, decreasing energy costs and conducting site evaluations. I will discuss site pilot deployment goals, testing parameters and data collection results. I will also discuss how the Beacon® connects to an online portal that can be accessed from any device and will send alerts instantly if the temperature or dissolved oxygen levels drop. Finally, I will review data points throughout the pilot programs via the online interface.



YELLOW PERCH CULTURED IN OHIO SPLIT PONDS

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Yellow perch (*Perca flavescens*) are cultured throughout the Midwest. Perch may be cultured for sale as either food or stock enhancement, with the latter being the most prominent. In 2018, Ohio State University received funding from the USDA NIFA North Central Regional Aquaculture Center to conduct split pond and intensively aerated (3.75 kw/1.00 ha) on-farm Extension demonstrations at two yellow perch facilities in central Ohio. Brehm's Perch Farm LLC (West Liberty, Ohio) modified three existing ponds into one split pond, and Millcreek Perch Farms LLC (Marysville, Ohio) conducted the intensively aerated demonstrations. Brehm's split pond ratio is approximately 25% fish culture area and 75% waste treatment area, for a total water surface area of 0.40 ha. The split pond utilized submersible pumps (Xylem, Goulds Water Technology, Seneca Falls, New York) to lift water into the fish culture area from the waste treatment area, and the turnover rate was 113% per day. For decades, these two farms have stocked newly feed-habituated yellow perch in May at approximately 98,842/ha. The ponds were stocked at twice the normal rate (approximately 197,684/ha) for the 2018 demonstrations. Water quality was mostly maintained within acceptable parameters throughout the project. Fish length/weight samples were conducted at the start of the demonstrations, approximately monthly during the production season, and again at harvest. In the fall, Brehm's 0.40 ha split pond had a net yield of 1,595 kg. Total amount of feed fed was 1,728 kg (Zeigler Bros., Inc., East Berlin, Pennsylvania) for a feed conversion ratio of approximately 1.08. Survival was estimated at approximately 90%. Significant discrepancies can be found when harvesting any perch pond due to the disproportionate growth rate between male and female fish, as well as perch that never habituated to feed and yet managed to avoid being cannibalized. Fish smaller than 10.16 cm are presumed to have never habituated to feed during the habituation process. Weight ranges for yellow perch at harvest may be as low as 75 perch/kg up to 12.5 or more perch/kg, and the perch in the split pond averaged 36.4 perch/kg.

At the conclusion of the funded 2018 on-farm Extension demonstration projects, Brehm's and Millcreek's historical results indicated to them that this work helped them achieve moderate to high survival rates and typical or higher than average individual lengths and weights. In addition to production parameters, Brehm's appreciated the ease of harvest and feeding. Producers were content enough with the results that they have continued to utilize these alternative pond-based technologies. In 2019, Brehm's connected two more ponds into an additional split pond (approximately 54% fish culture and 46% waste treatment), and in 2021, Brehm's built three more ponds which are now connected into another modified split pond. Stocking rates continue, when fish are available, to be double the normal historical rate. This presentation will highlight some of the Extension demonstration project results, photos and aerial views of the farm, and the farmer's thoughts on why they have continued to incorporate split ponds on their farm.

ALGAL-BASED AQAUCULTURE AT SANTA FE COMMUNITY COLLEGE FINALISTS IN THE 2022 DEPARTMENT OF ENERGY ALGAEPRIZE COMPETITION

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The AlgaePrize is a new competition from the U.S. Department of Energy's (DOE's) Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office (BETO) that encourages high school through graduate students from any US based school to present their ideas and develop them for commercial-scale algal cultivation and products. On April 20, 2022, the 15 finalist teams were announced and awarded $5,000 to fund the research and development of the proposed projects over the next year. Three of the fifteen finalist teams chosen are from the Santa Fe Community College Alternative Fuels and Algae Cultivation programs. The first finalist team project is a seaweed cultivation experiment utilizing brackish aquifer water in New Mexico to grow sea vegetables. This team intends to explore the potential of emerging marine aquaculture markets in landlocked desert regions. The second finalist team project investigates reduction in energy consumption using algae to treat agricultural wastewater for nutrient recapture and water recycling. The third finalist team is investigating the use of algae in phytoremediation capacity of an algal polyculture, from a legacy uranium mining site in New Mexico, to remove radionuclides from contaminated surface waters. The utility of algal based phytoremediation systems in addressing the impacts of other extraction industries is also being examined.

RAPID, COST EFFECTIVE SNP GENOTYPING USING STANDARD BIOTOOLS POWERFUL MICROFLUIDICS TECHNOLOGY

Luke Stewart, Jamel Dobbs, Roberto Spada

The use of single nucleotide polymorphism (SNP) genotyping with non-model organisms—those whose genomes are yet to be sequenced—has increased in importance as it provides robust, comparative data sets that can be easily shared across organism communities for a variety of purposes. Non-model organisms have the added burden of low available genomic sequence information requiring custom SNP assay development. Until recently, SNP genotyping technologies have been prohibitive to these communities due to the high cost of developing and running quality SNP genotyping panels. Standard BioTools SNPtypeAssays and the X9 Real-Time PCR System have addressed these barriers with a custom assay design service, cost-effective and high qualitySNP assays, and a high-throughput workflow minimizing hands-on time. The salmon research community has been specifically hampered by the cost barriers, andwould benefit from the technology for conservation and management purposes. Using chum salmon (Oncorhynchus keta) as an example, we describe a simple workflow using Standard BioTools SNPtypeAssays, the X9 System, and 96.96 Dynamic Array™ Integrated Fluidic Circuits (IFCs) for Genotyping to achieve cost-effective and rapid development of a SNP genotyping panel. Moreover, SNPtypeAssays provide significant cost savings for high-throughput, routine testing post panel development.

WHAT'S NEXT FOR AQUACULTURE MANAGEMENT IN THE PACIFIC ISLANDS

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The National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) Pacific Islands Regional Office (PIRO) is working 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. 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.

The management program is being designed to regulate, manage, and promote the development of an environmentally sound and economically sustainable aquaculture industry in Federal waters of the PIR. The program would enable NMFS and the Council to provide enhanced planning, coordination, and oversight of aquaculture in 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/.

INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) FOR LAND BASED SHRIMP PRODUCTION

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Saltwater aquaponics is a novel concept that is increasingly becoming a viable option in aquaculture science. Countries are looking for ways to use brackish water while increasing seafood availability in areas without a local source. The effluent created by these aquaculture systems can create major ecological and biological setbacks, but multi-trophic integrated aquaculture systems (IMTAs) are potential solutions to reduce the impact of nutrient waste. The objective of this study was to see if *Ulva rigida* had an effect on 2 specific nutrient levels in small aquaculture systems. Nitrogen is a limiting factor in saltwater while phosphorus is limiting in freshwater, and both can be destructive in watersheds and recirculating systems.

The study ran for a total of 11 days for ammonia testing, 7 days for phosphate testing and 20 days for shrimp population counts. The aquariums were cycled in anticipation of the *Ulva rigida* and *Palamonetes* shipment from Florida. A salinity range of 1.023-1.025 with a pH of 8.3, was targeted to reflect high end brackish aquaculture systems. Instant Ocean Marine Salt was used to maintain stable salinity. The shrimp were distributed as evenly as possible between six 20 gallon aquariums. All six aquariums had 5lbs of macro marine rock to aid in the beneficial bacteria growth, creating a microbial bed. Three (3) of the aquariums contained *Ulva rigida* while the other 3 did not. Phosphate and ammonia (ppm) were collected via API Saltwater Test kits from water samples from each aquarium.

Over 11 days ammonia was tested in each aquarium (n=30). The average ammonia was 0.17 ppm with *Ulva rigida*, but averaged 0.58 ppm in aquariums without it. These ammonia readings were significantly different between treatments.

Phosphate levels were also tested in each aquarium (n=15). The average phosphate was 0.13 ppm with *Ulva rigida*, but averaged 0.12 ppm in aquariums without it. The phosphate tests showed that there was little difference between the *Ulva rigida* present and *Ulva rigida* not present treatments..

In summary, seaweed such as *Ulva rigida* did have a significant effect on the nitrogen levels in the shrimp aquaculture systems, and has the potential to reduce waste to levels that would promote cleaner effluent water and more sustainable local seafood.

DEVELOPING A CARBON-FOCUSED LIFE CYLE SUSTAINABILITY ASSESSMENT FRAMEWORK TO ASSESS SCALING PATHWAYS AND TECHNOECONOMIC OPTIMIZATION OF KELP FARMING

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In the global marketplace for cultivated macroalgae, raw product is traded as a commodity regardless of the ultimate end. New kelp producing regions in the north Atlantic and northeast Pacific have nucleated from food-focused, community-scale starting points. However, recent research initiatives in the United States and European Union have incentivized commodity scale development for macroalgae biomass beyond niche-food products including animal feed, biorefinery processes, biofuel production and ocean carbon dioxide removal (Blue Carbon/CDR) which is the focus of this assessment.

Recent efforts have taken a technoeconomic analytical (TEA) approach to assess the viability of scaling production to achieve high-volume end uses of farmed kelp, including CDR. These efforts are informative but focus on largely theoretical production systems which inherently encompass high variability and uncertainty in model inputs. However, recent works by Coleman et al. (2022a,b) provide detailed "baseline" TEAs based in real world application of currently employed kelp farming technology from nursery to harvest. These efforts have highlighted the importance of realistic TEA's with integrated Life Cycle Assessments to account for emissions within the production system and the impact on the per unit cost of carbon sequestration.

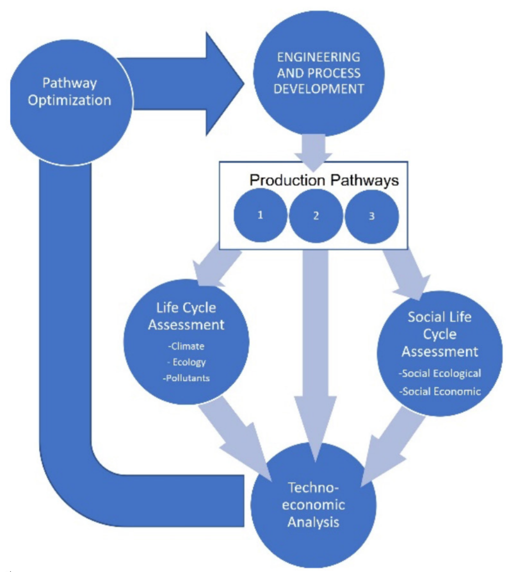

Figure 1. Lifecycle Sustainability Assessment Process Diagram.

Building from the Coleman et al. (2022) baseline model, we propose a framework for expanding the integration of LCA and TEA for modeling several CDR-focused kelp production scaling pathways from cradle to grave (in this case, nursery through sequestration) (figure 1). We will present a pathway to comprehensive Lifecycle Sustainability Assessments (LCSA) in which a Social-LCA (SLCA), TEA, and LCA are integrated. Even when optimized, macroalgae CDR will require considerable ocean space and potentially unforeseen social implications in addition to the ecological ones. Integrating the potential social-ecological and socio-economic impacts and benefits of different production pathways as well as of different carbon sequestration/avoidance related product end uses (direct sinking vs product substitution for example) will be important to achieving full sustainability of the sector.

References:

Coleman, et al. (2022a) "Identifying scaling pathways and research priorities for kelp aquaculture nurseries using a techno-economic modeling approach." *Frontiers in Marine Science* 9: 894461.

Coleman, et al. (2022b) "Quantifying baseline costs and cataloging potential optimization strategies for kelp aquaculture carbon dioxide removal." *Frontiers in Marine Science*: 1460.

A DUAL QCPCR ASSAY TO TEST FOR *Haplosporidium nelsoni* AND *Perkinsus marinus* DETECTION AND QUANTIFICATION IN WATERS AND OYSTERS OF GREAT BAY ESTUARY, NH

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Perkinsus marinus and *Haplosporidium nelsoni* are the main active pathogens causing Dermo and MSX, respectively, in wild and farmed eastern oysters *(Crassostrea virginica)* of Great Bay, NH. In this study, quantitative competitive PCR (QCPCR) was used to simultaneously detect and quantify both organisms in the water column and individual oysters of Great Bay Estuary. QCPCR requires co-amplification with a known quantity of competitor DNA, which shares most of the nucleotide sequence with the target DNA. Due to the known ratios of competitor and target, it allows for precise quantification of DNA present in samples. This study found both organisms were present in estuarine waters at three Great Bay sampling sites throughout the "typical oyster season," or when oysters are most active and spawning (June through November). Results also showed that both pathogens exhibited an oyster-effect, whereby pathogenic DNAs were more frequent and variable in water associated with oyster habitats than a non-oyster associated control site. This finding enhances understanding of disease-causing mechanisms of those organisms in New England estuaries and allows insight into where best to site oyster restoration areas. Combined with previous study of the consistency between the QCPCR and histological results of diseased oysters, the study also demonstrates accurate detection and quantification of oyster pathogens in both environmental and individual oyster samples.

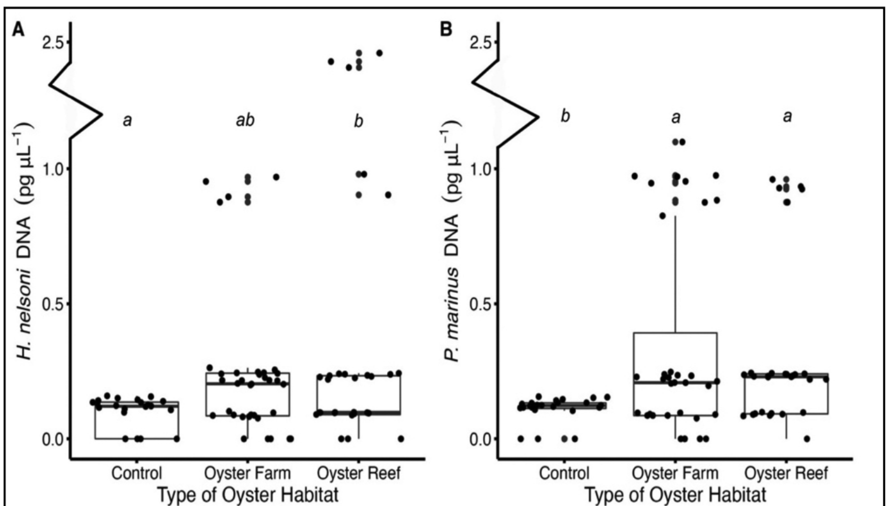

Figure 1. Comparison of A) *H. nelsoni* and B) *P. marinus* levels in the water column at an oyster farm, an oyster reef, and a control site in Great Bay Estuary, NH.

ALTERNATIVES TO ANTIBIOTICS: NEW AND OLD

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Antibiotics remain a critical tool in controlling bacterial diseases in commonly cultured species. However, certain limitations on the long-term feasibility of antibiotic use exist. Only three antibiotics are FDA approved for use in cultured finfish: oxytetracycline, florfenicol, and sulfadimethoxine. Even these three approved drugs can only be applied in specified cultured species when infected with certain bacterial agents. Moreover, these approved antibiotics are exclusively administered via medicated feeds with the exception of oxytetracycline. Given that bacterial agents often lower appetence in cultured finfish, oral administration may not prove effective. Additionally, antimicrobial resistance continues to occur despite steps taken to prevent its development. For these reasons, producers and veterinarians alike should reevaluate alternatives to antibiotic use in the aquaculture setting.

Novel therapies post-infection pose promising yet poorly underdeveloped methods to counteract bacterial agents. Bacteriophage and immunomodulatory therapies can be tailored to the bacterial species of concern. The targeted nature of these two therapies may confer a more precise treatment for the infection, but such an individualized approach may hamper widespread adoption in the industry. Prebiotics, probiotics, and phytobiotics may be utilized to address outbreaks, as these therapies alter multiple host pathways to discourage bacterial growth. While these three treatments show potential for development, their mechanisms of action are poorly understood even in human medicine. It should be noted that all novel therapies will suffer from many of the same problems antibiotics exhibit: prolonged FDA approval and limited routes of administration.

Previously established alternatives to antibiotic use primarily center on preventative medicine. Biosecurity protocols are vital to minimizing the introduction and proliferation of bacterial diseases at individual operations. Vaccination, while not yet mainstream, has proven effective in preventing economically disastrous outbreaks at larger farms. The industry should continue to emphasize such preventative measures because discouraging outbreaks suppresses the overuse of antibiotics and provides cost savings to farms. Finally, preventative steps represent the most practical alternatives to antibiotics in the near term given that novel therapies require lengthy FDA approval.

GLOBAL OVERVIEW OF *Seriola spp.* AQUACULTURE: ADVANCEMENTS AND REMAINING CHALLENGES

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Carangid marine fish species within the genus *Seriola* have been cultured on a commercial scale for nearly 75 years globally and are highly regarded by consumers and culinary professionals. Multiple species within this complex of yellowtail jacks have been identified as promising aquaculture candidates for further development in various regions and types of production systems. While much of the global *Seriola* production has historically utilized wild-caught juvenile seedstock for growout operations, there have been significant advances in hatchery technology of multiple *Seriola* species to allow for increased levels of closed-cycle production where fish are produced from eggs of captive broodstock. Each species of *Seriola* has its own unique life history, including specific thermal niches, energetic requirements, spawning dynamics, and larval rearing requirements. The differences extend to the marketplace, where species of *Seriola*, sizes of fish, specific preparations, and end-use markets all serve to impact the overall farm-gate price of harvested *Seriola*. Through combined analysis of all such factors, from biological to market-based considerations, the overall aquaculture performance potential of species can be assessed. Comparative analysis of the aquaculture performance dynamics of *Seriola* species reveals a multitude of key advancements in this industry while also highlighting critical challenges that remain within this sector of global marine aquaculture. Aspects of *Seriola* species-specific differences will be presented and discussed, including analysis of recent advances in comparative bioenergetics research and overall aquaculture performance potential of *Seriola spp*. throughout the world.

IMPACTS OF FOREIGN BUSINESS COSTS ON NORWEGIAN SALMON EXPORTS

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Norway is the world's second largest seafood exporter and more than 90% of the seafood produced is exported. The most valuable specie in Norwegian exports of seafood is farmed salmon, for which Norway is the world's leading producer. Salmon is also among the most traded fish species globally with the most advanced logistics.

There exist a large body of literature on international trade highlighting the importance of firm characteristics and trade costs for export. This is also true for research on seafood trade. It is well-known that exporters of seafood share common characteristics of exporters found in the general literature of international trade. Exporters are relatively few and compared to non-exporters they are both larger and more productive. A few firms account for a relatively large share of export value. It is well known that as trade costs increases export value will decline. Less is known about the actual composition of firm-level trade costs. In this paper we investigate how foreign business costs, costs that stem from e.g. laws, regulations, macroeconomic stability, and customs in the destination market, affects exports of salmon.

Foreign business costs are proxied by the index provided by the Fraser Institute, the Canadian public policy organization. This data source covers 124 different countries and is constructed using primary data sources such as the World Bank and IMF. Data from the Fraser Institute will include both an overall index for costs of doing business in different countries, as well as five different sub-components from the overall index. Our results indicate that improvements in foreign business costs increases the export of salmon from Norway, but the impact differs between types of the exporting firms. The effect from improved foreign business costs are strongest for firms that has their main activity related to wholesale and distribution of salmon. The effect is not as strong for producers that also handles export themselves. Further, we show that this effect is still present after controlling for traditional gravity variables such as geographic distance and the size of the economy in the destination country. Finally, the effects from the different sub-components of the index on export is investigated for different groups of exporting firms.

TOXICITY OF COPPER SULFATE TO LARGEMOUTH BASS FRY IN NATURALLY SOFT WATER

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Use of copper sulfate pentahydrate for fungus control on fish embryos is extremely effective and economical for farmers in waters with moderate or high alkalinity/hardness; however, fry toxicity in low alkalinity/hardness waters is a concern. In the present study, the acute toxicity of $CuSO_4$ to Largemouth Bass (LMB) fry in naturally soft water (i.e., 42 mg/L alkalinity, 41 mg/L hardness) was determined. Median lethal concentration (LC50) values at 24 h were 3.97 mg/L $CuSO_4$ for yolk-sac fry and 5.24 mg/L $CuSO_4$ for swim-up fry. Most importantly for farmers, the No Observed Effect Concentrations (NOEC) in the present study was 0.625 mg/L $CuSO_4$ for both stages of fry. In regard to the safe use of $CuSO_4$ for fungus control on LMB embryos, this research was done to demonstrate $CuSO_4$ toxicity to LMB fry in soft water.

ON DEMAND FEEDING AND THE RESPONSE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) TO VARYING DIETARY PROTEIN LEVELS IN SEMI-INTENSIVE POND PRODUCTION

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Feed is one of the primary costs associated with commercial production of Pacific white shrimp (*Litopenaus vannamei*). This high cost is the combined outcome of feed cost and feed management. As feeding technology evolves, specifically the use of passive acoustic monitoring (PAM) feeding systems, it is vital to reevaluate the optimal protein levels in diets for the best production outcomes. The use of acoustic monitoring adds another level of complexity to how shrimp respond to feed because it has the potential to automatically adjust feed offerings based on protein because of the shrimp's response. In this research, four diets with various protein levels (40, 35, 30, and 25%) were fed to shrimp which were stocked (0.045 g, 25 shrimp/m2) into 16 ponds (0.1 ha) and cultured for an 85-day production cycle. Shrimp were fed using the AQ1 passive acoustic monitoring system. Final individual weights were significantly smaller for shrimp fed the 25% diet (31.22 g) compared to all other diets. The total biomass of all ponds ranged from 7,037- 7,878 kg/ha for shrimp offered the 25%- 40% diets, respectively. Other than final weight, analysis of this and all other production data showed no differences between treatments (p>0.05). Whole-body analysis revealed significant differences in fat (p=0.0002), copper (p=0.018), and apparent net protein retention (p=0.0025). Analysis of economic values indicated a statistically significant difference between treatments for feed cost (p=0.020). The significantly lower individual weights from shrimp fed the 25% diet and the notably lower total biomass resulted in a subsequent difference in class size distribution. This ultimately led to a difference in the market value of shrimp ranging from $60,383 to $71,247. Although the 40% protein diet was significantly higher in cost it showed no differences in production or economic outcomes compared to the other diets. Overall, these results indicate that a 30-35% protein diet would be the most efficient for use in pond production of Pacific white shrimp under the culture conditions examined in this study.

	25%[1]	30%[2]	35%[1]	40%[1]	*P*-value	PSE[3]
g/week	2.49	2.72	2.68	2.73	0.27	0.074
Weight (g)	31.22	34.20	33.59	34.25	0.23	1.005
Feed Input (kg/ha)	8,193	8,593	8,658	8,806	0.42	36.60
Protein Fed (kg/ha)	2115^{c}	2659^{b}	2984^{b}	3679^{a}	0.0001	11.86
Survival (%)	88.57	87.22	91.46	83.56	0.93	6.024
Yield (kg/ha)	7,037	7,492	7,704	7,878	0.78	57.91
FCR	1.18	1.17	1.13	1.17	0.99	0.0678
ANPR(%)[4]	66.1^{a}	57.2^{a}	54.8^{ab}	43.0^{b}	0.0025	0.028
Feed Cost ($/ha)	12,559^{b}	13,670^{ab}	14,447^{ab}	15,960^{a}	0.02	58.96
Shrimp value ($/ha)	60,382	67,368	69,141	71,247	0.5832	552.2

[1]n=3
[2]n=4
[3]PSE: Pooled Standard Error
[4]Apparent Net Protein Retention

EVALUATION OF THREE DIFFERENT MICRODIETS FOR CALIFORNIA YELLOWTAIL *Seriola dorsalis*

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An important area of larval nutrition and feed research is the development of formulated microdiets. Reducing the need for live feeds during the larval phase of culture as well as transitioning larvae onto a well-formulated microdiet have the potential to improve growth and survival rates while reducing overall culture costs. Here we tested two experimental diets versus a commercial control.

Three microdiets were tested on California yellowtail (*Seriola dorsalis;* CYT) larvae, two experimental diets manufactured by Zeigler, Bros., Inc (Gardners, PA) and one by Otohime (OTO, Marubeni Nisshin Feed, Tokyo, Japan). Treatments were: experimental diet 1 (D1), experimental diet 2 (D2), and OTO. The two experimental microdiets used two different production techniques, D1 was manufactured with standard extrusion practices and D2 was manufactured as a soft-moist extruded pellet. At the start of the study, 16 dph larvae (5 larvae L^{-1}) were stocked into a recirculating system consisting of fifteen 320-L black conical bottom tanks, and the trial duration was 42 days.

Sinking rates were faster for both D1 and D2 diets compared to the OTO, across all particle sizes. Feeding incidence was similar for D1 and OTO at 26 and 30 dph, however at 34 dph the larvae in the OTO treatment had significantly higher feeding incidence than D1 or D2. Standard length (SL) and wet weight (WW) at 59 dph was similar between D1 (SL – 43.2 ± 5.5 mm; WW – 1.56 ± 0.57 g) and OTO (SL – 44.3 ± 5.4 mm; WW – 1.54 ± 0.58 g) and lowest in the D2 treatment (SL – 44.3 ± 5.4 mm; WW – 1.29 ± 0.48 g). Survival at 59 dph was not significantly different between the treatments but was highest in the OTO treatment (30.2 ± 3.8%).

We demonstrated that using the D1 diet to wean CYT can produce similar growth and survival to the commercial control, OTO. OTO is a commonly used weaning diet in the production of many marine finfish species, including *Seriola*. Even though feeding incidence and survival were not significantly different between the diets, OTO had higher feeding incidence at 26 dph and higher survival. This could be due to the limited size range of the feed particles in the experimental diets and the difference in sinking rates between the experimental and OTO diets. The slower sinking rates for OTO allow for a longer contact time in the water column for the larvae to feed on the diet, and the wider range of feed sizes allows for more of the population to survive through weaning. Continued development of microdiet formulation has the potential to have a significant impact on *Seriola* culture. Also, having local alternatives to international products, especially diets, is key to the development of a finfish industry in the United States.

EFFECTS OF POLY-B-HYDROXYBUTYRATE ON GROWTH AND IMMUNE RESPONSES OF FINGERLING AND ADVANCED STAGE NILE TILAPIA (*Oreochromis niloticus*) BASED ON IN VIVO AND IN VITRO APPROACHES

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Further research into the immunological capacity and growth potential of dietary supplementation of poly-β-hydroxybutyrate (PHB) is warranted to assess it as a novel growth enhancement and disease mitigation strategy. Previous observations reported in this laboratory have demonstrated potential beneficial effects of bacteria-derived PHB on growth and health of juvenile Nile tilapia. Therefore, the goal of two subsequent 8-week feeding trials was to replicate these evaluations in both commercially relevant advanced stage and fingerling Nile tilapia. Advanced stage Nile tilapia (~207 g/ fish initial weight) were fed a commercial diet [~33% crude protein (CP)] top-coated with carrier consisting of menhaden oil, carboxymethyl cellulose and alcohol, either with or without crystalline PHB dissolved in it to provide PHB at 1.0% of dry diet weight. Each diet was fed to 30 advanced stage Nile tilapia in triplicate 1200-L round tanks fashioned as a recirculating aquaculture system with well water trickled through to maintain optimal water quality. Tilapia were fed to apparent satiation twice daily based on visually assessed feeding activity. After 4 weeks of feeding, advanced stage tilapia exhibited significant ($P<0.05$) enhanced percentage weight gain and feed efficiency with PHB supplementation (Fig. 1). However, no statistical differences in any measured growth performance parameter or body condition indices [hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and fillet yield] were apparent after 8 weeks. *In vitro/ex vivo* analyses including superoxide anion production, bactericidal, and phagocytic activity of head kidney-derived leukocytes, and blood serum lysozyme are currently being determined and will be complete at the time of presentation. An additional feeding trial is currently being conducted in which eight fish meal- and fish oil-free isoenergetic and isolipidic diets containing 40% CP and 10% lipid from practical ingredients are being fed to Nile tilapia fry (0.9 g/ fish initial weight). To compare with the efficacy of the bacteria-derived PHB previously evaluated, dietary treatments in this trial include a basal diet to which two levels of crystalline PHB (0.0 and 1.0%), chitosan (0.0 and 0.5%), and nucleotides (0.0 and 0.5%) were supplemented in a 2 x 2 x 2 factorial design. Each diet is being fed to triplicate groups of fingerling Nile tilapia stocked at 15 fish per 30-L aquaria operated as a recirculating system. Fish are being fed to apparent satiation twice daily based on a percentage of group body weight which is determined weekly. This trial is currently in progress with a scheduled completion in January of 2023.

In conclusion, advanced stage Nile tilapia showed a dramatic increase in weight gain and feed efficiency after short-term (4 week) exposure to PHB supplementation; however, such effects diminished in the subsequent 4 weeks. Immune response analyses of advanced fish are currently underway, and responses of fingerling tilapia in the ongoing trial will be presented.

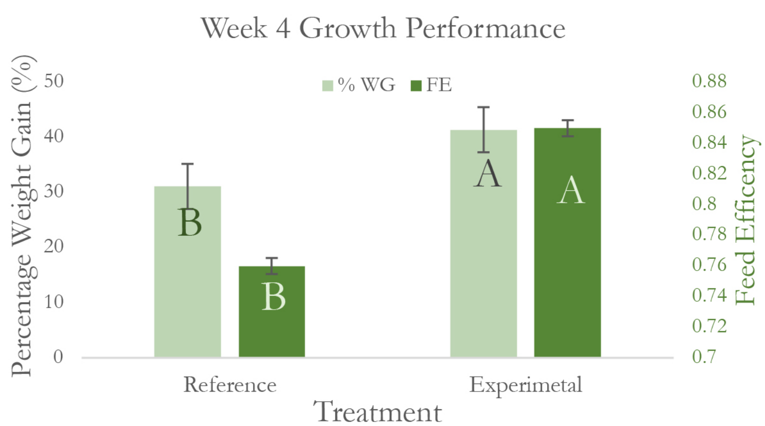

Figure 1. Week 4 growth performance of advanced stage Nile tilapia.

UTILIZING NEW FARMING LOCATIONS VIA 'DUCK-AND-COVER' AQUACULTURE

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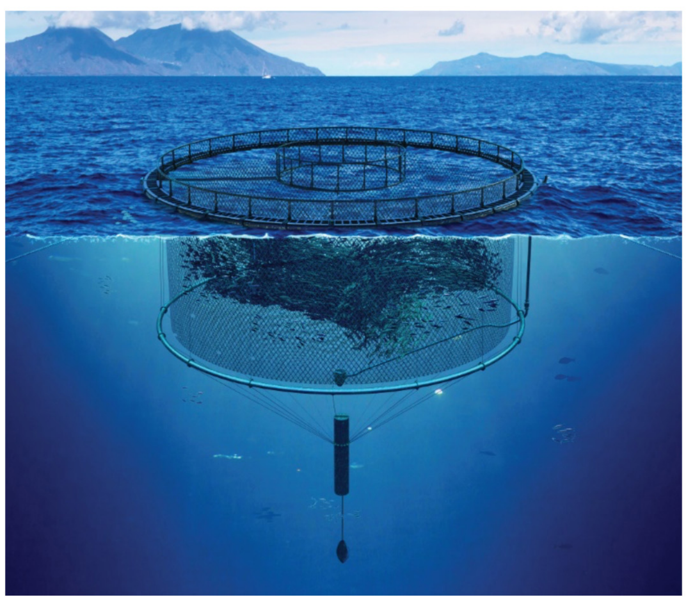
The availability of protected or semi protected waters for new net pen aquaculture installations is shrinking rapidly around the globe due to a number of factors. That has spurred significant growth in open ocean aquaculture, which requires robust infrastructure to farm fish safely and effectively in tough conditions offshore. A result of this growth is an increasing appetite for equipment designed to operate in the many locations around the globe that fall between protected sites and high-energy offshore environments.

A significant portion of our oceans have relatively mild conditions for most of the year but are plagued by occasional extreme events (e.g., hurricanes, cyclones, and typhoons) or short seasonal weather volatility. In these locations standard, surface-oriented operations can be run during the calmer times of year. But farm operators need the ability to protect their fish stocks and infrastructure when severe conditions are encountered. The ideal way to do this is with subsea infrastructure and with pens that are easily submersible. Innovasea calls this "duck-and-cover" fish farming and has been designing and building the equipment for it for the past five years.

In this talk we'll discuss the reasoning and benefits behind subsea and duck-and-cover aquaculture. The discussion will also touch on Innovasea's new SeaProtean pen, an affordable easy-to-submerge pen that was designed specifically for duck-and-cover sites.



FACTORS INFLUENCING U.S. RETAIL SEAFOOD MARKET SALES

Lianqun Sun* and Ganesh Kumar

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As the largest seafood market in the world, the United States retail markets are highly competitive and rapidly evolving. Consumer preferences evolve constantly as reflected by the changing dynamics of the seafood market as well as the increased diversity of seafood products sold in retail markets. In-depth analysis of retail scanner data collected from grocery outlets using Universal Product Codes (UPC) would provide a greater understanding of the retail market trends. Using weekly store-based scanner data (ScanTrack data) from AC Nielsen company from September 2016 through August 2021, this study analyzed how product characteristics affect seafood consumption in the retail market for general and specific seafood categories using regression techniques. The explanatory variables include price, seafood category (finfish, crustacea, mollusks, and others), farm-raised, season, promotional share, product form (frozen or refrigerated seafood, entrée, or others), region, value-addition, the effect of the pandemic period, and labeling characteristics. The regressions results show that price is the biggest indicator of quantity sold in the market, and their relationship are inverse, which means higher price results in lower quantity demand, and vice versa for general seafood as well as specific seafood categories, such as finfish, crustacea, and mollusks. In addition, farm-raised seafood species had a positive impact on seafood quantity sold in the retail market. Promotional practices also showed positive effects on retail seafood sales. Spring season was positively related to the quantity demanded in the retail market, for general seafood and finfish, but negatively related to crustacea and mollusks. The results also indicated that seafood consumption has increased during COVID-19 pandemic time. As the largest seafood consumption region, sales in the South region had a consistently positive effect on overall seafood sales as well as on. Conversely, the west north region had negative effects on seafood sales and specific seafood categories. Private labeling (store brands) also had a positive effect on seafood quantity in the retail market while products with no label or company label had a negative sign on seafood consumption.

TRENDS FOR U.S. CATFISH AND SWAI PRODUCTS IN RETAIL MARKETS

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Farm-raised catfish is the largest U.S. aquaculture industry. Over the years, the ingenuity of catfish processors has made catfish products available all over the U.S. including globally competitive retail marketplaces. U.S. catfish products face stiff competition from Vietnamese swai products in these diverse markets. This work elucidates the detailed nascent retail trends for U.S. catfish and swai products by evaluating weekly, store-based, AC Nielsen ScanTrack data from September 2016 through August 2021. The five-year analysis compares retail sales of U.S. catfish and swai products across national, regional, and city levels while providing key trends in product forms, quantities sold, promotional patterns, and price information. Results indicated an average annual growth rate of retail sales of 5.8% for U.S. catfish and 0.7% for swai. The South Central region had the greatest total retail sales for both U.S. catfish and swai products. Dallas/Fort Worth had the greatest total retail seafood sales for U.S. catfish products, followed by Chicago and St. Louis (Figure 1). Los Angeles was the most important city for retail swai sales. The 454-g U.S. catfish packs had a share of 77 and 68% in terms of volume and revenue while the same pack for swai had 48 and 28% volume and revenue shares. Frozen and refrigerated products dominated sales for both products in 2021. Swai products were characteristically priced lower than U.S. catfish products across all regions, cities, and study years, especially for larger packs. The study revealed the shift to increased at-home consumption of these two products during the pandemic years that have been reported for other seafood products.

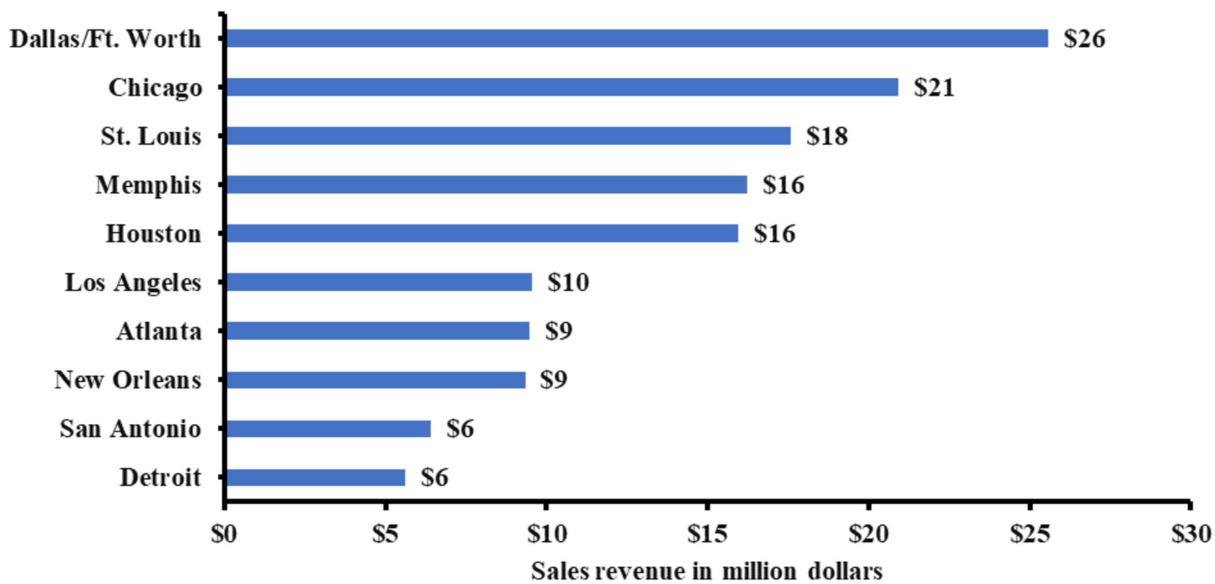

Figure 1: Top 10 cities for catfish by sales revenue, 2020-2021 (in million $).

MARKET TRENDS FOR TROUT PRODUCTS IN THE U.S. RETAIL MARKET

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Trout is an important U.S. aquaculture product. The seafood supply in the US is increasingly dominated by imported products. It is important for trout farmers, processors, wholesalers, and retailers to continually seek information on the latest trends in retail markets. This paper focuses on the retail market trends for trout, especially rainbow and steelhead trout products across national, regional, and city levels as well as key trends in product forms, quantity sold, sales under promotion, and price comparisons. The retail sales information was obtained from weekly, store-based AC Nielsen ScanTrack data from September 2016 through August 2021. The results show that trout sales in the retail market have increased steadily by 7% annually from 2016 to 2021, with the quantity increasing by a 6% annual growth rate. The South Atlantic region was the largest regional market for trout sales, followed by the Pacific, and South Central regions. The West North Central region, however, was the fastest-growing regional market in terms of sales and quantity. Seattle was the biggest city market for trout sales, followed by Portland, OR, and Atlanta in 2020-2021. Atlanta and Tampa had the highest growth rate for the quantity of trout sold (Figure 1). Steelhead trout occupied a 72% market share of trout sales, while rainbow trout had a 26% retail market share. The retail market share of steelhead trout steadily increased (sales and quantity), with an annual growth rate of 6% and 9%, respectively. Frozen products were the most commonly sold products (90%) mainly in small packs (454-g packs). The price for trout products remained relatively stable over the 2016-2021 period.

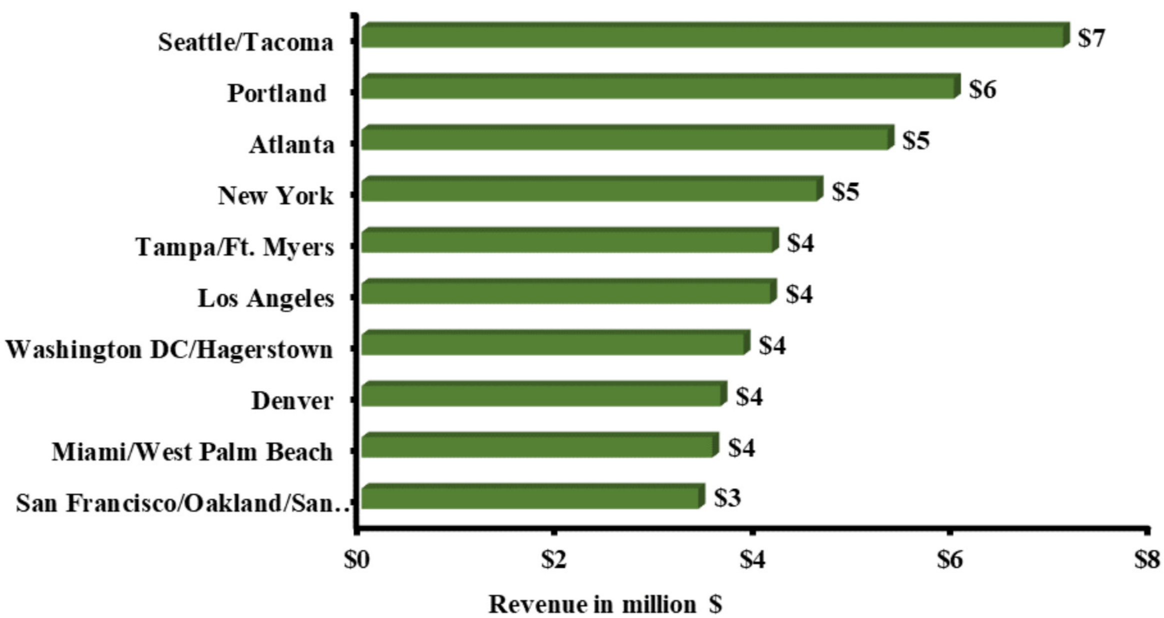

Figure 1: Top 10 cities for trout by sales revenue, 2020-2021 (in million $).

U.S. RETAIL MARKET TRENDS FOR TILAPIA PRODUCTS

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Tilapia products are the fifthmost sold product in U.S. seafood retail markets. This article focuses on grocery retail sales with special emphasis on tilapia market trends over the last five years by evaluating weekly, store-based ScanTrack scanner data from AC Nielson from September 2016 through August 2021. Results show that tilapia sales have been slightly declining in the retail market, with -1% annual growth rate. Such slightly declining trends were an exception among the top seafood products sold in retail markets such as shrimp, salmon, crab, lobster, and catfish which registered positive annual growth rates in sales. The South Central region is the largest tilapia regional market, followed by the South Atlantic and Pacific regions. Los Angeles, New York, and Dallas/Ft. Worth were the top three city retail markets for tilapia in 2020-2021(Figure 1). The majority (90%) of the tilapia products were sold as non-value added products. Frozen and refrigerated products dominated the retail sales (89%) of which fillets (76%) had the major market share (76%) in terms of sales value. Smaller 454-g packages of tilapia had 38% of the market share among all different package sizes, followed by 1.81-kg with 19%, and 908-g, with 15% revenue share. Fresh tilapia sold in the retail market has declined over the years while frozen tilapia sales have risen, with 9% and 78% market share, respectively in 2020-2021. Tilapia prices remained constant over the study period. An increased understanding of tilapia trends in the retail market would assist in the important development of effective marketing strategies in the United States.

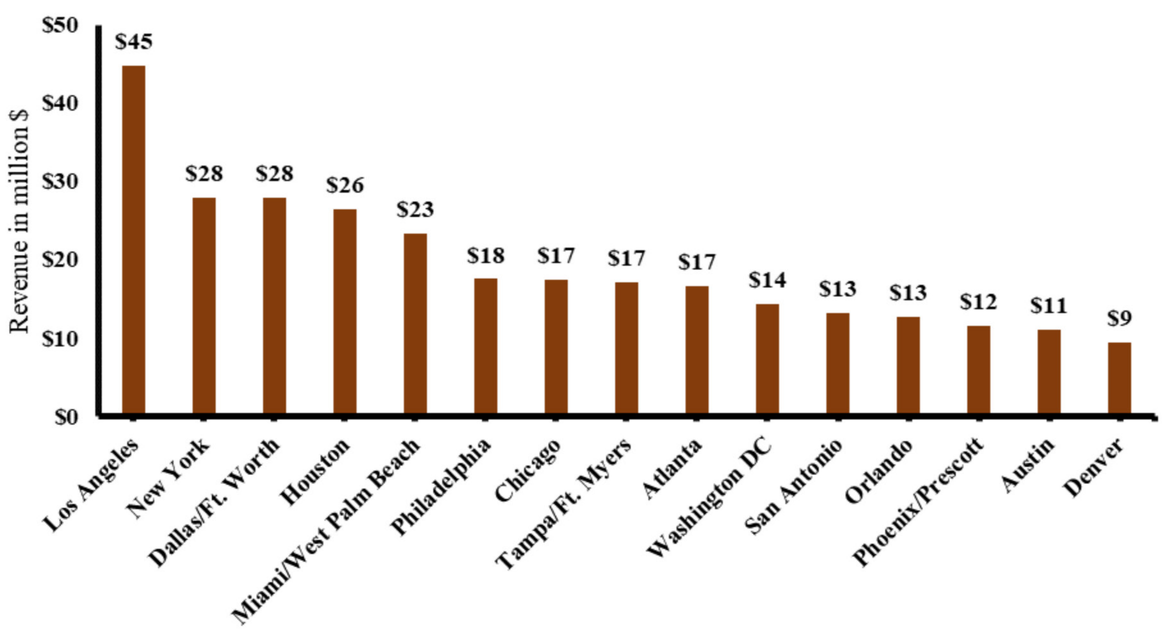

Figure 1: Top 15 cities for tilapia by sales revenue, 2020-2021 (in million $).

COMMERCIAL ENHANCEMENT OF BIVALVE HATCHERY SUSTAINABILITY THROUGHAPPLIED TECHNOLOGY APPLICATION

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Shellfish aquaculture, especially oysters, has provided valuable economic, ecological, and social benefits. Virginia is a leading producer of oysters along the U.S. Atlantic coast. In 2019, the aquaculture industry of Virginia produced more than $177 million for the commonwealth, while shellfish (e.g., oysters) contributed 84 percent of the total economic output. Oyster hatcheries are facilities that provide juvenile oysters for commercial production. The efficacy of larvae production in the oyster hatchery is primarily impacted by the utilized ambient water. However, many environmental factors can affect the quality and microbial safety of ambient water; a primary concern for oyster hatcheries is the loss of larvae due to disease outbreaks caused by bacterial pathogens, especially in the genus *Vibrio*. Therefore, developing innovations to ensure the quality and safety of ambient water is needed.

In this project, we have developed a prototype bioreactor system to clean and polish production water for direct reuse during oyster larval production. The system (as seen in the picture) can be integrated directly into standard hatchery operations, which include a typical drain/fill of larval tanks on a two-day cycle. Under standard operations, the larvae are usually resuspended in the water pumped directly from coastal waters or in a continuous supply culture system. While using our new technology, the water is sourced from a recirculating bioreactor. This bioreactor accomplishes converting nitrogenous wastes and organic material from microalgae and larvae. More importantly, it promotes the removal of fast-growing bacteria responding to larval culture, including potentially pathogenic bacteria (e.g., *Vibrio* spp.), by promoting populations of bacterivorous protists within the bioreactor. This technology supports bivalve hatchery operations "offline" when ambient water quality in coastal areas is impaired. Additionally, it remotes hatchery operations without direct access to surface water and out-of-season or head-start production.

The system is scaled up and in testing at a commercial hatchery currently. Water quality (e.g., temperature, dissolved oxygen, pH, salinity, total ammonia nitrogen, nitrite, nitrate, and alkalinity), seeds survival rate, and microbial analysis and community study (e.g., plate counts, DNA sequencing) were performed and evaluated. Our preliminary data indicated promising results compared to standard commercial single-use water management practices. Improved water quality and a significant reduction of *Vibrio* spp. were observed. The system has shown great potential in enhancing seed production consistency temporally, reducing reliance on coastal waters during poor ambient water quality, and providing improved options for out-of-season or early-season seed.

REARING ATLANTIC SALMON IN A FLOW THROUGH FACILITY DESIGNED FOR REARING PACIFIC SALMON

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The Michigan Department of Natural Resources (MDNR) has reared Pacific Salmon to support the sport fisheries of the Great Lakes for over 60 years. Lake Huron Chinook Salmon were depleting forage compounded by the latest invasive mussels taking out the bottom of the food chain. In the late 2000's the salmon fishery in Lake Huron had all but disappeared. The MDNR began experimenting with Atlantic Salmon in the Fish Production System. In 2012 MDNR took on Atlantic Salmon on a full production scale with the goal of producing 200,000 spring yearlings for stocking in Lake Huron. Only 106,000 fish were produced for stocking in 2012.

Today, ten years later, the MDNR is much closer to hitting the mark of 200,000 yearlings on an annual basis. Success took hard work, mentoring and retrofitting a portion of a facility designed for rearing Pacific Salmon. It took Michigan Department of Natural Resources years to get it right. The intent of the session is to describe todays current rearing conditions and discuss the lessons learned.

EXPLORING THE LIFE SUPPORT SYSTEM OF A MICHIGAN DEPARTMENT OF NATURAL RESOURCES FISH STOCKING UNIT

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Life support systems are critical to all aquaculture facilities. Life support systems on stocking units are just as critical to a program that transports live animals out of the facility. Michigan Department of Natural Resources understands the importance and is committed to stocking fish that are healthy and alive. Michigan is also committed to keeping their 19 stocking units modernized and up to date. These units are custom built and highly reliable. The intent of this session is to explore the life support system of a 3200 gallon stocking unit.

CONSERVATION PROPAGATION IN EASTERN NORTH CAROLINA

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Methods used to propagate conservation aquatic species differ from those used to produce species commercially. Conservation propagation is population driven, labor intensive, and feed variety is important. Feed rates, tank design, tank size, and densities differ compared to commercial production. In Edenton, North Carolina, hatchery biologists have partnered with the Raleigh Ecological Services Office, North Carolina Wildlife Resources Commission, and North Carolina State University to propagate several conservation species.

Two fish species are being propagated as host fish for endangered freshwater mussels. Mussels release glochidia (offspring) in the water column to attach to a fish gill. Some freshwater mussels tend to be specific to their host species. Research has shown the Tar River Spinymussel (*Elliptio steinstansana*) to be partial to the White shiner (*Luxilus albeolus*) and the Dwarf Wedge mussel (*Alasmidonta heterodon*) to the Johnny darter (*Etheostoma nigrum*). In 2019, a total of 1,600 juvenile fish were produced for Tar River Spinymussel hatcheries and programs. In 2022 (pilot year), 350 juvenile Johnny darters were produced and will be distributed to Dwarf Wedge mussel programs in winter of 2023. Two endangered aquatic species are also being reared at Edenton. Carolina Gopher frogs (*Rana capito*) are listed as endangered by the state of North Carolina and currently being reviewed under the Federal Endangered Species Act. In North Carolina, Gopher frog populations have decreased from twenty-three to seven known populations. In 2021 (pilot year), 116 Gopher frogs were released in the Croatan National Forest. Cape Fear shiners (*Notropis mekistocholas*), an endemic fish species, are also being propagated at Edenton. These fish are localized to the Cape Fear River basin and some of its tributaries. In 2020 (pilot year), no juvenile fish were produced. In 2021, 5 juveniles and their parents were released in the confluence of the Deep and Rocky rivers. In 2022, 517 juveniles and their parents were released in the Cape Fear River.

Struggles and successes are present in the production of each species. Johnny darters are specific to what color structure they lay their eggs, and trials were conducted to determine when and how to remove eggs from broodfish tanks. White shiners prefer larger groups to spawn with and need nests to successfully spawn. Modified minnow traps were not as successful at capturing newly metamorphed frogs as anticipated. Cape Fear shiners have eggs and fry that are nearly invisible! The tough egg and fry visibility causes struggles with retrieving, enumerating, and cleaning. Every conservation species is reared differently, however, they have the same goal: produce quality organisms, keeping body composition, morphology, and physiology in mind. This process starts at rearing and ends with a successful release. As knowledge and experience with these species is gained, rearing and release methods continue to be optimized.

BROODSTOCK CONDITIONING SALINITY IMPACTS SPERM QUAILTY IN EASTERN OYSTER, *Crassostrea virginica*

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Aquaculture demands for high value products, like the Eastern oyster (*Crassostrea virginica*), continue to rise. Many factors, including eyed larval production, limit commercial oyster output. To combat this, hatcheries spawn diploid females with tetraploid males to produce triploid offspring, which in many instances exhibit faster growth than diploid oysters and maintain meat quality during the reproductive season. Despite improvements, gametes produced in hatcheries are often of low quality. While it is known that abiotic factors, like temperature and pH, influence gametogenesis in Eastern oyster the effects of salinity need to be fully identified. In *C. gigas* low salinity negatively impacted fertility, which indicates inhibition in sperm motility. To expand on these ideas, this study investigates how salinity affects Eastern oyster gametogenesis and gamete quality for triploid production. Oysters were conditioned in recirculating aquaculture systems (RAS) at 10, 20, and 30 PSU for 30 days. Temperature and light mimicked oceanic conditions in the Gulf of Mexico. RAS contained 6 ×140 L tanks, each housing 15 oysters per ploidy. Oysters were opened and sex determined by observation of their gonads under a microscope. Semen was collected from tetraploid males, sperm density was determined using hemocytometer counts while viability was evaluated by flow cytometry. Sperm velocity (VCL) and motility were evaluated by computer assisted sperm analysis. Eggs collected from diploid females were assessed for fecundity and egg size. For both sexes, lipids were extracted and analyzed by gas chromatography. Histology samples will be processed and gametogenic development assessed based on a maturity stage index.

Sperm density tended to increase at higher conditioning salinities (1.35×10^9 at 10 PSU to 1.86×10^9 sperm/mL at 30 PSU), however this trend was non-significant. At 30 and 60 s post-activation of sperm, oysters conditioned at 10 PSU had lower VCL than other salinities (Fig 1A). Sperm motility was lower for oysters conditioned at 10 PSU (Fig 1B). Conditioning salinity also affected sperm viability with lowest values observed at 30 PSU (Fig 1C). Female fecundity tended to decrease when oysters were conditioned at higher salinities (2.74×10^6 at 10 PSU to 2.41×10^6 eggs at 30 PSU), however this trend was non-significant. Results, thus far, suggest 20 PSU as a promising conditioning salinity for sperm quality. Lipid and histology analyses will provide further support for this conclusion.

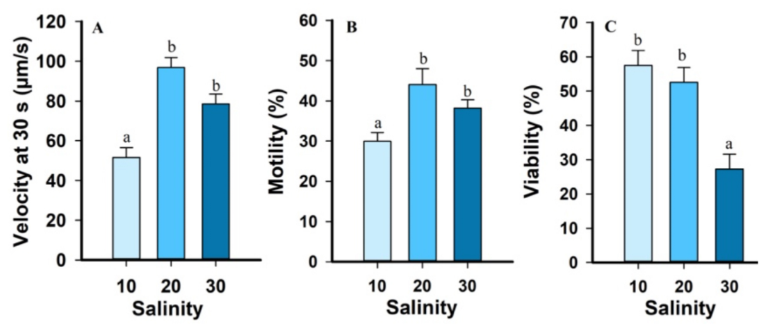

Fig. 1. Effects of conditioning salinity on sperm velocity (A), motility (B), and viability (C) in Eastern oyster tetraploid males (Crassostrea virginica).

THE FEASIBILITY OF PROCESSING FISH IN LOCAL FACILITIES FOR LOCAL FOOD SYSTEMS

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Small and medium-scale fish farmers in the Midwest are looking for opportunities to utilize local processing facilities to diversify their product offerings and boost their farm profitability. The COVID-19 virus has stressed this need. Commercial kitchens and on-farm fish processing plants are two potential facility types for fish processing. Commercial kitchen facilities are available across the region and are ideal for new or expanding small food businesses that do not have the financial resources to invest in a processing facility.

By venturing into processing, potential opportunities for fish farmers include supplying processed fish products to their community; stimulating new fish product development; improving the diversity of local aquaculture products; increasing sales of local aquaculture products; increasing awareness of supply and access to local aquaculture products; and increasing consumption of local aquaculture products. Bridging the gap between the farmers' desire to process fish and the demand for a suitable place to process fish and other aquaculture products is the task we face.

We aim to understand the feasibility of starting a fish processing business from local facilities using a "PEST" analysis approach. "PEST" stands for Political, Economic, Social, and Technological factors that need to be considered in market analysis. It is a concept in market research that companies use as a tool to track their operating environment or the environment they are planning to launch a new product or service in. The states of Illinois, Indiana, and Ohio are chosen as case studies because they belong to the North Central Region, whose aquaculture industry lacks processing facilities. By conducting a PEST analysis for these states, we discover political, economic, socioeconomic, and technological factors that would affect the feasibility of aquaculture processing. We also generate maps and frameworks regarding the location, function, and cost of seafood processing facilities available in the three states to help fish farmers' decision making. Lastly, a financial analysis is provided to help fish farmers develop individual strategic plans.

The research results will help fish farmers understand what it takes to process fish and other aquaculture products in commercial kitchens and/or in on-farm processing facilities to supply their local markets.

PARENTAL AND TEMPORAL INFLUENCES ON THE LARVAL RED DRUM *Sciaenops ocellatus* MICROBIOTA

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The environment, host, and microbial interactions influence fish microbiota structure. Despite many studies describing the microbiota of larval fishes, few have addressed potential parental contributions to these communities. This study fed broodstock red drum populations two diets: shrimp (Shrimp) and shrimp, squid, and sardines in equal proportions (ShSqSa). Larvae (7- and 10-days post-hatch) were collected from eleven spawning events throughout a year for microbiota analysis. Fatty acids were quantified in broodstock diets, eggs, and larvae.

Parental and environmental factors influenced larval bacterial assemblages. Larvae from broodstock fed ShSqSa had different communities than those fed Shrimp (Fig. 1), with discriminatory taxa belonging to the *Desulfobacterota* and *Actinobacteria*, respectively. In broodstock fed Shrimp followed by ShSqSa, the larval microbiota corresponded to that of ShSqSa-fed groups. Temporal differences in microbiota structure were apparent, with larval bacterial communities clustering by spawn month within each diet. Predicted microbiota function indicated greater potential for metabolism of α-linolenic acid (ALA) and arachidonic acid (ARA) in larvae from Shrimp and ShSqSa, respectively, corresponding with observed patterns in larval fatty acid composition (Fig. 2).

Arachidonic (ARA) and palmitoleic (POA) acids were significantly higher in eggs from Shrimp-fed broodstock but lower in the corresponding larvae; eicosatrienoic acid (ETA) showed the opposite trend. Operational taxonomic units positively associated with ARA and POA were negatively associated with ETA and vice versa, indicating a possible influence of microbiota on larval fatty acid composition. This study suggests a relationship between parental diet and bacteria associated with fish larvae, with possible effects on larval fatty acid metabolism, highlighting the complex interactions selecting for larval microbiota.

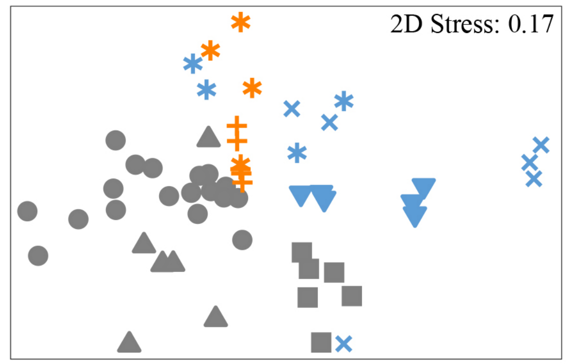

Fig. 1. Multidimensional scaling of red drum larval microbiota, grouped by adult diet (Shrimp: gray; ShSqSa: blue; Shrimp followed by ShSqSa: orange) and spawn month (symbols).

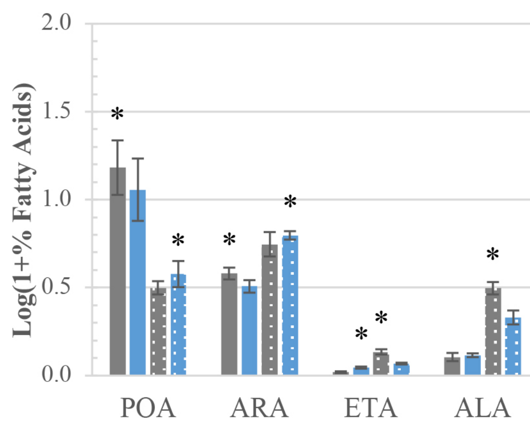

Fig. 2. Fatty acids significantly different in abundance by diet in both eggs and larvae. Gray, shrimp; blue, ShSqS; Solid, eggs; pattern, larvae.

IMPACT OF UPWELLER TYPE ON SUBSEQUENT GROWTH OF EASTERN OYSTER *Crassostrea virginica*

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The goal of this study was to compare growth and survival of eastern oysters (*Crassostrea virginica*) reared in silo and bottle upwellers. Oysters were maintained in their respective nursery systems until reaching R2 (retain on a 2 mm screen) at which point they were moved into the field to an adjustable longline system. Biweekly, oysters were measured (height, length, width) and mortalities estimated. Stocking density was reduced at each sampling point by removing slow growers. Sampling continued for six weeks. At the end of the trial, oysters were examined for backbend deformities.

Following the nursery period, silo reared oysters had greater shell height and length but a lower fan ratio than those reared in bottles. After 6 weeks, silo reared oysters reaching R22 or larger had greater shell height and width than bottle-reared oysters of a similar size (Table 1). Oysters between R16 and R22 from silos were significantly longer than those from bottles. Both rearing strategies resulted in equal proportions of oysters in each size class (Fig. 1) with equivalent cup ratios. R22 oysters from silo nurseries had higher Fan ratios after 6 weeks. There were no differences between rearing groups in mortality or deformities (i.e., backbends) after 6 weeks.

Overall, silo upwellers resulted in faster oyster growth than bottle upwellers. The size benefit was most apparent in the fastest-growing oysters. Size differences were generally small; therefore, optimization of bottle upweller parameters (i.e., water flow, stocking density, etc.) may reduce growth differences between these two nursery types.

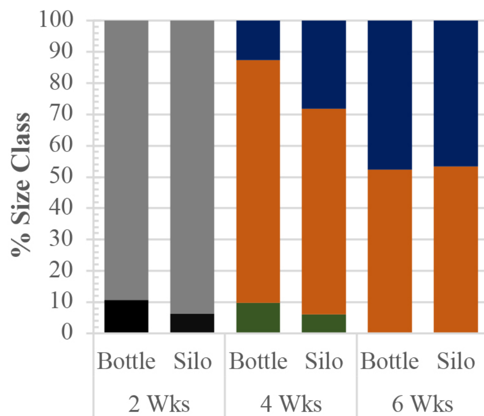

Fig. 1. Biweekly abundances of oyster size classes from each upweller. Black, R4; Gray, R6; Green, <R16; Orange, R16-R22; Blue, >R22.

Table 1. Size measurements after six weeks in the field. Superscripts denote significant differences within a size class.

Size Class	Upweller	Shell Height (mm)	Shell Length (mm)	Shell Width (mm)	Fan Ratio	Cup Ratio
R16-22	Silo	24.8 ± 5.8	17.1 ± 3.0^{A}	7.5 ± 1.8	1.45 ± 0.19	0.30 ± 0.08
	Bottle	24.1 ± 5.8	16.5 ± 3.0^{B}	7.2 ± 1.4	1.46 ± 0.20	0.30 ± 0.04
>R22	Silo	32.0 ± 5.8^{A}	21.4 ± 3.0	8.8 ± 1.4^{A}	1.50 ± 0.21^{A}	0.28 ± 0.05
	Bottle	30.6 ± 4.6^{B}	20.9 ± 2.7	8.4 ± 1.2^{B}	1.47 ± 0.19^{B}	0.28 ± 0.04

SMALL-SCALE EASTERN OYSTER *Crassostrea virginica* LARVAL PRODUCTION USING ALGAE CONCENTRATE

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Addressing larval rearing challenges for Eastern oyster (*Crassostrea virginica*) aquaculture requires replicated studies. Small-scale systems are useful for replication; however, most studies use live algae and, to our knowledge, have not compared performance to large-scale systems. To that end, the objective of this study was to optimize small-scale system production fed with algae concentrate. We tested a variety of aeration techniques (see Table) in small-scale, 17 L bucket systems. Control systems were 4000-L or 1000-L tanks (Fig. 1). Water quality was monitored, and larvae were counted volumetrically and measured for 14 days. Survival, growth, and percent set were compared among treatments and across time.

During trial 1, production requirements prohibited running replicated controls; however, bucket-reared larvae started setting at Day 10, with no differences between aeration treatments. Slow growth and high mortalities plagued all treatments in Trial 2. During Trial 3, bucket-reared larvae grew significantly faster than controls, with the 1 mL air injector and 2-inch airlift suffering fewer mortalities. In Trial 4, control larvae were larger than all bucket larvae at Day 10 and produced more setters by Day 14 than the 1 mL air injector and the no aeration treatment. We hypothesize this was due to lower nighttime temperatures than previous trials. However, all buckets had fewer mortalities than the control tanks.

This study indicates that performance of small-scale systems using algae concentrate are therefore feasible for replicated larval studies. These systems and the algae concentrate are inexpensive and simple to maintain and may provide demonstration opportunities for schools or individuals interested in hatchery management. Due to the simplicity of these systems, further adjustments can easily be made to improve growth, survival, and set rate of larval oysters.

		Larval size (mm)	Eyed Larvae (%)	Survival (Larvae/mL)
Trial 1	Control	158 ± 62	0.97 ± 1.6	2.0 ± 2.3
	2-inch airlift	154 ± 51	2.3 ± 1.5	4.3 ± 5.7
	4-inch airlift	146 ± 51	2.1 ± 1.7	2.4 ± 3.0
Trial 2*	Control	106 ± 30	1.33 ± 0.13^{A}	3.8 ± 0.8
	2-inch airlift	105 ± 17	0^{B}	2.3 ± 1.9
	4-inch airlift	106 ± 16	0^{B}	2.6 ± 1.9
	1 mL air injector	113 ± 21	0^{B}	2.6 ± 1.7
Trial 3	Control	161 ± 41^{B}	4.5 ± 1.1	2.7 ± 0.9^{BC}
	2-inch airlift	175 ± 45^{A}	6.4 ± 2.1	3.2 ± 1.3^{AB}
	4-inch airlift	180 ± 47^{A}	10.8 ± 3.5	3.0 ± 1.1^{B}
	1 mL air injector	174 ± 41^{A}	7.6 ± 8.0	3.5 ± 1.1^{A}
Trial 4	Control	164 ± 61^{A}	25.9 ± 12.6^{A}	2.6 ± 0.6^{B}
	1 mL air injector	155 ± 62^{B}	8.8 ± 13.7^{B}	3.4 ± 1.2^{A}
	5 mL air injector	158 ± 65^{B}	13.7 ± 16.7^{AB}	3.2 ± 1.1^{A}
	No aeration	152 ± 62^{B}	7.0 ± 5.9^{B}	3.2 ± 0.9^{A}

*There were interactive effects between day and treatment, so no statistical results are presented.

Fig. 1. Size of small-scale systems (17 L; front) compared to 4,000-L production tank (back).

EASTERN OYSTERS RESTORATION, RECRUITMENT, AND THEIR IMPACT ON SPECIES DIVERSITY AND WATER QUALITY IN REHOBOTH BAY, DELAWARE

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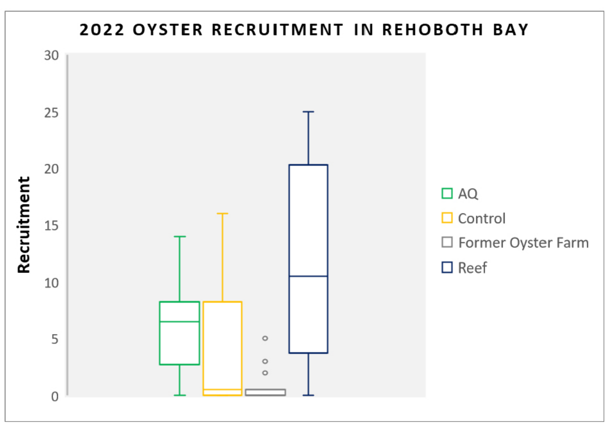
Rehoboth Bay, Indian River Bay, and Little Assawoman Bay are part of Delaware's Inland Bay system. Rehoboth Bay spans thirteen square miles and is connected to the Indian River Bay in the south, the Lewes-Rehoboth Canal to the north, and other freshwater bodies. Eastern oyster (*Crassostrea virginica*) 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 objective of this project is to understand how artificial oyster reefs and aquaculture farms can affect water quality, species diversity, and oyster recruitment in Rehoboth Bay. Two artificial oyster reefs, two oyster farms, two control sites, and one former oyster farm 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 data. Water samples are also collected and processed in the lab for nitrate and orthophosphate levels. 2021 results from Dr. Ozbay's lab show that artificial and oyster farms have the highest oyster recruitment and species richness, and species abundance, while oyster farms had the highest species diversity. 2022 field data is currently being analyzed.

Acknowledgments: We acknowledge NOAA LMRCSC Grant #NA16SEC4810007, National Science Foundation EPSCoR Grant No. 1757353 and the State of Delaware, and Dr. Gulnihal Ozbay's One Health Lab team



WATER SYSTEM VALVE IDENTIFICATION, DESCRIPTION AND SELECTION CRITERIA

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Every water system will use at least one valve. Choosing the correct valve for each application is important to ensure you can properly control the flow, pressure and distribution of the water in your systems. The type and sizes of valves selected for your system should be based on the following criteria: quality, materials of construction, Cv value, control characteristic, and performance requirement. We will present these characteristics for the following valves and more: ball, globe, diaphragm and butterfly valves.

If selected properly, your valve will provide you with long-term worry-free service in your water systems. We will concentrate on the important parts of each valve and explain why you should select a certain valve over another? What are the advantages to using an industrial valve over a "Home Improvement Store" style valve? What elastomeric should you use for different applications in your system? These are all good common questions that we will address in this presentation.

Reference: Asahi/America, Inc. Engineering Design Guide.

ASSESSMENT OF THE EFFECTIVENESS OF MOBILE RESPONSIVE FISH HEALTH DATABASE

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In order to significantly ameliorate mortalities on aquaculture farms due to diseases, it is important that trends of disease occurrences are monitored over time. Such data, however, is not commonly available for analysis due, in part, to non-digital recording of fish disease cases (e.g., hand written reports) and to a non-standard way of recording data even if it is digital. Due to this unavailability, a user-friendly, standardized online database was developed to improve fish health management by streamlining data entry, increasing data reporting, and enabling real-time monitoring and reaction to disease occurrences, as well as future study. With the use of a recently created mobile responsive clinical fish health database, this project will increase the efficiency of fish health services provided to the aquaculture industry (referred to as the Database; McKay, T.J. 2022), thereby reducing fish losses and maximizing net income of fish farmers. In order to increase the efficiency and effectiveness of the Database, beta testing feedback from a number of fish disease diagnostic laboratories that will input their laboratories' data into the Database will be collected via a survey. The Kentucky State University Fish Disease Diagnostic Laboratory and a number of other comparable (public and private) institutions, as well as a few in Great Britain and Europe, will be among the labs doing beta testing.

STIMULATION OF SOUTHERN FLOUNDER SPERM MOTILITY AND FERTILITY BY DIRECT ACTIVATION OF INTRACELLULAR SIGNALING PATHWAYS

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Development of commercial aquaculture for many marine flatfish species such as southern flounder is severely limited by poor male reproductive performance, especially reductions in sperm motility and fertility, but the underlying causes of these reductions and the mechanisms regulating sperm motility are largely unknown. Our previous studies have shown that acute treatment of southern flounder and Atlantic croaker sperm in vitro with two progestins, a teleost progestogen hormone (20β-S) and a specific agonist (Org-OD-02-0), causes rapid induction of sperm hypermotility and increased fertility through activation of progestin membrane receptor alpha (mPRα) coupled to a stimulatory G protein and increases in cAMP/Acy (adenylyl cyclase) activity. Progestin-induced hypermotility is also mediated through Egfr (epidermal growth factor receptor)/Mapk (mitogen-activated protein kinase)/Erk1/2 (extracellular-regulated kinase 1/2), and Pi3 (phosphatidylinositol-3-kinase)/Akt (protein kinase B)/PDE (phosphodiesterase) signaling pathways as well as increases in calcium levels in croaker sperm, but additional studies are required to determine whether they are involved in the motility response in flounder sperm.

In the present study the role of the Egfr/Mapk/Erk1/2 signaling pathways in progestin stimulation of flounder sperm hypermotility was investigated using specific pharmacological tools. Aliquots of freshly collected sperm, diluted in physiological saline, were preincubated with two EGFR inhibitors, AG1478, and AG825, and an Erk inhibitor U0126, for 30 min. prior to treatment with progestins for 1 min. and motility activation with a hyperosmotic medium. Sperm motility was observed under a microscope and recorded for 1 min. with a video camera and swimming speed was calculated using motion analysis software. Whereas treatment with the three inhibitors did not alter basal sperm motility, all of them significantly attenuated the hypermotility response to the progestins. Interestingly, 1 min. in vitro treatment with 100 nM human recombinant Egf, the Egfr agonist, mimicked the stimulatory effects of progestins on sperm motility, calcium levels, and fertility. The results suggest Egfr/Mapk/Erk signaling is involved in the hypermotility and fertility response to progestins in flounder sperm. The cAMP/Acy signaling pathway has been implicated in progestin upregulation of flounder sperm motility which is accompanied by a rapid increase in cAMP levels. The Acy activator, forskolin, mimicked the stimulatory effects of progestins on cAMP levels but its effects on sperm motility were not investigated. The present results show that direct in vitro treatment with 10μM forskolin for 1 min. mimics the stimulatory action of progestins on flounder sperm motility, calcium levels, and fertility.

These results demonstrate that flounder sperm motility and fertility can be directly stimulated by recombinant Egfr and forskolin and suggest they could potentially be used as pharmacological agents to enhance reproductive performance of flounder broodstock. This research was supported by USDA NIFA grant 2018-67015-27574 to PT.

A GUIDE FOR EFFECTIVE, LOW-COST COMMUNITY OUTREACH FOR U.S. SEAFOOD FARMERS AND FISHERMEN

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Poor public perceptions about fisheries and marine aquaculture, or seafood farming, can have detrimental impacts to livelihoods, economies, and the environment. Current efforts to engage 'public' audiences are aimed at consumers, but many of the social and political conflicts that come to light in permitting processes or debates over whether or not a fishery or farm should stay in operation come from broader audiences who may or may not eat seafood. Without broader public support, it will be harder for the U.S. to increase seafood production in domestic waters. Fishers, farmers, and other stakeholders can play a critical role to share their stories and contribute to the narrative and efforts to educate broader audiences about responsible U.S. seafood and the role it plays in supporting a more nutritious and sustainable food supply. But outreach and communications can be expensive and time consuming.

Much of the narrative about U.S. seafood is currently shared with broader public audiences by other stakeholder groups including NGOs, media, and others who may or may not represent the U.S. seafood community. Stronger engagement from the U.S. seafood community can support ongoing efforts to improve public perceptions about, and public support for expanding responsible seafood production in the U.S. The Aquarium of the Pacific worked with many stakeholders and consultants to develop a guide for U.S. seafood farmers and fishermen to more effectively leverage social media to share their stories, build trust, and contribute accurate information to the narrative about U.S. seafood. Content in the guide was informed by a series of qualitative and quantitative surveys, as well as test runs with social media influencers. We will share the methodology and results from the content testing and social media influencer insights that informed the development of the guide. This project was funded by the NOAA Saltonstall-Kennedy Grant Program.

OPEN TECHNOLOGIES CAN PROVIDE NOVEL APPROACHES TO SUPPORT DEVELOPMENT OF GERMPLASM REPOSITORIES TO SAFEGAURD THE GENETIC RESOURCES OF AQUATIC SPECIES

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Around the planet there are immense challenges that defy resolution despite offering tremendous opportunities. Development of germplasm repositories to protect, maintain, and distribute genetic resources of aquatic species is such a challenge. For example, the results of decades of selective breeding and millions of dollars of investment are typically maintained as live populations for fish and shellfish. This is risky and unnecessarily expensive. Thus, despite 70 years of cryopreservation research, fish and shellfish only have 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 drive multi-billion-dollar global markets for improved genetics. The lack of repository capabilities suppresses advances across aquaculture, conservation programs, natural fisheries, biomedical models, and addressing food security and poverty alleviation. Recognition of this as an immense challenge (not addressed by current approaches) is an important step towards resolving it. Because problems such as this are beyond the resources of single entities, new 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, and offers opportunities for management of aquatic genetic resources. 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 mechanism was in direct response to the limitations of proprietary development. The success of Linux provided impetus for other open-source projects, and the accrued experience has opened doors to expansion of distributed development for software. This spirit has emerged in renewed form with new consumer-level design and fabrication technologies that can enable study, production, distribution, modification, improvement, and commercialization (based on licensing agreements) of open hardware devices shared over the internet as computer-aided design (CAD) files. Fabrication can combine powerful tools such as microprocessors and microcontrollers, 3-D printing (of plastic, resins, and metal), printed circuitry, LEDs and fiber optics, laser etching and cutting, and CNC machining. As such, these diverse technologies provide a powerful alternative to traditional research and proprietary development by enabling combined efforts across multiple communities to establish and operate germplasm repositories and manage and eventually commercialize genetic resources of aquatic species.

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 has 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 the evaluation of a 4.5-ton tank, an 85-liter PolyGeyser® bioclarifier, and a 7.6 cm airlift driven by a 100 lpm linear air pump 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 8.25 kg/m^3 under less-than-ideal greenhouse conditions. During the Winter 2022 run, two systems were successfully harvested at 19 grams and a density of 9.2 and 10.1 kg/m^3. The Winter 2022 run incorporated localized sludge digestors driven by a pneumatic exchange to conserve salt as sludge discharge is eliminated. Research into the use of the localized sludge digester as a means of tertiary treatment is ongoing. 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.

CREATING OPEN-SOURCE ENVIRONMENTS FOR HARDWARE TO SUPPORT CRYOPRESERVATION AND REPOSITORY DEVELOPMENT FOR AQUATIC SPECIES

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Aquatic species represent billions of dollars of societal investment in establishment and characterization of biomedical animal models, global production of more than 110 million tons of food, and employment for more than 60 million people. The genetic resources of these species are typically maintained as live populations that are susceptible to disease, environmental degradation, and catastrophic events. Germplasm repositories with samples cryopreserved in a repeatable, controlled fashion with all the necessary associated information ensure recovery success years after collection, and they offer much-needed capability to preserve valuable genetic resources. The AGGRC has decades of experience in cryopreservation and repository development and more than a decade in pioneering of open hardware for these needs. The 'Cajun Ejector', an inexpensive device created for reproducible freezing using a standard shipping dewar, is an example of this type of work. This device was designed and prototyped in multiple versions, and it has been tested by aquatic biomedical stock centers and other users through workshops and direct interaction. Unfortunately, continued development and dissemination of such devices will not flourish without emergence of an open-source ecosystem. Such systems would maximize the reach and application of open hardware, and they would build communities of users leading to makers and developers.

A well-informed strategy to establish an Open Hardware Ecosystem is necessary to avoid obvious pitfalls. Plans can be based on the principles of business development by identifying the needs for resources and personnel. In addition, platforms will need to be developed to distribute fabrication files, training curriculum, user manuals, data collection worksheets, database structure, and other information necessary for users to interact with repositories. Specific needs are to: 1) determine the potential and constraints for transition of current germplasm repository users along the path of users-makers-developers; 2) evaluate size and characteristics of the target sector to determine the suitability of expanding current communities to new users and sectors; 3) develop process maps and simulation modeling for analysis of open-source environments specifically designed for open hardware distribution, collaboration, and training, and 4) develop a plan to establish a sustainable distributed infrastructure to support user training and operation of open hardware including file storage and sharing, workflow standards, design for open fabrication, licensing, and overall continuity of the open hardware environment. Organizational plans to ensure sustainability of open hardware platforms should consider a broad range of possibilities including fee-based distribution and memberships. Licensing systems such as Creative Commons can be used, and traditional patenting and licensing would enable open sharing of advanced technologies while maintaining protection of intellectual property. Open hardware will allow stock centers, laboratories, academic institutions, aquaculture producers, and conservation agencies to follow standardized and repeatable processes that can be linked to collection of relevant data. This will reinforce community-level solutions to longstanding problems, and it will ensure essential long-term preservation of genetic diversity.

LEVERAGING NEXT GENERATION SEQUENCING FOR AQUATIC VIRUS DISCOVERY AND CHARACTERIZATION IN CPE-POSITIVE VIRUS CULTURES OF UNKNOWN ETIOLOGY

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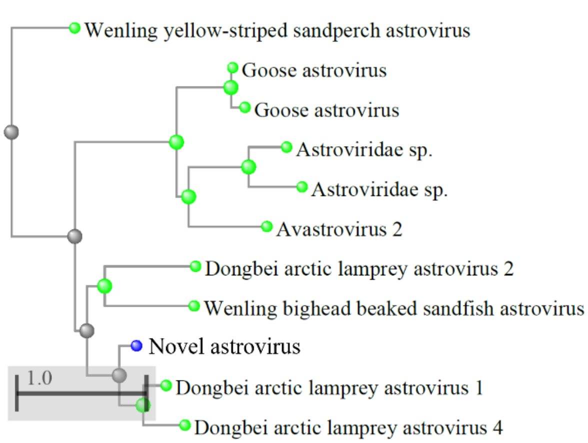
Aquaculture is one of the fastest growing sectors of global food production, and as such, it is critical to safeguard food security through timely detection, prevention, and control of infectious diseases that threaten the industry. A bottleneck in rapidly detecting novel diseases exists, which results in delayed response measures and further agricultural losses. Metagenomic sequencing offers unbiased detection of unculturable, novel, and under-characterized agents previously undetected by traditional methods. Here we present a case in which metagenomic sequencing identified a novel virus producing cytopathic effect (CPE) *in vitro* during routine surveillance testing and known agents were not detected by validated assays. We discuss how this technology can be applied to clinical veterinary diagnostics in an outbreak scenario when the infectious agent is not determined by existing validated assays.

Tissue pools from clinically normal bass and minnow were submitted to WADDL for annual health screening by virus culture. CPE developed from all pools in the first and filtered second passage on EPC cells. Cells were harvested and submitted for Blue Book PCR testing for IHNV, VHSV, IPNV and SVCV. Additional PCR tests were ordered from a secondary lab, including Fathead Minnow nidovirus and picornavirus, golden shiner virus and white sucker virus. No known viruses were detected. Using metagenomic sequencing, a novel astrovirus was discovered which bears similarity to Dongbei arctic lamprey astrovirus. This astrovirus only present in cultures with CPE and was not detected in normal, uninoculated cell culture controls.

Thus far, a number of novel fish astroviruses have been discovered by metegenomic sequencing of apparently healthy fish. Based on the assembled genome, a novel astrovirus PCR assay was developed as a rule-out diagnostic test for future CPE results of unknown etiology. This pipeline of sequencing for pathogen discovery can also be applied directly to tissue for the detection of unculturable agents, reducing turnaround time significantly. The use of this technology in a clinical setting is still under development, but the potential application is broad. Improved metagenomics could significantly improve time to detection, intervention, and prevent further losses for many sectors of animal production and disease prevention.



INVESTIGATION OF MONKEYFACE PRICKLEBACK *Cebidichthys violaceus* FOR COMMERCIAL FINFISH AQUACULTURE WITH ALTERNATIVE FEEDS

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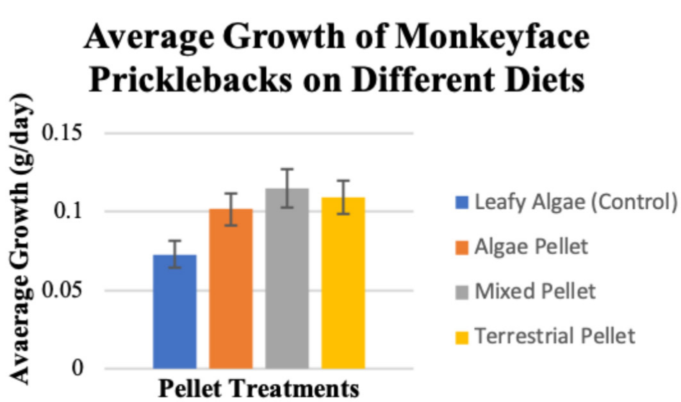
As aquaculture production continues to produce more seafood than wild fisheries landings, diversification of finfish aquaculture is an important strategy to increase resiliency in food systems and provide farmed alternatives to some unsustainable fisheries. Unlike fisheries, finfish aquaculture is dominated by relatively few species with some being associated with environmentally unsustainable practices such as the use of wild fish derived feed ingredients and reliance on juvenile wild fish stocks to supply aquaculture grow out operations. Of interest to this study is the declining freshwater eels (unagi) fishery due to overfishing. While aquaculture practices may supplement the fishery, full life cycle sustainable unagi aquaculture has been difficult to achieve thus far due to their carnivorous diet and complex lifestyle.

Monkeyface pricklebacks (*Cebidichthys violaceus*) have potential as an alternative cultivated finfish, possessing a number of characteristics warranting an assessment of the species that includes herbivory, sedentary behavior, no fishery competition, and a possibility to be substituted for the declining fishery product-unagi. Though not technically an eel, *C. violaceus* is eel-like in appearance and is reported to taste like unagi. Their herbivorous ecology has the potential to facilitate the use of fish free feeds. However, it is unknown how efficiently *C. violaceus* will grow on a formulated feed, and if any diet ingredients cause adverse health effects. To test this, we conducted a controlled feed study by rearing juvenile pricklebacks on whole seaweed (control) and three different formulated pellet diets with varying amounts of seaweed and soy protein concentrate: algae pellet (3 types of seaweed), a mixed pellet (seaweed and soy protein concentrate), and a terrestrial plant pellet (soy protein concentrate and 10% Nori for palatability). Throughout the four-month study, we measured individual growth, the metabolic cost of digesting a pellet (specific dynamic action), and digestion efficiency.

Preliminary results indicate growth rates of monkeyface pricklebacks were statistically similar among the feed treatments, although growth tended to be highest on the mixed pellet feed. Additionally, the feed conversion ratios (FCR) between pellet treatments were lower on the soy-based pellets. In effect, monkeyface pricklebacks are capable of growing on a pelleted feed that is fish free.



GROWTH PERFORMANCE AND NUTRIENT UTILIZATION IN THREE GENERATIONS OF UNKNOWN BELUGA STURGEON (*Huso huso*) POPULATIONS

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Beluga sturgeon is a valuable fish in the world. But the species is presently critically endangered, which makes researching to improve its production critical toward sustainable fish production. Optimum dietary requirements for developing commercial diets to enhance beluga sturgeon metabolic capacities during culturing are unavailable. Presently, Beluga farmers either use commercially available diets intended for other fishes or use diets formulated based on the dietary requirements of other freshwater fishes. To surmount the challenge of Beluga taking 8 - 10 years to reach maturity, there is the need to develop diets that specifically meet the requirements of the fish at various stages of development. Hence, this study investigated nutrient utilization and gut health of beluga sturgeon.

Three unknown Beluga sturgeon populations (2012, 2013, and 2018) maintained on identical diets and hatchery conditions but differed by age were evaluated. Samples of blood, tissues and gut samples were collected for proximate compositional analyses. Tissue and gut samples were used for histopathological and microbial analyses. The results show that weight ($P=0.0000250$), hepatosomatic index ($P=0.000147$), and Visceral somatic index ($P=0.000927$) were significantly different between the years. Fish from the 2018 population were different and had a higher weight than fish from 2012 ($P=0.0012$) and 2013 ($P= 0.000019$). Three histological sections of each fish evaluated for all microscopic pathology show mucosal goblet and epithelial cell hyperplasia, chronic, multifocal, minimal to moderate, with minimal to mild lymphocytic inflammation for all the fish from 2012, 2013, and 2018. Differences in the presence or absence of pancreatic tissue lymphoid follicles and coelomic adipose tissue were observed for all the fish. The observed pathological differences in the fish could be attributed to decreased nutrient absorption and utilization. Other analyses of the tissues and gut samples are ongoing and will be presented.

A NEW AIRLIFT FOR AQUAPONIC SYSTEMS

Huy Tran, Nam Tran, Bosonto Kristo (Chris) Gupta

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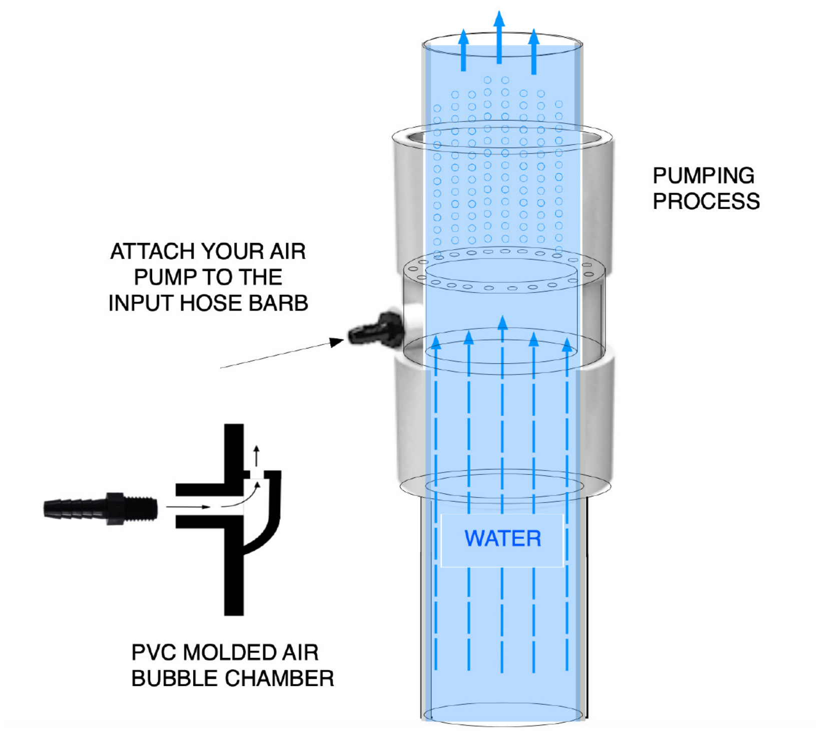
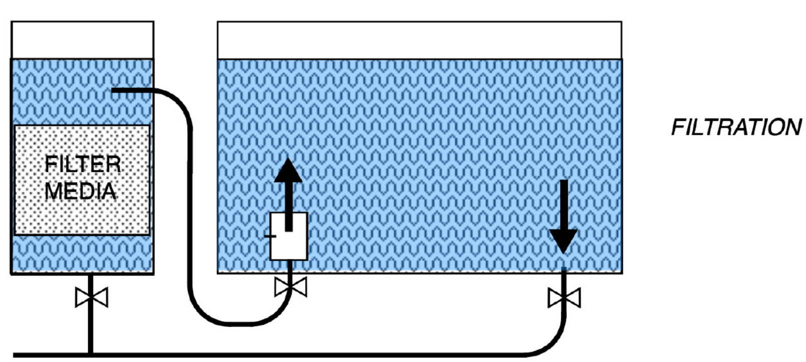
Testing new airlift design for aquaponic applications.

An airlift design to optimize the efficiency in low head aquaponic systems. Airlift applications includes fish culture tanks, mechanical filtration, biological filtration, mineralization, aeration, DWC, media/wicking beds, and water circulation.





ANTS ARE DESTROYING YOUR AQUAPONIC GARDEN PLANTS BY NURTURING THE PERFECT APHID COLONIES, TESTING CONTROL METHOD WITH BOTANICIDE

Huy Tran, Nam Tran, Bradley Todd

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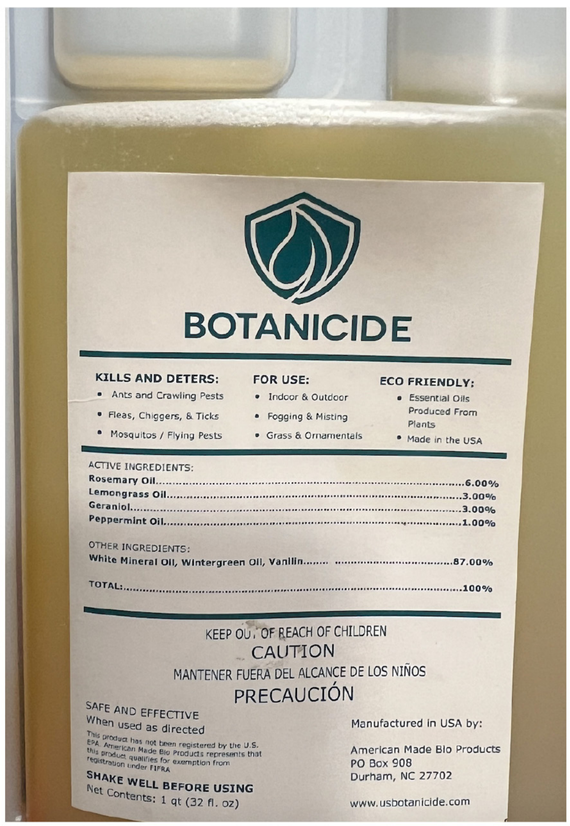
Testing viability of using Botanicide as a control agent for *Pheidole megacephalsa*

Crops are routinely damage by aphids in aquaponic operations. Looking at the effectiveness of Botanicide in controlling farmer ants before having to deal with pest management for aphids. Applying Botanicide in different areas of your operation and looking at the effectiveness of each application site. We also did a LC 50, lethal concentration, to ensure safety of our aquatic organism.



A NEW AIRLIFT DESIGN FOR LOW HEAD RAS

Huy Tran, Nam Tran, Bosonto Kristo (Chris) Gupta

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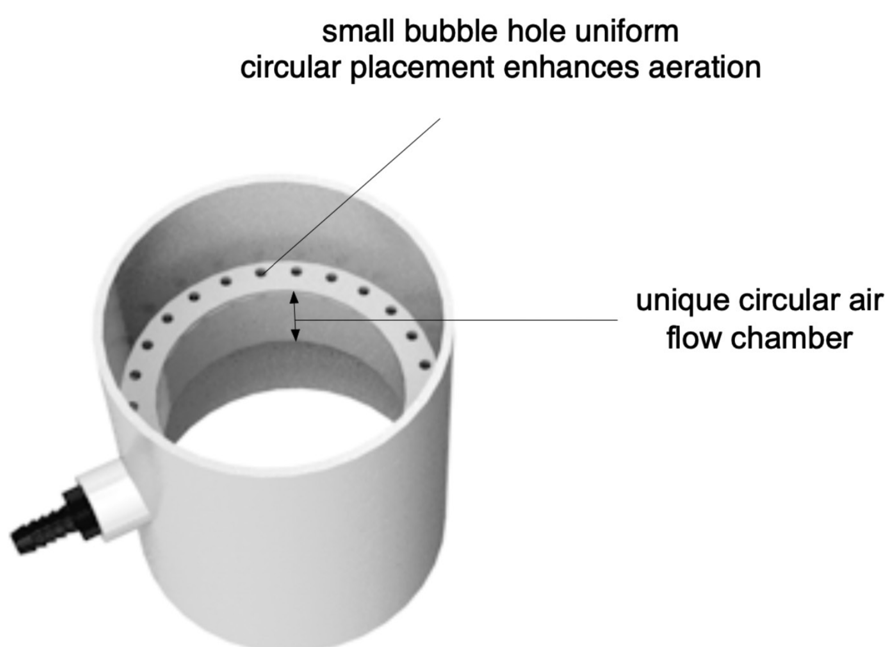
A look at a new airlift design and performance for, small, low head RAS systems.

An airlift design to optimize the efficiency using air to move water in a small RAS system. This design reduces the slippage of a conventional airlift system while maintaining efficient lifting capability by using less CFM than traditional airlift designs. Airlift applications includes circulation of fish culture tanks, mechanical filtration, and biological filtration.



DEVELOPING SMALL-SCALE CULTURE SYSTEMS AND PROTOCOLS FOR ENDANGERED DELTA SMELT *Hypomesus transpacificus*

Yi-Jiun Jean Tsai*, Luke Ellison, Troy Stevenson, William J. Mulvaney, Evan W. Carson, and Tien-Chieh Hung

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A refuge population of Delta Smelt has been cultured in a conservation hatchery (Byron, CA) since 2008 to help protect the imperiled species from extinction. Culture techniques for this population have thus far focused on maximizing genetic diversity and fish production, which includes rearing multiple families within the same tanks. However, Delta Smelt production, associated research projects, and related conservation measures would benefit from also being able to rear fish on a smaller scale, such as in individual family groups. Accordingly, new culture systems and protocols have been developed for rearing small numbers of Delta Smelt embryos (3─10 days post-fertilization), early-stage larvae (0─40 days post-hatch, dph), and late-stage larvae (40─80 dph).

We evaluated the performance of these small-scale culture systems across two studies. In Tsai *et al*. (2022), we found that the hatching rate of embryos incubated in a small-scale system did not differ from that of embryos incubated in standard facility systems. Early-stage larvae reared in a small-scale system did not differ in survival rate, but were longer and heavier than facility-reared larvae. In contrast, late-stage larvae reared in a small-scale system had lower survival and were shorter and lighter than facility-reared larvae. To help identify the cause of poor late-larval outcomes, we conducted a second study examining the effects of stocking density on the survival, length, and weight of late-stage larvae reared in a small-scale system (Tsai *et al*. Submitted). We found no effect of density on any of these measures and therefore could not identify the reason for the poor performance of late-stage larvae in the first study. However, across all densities, the survival and size of larvae reared in Tsai *et al*. (Submitted) did not differ from that of facility-reared larvae (Tsai *et al*. 2022). Thus, with optimization of rearing protocols (Tsai *et al*. Submitted), the small-scale systems are effective in culturing Delta Smelt from embryo to 80 dph. Based on these results, we will expand the development of small-scale Delta Smelt culture to include juvenile and adult life stages.

TABLE 1. Survival (%), length (mm), and weight (mg) of embryos and larvae reared using standard facility and small-scale culture systems in Tsai *et al.* (2022) and Tsai *et al.* (Submitted). Shown are means ± SE.

Life Stage	Standard Facility Systems			Small-Scale Systems		
	Survival	Length	Weight	Survival	Length	Weight
	Tsai *et al.* (2022)					
Embryo	96.9 ± 0.4	—	—	97.3 ± 0.7	—	—
Early-Stage Larvae	74.7 ± 2.6	15.0 ± 0.2	8.8 ± 0.4	71.3 ± 3.2	15.7 ± 0.2	10.3 ± 0.4
Late-Stage Larvae	71.8 ± 4.0	24.5 ± 0.4	64.9 ± 3.6	54.6 ± 4.7	23.2 ± 0.2	51.6 ± 2.0
	Tsai *et al.* (Submitted)					
Late-Stage Larvae				79.6 ± 1.6	25.1 ± 0.2	69.4 ± 2.6

ALTERNATIVE METHODS FOR DEAD EGG REMOVAL FOR THE CONSERVATION OF DELTA SMELT (*Hypomesus transpacificus*) IN HATCHERY

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Delta smelt (*Hypomesus transpacificus*) is an endangered small fish species inhabits the upper San Francisco Estuary, California. The UC Davis Fish Conservation and Culture Laboratory (FCCL) has been culturing a refuge population of Delta Smelt to prevent them from extinction. Currently, FCCL produces more than 600,000 eggs annually to achieve the goal and to propagate fish for wild fish supplementation. To obtain an optimal hatching rate, FCCL has been implementing a standard operation procedure that requires staff to manually take out dead eggs during the incubation processes to prevent fungal infection. The procedure is particularly labor- and time-consuming. Therefore, alternative methods are highly demanded.

In this study, two alternative methods were tested, including separating eggs by the specific gravity (SG) difference between live and dead eggs and by introducing ramshorn snails (*Helisoma anceps*) to identify and consume dead eggs. By far, separating eggs by the difference of SG shows promising outcome. The time needed to separate eggs at different SG is shown in Fig.1. A follow-up study for the effect of solution used on the hatching will be conducted. Also, a device for the separation will be designed accordingly. For the method of using snails to remove the dead eggs, preliminary results have shown that snails preferred to consume dead eggs rather than live ones (Fig.2). Putting together, either method shows the potential to be an alternative method to improve the current operation.

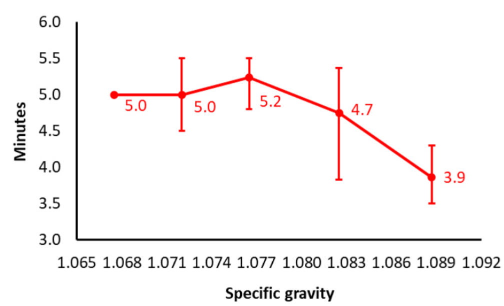

Figure 1. Time needed to separate eggs in solutions with different specific gravities

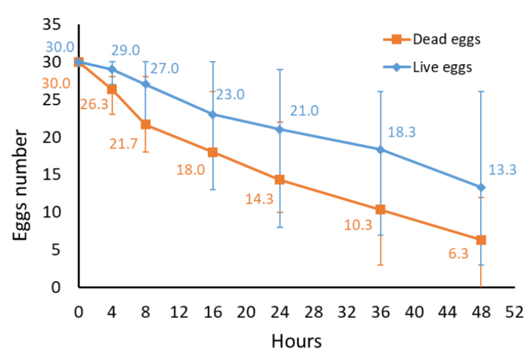

Figure 2. Egg consumption by snails. Error bars indicate S.D.

BEST MANAGEMENT PRACTICES AND ESCAPEMENT OF ORNAMENTAL FISH FROM AQUACULTURE FACILITIES IN FLORIDA

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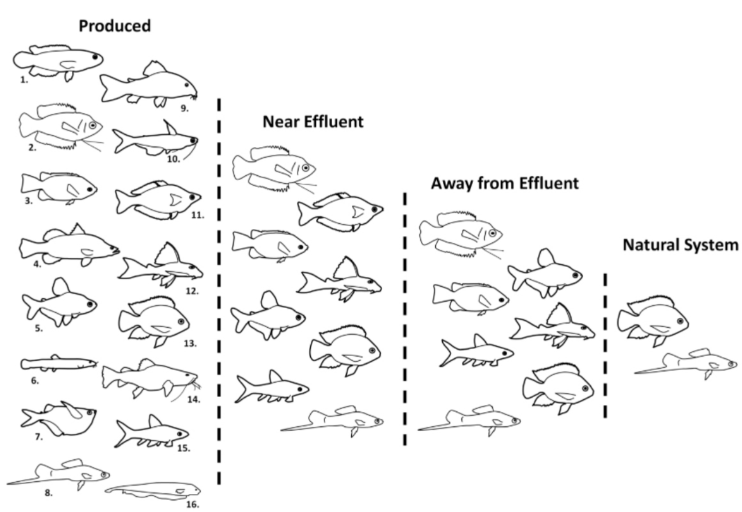
Tropical ornamental aquaculture is an important industry in the state of Florida. While the industry is an important economic sector in the state of Florida, escapement of non-native organisms has resulted in criticism that the ornamental aquaculture industry is essentially unregulated. This is not the case, aquaculture in Florida is regulated by multiple agencies with main authority residing within the Florida Department of Agriculture and Consumer Services (FDACS). The goal of the present study was to evaluate FDACS Best Management Practices (BMP) regarding non-native species.

Our specific objectives were to 1) evaluate the inspection process, 2) examine BMP compliance, 3) identify the critical control points for fish escapement, 4) analyze the stages of invasion and 5) determine the effectiveness of current aquaculture BMPs following evaluation of the above objectives. We conducted site visits with FDACS compliance inspectors and bird surveys on farms, sampled fish immediately adjacent to farms and sampled fish across the broader environment in west-central Florida.

Our findings suggest a coherent inspection process, strong compliance by producers and rapid correction of non-compliance issues. Escape in effluents is the dominant pathway of introduction; all other possible pathways were unimportant. Presence of a detention pond or absence of effluents is the most important factor increasing compliance. Native fish dominated the catch across the sampled landscape from adjacent to farms out into the larger, more natural aquatic systems. Despite the occurrence of some non-natives adjacent to facilities, the number and diversity of fish declined rapidly with distance from the farm such that non-native ornamentals were rare to non-existent in the broader landscape. Our results suggest the mandatory inspection process is fundamentally sound with a coherent regulatory structure that facilitates cooperation between FDACS and farm operators.

Figure 1. Fish families produced and captured near sources of ornamental fish production in Florida.



1. Anabantidae, 2. Osphronemidae, 3. Helostomatidae, 4. Latidae, 5. Characidae, 6. Cobitidae, 7. Gasteropelecidae, 8. Poeciliidae, 9. Callichthyidae, 10. Pangasiidae, 11. Melanotaeniidae, 12. Loricariidae, 13. Cichlidae, 14. Pimelodidae, 15. Cyprinidae, 16. Apteronotidae

STATE SURVEY TO IDENTIFY RISK PRACTICES AND RESPONSES TO LACEY ACT CHANGES FOR INJURIOUS WILDLIFE

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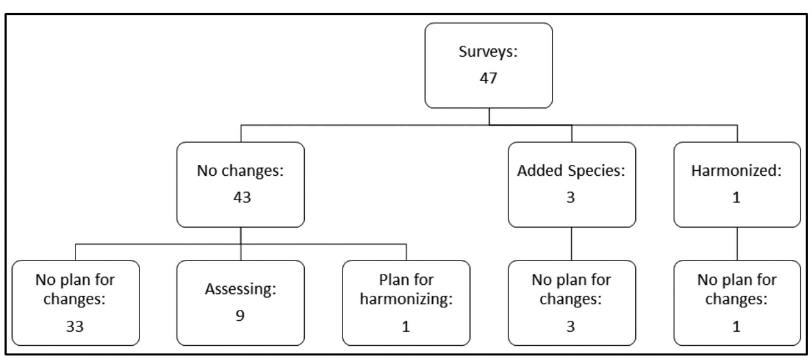
Under provisions of the Lacey Act, the USFWS not only regulated the importation of injurious wildlife into the United States and its territories, but it was also further interpreted since the 1960s as banning interstate movement within the continental United States. Following a challenge by the U.S. Association of Reptile Keepers, the U.S. Court of Appeals for the District of Columbia on April 7, 2017 affirmed a lower court ruling that the government lacks authority to regulate shipment of injurious species across state lines. Thus, depending on the outcome of state agency decisions following this court ruling, and potential changes to state regulation, there may be increased uncertainty and complexity for aquaculture producers if regulations are added on a state-by-state basis. Our goal was to identify anticipated changes at the state level, the strengths and weaknesses of these and other identified policy/regulatory approaches, to provide clarity to producers. We used a formal phone survey with a standardized questionnaire to identify current and anticipated regulatory practices. The phone survey was directed to state Aquatic Nuisance Species coordinators.

Following the Lacey Act ruling, 43 states made no changes, 3 added species to their prohibited list, and 1 state harmonized regulations with USFWS injurious wildlife lists. Of the 43 states yet to make changes, 33 had no plan for changes, 9 were in the process of assessing their options, and 1 had plans for harmonization with USFWS lists. With respect to procedures for evaluating invasiveness risk of aquaculture species, 32 states had no standardized procedure, 13 had a standardized process, and 2 used common processes but were not standardized. Further, 31 states had no intention of changing process and 16 were interested in changing or adopting a process. Ultimately, this survey indicated there were few regulatory changes following the court ruling in 2017.

Figure 1. State responses to Lacey Act changes. Few states added species to injurious wildlife lists or made changes following the court ruling.



INNOVATIONS IN AQUACULTURE WORKFORCE DEVELOPMENT PROGRAMMING IN MAINE AND BEYOND

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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 in collaboration with Cooperative Extension developed an aquaculture micro-credentialing pathway for youth and adults. 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.

DATA ANALYSIS OF WATER TEMPERATURES FOR MOLLUSCAN MARICULTURE

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Mariculture is defined as the cultivation of marine organisms for consumption and integration for pharmaceutical to cosmetic purposes. The lion's paw scallop (*Nodipecten nodosus*) is indigenous to the Caribbean region and is regarded as a potential subject for molluscan mariculture. Low population rate is a contributing factor to the Caribbean's minimal aquaculture activity, resulting in the absence of local scallop consumption in some areas. Predation, diseases, natural catastrophes, and extreme temperatures are all factors that contribute to the low survival rates of these species in aquaculture.

An undergraduate exploratory research study was conducted to analyze temperature values within the marine environment for determining the culture potential of such species, especially in the United States Virgin Islands (USVI). Therefore, the following question was proposed: Would the water temperature conditions within the USVI be appropriate for the scallops to thrive? A total of 10,978 data points provided by the Department of Planning & Natural Resources (DPNR) were analyzed by computing the mean, standard deviation, and coefficient of variance per month between 2010-2021.

The results demonstrated a similar pattern between each island independently in comparison to the overall view as a territory. Each island's temperatures exceeded two degrees above the baseline temperature (ranging between 28◦C - 30◦C) starting from May through September, before slowly declining towards December (See Table 1). Integrating mariculture into the island's economy would be favorable, and could potentially generate employment, revenue, and exports. Based on this analysis, the scallops would need to be cultured at greater depths in comparison to the water's surface, and culture conditions could be closely monitored to ensure the scallops' survival.

	ALL THREE ISLANDS	
	MEAN ± ST. DEV	CV (%)
January	26.94 ± 0.51	1.90
February	27.39 ± 0.77	2.80
March	27.21 ± 0.77	2.82
April	27.06 ± 0.60	2.20
May	28.18 ± 0.89	3.17
June	28.89 ± 0.92	3.17
July	29.16 ± 0.52	1.78
August	29.47 ± 0.61	2.07
September	29.87 ± 0.54	1.80
October	29.33 ± 0.44	1.51
November	29.06 ± 0.34	1.17
December	27.79 ± 0.48	1.72

Table 1. An overview of the average water temperatures computed for the USVI per month.

CELLULOSE NANOMATERIALS: A NOVEL ADJUVANT AND DELIVERY SYSTEM FOR AQUACULTURE VACCINE APPLICATIONS

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Disease outbreaks are a major impediment to aquaculture production and are forecasted to continue as the industry grows and the climate warms. Vaccines are integral for disease management in aquaculture but they can be expensive, vary in effectiveness, and come with adjuvant-induced adverse effects causing fish welfare issues and negative economic impacts. The goal of this interdisciplinary project is to develop a new generation of vaccines for sustainable aquaculture. Our project uses novel nanomaterials produced from renewable wood fiber as depots/adjuvants in vaccine formulations to modulate the immune response of Atlantic salmon in a biocompatible, environmentally friendly, and cost-effective manner.

Our interdisciplinary research team is elucidating the role of cellulose nanomaterials (CNM) as a vaccine depot and mobile immunostimulant, the extent of CNM migration *in vivo*, and the efficacy of CNM bound antigen as an immunostimulant for protection against two Atlantic salmon pathogens. To accomplish this, we have prepared and conducted *in vitro* characterizations of CNM hydrogels and CNM/antigen (vaccine) formulations by using fluorescent CNM variants (CNM-FL) and *in vivo* durability and migration using FL microscopy. Additionally, we assessed safety of CNM hydrogel formulations *in vivo* as a vaccine depot in Atlantic salmon. The next phase of our work will involve conducting *in vivo* studies to quantify the antibody kinetics in vaccinated fish serum using enzyme-linked immunosorbent assays. We will also evaluate the efficacy of the CNM vaccine(s) in protecting against *Vibrio anguillarum* in Atlantic salmon by performing a pathogen challenge study.

Our results to date will be reported and discussed.

PERSISTENCE OF VIRULENT *Aeromonas hydrophila* AND *Edwardsiella ictaluri* IN COMMERCIAL CATFISH POND SEDIMENTS

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Virulent *Aeromonas hydrophila* (vAh) and *Edwardsiella ictaluri* (ESC) are two of the most prevalent pathogenic bacteria in west Alabama commercial catfish aquaculture and are responsible for chronic bacterial outbreaks. To further understand chronic vAh and ESC pathogenesis, investigating the ability of these bacteria to persist in the bottom sediments is paramount. Sediments from four ponds were collected at six points and combined to form a single composite sample, and used for persistence trials. Firstly, 600 g of each composite sample was autoclaved thrice for 60 minutes to ensure sterilization. Alkalinity, soil organic matter content, prevalent cation concentrations, pH, and cation exchange capacity were measured both before and after autoclaving for all four sediments. The vAh isolate (ML-09-119) and the ESC isolate (S97-773) were cultured in tryptic soy broth (TSB) for 24 h, and brain-heart infusion broth (BHI) for 48 h respectively, non-concurrently at 28°C. After incubation, each bacterial culture was centrifuged and resuspended in 1.0 X pH-adjusted PBS to an optical density of 0.2 at 550 nm. This resulted in inoculum concentrations of $8.67 \pm 0.65 \times 10^7$ CFU/mL and $6.4 \pm 0.73 \times 10^7$ CFU/mL (Mean ± SE) for the vAh and ESC trials, respectively. A combination of 200 g of sterilized sediment, 20 mL of bacterial inoculum, and 500 mL of sterile dechlorinated city water were mixed thoroughly for 60 minutes. The sediment treatments were housed in three 37 L glass aquaria, each separated into four chambers, using glass dividers sealed with aquarium silicone. Then, the total chamber volume was increased to 8 L of dechlorinated water, aerated for alternating cycles (12 h on:12 h off) to simulate a production pond, and maintained at 28 and 25°C for the vAh and ESC trials, respectively. Water alkalinity, hardness, pH, phosphorus, TAN, NO_2^-, and NO_3^- concentrations were measured weekly. Bacterial enumeration was conducted by first centrifuging 1 g of extracted sediment, resuspending the pellet in 0.1 X PBS, conducting seven 10-fold serial dilutions in a 96 well plate, then plating diluted inoculum onto ampicillin dextrin agar and *E. ictaluri* medium for the vAh and ESC trials respectively. Agar plates were incubated at 28°C for 16 hours in both trials. In a pilot persistence trial, vAh colonies were still viable after 113 days post inoculation despite an over 95% decrease in the initial CFU/g population. In the vAh trail, colonies were identified via PCR confirmation, and the 95% population decreased occurred by 28 days post inoculation. Additionally, a background population of *Psuedomonas spp*. began appearing in one of the sediment types at 48 hrs post inoculation. In the ESC trial, CFU/g of positive colony population decreased below 95% 14 days post inoculation. One of sediment types was acquired from a recently renovated pond, with a sediment pH of 4.98. This low soil pH caused a rapid decrease in CFU/g of vAh and ESC when inoculated into that specific sediment. Future research on virulence, gene expression, and interactions between pathogenic and primary successional soil bacteria are necessary to understand specific mechanisms and strategies which allow vAh and ESC to persist and how soil chemical properties can influence pathogenic bacteria in pond bottoms.

DETECTION OF *Vibrio parahaemolyticus* IN POST-LARVAE OF WHITE SHRIMP *Litopenaeus vannamei* COLLECTED IN THE TRANSPORT TANKS, PRIOR TO SOWING IN MEXICO

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Shrimp farming is an extremely important sector in food production worldwide, considering that the biggest problem they face is disease outbreaks. Various infections are attributed to the genus Vibrio, which cause significant mortalities from the first days of cultivation, managing to eliminate up to 100% of the population. The strains responsible for transmitting this disease are those that encode the binary toxins Pir A and B. The objective of this study was to determine the presence of toxigenic *Vibrio parahaemolyticus* in white shrimp (*Litopenaeus vannamei*) post-larvae collected in transport tanks from laboratories, using bacteriological, molecular and histopathological methods, achieving a mitigation of mortalities in the first days of culture in Mexico.

Detection of toxigenic *V. parahaemolyticus* was carried out using four processes. The last process was carried out in three phases (Fig. 1).

13 cases were obtained from six laboratories located in the states of Sonora and Sinaloa. Six were positive for toxigenic *V. parahaemolyticus* (Fig. 2).

The hepatopancreas is the organ most affected by *V.parahaemolyticus*, causing massive and acute cell detachments. With histological methods, the degree or phase of the lesion can be observed (Fig. 3).

It is concluded that white shrimp (*L. vannamei)* post-larvae are infected with the bacterium *V. parahaemolyticus*, in larval stages before they are entered into any culture system or have any contact with grow-out ponds.

Figure 1. Diagram of the detection process of toxigenic *V. parahaemolyticus.*

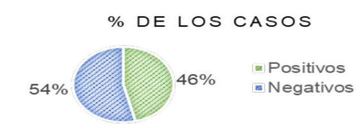

Figure 2. Percentage of positive and negative cases to toxigenic V. parahaemolyticus.

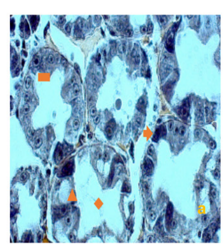

Figure 3. Acute or initial phase of a V. parahaemolyticus infection. Deficiency in the differentiation of F, R, B cells (arrow, rectangle) and great absence of vacuoles (triangle), deformed lumen (rhombus)

THE VALUE CHAIN ANALYSIS OF TROUT AQUACULTURE IN TURKEY

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The main goal of this study was to reveal the basic and supportive activities that should be included in the value chain for trout farming in Turkey and to perform a value chain analysis of trout farming. The concept of value chain was first used in the 1960s and 1970s by analysts who charted development paths for mineral-exporting economies. Since the 1990s, value chain analysis has been widely applied across other industries. The value chain consists of the activities required from the inception of a product or service (from the idea and design stage) through the different production stages (by combining the inputs of physical transformation and various producer services), to reaching the end consumers and eliminating it after use (including recycling). The value chain; consists of two main parts: primary activities (incoming logistics, operations, outgoing logistics, marketing and sales, services) and supporting activities (firm infrastructure, human resources method, technology development, supply). The value of this work; was to reveal the value chain map of the trout farming sector and determine the product flow along the value chain across the various stages. This study was the first of its kind within the field of fisheries among the value chain analysis applications in the national literature.

The knowledge developed by this study consists of primary data and secondary data obtained from interviews with stakeholders in the trout farming sector in inland waters (lakes and streams). First, the value chain of the trout farming sector was mapped, and the main actors and stakeholders in the sector were identified, and the entire product flow from production to the end consumer was defined. The basic and supporting activities in the value chain of trout farming in Turkey were determined based on the value chain model of Porter (1985). Five basic activities and their sub-dimensions and four supporting activities and their sub-dimensions in the value chain model of Porter (1985) were examined, supported by the information obtained from the actors and stakeholders in the trout farming sector in our country.

CARBON DIOXIDE REMOVAL THROUGH SEAWEED AQUACULTURE: ASSESSING THE EVIDENCE FROM LIFE CYCLE ASSESSMENTS

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In addition to its economic benefits, the role of seaweed farming as an important purveyor of ecosystem services is being increasingly recognized. A scientific narrative advocating for the large-scale expansion of the seaweed aquaculture industry in recognition of the relatively high removal rates of atmospheric carbon dioxide (CO_2) during biomass growout has recently been articulated. As a climate solution, the idea has catalyzed investment from the private sector on projects aimed at the development of 'carbon negative' seaweed products such as food, feed, extracts and fertilizers. Nevertheless, a scientific consensus on the true potential of seaweed farming as a Carbon Dioxide Removal (CDR) solution is yet to emerge as uncertainties regarding the permanence of carbon storage in culture facilities are still being resolved.

To improve our understanding of the CDR potential of seaweed aquaculture, a meta-analysis of Life Cycle Assessments (LCA) and carbon sequestration models published during 2013-2022 was conducted to arrive at standardized estimates of the Global Warming Potential (GWP) of the activity. The analysis revealed that while substantial CDR can be achieved though dissolved and particulate carbon exported to sediments and the deep sea, benefits are much more apparent if substitution of fossil fuel products (gas, fertilizers) is embedded into the LCAs. Non-parametric tests indicated that removal through both pathways potentially exceed the emissions associated with culture activities (Figure 1). Although the CDR potential of seaweed aquaculture was confirmed by the meta-analysis, updated assessments are recommended to further reduce the variability in the estimates of positive emissions and removal rates.

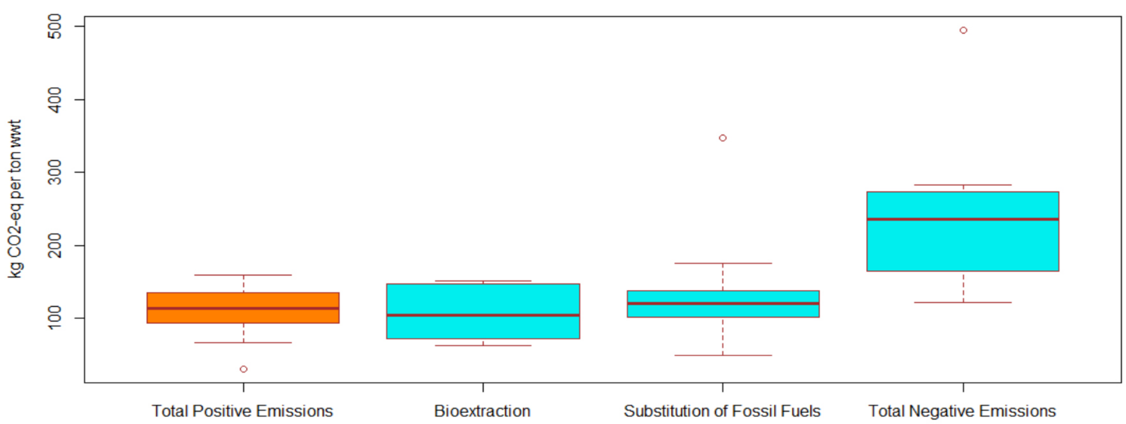

Figure 1. Positive (cultivation, drying/preservation) and negative (bioextraction, substitution of fossil fuels) CO_2 emissions (kg CO2-eq per ton of wet weight) resulting from seaweed aquaculture and associated industrial processes.

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.

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.

EFFECTS OF DIFFERENT PROTEIN SOURCES AND VARYING PROTEIN:LIPID CONCENTRATIONS ON GROWTH, BODY COMPOSITION, AND GUT HISTOLOGY OF YELLOWTAIL SNAPPER *Ocyurus chrysurus*

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Yellowtail snapper, *Ocyurus chrysurus* is a marine snapper that is relatively easy to spawn and rear the larveagiving it potential as a mariculture species or research model for other snapper species. Hence, there is an interest in identifying diets that are appropriate for its culture. The utilization of practical diets and its effects on fish growth performance and body composition is currently poorly understood. Two trials were conducted to observe the effects of practical diets for yellowtail snapper having different protein sources and varying protein:lipid ratios. A 14-week growth trial (mean initial weight of 2.03 ± 0.06g) was conducted in 24 (730 L) with two series of experimental diets. First set of diets with varying levels of different protein sources such as fishmeal (F), poultry meal (P), and soybean meal (SBM) namely F30:P0:SMB20, F15:P15:SMB20, F0:P30:SMB20, and F15:P0:SMB40 were formulated to have 40% protein and 10% lipid levels. The second set of diets were formulated with varying concentrations of protein and lipids namely -- low protein:average lipid (Pro36:Lip10), average protein:low lipid (Pro40:Lip6), average protein:average lipid (Pro40:Lip10), and high protein:high lipid (Pro44:Lip14) levels. Survival showed significant differences (p<0.05) among all dietary treatments, with highest survival in fish fed F0:P30:SMB20 and low protein:average lipid (Pro36:Lip10) levels. Histological measurements of distal intestine diameter showed significant difference (p<0.05) among fish fed F30:P0:SMB20, F0:P30:SMB20, and F15:P0:SMB40. However, no significant differences (p>0.05) were observed in histological measurements of mucosal length, and thickness of the mucosa, lamina propria, submucosa, and serosa, as well as in the histological scoring of the lamina propria folds, connective tissue, and large vacuoles. Results show that no adverse effects on growth performance and gut histology were observed in yellowtail snapper when fed with diets containing low levels of different protein sources. The second 10-week growth trial (mean initial weight of 3.40 ± 0.05g, in 36 [82.9L] tanks) was conducted to optimize lipid concentrations, following low protein levels from the previous trial. Four diets were formulated to contain 36% protein with varying lipid concentrations of 7% (Pro36:Lip07), 10% (Pro36:Lip10), 13% Pro36:Lip13, and 16% (Pro36:Lip16). Commercial diet (Otohime EP3) having 48% protein and 10% lipid concentration was used as a reference diet. Results showed significant differences (p<0.05) in weight gain and thermal growth coefficient values, with Pro36:Lip13 having the highest weight gain, survival, and FCE. Consecutively, whole fish body composition showed significant differences (p<0.05) in moisture and fat content. Over-all results show that diets containing 36% protein and 13% lipid concentration levels were observed to be the optimal level for yellowtail snapper diets, showing no adverse effects on growth performance and body composition. The current study serves as initial findings posing promising potential in the development of the relatively obscure culture of the yellowtail snapper.

THE USE OF INTACT PROTEINS AND PURIFIED AMINO ACIDS IN DETERMINING THE METHIONINE REQUIREMENT IN PRACTICAL DIETS OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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As there is no consensus on the efficacy of purified amino acids in shrimp feed, two approaches were conducted to determine the methionine requirement in practical diets for *Litopenaeus vannamei*. The first approach used intact proteins in producing both a deficient and a replete diet that was then co-mixed in creating varying levels of methionine using intact protein. The second approach was to use the deficient diet supplemented with graded levels of pure methionine. Hence, three main diets were made, including a deficient basal diet (B, 0.48% methionine, Lentil meal-based, AGT Food and Ingredients, Inc. Saskatchewan, Canada), a dipeptide Met (Met-Met)-supplemented diet (M, 0.85% methionine), and a replete diet containing CPC (C, 0.84% methionine, Corn Protein Concentrate, Empyreal 5 TM Cargill Corn Milling, Cargill Inc., Blair, Nebraska, USA). Ten experimental diets were produced by blending the deficient diet with the replete diet resulting in graded levels of methionine namely B100, B70:M30, M100, B90:C10, B80:C20, B70:C30, B60:C40, B40:C60. B20:C80, and C100, Test diets were then fed to shrimp (15/aquaria) in 60 randomly-assigned aquaria (55.8 L) with a mean initial weight of 0.23 ± 0.0001g over a a six-week growth trial. All diets were formulated to be isonitrogenous and isolipidic (36% protein and 8% lipid, as is), with the basal diet formulated with fishmeal and lentil meal as primary protein sources and whole wheat as a carbohydrate source. Significant differences (p<0.05) were observed in weight gain as well as whole shrimp body amino acids namely Cysteine, Glycine, Threonine, and Taurine. The optimal dietary methionine requirement of *L. vannamei*, estimated by a two-slope broken-line regression analysis model based on weight gain% was 0.58% of the dry diet (corresponding to 1.61% of dietary protein on a dry-weight basis). Such findings are crucial in formulating cost-effective practical diets and utilizing intact proteins or purified amino acids for juvenile *L. vannamei*.

You may want to workin a sentence with the range of levels for both diet sets. Maybe where you talk about the Basal and two replete diets…

Hence, three main diets were made, including a deficient basal diet (0.?? % methionine, Lentil meal-based, AGT Food and Ingredients, Inc. Saskatchewan, Canada), a dipeptide Met (Met-Met)-supplemented diet (??? % methionine), and a replete diet containing CPC (??? % methionine, Corn Protein Concentrate, Empyreal 5 TM Cargill Corn Milling, Cargill Inc., Blair, Nebraska, USA).

Basal	BM70: 30	M10 0	B90:1 0	B80:2 0	B70:3 0	B60:4 0	B40:6 0	B20:8 0	B0:10 0

REPRODUCTIVE CYCLE OF THE PACIFIC PORGY *Calamus brachysomus* IN SANTA ROSALÍA, BCS, MÉXICO

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Calamus brachysomus, commonly known as Pacific porgy, is the only member of the Sparidae family that inhabits the Gulf of California. In Baja California Sur (BCS), its catch has increased in the last years. It has previously been considered as a candidate for aquaculture, which increases the need to know basic aspects of its biology. The objective of this research is to describe their reproductive cycle and obtain the gonadosomatic index from samples obtained from Santa Rosalía, BCS.

From July 2015 to July 2016, specimens were captured monthly for a total of 265 samples (147 females and 118 males). From these, the gonadosomatic index (GSI=gonad weight/body weight) was obtained. In addition, 1 cm^3 sections of the gonad were analyzed by histological techniques to obtain the reproductive cycle.

Females measured between 16.5 and 36.5 cm TL (mean ± standard deviation, 24.5 ± 3 cm), and males measured between 15 and 37 cm TL (24 ± 3.1 cm). Capable spawning females were identified in 11 of the 13 months analyzed. This extended spawning period coincides with the reported for other members of the family Sparidae. The GSI trends showed a peak from May to July in both sexes, which coincided with the highest percentage of organisms with spawning capacity. This suggests that the GSI is a reliable indicator to monitor maturation events in this species. The peak of reproductive activity was found at a sea surface temperature of 21 to 28 °C.

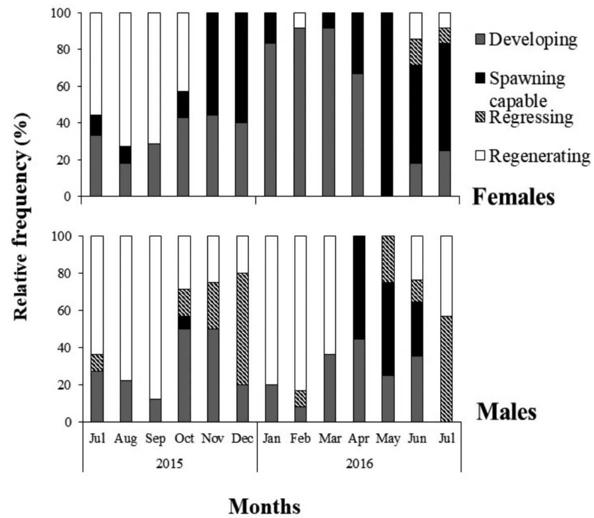

Figura 1. Ciclo reproductivo de hembras y machos de *Calamus brachysomus* en Santa Rosalía, BCS, México.

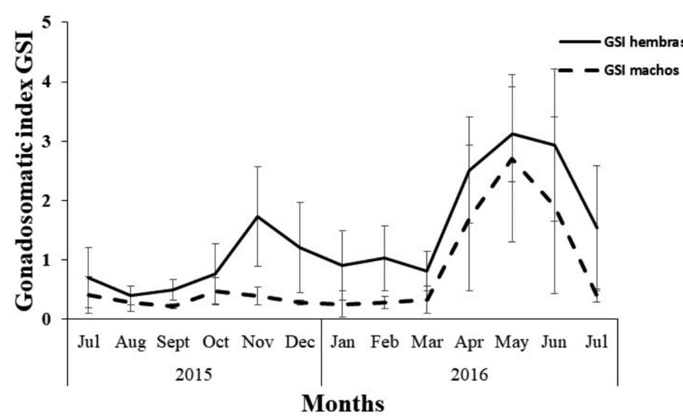

Figura 2. Índice gonadosomático de hembras y machos de *Calamus brachysomus* en Santa Rosalía, BCS, México.

LESSONS LEARNED FROM A PUBLIC RELATIONS CAMPAIGN FOR MAINE AQUACULTURE

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Maine's aquaculture industry overall represents nearly 200 farms and more than 700 farmers who produce premium seafood–fin fish, shellfish, and sea vegetables–in Maine's cold, clean waters. The sector has been steadily growing in recent years, and its total economic impact has nearly tripled – from $50 million in 2007 to $137 million in 2014 – according to the latest Maine Aquaculture Economic Impact Report. Sustainable aquaculture has tremendous potential to bolster our coastal economy, providing good jobs, local food security, and diversification opportunities for working waterfront families.

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 launched a public relations campaign in 2022 with the goal of raising awareness of sustainable aquaculture in Maine and beyond.

The association employed the help of a local public relations consultant over a 12-month period to initiate the campaign in collaboration with MAA staff. During the course of the campaign, the project generated 27+ articles and 15,000,000+ potential reach. This talk will cover the basics of public relations, including crafting a PR plan, key messages, media training for farmers, press releases, pitching and media relations, media tours, and metrics. While this project was unique, the strategies, challenges, and lessons learned 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.

EFFECT OF FORMULATED DIETS ON THE GONAD QUALITY OF THE SEA URCHIN *Tripneustes depressus*

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The sea urchin *Tripneustes depressus* is a potential fishing resource on the coast of Baja California Sur due to the quality of its gonads for human consumption. An alternative to increase gonadal quality in culture is to control the nutritional balance of the feed. The effect of different formulated diets on the quality of *T. depressus* gonads was evaluated.

Three balanced feeds were formulated and elaborated, two based on previously selected algae (*Eisenia arborea*, *Sargassum sinicola*), and a mixed one combining both. Sixty organisms (8-11 cm in diameter) previously acclimatized without feeding (three replicates of 20 organisms for each treatment) and three controls with each type of natural algae were placed in the laboratory. The organisms were fed *ad libitum* for three months. At the end of the experiment, the quality of the gonads was evaluated for each treatment, considering characteristics such as gonadal weight, color, texture, firmness, and flavor. Additionally, to know the initial conditions, 20 wild organisms recently collected and 20 after the acclimatization period were sacrificed, as well as at the end of the experiment another 20 wild organisms were sacrificed.

The highest gonadal weights were observed in the wild organisms before and after the experiment, as well as in the treatment with *E. arborea*, with significant differences being found between these and the rest of the treatments ($H=165.46$; $p<0.0001$). When analyzing the quality characteristics by treatment, we can observe the highest percentages of "excellent" and "very good" gonads in the organisms prior to and at the end of the experiment, as well as in the treatment with *E. arborea*, finding significant differences between these and the rest of the treatments (Color: $H=105.93$, $p<0.0001$; Texture: $H=128.06$, $p<0.0001$; Firmness: $H=135.90$, $p<0.0001$; Taste: $H=78.37$, $p<0.0001$). In particular, the color parameters showed high values of L and low values of a in all treatments, and higher values of b in the organisms before and after the experiment, as well as in the treatment with *E. arborea*. The ΔLab values were lower in the organisms before and after the experiment, as well as in the treatment with *E. arborea*, which corresponds to gonads with better coloration, presenting significant differences between these and the rest of the treatments (L: $H=34.85$, $p<0.0001$, a: $H=59.13$, $p<0.0001$, b: $H=123.10$, $p<0.0001$, ΔLab: $H=99.65$, $p<0.0001$).

Apparently, the use of algae, specifically *E. arborea*, was favorable in the formulation of balanced food in *T. depressus*, obtaining good quality gonads when using this species of algae naturally. The commercial quality of the gonads of this species could represent a viable alternative for the development of sea urchin aquaculture in Mexico.

on THE ENVIRONMENTAL SUSTAINABILITY OF MARICULTURE SYSTEMS WITH ALTERNATIVE FUELS: LCA CONSIDERATIONS

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Aquaculture is expanding sector on the global level resulting in higher concerns for its sustainability. Generally, sustainability can be defined as a process of meeting the needs of the present without compromising the ability of future generations to meet their needs, and it is considered to be constituted of three pillars: the economy, society and environment. This work considers environmental pillar of sustainability of mariculture systems, with a goal to investigate effect of alternative powering options in mariculture on its lifetime environmental footprint. In this sense the alternative mariculture system from Koričan at al. (2022) has been considered. This work, as a result of INTEL-MARIC project, conducted at UNIZAG FSB considers set of alternative fuels (LNG, Methanol, Biodiesel) and associated emissions (CO_2, NOx, SO, and PM) of the mariculture system. The life-cycle assessment (LCA) indicates that significant environmental benefits are possible in case of alternative mariculture system layouts and by use of alternative fuels like for instance methanol or biodiesel, Figure 1.

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Acknowledgement

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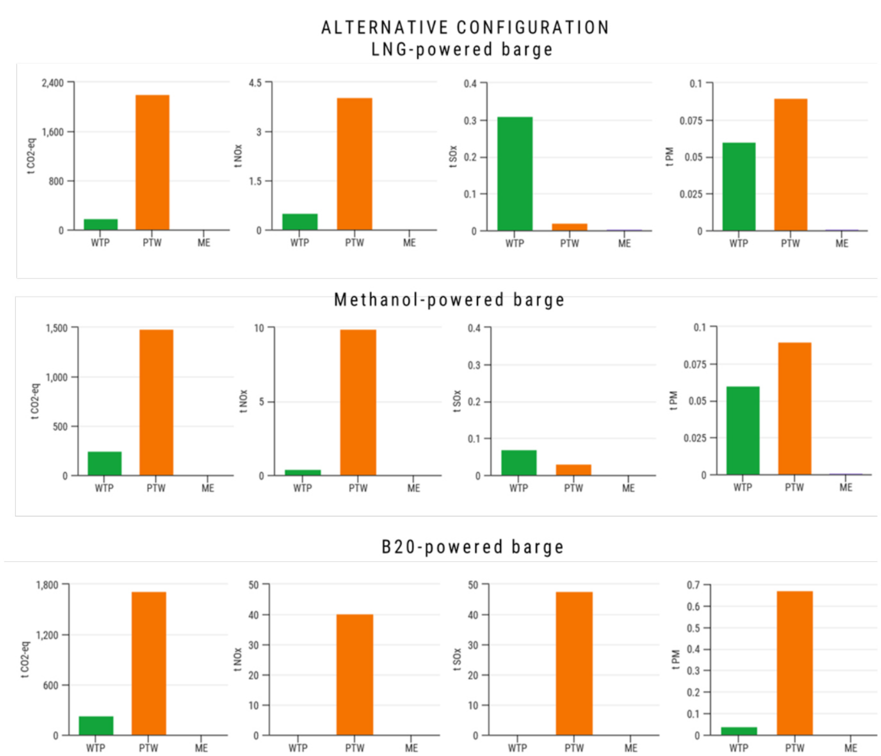

Figure 1. LCA results of mariculture systems powered by different fuels (WTP – well to pump phase, PTW – pump to wheel phase, ME – manufacturing emissions)

ASTAXANTHIN FROM *Haematococcus pluvialis* AS SUBSTITUTE FOR SYNTHETIC ASTAXANTHIN IN ATLANTIC SALMON (*Salmo salar)* PIGMENTATION

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In Atlantic salmon, the specific red-pink flesh color is formed by the accumulation of astaxanthin from food. While wild salmon get their pigment from their prey, farmed Atlantic salmon needs supplemented formulated diets. Nowadays, the share between synthetic- and natural sources is approximately 90:10, with an increasing interest in new natural sources. The cost of carotenoid supplements accounts for 6-10% of the total feed cost, whereas the deposition for flesh pigmentation in salmonids is less than 22%. Natural astaxanthin from algae *Haematococcus pluvialis* contains only 3S,3'S stereoisomer, the predominant pigment in wild salmon, suggesting an improved biochemical effect compared to the synthetic form. In addition, some experimental diets containing algae-extracted astaxanthin have shown improved immune responses and stress tolerance in many aquatic species. Therefore, our study was performed to understand whether astaxanthin from *H. pluvialis* could contribute to enhancing astaxanthin assimilation, potentially boosting growth, and immune responses.

A 108-day feeding experiment was conducted in a flow-through system with seawater taken from a depth of 250 m of eighteen 800 L-tanks including three diets: AS (synthetic astaxanthin), AW (whole algae astaxanthin), and AX (algae-extracted astaxanthin) (3 x 6 replicates). Twenty fish (approximately 652 g) were stocked in each tank and fed twice a day. Environmental conditions were maintained in the optimal range of Atlantic salmon. Three fish were sampled from each tank on days 30, 60 and 108 (results not yet analyzed) to assess growth performance, visual flesh color, and astaxanthin content in Norwegian Quality Cut. On Day 60, liver and pyloric caeca in each treatment (n = 4) were collected for RNA extraction and sequencing for differential expression analysis. No significant difference in neither color assessment nor growth performance was observed after 30 days (Fig. 1). After 60 days, the fish weight in AS and AW is similar and higher than in AX, but no significant difference ($P > 0.05$). In AW, astaxanthin content was highest and significantly different from AX.

Regarding transcriptomics, differentially gene expression in both the tissues was more pronounced when comparing the timelines than the comparisons between experimental diets (Fig. 2). Gene Ontology (GO) annotation was mainly distributed in two categories: molecular function and biological process. On Day 60, enriched GOs and KEGG pathways were involved in the biosynthesis and metabolism of steroids, lipids, and fatty acids compared to Day 0. Particularly, *metalloreductase step4* gene in the liver was upregulated in AW compared to AS, suggesting control of lipogenesis, glucogenesis, and fat content. In both AW and AX, *H-2 class I histocompatibility antigen, Q8 α-chain* gene in the pyloric caeca involved in immune responses was upregulated.

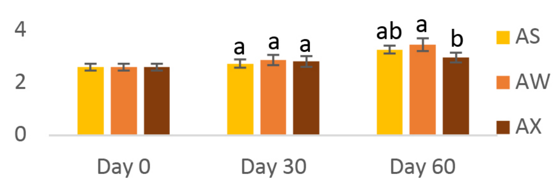

Fig. 1. Astaxanthin concentration (mg/kg) between three diets (Mean ± SD with different letters were significantly different P < 0.05).

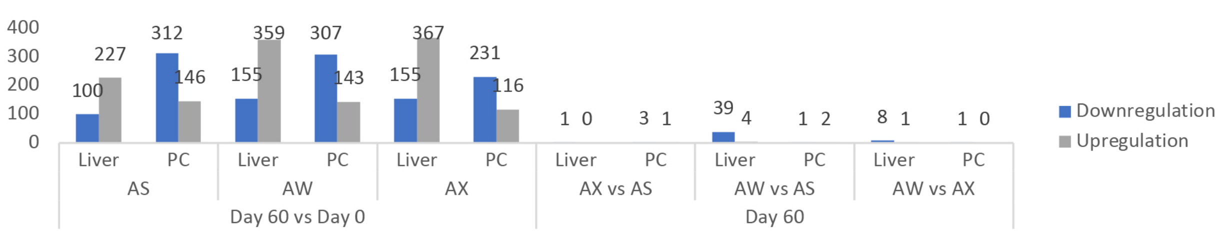

Fig. 2. The numbers of differentially expressed genes between days 0 and 60, and the three diets on day 60.

DEVELOPMENT OF GENETIC MARKERS FOR SEXING COBIA (*Rachycentron canadum*)

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Sex identification of fishes is critical for understanding life histories, developing successful management strategies of wild stocks, and for aquaculture industry applications. For some large species without obvious phenotypic sexual dimorphism, sex can sometimes only be confirmed via dissection or invasive gonad biopsies, which prompts significant interest for the development of non-lethal and less invasive approaches. Cobia (*Rachycentron canadum*) has become a species of concern in South Carolina due to heavy fishing pressure on an inshore spawning aggregation over the last two decades. This distinct population segment's (DPS) numbers have declined to levels of conservation concern and the State of South Carolina has implemented management actions, including harvest reduction and stock enhancement within inshore waters. To maintain an efficient, effective, and responsible marine stock enhancement program, determining the sex of broodstock, hatchery produced fish, and the wild population is therefore imperative.

The goal of this project was to develop and test a non-lethal and minimally invasive sex identification tool for Cobia. Here, we used whole genomic and molecular approaches to identify and validate sex-specific genetic fragments. Using long-read sequencing (Pacbio) to generate reference genomes and re-sequencing data (Illumina) from male and female Cobia, we designed primers for PCR assays that target sex-specific genetic regions and tested them on tissues from known-sex individuals. Results showed that males are consistently heterozygous at those regions, whereas females are homozygous (Figure 1) supporting a genetic-based sex identification and putative sex determination system in Cobia. These markers are currently being incorporated into our multiplexed PCR panels used for genetic tagging of hatchery fish and population genetics.

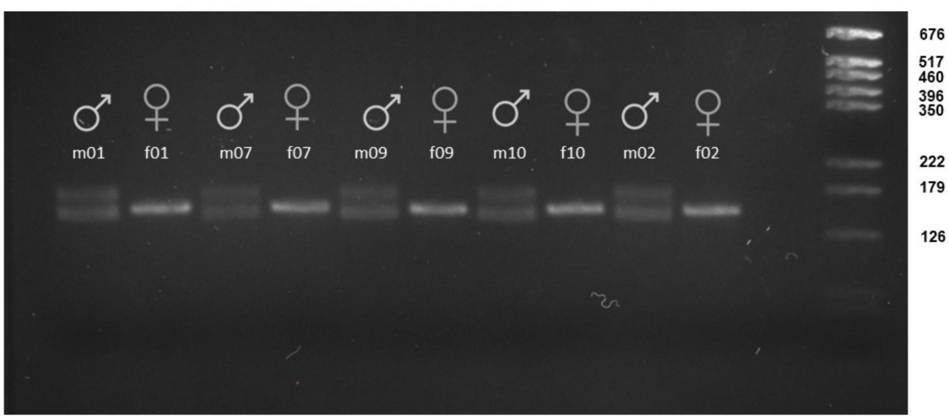

Figure 1. Agarose gel image of PCR products of male and female cobia using one of our new sex identification markers

WOMEN ASPIRE AND EXCEL THROUGH AQUACULTURE CAREERS

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When I introduce myself as a Kentuckian, people often ask me about bourbon and horse racing. When I mention that I do aquaculture research, I tend to get many questions about how I began and why I've decided on this career path. People can sometimes assume that since Kentucky is not on the coast, that aquaculture research and production does not occur here. However, Kentucky is the only state that is bordered by rivers on three sides and there are numerous lakes and streams throughout the state. Some of my fondest childhood memories were fishing with my papaw and flipping creek rocks to find critters. While the aquaculture industry in Kentucky may not be as extensive as it is in other states, there is value in the products grown here and the Aquaculture Research Center (ARC) at Kentucky State University has contributed valuable research to the field for nearly four decades.

My love for science began in elementary school; I remember being so proud of my science fair projects and asking for a microscope and experiment kits for Christmas. After high school, I enrolled as a Biology major at Kentucky State University but changed to an Agriculture, Food and the Environment major when the program began. As a junior, I started working at the ARC as part of an undergraduate course requirement. I instantly fell in love with the research and knew that this field could satisfy my love of water and food production. I was granted one year of tuition for graduate school and was so grateful for this opportunity. If I am being honest, I never expected that a small town country girl like myself could earn a Master's degree. As a first generation college graduate I knew my family was proud but I felt somewhat alone on my journey. Most of the girls I went to high school with already had six year olds by the time I finished grad school. So as society continues to pressure us all I often wonder how can we be expected to push ourselves to our fullest potential if we don't have examples of what that even looks like.

Young women interested in science, technology, engineering and math (STEM) careers need examples that are representative of themselves. Women that are harvesting ponds and surveying rivers by day while feeding their families and rocking their babies at night. This industry requires a lot of strength; physically and mentally. I hope to see more women take on active production and facility management roles. Women have diverse skill sets that make us efficient leaders and diligent researchers and we absolutely have a place in the aquaculture field. I encourage the women of aquaculture to find talented peers that they can look up to and see themselves in. The many incredible women that I have crossed paths with in this industry have left lasting impressions by showing me that I am capable of so much more than I ever thought possible.

STUDENTS DEVELOP FOOD LITERACY AND CULINARY SKILLS THROUGH THE USE OF CLASSROOM AQUACULTURE EXTENSION PROGRAMMING

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Classroom aquaculture extension programs can be used to teach complex science, technology, engineering and math (STEM) concepts and increase student awareness of sustainable food production systems. Making real-world connections between where food comes from and how to make nutritious meal choices is essential for all consumers; aquaculture curricula can allow youth to develop food literacy skills earlier.

Kentucky State University K-12 Aquaculture Extension team delivers educational aquaculture programs, aquaponics systems and aquaponics curriculum to central Kentucky High schools. Locust Trace AgriScience Center in Lexington, Kentucky is one of the 12 public high school that have been a part of the "Aquaponics in the Classroom" program. An aquaponics system was donated to the school as part of a grant project and the students participated in a 12-week aquaponics project based investigation (APBI) where they monitored fish health, water quality parameters and plant growth. This school also has an approximately 1,000 gallon recirculating aquaculture system (RAS) in their greenhouse that is used to demonstrate more intensive fish rearing protocols.

Kentucky State University donated approximately 40 channel catfish (*Ictalurus punctatus*) weighing approximately 680g each for stocking into the greenhouse RAS. Students helped with stocking the fish into the cycled RAS and monitored fish health for 2 weeks. In addition, an anatomy and filleting demonstration was given on the stocking day to introduce the students to dissection. After harvesting the fish, the students participated in a "Hook to Cook Guidebook" activity as a part of their final examination. Students worked together to dissect, identify internal anatomy and fillet the fish. The students gave presentations where they described the process of producing catfish and then cooked the catfish into healthy recipes. A post-interview with the teacher revealed that her students really enjoyed this activity and they had good retention of the information. Students were encouraged to try fish products, think about the sources of their foods and develop food preparation and culinary skills. Hands-on aquaculture extension activities can make lasting impressions on students by allowing experiential learners to immerse themselves in the material and apply these lessons when faced with STEM problem-solving challenges.

EMPOWERING SEAFOOD SERVERS TO TELL THE STORIES OF AQUACULTURED OYSTERS: UPDATE ON THE OYSTER ESSENTIALS TRAINING PROGRAM

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Oyster aquaculture in the southern United States (especially from North Carolina around to Texas) has expanded rapidly in the past decade, prompting concerns about market saturation. To address this concern, we are conducting a two-year program that targets seafood restaurant professionals to increase their understanding of the differences among oyster varieties and their ability to answer customer questions. Trainings are being conducted in eight southern US coastal states as well as six 'foodie' cities across the US. In the training, servers are given an 'oyster compass' as a tool to help remember how to differentiate oysters. They also participate in real-time polling to evaluate any change in knowledge due to the training.

We are testing the hypothesis that trained, more knowledgeable seafood servers will increase sales of aquacultured oysters by comparing sales pre- and post-trainings. In addition, we are conducting interviews with participants to assess the value of the trainings beyond sales.

An overview of the training will be provided, and results of trainings to date will be presented. Trainings have been conducted in Louisiana, Mississippi, Georgia, and Virginia, as well as in Atlanta, Baltimore and Washington, DC. Additional trainings will also be held in Alabama, Florida, South Carolina, and North Carolina.

THE SHELLFISH AQUACULTURE PROGRAM AT THE VIRGINIA INSTITUTE OF MARINE SCIENCE

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The Virginia Institute of Marine Science (VIMS) has a long history in shellfish aquaculture research and extension, dating back to foundational work at the Eastern Shore Laboratory in Wachapreague, VA (USA). Work by Michael Castagna and many others helped lead to the start of the farmed hard clam (*Mercenaria mercenaria*) industry. A breeding program led by Stan Allen was integral to the development and growth of the Eastern oyster (*Crassostrea virginica*) aquaculture industry in the region. The Marine Advisory Program has worked closely with the shellfish aquaculture industry, focused on addressing the needs of Virginia stakeholders.

Currently, VIMS is working to coordinate and expand its shellfish aquaculture research and extension work through increased collaboration and communication internally, building on external collaborations and, most critically, listening to and collaborating with stakeholders. The current focal areas in VIMS' shellfish aquaculture program will be discussed, as well as recently built facilities.

Looking forward, VIMS will seek to continue to advance and support a thriving sustainable shellfish aquaculture community in Virginia and the United States, through globally relevant shellfish aquaculture science, outreach, and education.

FACTORS SHAPING THE MICROBIAL POPULATIONS PRESENT ON FISH AND FISH PRODUCTS

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Catfish is one of the leading finfish species produced in the U.S., with 94.6 % of total food-size fish sold after processing. The control of microbial contamination during processing is a key factor for ensuring the microbial safety, quality, and shelf life of fish products. Unfortunately, how microorganisms present in the processing environment (e.g., equipment and line workers) and fish harvesting and processing seasons shape the microbial compositions of fish and fish products remains largely uninvestigated. To address this need, this research team applied the amplicon sequencing approach to characterize microorganisms present on the intact fish skin, in fish intestine, on fish fillets, and in environmental samples collected from catfish processing plants to categorize factors determining the microbial compositions of fish and fish products based on their significance.

Three catfish processing facilities in Southeast U.S participated in this study and two visits were conducted for each plant representing the Spring and Fall harvesting and processing seasons. A total of 70 environmental swab samples were collected representing the microbial populations present on the blade of de-heading machines, conveyor belts, worker gloves, floors of the sorting, processing, and packaging areas and the exterior of bins used for holding and transferring of final fish products. In addition, swabs (n= 46 for fish and n= 46 for fillet) were used to collect microbes present in the intestine, on the skin of intact fish and on the fish fillets. DNA was extracted from swab samples using QIAGEN DNeasy PowerSoil Pro Kits, followed by library preparation and sequencing.

Figure 1A shows that fillet samples, although grouped separately from other sample types, are closer to the centroid of environmental samples than fish samples, indicting that the processing environment has a larger impact on the microbial composition of final fillet products. Both season and facility have an influence on shaping the bacterial communities of environmental samples (Figures 1B-C). Samples collected from Facility 3 formed a distinct cluster compared with other two facilities (F1 and F2).

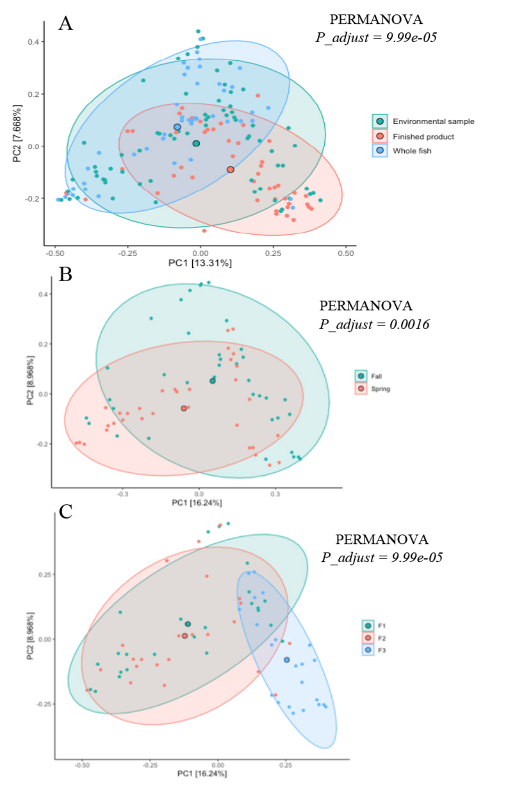

Figure 1. Principal Coordinates Analysis (PCoA) of A. all samples separated by their origins. B. environmental samples separated by season. C. environmental samples separated by processing facilities.

THE IMPACT OF FLORFENICOL TREATMENT ON THE MICROBIAL POPULATIONS ASSOCIATED WITH LIVE CATFISH

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Florfenicol has been approved by the U.S. Food and Drug Administration to control the infection caused by *Edwardsiella ictaluri* in catfish. However, there is limited information about the impact of such treatment on the native microbiota associated with catfish. As the microbial composition and abundance in catfish may directly influence the catfish's health and growth, it is crucial to understand how therapeutical florfenicol treatment impacts the microbial populations associated with catfish and in its rearing waters.

Eight 35-gallon tanks were set up for this study with 25 fish in each tank and hold at 25 °C (a temperature at which ESC typically occurs). Five fish were taken from each tank before and after the treatment as well as at the end of withdrawal period. Microorganisms present on fish gill and skin, and in the intestine were collected by using FLOQSwabs. Their abundance and diversity were analyzed by direct plating and 16S rRNA sequencing.

Both total aerobic and anaerobic counts in the intestine significantly ($P > 0.05$) increased at the end of treatment and continued increasing at the end of withdrawal period. The abundance of bacterial phyla, such as *Patescibacteria*, *Fibrobacterota,* and *Proteobacteria*, present in the intestine of catfish was altered by the florfenicol treatment. Results indicated that the impact of florfenicol on the native microorganisms is site-dependent with bigger impacts observed in the intestine, and the antibiotic treatment could generate prolonged impact on the antimicrobial resistant profiles in catfish (Figure 1). This study provides valuable information on the impact of florfenicol treatment during catfish production, which is needed for ensuring the sustainability of aquaculture production and for improving food safety.

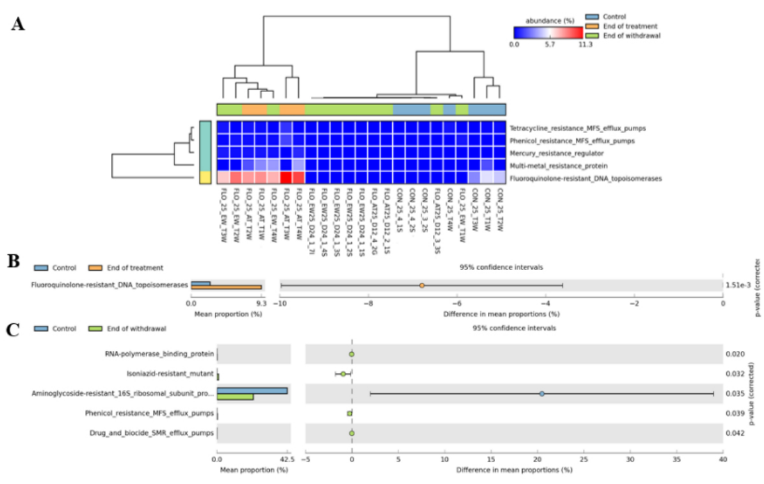

Figure 1 AMR profiles in fish samples. X-axis represents the AMR profiles as annotated to MEGARes database in different samples, while Y- axis stands for the different samples (A). Extended error bar plot indicating the AMR profiles changed due to the florfenicol treatment at the end of treatment (B) and at the end of withdraw (C). Points and bars indicate the difference between florfenicol-treated and untreated samples.

FITNESS AND TRANSCRIPTOMIC ANALYSIS OF PATHOGENIC *Vibrio parahaemolyticus* IN SEAWATER STORED AT DIFFERENT OYSTER HARVESTING TEMPERATURES

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Vibrio parahaemolyticus (*Vp*) is an important foodborne pathogen worldwide and has been one leading cause of seafood-associated human gastroenteritis in the United States. However, knowledge gap still exists about the fitness and survival mechanisms of *Vp* in pre-harvest environment.

Two pathogenic ATCC strains of VP43996 (*tdh*+) and VP17802 (*trh*+) were used as the model organisms. Overnight fresh and washed cultures were inoculated into autoclaved natural seawater and stored at 10 °C for 10 days and 30 °C for 5 days (representing the average seawater temperatures in winter and summer months). The surviving *Vp* was determined by plating inoculated seawater onto thiosulfate-citrate-bile salts-sucrose agar (TCBS). Total RNA was extracted from the artifically-inoculated seawater 2 hours after inoculation and on Day 5 for samples stored at 10 and 30 °C by using the Qiagen RNAeasy mini kits. cDNA library was prepared by using the Qiagen QuantiTect Reverse Transcription Kit and sequenced with the Illumina Hiseq platform to produce 2 x 50 bp pair end short reads. Transcriptomics analysis was conducted to profile the transcriptome of *Vp* at different harvesting water temperatures.

The survival of *Vp* in artificially-inoculated seawater at two storage temperatures are illustrated in Figure 1. At 10 °C, the *trh*+ strain survived with greater numbers than the *tdh*+ strain. At 30 °C, the viable counts of both *Vp* strains reached plateau after 24 hours and remained at ~6.8 Log CFU/ml for the rest of the storage time. Compared with the strains stored at 30 °C, the Gene Set Enrichment Analysis based on the Kyoto Encyclopedia of Genes and Genomes (GSEA-KEGG) in Figure 2 provides biological insights of *Vp* persisted in seawater at 10 °C. It was found that both the histidine metabolism and biofilm formation metabolism pathways were significantly downregulated for both *Vp* strains at 10 °C.

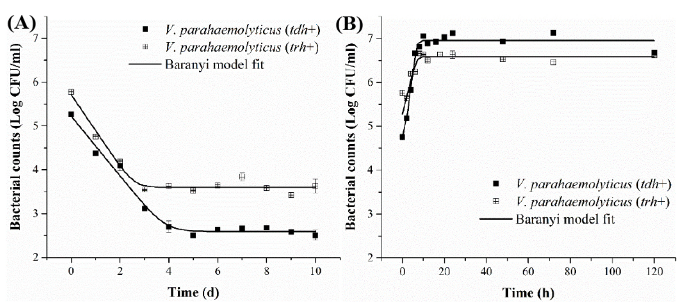

Figure 1. (A), The survival of two *Vp* strains in natural seawater at 10° C; (B), the growth of two *V. parahaemolyticus* strains in natural seawater at 30° C.

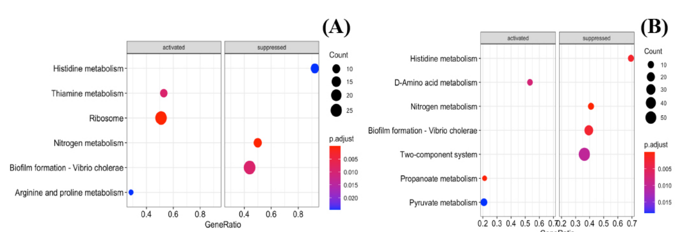

Figure 2. Gene set enrichment analysis against Kyoto Encyclopedia of Genes and Genomes database (KEGG) at 10 °C for *Vp* harboring *tdh* (A) and *trh* (B) genes.

THE ROLE AND APPLICATIONS OF EPIGENETICS IN AQUACULTURE

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As aquaculture and livestock production and human demands increase, much effort has been focused on improving production efficiency and heath wellbeing of agricultural animals and fish, as well as their sustainability using genetic approaches, which have explained part of the phenotypic variability of economic traits. Mounting evidence from epigenetic research in humans and animals has demonstrated that epigenetics plays a complementary role to genetics, touching many aspects of biological processes such as reproduction, early development, disease, growth, and nutrition. The role of epigenetics in aquaculture and aquatic animals is similar to other vertebrates in principle, even for embryonic development. As epigenetics in aquaculture is behind other livestock and animals, in this paper we will summarize the roles and applications of epigenetic processes in reproduction and early development, health and wellbeing management, and nutrition and growth advancement, and sustainability enhancement from different taxa to diverse teleosts, aimed to provide insights into the potential roles of epigenetics in aquaculture and aquatic animals, based on most recently research progress.

New findings regarding epigenetics being made in Metazoa have the potential to contribute to future applications related to the wellbeing and productivity of farm animals and aquaculture species with minimal environmental impact. However, the roles of epigenetic mechanisms and their potential in the aforementioned aspects are far from being fully understood. Thus, more studies are needed to generate/identify comprehensive data and fill the knowledge gaps concerning the contributions of epigenetics to reproduction, growth, nutrition, health, and the immune response of farm animals and aquaculture species.

To generate comprehensive and meaningful data, eventually epigenome-wide association studies (EWAS) will need to be implemented in farm animals and aquatic species. For instance, array-based DNA methylation has been used for EWAS in human. Meanwhile, identifying epigenetic effects on production traits under the impact of multiple factors is also worth investigating. This will facilitate epigenetic biomarker development and utilization in farm animal management. In addition, comprehensive exploration of the impacts of transgenerational epigenetic inheritance on domestication and selective breeding is needed. This will further facilitate understanding of currently identified effects and applications of epigenetics in sustainable production. Moreover, there is a need for studying the correlation between epigenetics and related physiologic responses. This could enhance our knowledge of the regulation of key production traits of farm animals and aquaculture species. For detailed information on progress, knowledge gaps, challenges, and prospects in epigenetics with aquaculture and aquatic species, please see related chapters in the book “Epigenetics in Aquaculture”.

EFFECTS OF FEED LOADING RATES ON GROWTH OF BIBB LETTUCE (*Lactuca sative)* WITH NILE TILAPIA (*Oreochromis niloticus*) IN AQUAPONIC SYSTEMS

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Fish feed is a highly variable recurring expense for aquaponic farmers. Small farmers are more susceptible to price instability as they commonly pay full retail prices for feed. Financially viable aquaponic businesses require maximizing output while minimizing expenditures. Since fish feed is the primary nutrient input for both fish and plant growth, it is important that the "feed to plant ratio" is sufficient but also economical. The standard loading rate for most aquaponic systems is 60 grams of feed/ m^2 plant area/ day (g/m^2/day). However, it is unclear if this loading rate is the most cost effective.

This study compared a range of feed loading rates for aquaponics. Utilizing six replicated systems, a range of "feed to plant" ratios were evaluated in terms of total biomass production of Bibb lettuce, *Lactuca sativa var. capitate,* over three production cycles (61 days). Nile tilapia, *Oreochromis niloticus,* were fed at loading rates of 15, 30, 45, 60, 75, and 90 g/m^2/day based on the growing area of the hydroponic unit (2.7 m^2).

Using the average plant biomass (g) from two production cycles, a significant polynomial regression was developed to predict the expected plant biomass based on a set feed rate. (Figure 1). Results indicate that 60 g/m^2/day will result in the highest plant biomass. A partial budget analysis was then used to compare the projected plant/feed relationships. Even with slightly lower plant production, loading rates of 15, 30, and 45 g/m^2/day increased annual profits compared to the Control (60 g/m^2/day). This model indicates that 30 and 45 g/m^2/day may slightly lower plant production but could be more cost effective overall.

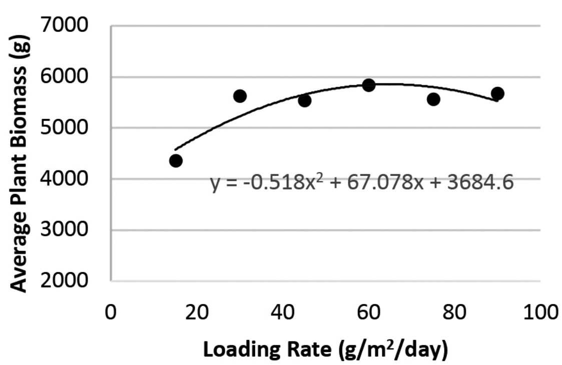

Fig 1: Avg plant biomass of different feed loading rates were used to create a predictive trend line of feed:plant ratios.

Table 1: Partial Budget Analysis - Feeding 15, 30, and 45 g/m2/day resulted in increased profits (yr) compared to 60 g/m2/day.

Feed (g/m^2/day)	Pl. Prod/yr (g)	Net change $ (yr)	Benefit:Cost
15 vs 60	4574.22	$5.29	1.007
30 vs 60	5230.74	$30.22	1.039
45 vs 60	5654.16	$28.46	1.035
75 vs 60	5801.7	-$55.16	0.940
90 vs 60	5525.82	-$137.02	0.859

BUSINESS AND ECONOMIC PLANNING FOR SEAWEED AQUACULTURE SYSTEMS IN THE UNITED STATES

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This presentation will provide an overview of a 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.

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. ***This presentation will share business planning and management tools developed to date.***

ALTERNATIVE METHODS FOR SMALL SCALE TILAPIA CULTURE

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Tilapia are warm fresh-water fish native to Africa and Middle East that are cultured all over the world. Nile Tilapia (*Oreochromis niloticus*) is the third most produced finfish in global aquaculture because they are a hardy, fast-growing, omnivorous fish, capable of utilizing lower cost feed ingredients. Tilapia is cultured both indoors and outdoors depending on rearing environment and regional climate. Nile tilapia grow best at temperatures around 27-31C, but the seasonal Kentucky climate limits outdoor production to May through September. However, the use of existing ponds has the potential to reduce initial costs for small-scale production of tilapia, which can allow for this limited growing season to still be profitable. In-pond cages and out-of-pond raceway culture methods have the potential to repurpose ponds unsuitable for traditional pond aquaculture.

The development of innovative methods to culture tilapia can provide data on the efficiency of different culture systems and optimal fish growth using existing ponds that are otherwise inadequate for fish farming. This presentation demonstrates how to build in-pond cages and out-of-pond tanks (either single or in series) and provides cost analysis of these easily constructed culture containers.

In-Pond Cage

Using PVC, screen mesh, and flotation mechanisms, these cages can be easily built and installed in existing ponds (Figure 1). Stocking and harvesting efficiency can be increased with the use of pulleys or by attaching them to docks. The addition of automated or demand feeders can reduce physical labor of daily feedings, especially for those farmers with special needs and mobility challenges.

Out-of-Pond Tank

Intermediate Bulk Containers (IBC totes), PVC, and a pump can be used to make a raceway culture set up (Figure 2). Water will be pumped from the pond into the tanks then back to the pond. These can also be set in a series where gravity flow distributes fresh water from one tank to another. This allows for use of ponds that are not suitable for traditional pond culture

These small-scale alternative production methods have the ability to be scaled up for increased production, and they have the potential to provide increased opportunity for much needed expansion of US Tilapia Aquaculture. Topic Area: Aquaculture Extension, Tilapia production

DIAGNOSING FISH DISEASES, RECOMMENDING TREATMENTS, AND EVALUATING FISH FARMERS' TREATMENT PREFERENCES AND PERCEPTIONS OF DIAGNOSTIC SERVICES

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Aquaculture practices in the United States has increased significantly over the decades, and with increased production, the knowledge of raising fish in aquaculture has also increased. Specific areas in the United States have a well-established aquaculture infrastructure and therefore can access resources, such as disease treatments, easier, whereas limited-resource states have a minimally developed infrastructure and therefore may have a more difficult time accessing appropriate disease treatment options. Medicated feed treatments are essential when trying to treat internal bacterial infections, but this option may not be accessible for many limited-resource fish farmers. A questionnaire was given to fish farmers in limited-resource and large-scale aquaculture states to compare infrastructure and find any gaps within the industry. During the survey, 77.8% of participants in limited-resource states mentioned that they often treat with an external chemical treatment rather than medicated feed when an internal bacteria has been diagnosed due to how long it takes to obtain the medicated feed. Of the limited-resource fish farmers that used medicated feed, the majority, 22.2%, stated that it took between 11-15 days for the feed to arrive at their farm, and in large-scale aquaculture states the majority (66.5%) of participants stated that they could obtain the medicated feed within 0-4 days after diagnosis. If treatment options were more accessible in limited-resource aquaculture states, then fish farmers may choose the more appropriate treatment option when disease outbreaks occur and relieve or eliminate the pathogen before higher mortality occurs.

SPAWNING DATE OF RAINBOW TROUT PRODUCED BY FERTILIZING EGGS FROM A SUMMER-SPAWNING LINE WITH CRYOPRESERVED MILT FROM A WINTER-SPAWNING LINE

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Rainbow trout lines selected to produce gametes at different times of the year and commercially cryopreservation services for rainbow trout semen provide an opportunity for extending the availability of gametes. Nevertheless, little data are available on the effect of crossing fish with different spawning dates on the spawning date of the offspring. Egg lots from females ovulated in August were divided in half and fertilized with milt from males from the same population ('purebred') or cryopreserved milt from two males from a winter-spawning population, collected in February ('hybrid'). Five crosses or families were made for each group and a total of 343 females from the purebred group and 244 females from the hybrid group were observed for spawn date.

The spawning windows of both the purebred and hybrid groups lasted around three months. In addition, each family took about nine weeks for the middle 10-90% of the individual females to ovulate. However, whereas 50% of the purebred fish had ovulated by the end of August, spawning of the hybrid progeny peaked in November, on average about 10 weeks after the purebred families. Furthermore, there was little overlap in the spawn dates between the purebred and hybrid females indicating they are two distinct egg producing populations. The hybrid progeny did not spawn in May which is also midway between February and August. The hybrids exhibited a classic phenotypic distribution pattern of heterozygous F1 hybrids. These results support hybridization of lines with different spawn dates by using cryopreserved sperm enables valuable strategies for maximizing hatchery production of year-round seedstock, and for quantitative trait loci (QTL) mapping of spawning date in rainbow trout. The F1 hybrids were crossed to produce an F2 generation for future genetic analysis to map QTL for spawning date.

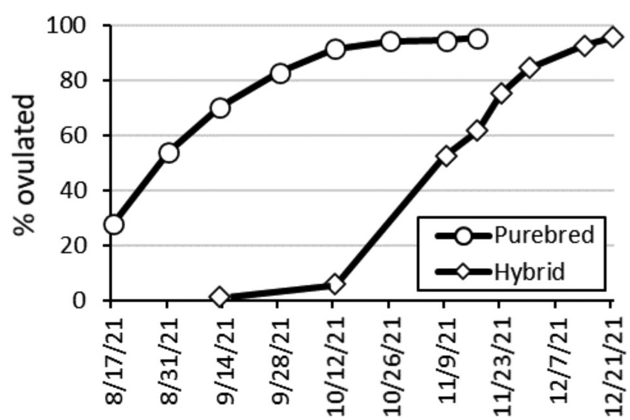

Fig 1. Spawn date of females from purebred and hybrid crosses.

EVALUATING ENVIRONMENTAL VARIABLES THAT INFLUENCE POND DISSOLVED OXYGEN TO INFORM PREDICTION MODEL DEVELOPMENT

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Globally, pond aquaculture is the most common method of cultivating finfish for human consumption. Growing populations and increased seafood demand has led to the intensification of pond aquaculture to the point where these systems are on the very limits of their carrying capacity. With this comes a heightened risk of a pond oxygen crash caused by the destratification of the water column or an algae die-off. These crashes are a leading cause of fish kills on pond farms due to the decline in dissolved oxygen (DO) that results. Current methods used on farms to monitor DO levels and prevent crashes are labor intensive, expensive, and reactionary rather than preventative. Being able to better monitor and predict DO levels would be hugely beneficial to the industry and its development.

The Hybrid Aerial/Underwater roboticS System (HAUCS) is an Internet of Things development for fish farms with the aim of improving DO monitoring efficiency and effectiveness by using autonomous sampling platforms to collect data to support a machine learning based DO prediction algorithm. This study focuses on the relevant environmental data that can be used to develop a robust prediction algorithm. The use of digital imagery as a proxy to algal bloom concentration data was also investigated. Data was collected June-September 2021 using ponds at Aqua Blue Cichlids in Fellsmere, FL. DO and water temperature were monitored 24/7 in each pond. A weather station on farm was used to collect air temperature, atmospheric pressure, wind speed/direction, solar irradiance, and rainfall. Images of the water's surface were taken in the mornings and afternoons using a digital camera. The color information from the images was used in correlation comparisons with the weather variables, algal concentration, and DO.

Significant correlations ($P < 0.05$) were seen between DO minimum and water temperature, algal concentration, sunlight irradiance, and rainfall (Table 1). For the image data, significant correlations were seen between DO minimum and some color components (e.g., RGB). Additionally, several correlations were seen between some image components and weather variables such as solar irradiance, windspeed, rainfall. Some correlations were seen between one image component and algal concentrations.

Table 1- Statistically significant Pearson's correlation results (p<0.05) for DOmin data and weather variables

DO_{min} Significant Correlations			
Parameter	r^2	*p* value	n
DO_{max}	0.31258	<0.0001	168
DO_{avg}	0.77949	<0.0001	168
Water Temperature	0.41267	<0.0001	168
Chl-*a* AM	0.29875	0.0042	90
Average Solar Irradiance	0.15347	0.0470	168

REVIEW OF NOAA SEA GRANT'S INVESTMENTS IN SUSTAINABLE US AQUACULTURE

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This presentation will provide an overview of NOAA Sea Grant's Aquaculture Portfolio and its over 50 year history with respect to support of US coastal, Great Lakes, and marine aquaculture research and extension activities. Funding mechanisms will be discussed and past and current examples of supporting sustainable and responsible aquaculture development in the US will be presented. In addition, potential future activities focused on support of aquaculture in the US will be discussed.

EFFECT OF SOY PROTEIN PRODUCTS AND GUM INCLUSION IN FEED ON FECAL PARTICLE SIZE PROFILE OF RAINBOW TROUT

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Replacement of fishmeal (FM) with alternative plant proteins, especially soybean meal (SBM), can cause a diarrhea-like symptom in rainbow trout (RBT), characterized by very fine fecal particles. These fines do not settle out in raceway effluent for collection and can contribute to pollution of receiving waters. In this study, two experiments were conducted. Experiment 1 examined effects of nine protein sources (sardine meal, menhaden meal, soy protein concentrates (SPC) (three types), SBM (regular and high protein), corn protein concentrate (CPC), and poultry by-product meal (PBM)) on fecal particle size distribution. Results showed that all five soy-based diets produced feces in RBT having 75.7–89.3% fines and only about 1.0% large particles, while the remaining four diets yielded feces having a balanced particle size distribution. Oligosaccharides present naturally in soy products, thought to contribute fecal fines, were not correlated to fecal particle size classes (Table 1). Instead, high crude fiber content in soy-based diets was likely responsible for unbalanced fecal particle distribution in RBT. Experiment 2 examined if improvements in formulation could reduce the negative effect of soy-based ingredients. Eight practical diets (FM, SPC, SPC + 0.3% guar gum, PBM + CPC, PBM + CPC + 20 or 30% SPC, and PBM + CPC + 20 or 30% SPC + 0.3% guar gum) were formulated to contain 40% protein and 20% lipid. Results showed that diets containing mixtures of PBM, CPC, and 20% or 30% SPC plus guar gum produced trout feces with the highest percentage of large particles and lowest of fines, while the diet containing SPC alone (56%) plus guar gum resulted in trout feces having the highest content of mid-size particles. It was concluded that crude fiber in soy protein products (SBM and SPC) caused undesirable fecal particle profiles in RBT, and the addition of guar gum could significantly alleviate this negative effect.

Table 1. Regression analysis of select dietary components versus fecal particle size.

		Dietary Carbohydrate Component (Dry-matter basis, g/100 g)						
Particle Size Class	Statistic	Starch	Crude Fiber	Sucrose	Oligosaccharides[1]	Lipid	Crude Protein	Ash
Fines	r	-0.63	0.91	0.6	0.56	0.46	-0.65	-0.52
	P-value	0.06	<0.001	0.09	0.12	0.22	0.08	0.15
	Effect	N/I[2]	Increase	N/I	N/I	N/I	N/I	N/I
Mid-Size	r	0.32	-0.66	-0.55	-0.58	-0.07	0.54	0.64
	P-value	0.4	0.05	0.12	0.1	0.87	0.13	0.07
	Effect	N/I	Decrease	N/I	N/I	N/I	N/I	N/I
Large	r	0.71	-0.91	-0.54	-0.47	-0.59	0.60	0.39
	P-value	0.03	<0.001	0.14	0.2	0.09	0.08	0.30
	Effect	Increase	Decrease	N/I	N/I	N/I	N/I	N/I

REPLACEMENT OF DIETRAY FISH PROTEIN WITH BACTERIAL SINGLE CELL PROTEIN RESULTS IN DECREASED ADIPOSITY COUPLED WITH LIVER EXPRESSION CHANGES IN FEMALE *DANIO RERIO*

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The common zebrafish (*D. rerio*) is an important preclinical biomedical model. The developmental, physiological and genomic platforms are well characterized in zebrafish, consequently, zebrafish can be used in understanding health related impacts of novel and sustainable feed ingredients with results directly translatable to humans. In an effort to assess the impact of sustainable bacterial single cell protein ingredient replacement for fish protein on zebrafish, we conducted a 14-week feeding trial on 31-day post fertilization zebrafish (10 tanks per diet, 5 fish per liter density). Zebrafish growth was assessed every 2 weeks during the feeding trial and at study termination fish were assessed for body composition, reproduction, and female liver expression (RNAseq with confirmatory rtPCR). Male and female zebrafish had no differences in terminal body weight, though female body lipid content was reduced in the bacterial protein diet. There were no differences between diets for reproduction based on spawning success, total eggs produced, or egg viability 24 hours post fertilization. Female RNAseq results for fish receiving the bacterial source protein (limited to the top 15% of expressed genes), found 43 differentially expressed genes (22 down-regulated, 21 up-regulated) with impacted gene ontologies related to cholesterol biosynthesis, metabolism, and protein processing in the endoplasmic reticulum. Expression changes are hypothesized to be related to the beta glucan content of the bacterial protein source, which can impact cholesterol and bile acid homeostasis. These data can support development of sustainable open-source diets for the zebrafish community.

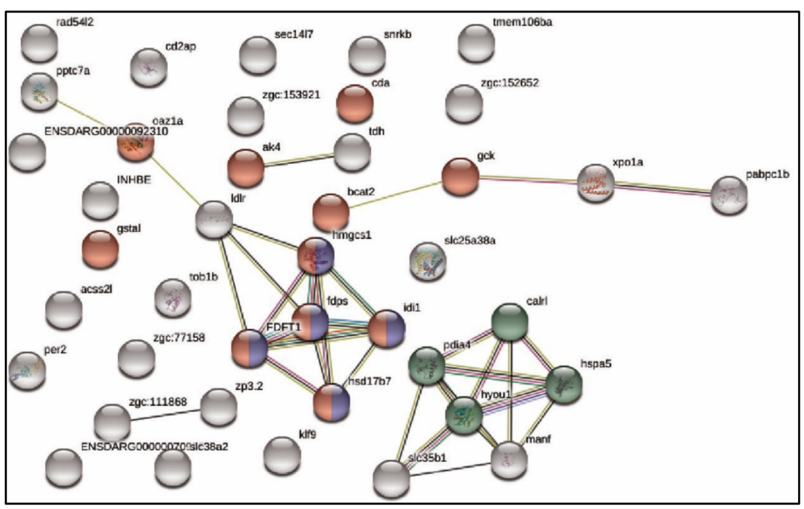

Figure 1. Visualization of the coexpression network of the top 15% of differentially expressed genes between female zebrafish fed bacterial and fish based protein diets. Red = metabolism related, blue = cholesterol biosynthesis, and green = protein processing in the endoplasmic reticulum.

MONITORING WATER QUALITY IMPACT OF REMOTE UNDERWATER VEHICLE USE WITHIN AN OYSTER LEASE

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Most wild oyster populations globally declined precipitously throughout the 20th century. The shellfish aquaculture industry has grown rapidly to meet the growing demand for seafood. Although shellfish aquaculture is one of the most sustainable sources of animal protein, it still uses antiquated equipment, such as dredges, that may impact water quality and benthic habitats within and adjacent to on-bottom leases. Dredges are commonly used to manage and harvest on-bottom oyster, yet they lack precision. Dredges indiscriminately collect crops and may damage smaller, less mature individuals. Compared to other methods (i.e., diver or tongs) they also may fail to collect many available oysters. Additionally, dredging disturbs bottom substrate, creating suspended sediment plumes (SSPs) that temporarily attenuate light and may impair water quality.

To improve crop management and increase sustainability, a smart sustainable shellfish aquaculture management (S^{3}AM) framework that utilizes a remote underwater vehicle (RUVs) and advanced software for crop detection, automated navigation, and other features is currently being developed. This technology is unproven and comparing its usefulness and ecological footprint to traditional methods is critical for approval and adoption from industry members and other stakeholders.

To lay the groundwork for this comparison we conducted a study to better understand natural variability on active oyster leases and developed methods for characterizing SSPs. Working in and adjacent to active oyster leases in Chesapeake Bay, a YSI EXO2 Sonde routinely measured background dissolved oxygen, chlorophyll, and total dissolved solids. These measurements were mapped into a 3D spatial grid to provide researchers with more information how water quality changes with depth in on-bottom oyster leases. To characterize SSPs from dredging activity, we measured the sedimentation and direction of the plumes using sediment traps and an array of light attenuation sensors placed radially at multiple depths near the impact site. The concentration of the plume was measured with the EXO2 in TSS values.

Novel data generated by this experiment includes spatial variability of conditions within leases, and ecological footprint of advanced RUV technology within an aquaculture setting. The overall goal of this project is to improve the understanding of how RUV technology can enhance sustainable shellfish aquaculture.

ADDITION OF MICRO-C AS A CARBON SOURCE FOR DENITRIFICATION IN BEAD FILTERS

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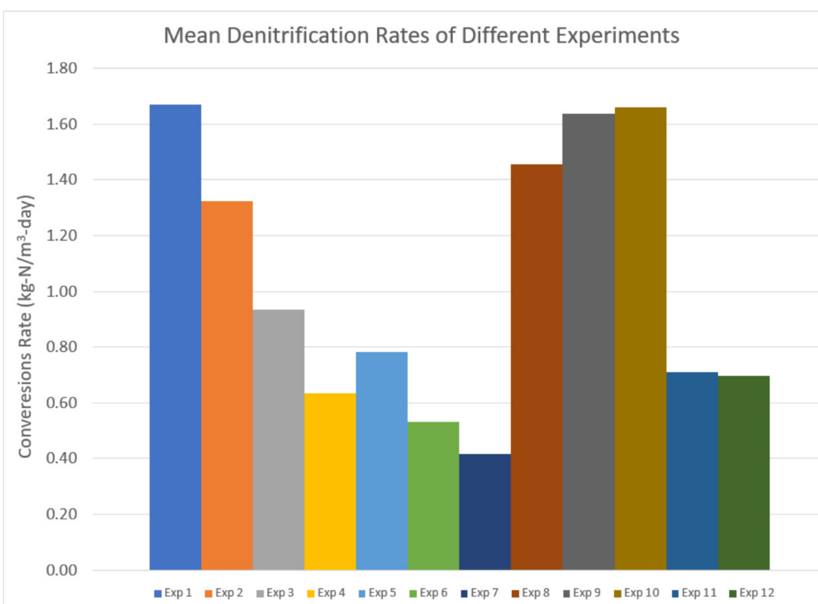
Removal of nitrates requires a carbon source for heterotrophic, denitrifying bacteria to consume. Ethanol and methanol are common dosing agents used to provide such a source. Micro-C 2000 is a commercially produced, concentrated, glycerin-based carbon source for biological processes. Suitable for denitrification, Micro-C 2000 is a safer alternative for small facility denitrification. Since it is nonflammable, it avoids stringent storage requirements. The objective of this study was to examine the effectiveness of Micro-C 2000 supporting denitrification in a floating bead format.

A series of studies were conducted to determine warm water denitrification rates sustainable by Micro-C 2000 fed into a bed of enhanced nitrification media. Nitrate enriched, aerobic freshwater from a 1200-gallon reservoir was injected with Micro-C 2000 into a 3 ft^3 (85 L) Bubble Bead® filter and discharged. Hydraulic residence time (20-60 minutes) in the unit was manipulated by variations in flow rate to achieve desired redox ranges (-100 to -500 mV). Impact of nitrate concentration, Micro-C 2000 feed rate, retention time, and redox potential on conversion rates were examined. Discharge concentrations of nitrate and nitrite were also examined.

Denitrification rates ranged from 0.5 to 2.5 kg-N/m^3-day across several conditions of operation. Best performance occurred when redox was less than -250 with excess Micro-C 2000. Flow rates ranged from 900 to 1800 mL/min and excess Micro-C 2000 feed rates ranged from 15 to 60 mL/min. Nitrate and nitrite values of less than 2 mg/L were obtainable with the high conversion rates. A denitrification sizing of 1 kg-N/m^3-day (SF=1.5) appears appropriate for this type of application.



CHARACTERIZATION OF CO-INFECTIVE BACTERIAL PATHOGENS VIRULENT *Aeromonas hydrophila* AND *Flavobacterium covae* IN CHANNEL CATFISH *Ictalurus punctatus* PRODUCTION

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Catfish farming is of great economic importance for Southern U.S. agriculture and is the largest production sector within U.S. aquaculture. *Flavobacterium covae* and virulent *Aeromonas hydrophila* are prevalent bacterial pathogens within this industry that can cause high mortality in production ponds. In addition, bacterial co-infections in catfish production may go unreported or misdiagnosed, resulting in a lack of proper mitigation or inadequate treatment. These polymicrobial infections are predicted to increase disease severity and farmed fish mortality.

A preliminary assessment of *in vivo* bacterial co-infection with virulent *A. hydrophila* (ML09-119) and *F. covae* (ALG-00-530) was conducted in juvenile channel catfish (*Ictalurus punctatus*). For the pathogen challenge, catfish were divided into seven treatment regimes: 1) mock control; 2 and 3) full and half doses of virulent *A. hydrophila*; 4 and 5) full and half doses of *F. covae*; 6 and 7) co-infective full and half doses of virulent *A. hydrophila* and *F. covae*. In addition to the mortality assessment, head kidney and spleen tissues were collected to evaluate immune gene expression and quantify bacterial load using qPCR. At 96 h post-challenge (hpc), the full-dose, single virulent *A. hydrophila* infection (immersed in 2.3×10^7 CFU mL^{-1}) resulted in final cumulative percent mortality (CPM) of 28.3 ± 9.5 %. The full-dose *F. covae* group (immersed in 5.2×10^6 CFU mL^{-1}) was 23.3 ± 12.9 %. When the single pathogens were compared to the polymicrobial infections, the co-infective full-dose combination (98.3. ± 1.4%) and half-dose combination (76.7. ± 17.1%) significantly increased mortality ($P < 0.001$).

Sera lysozyme content between treatment groups was not significantly different; however, differences were observed across time. At 12 hpc, a significant increase ($P<0.002$) in lysozyme content was observed compared to 6, 48, and 96 hpc. Conversely, lysozyme content at 96 hpc significantly decreased ($P<0.001$) compared to 6, 12, 24, and 48 hpc. An increase in expression of three pro-inflammatory cytokines (*tnfα, il8, il-1 β)* occurred at 48 hpc. For instance, *tnfα*,demonstrated a significant increase in expression at 48 hpc ($P < 0.001$) followed by a significant decrease at 96 hpc ($P < 0.001$).

Trial results emphasize the importance of evaluating co-infections and demonstrate dramatic increases in mortality when two pathogens are combined, even at half-doses. The synthesis of these mortality and health metrics will aid fish health diagnosticians and channel catfish producers in developing therapeutants and prevention methods to control bacterial co-infections better.

USE OF IRON FORTIFIED DIETS TO PROMOTE RED BLOOD CELL PRODUCTION IN ANEMIC CATFISH

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Channel catfish anemia (CCA) is a disease of unknown cause that has plagued the catfish industry since its inception. While normal packed cell volumes (PCV) of catfish range from approximately 20-40% (with higher PCVs in summer), anemic catfish have extremely low PCV, often with values of 5% or less. Clinically affected fish are often lethargic and show signs of respiratory distress despite adequate dissolved oxygen concentrations. The disease can result in chronic losses of 500-2000 lbs/acre, or acute losses with mortality approaching 100% in catastrophic outbreaks. The cause of CCA has not been identified and there are no known associations with infectious agents or environmental conditions. Histologically, there are abundant immature blast cells present in the head kidney, which coupled with low PCVs suggests arrested maturation of the erythroid lineage. Research was conducted to determine if iron fortified diets could promote red blood cell production in severely anemic fish. Anemic fish were collected from a commercial farm and transported to the NWAC research facility where a commercial diet fortified with varying levels of ferrous sulfate pentahydrate was delivered to anemic fish twice weekly by gavage. PCVs increased in a dose response fashion and after two weeks PCV levels returned to near normal levels. In comparison, the PCV of anemic fish fed non-fortified diets continued to decline over the same period. Similar results were observed in two commercial field trials where diets fortified with ferrous sulfate (500 ppm Fe) dramatically increased population level PCVs after 18-21 days. With few exceptions randomly sampled fish had PCVs greater than 20% and no severely anemic fish (PCV <10%) were identified. In comparison, there was no change in PCV values of anemic populations fed non-fortified diets over the same period. A two-year production trial was conducted in experimental ponds to ensure iron supplements did not increase iron concentrations in edible tissue or cause adverse effects on fish health. Healthy fish were fed diets supplemented with 0, 30, 700, and 1400 ppm Fe without any measurable differences in fish production, PCVs or iron levels in the fillet. While standard commercial catfish feeds far exceed the nutrient requirements for iron established by the National Research Council, data indicates increasing dietary iron in response to the onset of anemia promotes RBC production without measurable adverse effects on foodfish production, fish health or product quality.

COMPARING THE GROWTH OF XERIC TREES IN SOIL VS. AQUAPONICS

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Accelerated growth for sapling production could provide a potential solution to fighting climate change while conserving water, as well as contribute to habitat restoration efforts. Our hypothesis was that aquaponics will increase the growth rate of shoots and roots in their early stages. The growth of xeric trees was compared using traditional soil methods vs aquaponic conditions. Three trials were conducted over the course of one year using seven different species. We conducted two trials during the summer and one trial during the fall/winter in the controlled environment greenhouse at Santa Fe Community College. All varieties were started from seed and planted on the same day. In all three trials, tree performance under aquaponic conditions out-performed conventional soil treatments. In this preliminary study, data from all three trials support our hypothesis. Additionally, we observed that transplanting the saplings from water media to soil stressed the trees. Proper transplanting and hardening off from greenhouse to outdoor conditions should be optimized for successful aquaponic production of xeric tree.

ZONED LEASING IMPACT ON SHELLFISH AQUACULTURE INDUSTRY DEVELOPMENT: A CASE STUDY ON FLORIDA'S AQUACULTURE USE ZONES

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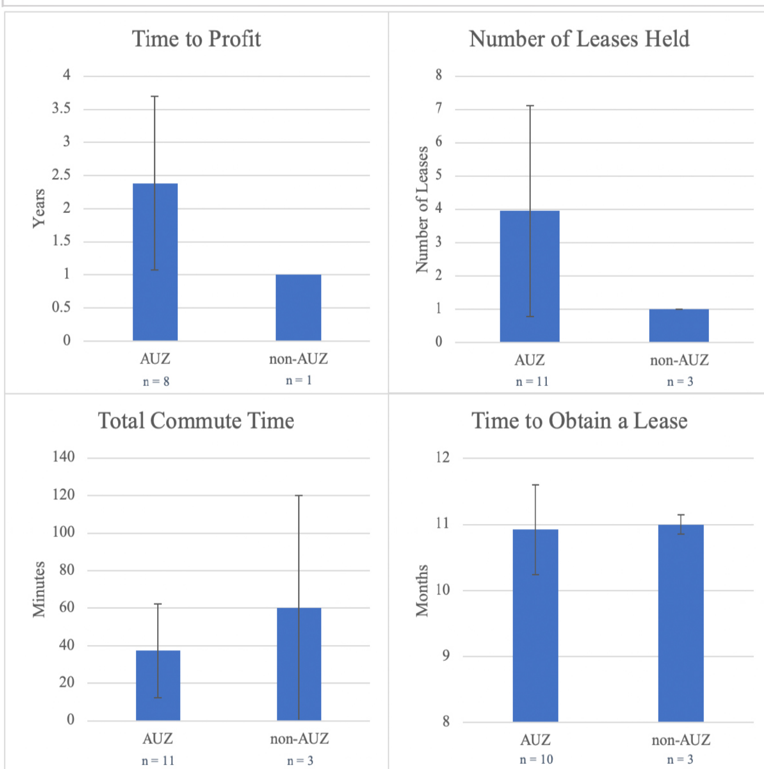
An important step in establishing or growing a shellfish aquaculture operation is obtaining a lease. While many states allow aquaculturists to find their own lease location, this process is often a time-intensive and expensive process which can be a barrier for new entrants to the industry. Zoned leasing is a way for state agencies to pre-approve large areas for shellfish aquaculture then divide the areas into smaller parcels. This theoretically lowers the barrier to entry that can arise from shellfish aquaculture leasing.

This project utilized semi-structured interviews with shellfish aquaculturists in Florida to investigate the impact of zoned leasing on individual businesses. Florida was chosen because it is both the first state in the United States to implement a zoned leasing system and the state with the greatest utilization of the system. This study found that zoned leasing can support new entrants and industry growth if specific additional factors are also considered. These include: establishing zones in locations with the necessary physical and environmental characteristics for the expected type of shellfish aquaculture; allowing space specifically for new entrants; making a streamlined process for industry participants to select their own lease site if zoned opportunities are not available; and needs for on-land infrastructure development and ample seed supplies to support the growing industry.

Figure 1. Time to Profit, Number of Leases Held, Total Commute Time, and Time to Obtain a Lease for AUZ and non-AUZ Leaseholders.



PREDICTION OF FILLET WEIGHT AND FILLET YIELD FROM BODY MEASUREMENTS AND GENETIC PARAMETERS IN A COMPLETE DIALLEL CROSS OF THREE NILE TILAPIA STRAINS

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In this study, the objective was to investigate whether non-lethal methods, utilizing body measurements, could be used to efficiently predict fillet weight and genetic analysis for in a complete diallel cross of three Nile tilapia strains. As in females, early sexual maturation was widespread, only 958 male fish from 81 full-sib families were used, both for prediction of fillet traits and in genetic analysis. The prediction equations from body measurements were established by a forward regression analysis, choosing models with the least predicted residual error sums of squares (PRESS).

The results revealed that body measurements on live Nile tilapia is well suited to predict fillet weight, but not fillet yield (R^2= 0.945 and 0.209, respectively), but both models were seemingly unbiased. The genetic analyses were carried out with bivariate, multibreed models. Body weight, fillet weight and predicted fillet weight were all estimated with a heritability ranged from 0.23 to 0.28, and with genetic correlations close to one. Contrary, fillet yield was only to a minor degree heritable (0.05), while predicted fillet yield obtained a heritability of 0.19, being a resultant of two body weight variables, known to have a high heritability. The latter trait was estimated with genetic correlations to body weight and fillet weight traits larger than 0.82.

No significant differences among strains were found for their additive genetic, reciprocal or heterosis effects, while total heterosis effects were estimated positive and significant ($P < 0.05$). As conclusion, prediction of prediction of fillet weight based on body measurements is possible, but not for fillet yield.

Table 1: The regression variables entering the prediction equation for fillet weight through the first six steps of forward selection.

		Training data set				Validation data set			
Step	Prediction equation[1)]	R^2	R^2-adj	AIC	RMSEP	RMSEP	PRESS	R^2_p	Bias
1	-5.4+0.36BW	0.936	0.935	2728.7	4.19	4.06	12378.1		
2	-18.4+0.32BW+7.7BT	0.940	0.940	2654.1	4.04	3.94	11503.6		
3	-7.1+0.35BW+9.5BT-8.1HT	0.943	0.943	2611.6	3.95	3.82	11153.3		
4	0.74+0.37BW+9.5BT-6.6 HT-2.9HL	0.944	0.944	2591.1	3.92	3.72	11100.5		
5	1.74+0.38BW+8.5BT-5.7HT-2.0HL-1.1BD	0.945	0.945	2579.8	3.90	3.67	11016.5	0.94	0.01
6	6.3+0.39BW+8.6BT-5.1HT-2.4HL-1.3BD -0.44CL	0.945	0.945	2579.4	3.90	3.67	11062.0		

Table 2 Estimates with standard errors (± s.e.) of genetic parameters for body weight

Traits	σ^2_p±s.e	BW5±s.e	BW7±s.e	FW±s.e	PFW±s.e	FY±s.e	PFY±s.e
BW5	1578 ± 72	**0.28 ± 0.06**	0.98 ± 0.04	0.93 ± 0.05	0.98 ± 0.05	0.04 ± 0.3	0.86 ± 0.10
BW7	1815 ± 98	0.49 ± 0.04	**0.27 ± 0.07**	0.99 ± 0.01	<1.0 ± 0.00	0.26 ± 0.3	0.86 ± 0.08
FW	257 ± 14	0.44 ± 0.04	0.96 ± 0.00	**0.23 ± 0.07**	0.99 ± 0.07	0.39 ± 0.2	0.82 ± 0.08
PFW	242 ± 13	0.47 ± 0.04	0.99 ± 0.00	0.96 ± 0.04	**0.26 ± 0.07**	0.26 ± 0.1	0.88 ± 0.06
FY	6.3 ± 0.3	0.09 ± 0.04	0.33 ± 0.04	0.57 ± 0.03	0.36 ± 0.04	**0.05 ± 0.04**	0.10 ± 0.30
PFY	1.3 ± 0.1	0.19 ± 0.05	0.64 ± 0.03	0.70 ± 0.03	0.71 ± 0.02	0.49 ± 0.04	**0.19 ± 0.06**

MENTORING A BEGINNING SMALL-SCALE CATFISH FARMER - YEAR ONE

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Mentoring a small-scale poultry/catfish grower with the Beginning Farmer Mentors Program began during summer of 20220. This program is a joint effort between the Kentucky Office of Agricultural Policy and the Kentucky Agricultural Finance Corporation. Securing the farm loan for the catfish production was contingent on having a mentor for guidance. Located in western Kentucky, the 20-year-old farm consists of 4 broiler houses to contract grow chickens and 25 acres of water divided into six catfish. Although the farmer had some experience with raising poultry, he had no experience growing catfish and is the farm's only full-time employee. Part time help is hired from the surrounding community for fish harvests.

Four-to-six-inch-long fingerlings were stocked in June of 2020 with 5,000 fish stocked per acre. Fish were fed most days to satiation with a 32 – 28% catfish diet. Harvesting 1 – 4 lb. fish began during late spring of 2022. At this time, additional fingerlings were stocked at approximately 6,000 fish per acre. To date an estimated 60,000 to 70,000 lbs. have been harvested. Fish are sold to live haul truck drivers for stocking in fee fishing operations at prices of $1.30 to $1.35 per pound.

Production challenges consist of ponds in need of renovation which make seining difficult. Paddlewheel aerators and fish harvest equipment are worn and require frequent repair. Incidence of disease and low dissolved oxygen caused some loss of fish during the spring. Acceptance of the importance of testing water quality on a regular basis remains a challenge to effectively communicate. Cash flow issues have occurred where revenue from catfish sales have paid for poultry production costs.

Positive developments include temporary fish harvesting labor has been readily available. Live catfish prices remain strong, which is critical for the success of small-scale growers. Demand for catfish exceeds local supply and allows producers to market their fish at competitive prices. Ideally, 20,000 to 30,000 lbs. of market sized fish would be held over winter to sell during the early spring of 2023. Catfish growth will resume in late spring when water temperatures warm.

THE IMPACT OF CLIMATE CHANGE ON PRECIPITATION IN ARKANSAS

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Data collected from US EPA website, where trends in pollutant concentrations, atmospheric deposition, and ecological effects were monitored due to changes in air pollutant emissions 2000 – 2020 in Caddo Valley, AR.

Partial Least Square (PLS) model and Principal Component Regression (PCR) were developed using 25 explanatory variables to predict precipitation. The goodness-of-fit of predictive models were assessed using calibration coefficients of determination and Root Mean Square Error of Prediction (RMSEP). By using the four PLS components, the and RMSEP for the predictive PLS model were 0.85 and 22.77, respectively. By using the four PCR components, the and RMSEP for the predictive PCR model were 0.87 and 25.82, respectively, indicating that both the PLS and the PCR models fit very well.

DIETARY IRON SUPPLEMENTATION CAN INCREASE HEMATOCRIT LEVELS FOR CATFISH (*Ictalurus* sp.) BUT DECREASE THEIR RESISTANCE AGAINST *Edwardsiella ictaluri*

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Supplementation of iron sulfate in plant-based feeds has been a common practice to mitigate idiopathic catfish anemia. Two separate feeding trials evaluated the supplementation of iron in feeds for hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) and channel catfish (*I. punctatus*). For the first feeding trial, four experimental diets were formulated with practical ingredients and supplemented with ferrous monosulfate to provide 0, 500, 1000, and 1500 mg of iron per kg of diet. Groups of 16 hybrid catfish juveniles (~22.4 g) were stocked in each of 20, 110-L aquaria (n=5), and experimental diets were distributed in a completely randomized block design. Fish were fed to apparent satiation for 12 weeks, and at the end of the study, production performance, survival, condition indices, and protein and iron retention were unaffected by the dietary treatments. Hematocrit levels as well as the iron concentration in the whole-body presented a linear increase as the concentration of iron increased in the diets. The remaining fish from the feeding trial were challenged with a virulent strain of *Edwardsiella ictaluri* through immersion at 1.2×10^7 CFU/mL for 1 h. The mortality rate was not significantly different but appeared to be higher for the dietary groups treated with iron-supplemented diets. Intestinal histology samples are being processed, and results are pending.

A follow-up trial conducted with channel catfish evaluated the supplementation of 1000 mg/kg, and a basal diet served as a control. The iron supplementation level was chosen based on the previous study, and what has been practiced by the catfish feed industry. Groups of 30 channel catfish juveniles (~13.3 g) were distributed in each of 16, 110-L aquaria (n=8), and experimental diets were distributed in a completely randomized block design. After 90 days of feeding, production performance, survival, condition indices, total cell count in the blood, plasma protein, and whole-body proximate composition were unaffected by the dietary treatments. Blood hematocrit increased when fish were fed 1000 mg/kg of iron. The transient microbiome did not present differences for the alpha and beta diversity metrics, nor for testing of differentially abundant bacterial taxa. The remaining fish were exposed to a bath exposure of *E. ictaluri* (3.2×10^8 CFU/mL) for 1 h, and fish fed the iron-supplemented diet had a significantly lower survival (27%) when compared to the control group (43%). These two trials indicate that iron supplementation above their established requirement level can increase their hematocrit levels, but also increase susceptibility to *E. ictaluri*.

STUDY OF INITIAL NUTRIENT REQUIREMENT FOR 10 DIFFERENT VEGETABLE CROPS IN AQUAPONICS

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Aquaponics is a form of aquaculture that integrates soilless crop production (hydroponics) to raise edible plants and fish. The fish are fed and excrete waste, which is broken down by bacteria into nutrients. Plants utilize some of these nutrients and in the process filter the water in the system. According to former study, we found that initial nutrient levels may make a big influence on crop growth rate, so during this study fish were fed once per day under two feeding scheme (daily uniform feeding (DUF) and daily increasing feeding (DIF) by 1% fish FW) in order to figure out initial nutrient requirement for aquaponics crops. Two feeding schemes had the same total monthly feeding amount, so initial nutrient level (60 g and 40 g/day) and nutrient accumulation rate (0 g and 5 g increment/day) were the only variances. Ten different vegetable crops (pac choi, mizuna, mustard, amaranth, bekana, Swiss chard, chia, basil, lettuce, and tomato) were planted under cultivation of Blue tilapia (*Oreochromis aureus*) in recirculating aquaponics systems over a 30-day trial. The mean feed conversion ratio (FCR) values for two feeding schemes were 1.08±0.65 and 0.92±0.26, respectively. The electrical conductivity (EC) values of two feeding schemes increased over time. DUF showed higher EC values although there was no significant difference compared to DIF. Orthophosphate, total ammonium nitrogen (TAN), nitrate, pH and dissolved oxygen (DO) levels were not significantly different between treatments. Data showed that DUF resulted in a significant lower water consumption than DIF after 15 days of transplant, which may a result of lower Gs (transpiration rate) values. There were no significant differences in weekly growth rate (height, leaf length, leaf number, SPAD) and harvest fresh weight between treatments. The instantaneous photosynthetic rate of pac Choi, swiss chard, lettuce, and amaranth in DUF increased over time and showed significant higher values than DIF by the end of study. Results of this study showed the potential effect of initial nutrient level on the aquaponics production of vegetable crops.

SEX DIFFERENTIATION AND REVERSAL OF JUVENILE YELLOW PERCH

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Yellow perch (YP, *Perca flavencens*) exhibit sexually dimorphic growth, with females growing faster, maturing later, and reaching a larger size than males. Production of monosex fish using sex control technology has become essential for the improvement of aquaculture productivity. Multiple studies indicate that hormonal treatment for sex reversal in most of fish species need to initiate before 35 days post-hatching (dph). The aim of this study was to determine the latest labile period or time during gonad development for the treatment success of the synthetic steroid hormone 17α-methyltestosterone (MT) in the yellow perch older than 35 dph.

Juvenile fish were subjected to two dietary concentrations of methyltestosterone (20 and 50 μg MT/g feed) for 60 days in three (3) age groups of 38, 46, and 67 dph, where control group were fed with standard commercial feed. Following a 10-month on-growing period, sex phenotypes were determined by gross and histological gonad morphology. Results showed the juvenile YP responded to the exogenous hormone when it was applied at 38 dph for both low and high dose of MT resulting in 100% males. At 46 dph, only high dosage of MT resulted in 100% males. Both MT-treated at 38 and 46 dph significantly differed (P<0.01) from the expected normal population of male: female (1:1). MT-treated at 67 dph resulted in 37% and 25% intersex fish for both the low and high dosage group, respectively. MT-treated at 38 and 46 dph promoted growth and showed significantly heavier mean body weight (P<0.05) compared to control. The gonadosomatic index (GSI) of MT-treated at 38 and 46 dph was significantly lower than control. This study provides the first evidence that juvenile YP can be successfully masculinized when the treatment is initiated at the age of up to 46 dph.

In conclusion, the dosage and time of MT-supplemented feed initiation influences the percentage of sex-reversed males and promotes growth. Where using higher dosage MT at 50 mg/kg feed could effectively broaden the efficacy of sex-reversal up to 46 dph initiation in yellow perch, nevertheless the higher concentrations of MT have evidenced a negative effect on the gonadal development of fish. Hence, the initiation of low dosage at 20 mg/kg of MT-supplemented feed at 38 dph for 60 days could be suggested in an attempt to produce all-male fish stocks of yellow perch, as it had effectively produced 100% sex-reversed males, promoted growth, and at the same time reduced the total amount of MT used. The identification of the labile period of sex determination that is manipulated by MT dosage and age of treatment would be a valuable progression toward optimizing commercially viable regimes for sex reversing YP for aquaculture industry. The findings are important for sex control in aquaculture and assessment of potential effects of environmental pollution on sex ratios of wild populations.

APPLICATIONS OF PARENTAL BASED TAGGING TO RESTORE KOOTENAI RIVER WHITE STURGEON AND BURBOT

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The Kootenai River White Sturgeon *Acipenser transmontanus* and Burbot *Lota lota maculosa* were once abundant in the Kootenai/ay River Basin in Idaho and Montana, USA, and British Columbia, Canada. Historically, the native fishes provided important cultural resources for indigenous peoples, and remain so today throughout the basin despite significant population decline. Kootenai White Sturgeon are listed as endangered in both countries due to cumulative effects of habitat destruction and of Libby Dam hydro-power operations in Montana that have resulted in persistent recruitment failure since the 1970's. Due to similar causes, Burbot were functionally extirpated by the 1990's. In 1988, the Kootenai Tribe of Idaho recognized the lack of White Sturgeon natural recruitment and started an experimental aquaculture facility to determine the feasibility of using wild broodstock to artificially spawn and rear year classes to reverse population decline. The Kootenai River Native Fish Conservation Aquaculture Program (KRNFCAP) began rearing fish during 1990-1992, and has been successfully releasing annual year classes since 1997. Following the Sturgeon program, a formal Burbot program feasibility evaluation was initiated in 2003. Since its inception, the conservation aquaculture program has boosted the Burbot abundance estimates from 50 adults in 2002 up to 50,000 adults. The KRNFCAP is part of multi-agency and stakeholder collaboration, and is one component of the greater Kootenai Ecosystem Restoration.

The KRNFCAP is guided by an annual adaptive management framework. As part of a large collaboration, the conservation hatcheries are used to 1) avoid extirpation and rebuild the species abundance to jump-start natural recruitment, and support culture and recreational harvest; 2) spawn, rear, and release early life stages across habitat types/conditions to determine causes of recruitment failure; and 3) spawn, rear, and release fish in a manner that supports post-release monitoring, research, and evaluations. The presentation will describe the applications of Parental Based Tagging that supports post-release monitoring and evaluation and guides adaptive management of habitat restoration projects.

EFFECT OF PROTEIN REDUCTION WITH INDISPENSABLE AMINO ACID SUPPLEMENTATION AT DIFFERENT LEVELS IN PRACTICAL DIETS OF NILE TILAPIA, *Oreochromis niloticus*

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A fifteen-week growth trial was conducted to investigate the effect of reducing dietary protein levels in Nile tilapia *(Oreochromis niloticus)* with dietary supplementation of commercially available synthetic amino acids at different levels (100 and 120% NRC, 1993) on growth performance, whole-body composition, morphometric indices, serum metabolites, immune and antioxidant biomarkers. The experiment consisted of six isoenergetic (3000 Kcal DE/Kg) dietary treatments with two replications per each. The first four groups of diets were formulated to contain different levels of dietary protein (32, 30, 28, and 26%) in which the indispensable amino acid (IAA) profile covered the IAA requirements for Nile tilapia as recommended by the NRC (1993), except for the low protein diet (26%) in which methionine was supplemented. The last two groups of diets were formulated to contain 26 and 28% crude protein in which IAAs were adjusted to be 120 % of the NRC requirements. By reducing dietary protein levels to 26%/IAA/100%NRC, final body weight (FBW), weight gain (WG), weight gain percent (WG %), specific growth rate (SGR), protein efficiency ratio (PER), and feed intake (FI) were significantly reduced, while feed conversion ratio (FCR) was significantly increased. Indispensable amino acid supplementation at 120% of the NRC requirements to low protein diets (26 and 28%) resulted in a significant improvement in all growth performance parameters. Reducing dietary protein with IAA supplementation did not affect whole-body moisture and crude protein, while fat and ash content were significantly increased. Serum total protein (TP), albumin (ALB), glucose, and serum superoxide dismutase (SOD) activity were not significantly altered by lowering dietary protein levels to 26%, while serum triglycerides (TG), cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine concentrations showed a significant increase. Serum IgG, IgM, and reduced glutathione (GSH) showed a significant improvement with IAA supplementation at 120% of the NRC requirements to 26 and 28% protein diets. In conclusion, dietary protein level could be lowered to 26% in Nile tilapia diets with supplementation of IAA at 120 % of the NRC requirements while maintaining comparable growth performance, feed utilization, immune and antioxidative responses.

EXTRACTION YIELD, AMINO ACID COMPOSITION, AND FUNCTIONAL PROPERTIES OF PROTEIN EXTRACTED FROM SORGHUM BY ALKALINE AND NAOH-ETHANOL-REDUCING AGENT METHODS

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FAO estimates that by 2050 food production must increase by 70% and meat production by 100% to address global demand. To meet this demand without exacerbating the environmental and public health costs of animal agriculture, growth in sustainable and alternative animal food products is critical. The overall goal of this project is to develop research-scaled novel protein concentrates to enhance value-added product marketing opportunities. The objective of this research was to compare the protein extracted with two methods, including alkaline and NaOH-Ethanol-Reducing agent methods regarding the extraction yield, and amino acid profiling to select the method with the higher extract yield and then the characterization of protein extracted with the selected method. The alkaline (NaOH) method showed an extraction yield of 1%, which was about 7.2% of the total protein content. Meanwhile, the protein content of the protein isolate was 56.11%, while the NaOH-Ethanol-Reducing Agent method revealed an extraction yield of 7.55%, which was 58.1% of the total protein. The protein content of sorghum protein isolate in this method was 88.83% which was significantly different from the NaOH method. Furthermore, the results from the amino acid composition analysis of sorghum showed that glutamic acid (2.82% w/w), leucine (1.82% w/w), and alanine (1.22% w/w) were the predominant amino acids, while protein extracted by NaOH from same sorghum, revealed that glutamic acid (7.44% w/w), arginine (5.06% w/w), and leucine (4.16% w/w) were the main amino acids, respectively. The amino acid composition analysis also revealed that despite the NaOH method, the protein extracted by the NER method did not show amino acids Taurine and Hydroxyproline. Moreover, glutamic acid, leucine, and alanine indicated the highest amount of 22.42%, 14.65%, and 9.43%, respectively, among other amino acids. The NER method was finally selected for functional properties evaluation as it showed a higher extraction yield and protein purity. The protein produced by NER method was further analyzed for its functional properties. The solubility of sorghum protein was evaluated at different pHs from 2 to 11, in which the highest protein solubility was at pH 8 to 11, while pH 2 to 6 revealed the lower solubility. Additionally, the water holding capacity (WHC) and oil holding capacity (OHC) of NER-generated protein also were evaluated, and the WHC was around 178% (1.78), which was lower than soybean and mung beans with 3.33 and 3.00, respectively, while the oil holding capacity of sorghum protein was 424%% (4.44), which was significantly higher than soybean and mung beans with 3.45 and 3.00, respectively.

Table 1. The extract yield and protein content of sorghum in NaOH and NaOH-Ethanol-Reducing agent (NER) Methods

Method	Extraction Yield (%)	Protein content of Extracted Isolate (%)
NaOH Method	1.00	56.11
NER Method	7.55	88.83

TOWARD UNDERSTANDING FACTORS ASSOCIATED WITH OUTBREAKS OF MOTILE *Aeromonas* SEPTICEMIA IN CHANNEL CATFISH (*Ictalurus punctatus*)

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Since the first outbreak of motile *Aeromonas* septicemia (MAS) in farmed catfish in southeastern United States in 2009, recurring outbreaks of MAS had resulted in the loss of millions of pounds of food-size fish annually. Fish mortalities in ponds ranged from 5 to 100%. A new pathotype of *Aeromonas hydrophila*, named virulent *A. hydrophila* (vAh), was identified and attributed to the cause of the outbreak. However, information is limited as for what nourished vAh population in pond water to reach a threshold level that caused acute outbreaks of MAS in fish.

In this study, the effects of nutrients in water on proliferation of vAh and severity of MAS in channel catfish were investigated. Results of the study revealed that both nutrient-rich tryptic soy broth powder (TSBp, the microbiological growth medium) and the commercial fish feeds supported vigorous growth of vAh in water (Figure 1). By addition of 6 g TSBp or 6 g of fish feed to 15 L of water, vAh multiplied from 2.4 x 10^5 CFU/mL to approximately 4.7 x 10^8 CFU/ml and 2.0 x 10^8 CFU/mL, respectively, at 24 h post inoculation (hpi). Challenge of fish in the vAh-propagated water at 24 hpi resulted in approximately 96% and 73% mortality, respectively. Finding s of this study suggest that, when fish were under environmental stresses and had poor appetite for feed supplied in the pond, the unconsumed feed provided vAh ample nutrients to propagate instantly and rapidly, which would result in outbreaks of MAS. Methods of culture of vAh *in situ* performed in this study was also shown to be useful for mimicking vAh growth dynamics in response to nutrient status in relatively natural environment.

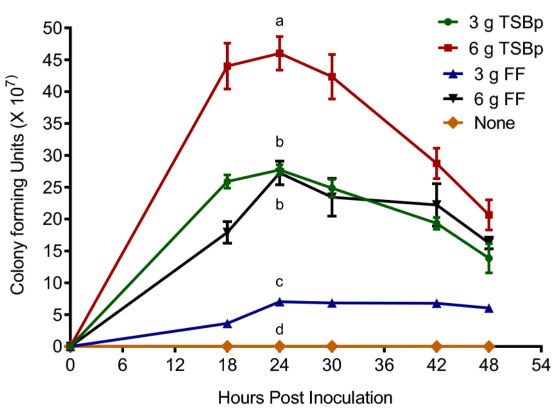

Figure 1. Growth dynamics of *A. hydrophila* in water supplemented with different media. A. hydrophila was inoculated in approximately 15 L of water held in an aquarium tank with initial concentration at 2.4 x 10^5 CFU per mL of water. Significant differences among means at 24 h post inoculation were marked with different superscript letters (p<0.05). TSBp: tryptic soy broth powder; FF: fish feed pellets; and None: nothing added

U.S. CONSUMER PREFERENCES AND ATTITUDES TOWARDS SEAWEED AND VALUE-ADDED PRODUCTS

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Seaweed, a novel food in western cuisine, is gaining popularity as consumers seek more healthy food options and alternative protein sources. Seaweed farming in the U.S. is an emerging and fast-growing aquaculture segment that offers fishermen, farmers, and food processors an opportunity to diversify operations or start a new business venture while providing ecosystem benefits to the ocean, including improved water quality. To help farmers better evaluate seaweed's market potential and develop strategies to enter or expand the market, it is necessary to gain a comprehensive understanding of U.S. consumers' preferences and attitudes towards seaweed and value-added products.

A few studies have investigated consumer preferences for name labeling, purchasing venues, production methods, production origin, and certification claims of seaweed products using samples in a specific state/region in the U.S. or foreign countries such as France. Other studies have analyzed consumer perceptions, attitudes, and purchase intentions towards seaweed products in Sweden, Spain, and Canada through in-person surveys or lab tastings. There is limited research on U.S. consumers' preferences and attitudes towards seaweed products, especially using a large-scale national sample. No existing studies distinguish between seaweed consumers and non-consumers and investigate their purchase intentions separately, an important perspective for seaweed farmers to evaluate consumer segments and make decisions on marketing strategies.

In this study, we aim to contribute to the aquaculture literature and industry by providing a comprehensive understanding of U.S. consumers' preferences and attitudes towards seaweed and value-added products. In particular, we study the consumers who consume seaweed and those who do not consume seaweed separately to compare their attitudes and examine the characteristics and factors affecting their attitudes. Our specific objectives include three perspectives. First, we explore the characteristics of U.S. seaweed consumers and non-consumers, including their knowledge, perceptions, and preferences for seaweed products. We also summarize the factors that the non-consumers perceive as barriers for them to consuming seaweeds and the factors that may encourage them to try seaweeds. Second, we compare the intentions to try new products flavored with or containing seaweeds between seaweed consumers and non-consumers and investigate the factors affecting their purchase intentions. Third, we provide suggestions about potential marketing strategies for the seaweed industry stakeholders to expand the seaweed market to meet consumers' needs.

THE IMPORTANCE OF CREATING SUPPORTIVE TECHNOLOGY FOR RAS AND FLOW-THROUGH SYSTEMS

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Land-based aquaculture is often an overlooked industry despite the widely held projection it is to experience significant growth within the next decade. One of the main appeals for RAS and flow-through systems is the plausibility of precision aquaculture. Partnering with RAS tech companies developing new technology for these underserved systems will help improve the return on investment required to build RAS facilities.

RAS offers a level of control that will help farms produce more by optimizing their operation beyond what is technically possible in the ocean. Supporting the creation of technology for tools like data retrieval and fish monitoring may be the move needed to help the industry grow and thrive. Supporting companies that can automate processes like feed disbursement, appetite monitoring, and biomass estimation will make land-based aquaculture much more appealing for investors and small companies looking to scale.

Many large-scale companies have begun to see these advantages and have begun to extend their post-smolts' stay in tanks. Seafood giants like Grieg have used this as a strategy to increase production in recent years. New and established companies in the land-based aquaculture industry are likewise projected to experience significant growth: Atlantic Sapphire is expecting to produce 25,000 metric tons of salmon per year in their next phase while Nordic Aquafarms recently gained key approvals for a huge expansion project in California and Maine.

Many aquatech companies focus on ocean-based farms to increase their profitability due to the larger addressable market, yet technology created for ocean-based aquaculture is not often transferable to RAS. Challenges like poor visibility due to stock density and water quality damage from overfeeding are not common issues in ocean-based aquaculture and therefore not understood by ocean-based tech companies.

Creating new technology that focuses on a burgeoning area of aquaculture requires a certain level of skill and innovation that is not a necessity in traditional aquaculture farming practices where technology continues to be developed and refined through decades of experience and shared knowledge. Developing new solutions for young companies that are not immediately profiting from investments is difficult and demands patience.

ReelData entered this industry in 2019 and found an oversaturated ocean-based market where dozens of tech companies had been dominating for decades. Upon further research, they found that the small and burgeoning industry of land-based aquaculture had yet to obtain support from well-known tech companies currently thriving in the ocean-based industry. With a highly creative and technical team, they were able to find early success with some of the biggest companies in land-based aquaculture including Atlantic Sapphire, The Kingfish Company, and Salmon Evolution.

The summer of 2022 might be an indication of what is in store for the land-based industry, as several companies announced the successful acquisition of permits for large facilities to be created across the globe. With these approvals secured, the aquaculture industry can be sure to experience a large surge in technology being developed to help support these farms and the many-thousand metric tons of fish they are projected to produce. As these farms become more established and begin to scale, tech companies will become increasingly more important for their ability to streamline aquaculture production.