Fixin' to Farm More Seafood

AQUACULTURE AMERICA 2021

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WELCOME

Welcome to Aquaculture America 2021! On behalf of the conference sponsors — the United States Aquaculture Society, the National Aquaculture Association (NAA) and the Aquaculture Suppliers Association (ASA), it is my pleasure to welcome you back to in-person conferences and to San Antonio, TX.

This year's theme is "Fixin' to Farm More Seafood" which I hope resonates with all of our attendees in one way or another. Over the last few years, we have watched as many aquaculture production metrics continue to increase annually. Yet, whether you are a farmer, a student, a researcher, a producer, an educator, an advocate, a commercial, private or public industry member, or yes even a regulator, you have done much more than watch, you have led in this progress. One of my favorite leadership quotes is "the role of leadership is to identify opportunities and remove obstacles". This is what this year's theme means to me as we work together to continue the increase in aquaculture production of all kinds both domestically and globally. Regardless of your individual roles or whether you lead from the front, the back, the side, or the top, what opportunities can you identify and what obstacles can you work to remove? This year, our Plenary speakers are a group of brothers from Kansas who operate what we think of as a traditional terrestrial farm. What lessons have they learned growing up and developing their business in an ever-changing world? What lessons can terrestrial agriculture teach us as many aquaculture sectors go through growing pains themselves? And what opportunities (and obstacles) are there in these shared spaces?

Of course, I would be remiss not to mention the COVID-19 pandemic. Many of us have not attended an in-person conference (and maybe not even travelled) in 16 months or more and many of us are seeing each other for the first time in much longer than expected. Production has been harmed, research projects upended, student graduation timelines extended, and lives and livelihoods changed. We will also hear from Dr. Carol Engle during the Plenary about "U.S. Aquaculture and the Pandemic: Impacts, Near Misses, The Future?", a topic we are just beginning to scratch the surface of and one that will continue to have ramifications on many facets of our daily industry operations.

Finally, I want to thank the other members of the Steering and Program Committees: Josh Patterson, Abigail Bockus, Matt Hawkyard, and Paul Zajicek. And as always, and maybe more so this year than most, John and Noah Cooksey and their staff for keeping the ship righted. Decisions on how to alter the conference schedule and program due to the pandemic were never made lightly. This group's determination and persistence, coupled with the flexibility you all have shown, are greatly appreciated, and have allowed us to be here this week to fully re-engage and be "Fixin' to Farm More Seafood" now, more than ever.

We hope you enjoy the conference, the trade show, the receptions, happy hours, and all that the history and culture of San Antonio have to offer.

Aaron M. Watson, Ph.D.
Steering Committee Chair
United States Aquaculture Society Vice President 2020-2022

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AQUACULTURE AMERICA 2021

Fixin' to Farm More Seafood

ABSTRACTS

FISH CELL CULTURES AND THEIR APPLICATIONS

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Cell cultures are employed in diverse research fields such as virology, physiology, toxicology, immunology, oncology, genetics, and pharmacology. These systems can be utilized for pathogen detection, confirmation, and characterization especially of viruses. It is also applicable in the case of intracellular bacteria, myxosporean or microsporean parasites. Fish cell cultures have gained more popularity in recent years and have prominent roles in viral disease diagnosis. Since treatment options are limited in the case of several viral diseases, early disease diagnosis and prophylactic measures are key for successful fish health management. Propagation of fish viruses *in vitro* using cell cultures is imperative in advancing research on viruses and to facilitate disease management strategies such as vaccines and antiviral agents. Moreover, potential host range of pathogens via cell culture susceptibility, virus-host cell interactions, and virus localization studies using cell cultures provide a better understanding of the viral pathogenesis. Availability of appropriate or host-specific fish cell cultures for propagation of viruses and disease diagnosis is very limited, which is a major concern in this area. The wide array of applications exemplifies the versatility, cost-effectiveness, and high potential of fish cell cultures in various research fields. The recent swift growth observed in research employing cell cultures is definitely an outcome of the progress in this sector and also due to increasing ethical demands for reduction in the use and replacement of animals in research. In near future, innovations in 3D cell culture and CRISPER-Cas9 genome editing will further enhance the research prospects of fish cell cultures.

EFFECTS OF PLANT-DERIVED AQUAFEED AND TEMPERATURE ON GROWTH, NUTRIENT UTILIZATION EFFICIENCIES, AND MITOCHONDRIAL FUNCTION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Rainbow trout (*Oncorhynchus mykiss*) are the most widely farmed trout in the world. Rainbow trout are carnivorous cold-water species that thrive in water temperatures ranging from 13°C to 18°C. The major challenges trout farmers currently face are expensive dietary protein and lipid sources; and rising global temperature.

A 4x2 factorial experiment was conducted to evaluate the effects of two dietary protein sources (fish meal and plant-based protein), two dietary lipid sources (100% camelina oil and a mixture of 50% fish meal and 50% camelina oil), and two water temperature regimens (14°C and 18°C) on the growth performance, nutrient utilization efficiencies, whole-body proximate composition, and mitochondrial enzyme complex activities in rainbow trout (*Oncorhynchus mykiss*) over a 150-day rearing period. Juvenile rainbow trout (average weight ± SD, 40 g ± 1.0 g) were randomly assigned to 24 glass aquaria (152 L) with 13 fish per aquaria. The 24 aquaria were randomly assigned to eight treatments with three replicates per treatment. The fish were fed to satiation twice daily for the entire duration of the experiment. Morphometric and quantitative measurements were taken every 30 days. Tissue was extracted from fish at the end of the experiment. The experimental results indicate that growth performance parameters such as feed efficiency (FE), and feed intake (FI) were significantly affected by diet ($P<0.05$). Whole-body proximate composition was not significantly affected by temperature ($P>0.05$) except for ash which was significantly affected ($P<0.05$). Nutrient utilization efficiency parameters such as protein efficiency ratio (PER), lipid efficiency ratio (LER), and protein productive value (PPV) were all significantly affected by diet ($P<0.05$). The results show that temperature had a significant main effect on complexes I, II, IV, V and citrate synthase in the intestine; complexes I, II, and citrate synthase activities in the muscle; and complexes I, II, and III activities in the liver ($P<0.05$). Diet had a significant main effect on complexes I, II, IV, V, and citrate synthase activities in the intestine; complexes I, II, III, IV, V, and citrate synthase activity in the muscle; and complexes I, II, IV, V, and citrate synthase activities in the liver. Temperature x diet interaction had significant interactive effects on citrate synthase activity in the intestine, complex II activity in the muscle, and complexes I and II activities in the liver.

Table 1: Degree of differential respiratory enzymatic (Complex I (CI), Complex II (C II), Complex III (C III), Complex IV (C IV), Complex V (C V) and Citrate Synthase (C S) activities in the muscle of rainbow trout fed practical diets for 150 days.

Muscle complexes							
Temperature °C	**DIET**	**C I**	**C II**	**CIII**	**C IV**	**C V**	**C S**
		Milliunits mg $^{-1}$ mitochondrial protein					
Individual treatment means							
14 °C	**DIET 1**	7.971	34.421^{b}	54.780	30.599	26.319	59.968
14 °C	**DIET 2**	7.780	31.767^{c}	59.292	28.800	23.252	59.872
14 °C	**DIET 3**	6.521	26.668^{d}	57.908	25.317	23.401	50.709
14 °C	**DIET 4**	5.654	26.186^{d}	59.335	20.609	19.805	35.760
18 °C	**DIET 1**	8.628	37.283^{a}	53.458	31.724	27.587	62.189
18 °C	**DIET 2**	7.971	35.132^{ab}	60.148	29.707	24.539	59.839
18 °C	**DIET 3**	7.267	32.635^{bc}	61.788	26.915	26.105	55.341
18 °C	**DIET 4**	6.331	25.777^{d}	56.887	23.378	22.097	37.811
Main effect means							
14 °C		6.966^{a}	29.760^{a}	57.829	26.331	23.194	50.856^{a}
18 °C		7.549^{b}	32.707^{b}	58.070	27.931	25.082	53.795^{b}
	DIET 1	8.268^{a}	35.852^{a}	54.119^{b}	31.162^{a}	26.953^{a}	61.078^{a}
	DIET 2	7.875^{b}	33.449^{b}	59.820^{a}	29.254^{a}	23.895^{ab}	58.413^{a}
	DIET 3	6.894^{c}	29.652^{c}	59.848^{a}	26.116^{b}	24.753^{a}	53.025^{b}
	DIET 4	5.992^{d}	25.982^{d}	58.111^{a}	21.993^{c}	20.951^{b}	36.786^{c}
ANOVA: P values							
Temperature		<.0001	<.0001	0.7934	0.0932	0.1041	0.0122
Diet		<.0001	<.0001	0.0002	<.0001	0.0060	<.0001
Interaction (T X D)		0.2201	0.0079	0.0901	0.8961	0.9584	0.8407

Means represent average values of three tanks, The LSD procedure was conducted for individual means because the two-factor interaction was significant. The LSD procedure was conducted for main effect means because the two-factor interaction was not significant. Main effect means and individual treatment means within a column followed by different letters found to differ at 0.05 probability level.

INTERACTIVE EFFECTS OF TEMPERATURE REGIMENS AND DIETARY COMPOSITION ON RAINBOW TROUT (*Oncorhynchus mykiss*) GUT MICROBIOME

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The microbiomes of fish represent complex communities consisting of protists, yeasts, bacteria, archaea, and viruses. These communities populate various regions in the body of fish such as, the skin, gastrointestinal tract, and the gills. The diversity and composition of organisms present in the microbiome are determined by several factors which include, diet, temperature, host genetics, and seasonality. In recent times, the gut microbiota has attracted the attention of nutritionists and health scientists. This is because of the significant role they play in host metabolism, immune response, and health.

A 4x2 factorial experiment was conducted to evaluate the effects of two dietary protein sources (fish meal and plant-based protein), two dietary lipid sources (100% camelina oil and a mixture of 50% fish meal and 50% camelina oil), and two water temperature regimens (14°C and 18°C) on the gut microbiome in rainbow trout (*Oncorhynchus mykiss*) over a 150-day rearing period. Juvenile rainbow trout (average weight ± SD, 40 g ± 1.0 g) were randomly assigned to 24 glass aquaria (152 L) with 13 fish per aquaria. The 24 aquaria were randomly assigned to eight treatments with three replicates per treatment. The fish were fed to satiation twice daily for the entire duration of the experiment. Intestinal digesta was extracted from fish at the end of the experiment. The results indicate that temperature affected the gut microbiome diversity and some metabolic functions ($q<0.05$). In the gut of fish reared at 18°C, there was a higher abundance of genes involved in environmental information processing, metabolism, and cellular processes. The dominant phyla were Proteobacteria and Firmicutes. The most dominant order was Mycoplasmatales. The results also show that temperature x diet interactions affected microbial functional diversity; higher temperature and higher inclusion of dietary plant ingredients correlated a higher abundance of functional genes involved in carbohydrate metabolism, virulence and diseases, and regulation and cell signaling. The results did not show any consistent significant effect of diet on the diversity and function of the rainbow trout gut microbiome. The higher mitochondrial complex activities and numerically lower growth performance and nutrient utilization efficiency (not significant at $p>0.05$) observed in fish reared at 18°C could suggest that the rainbow trout used in this experiment generated significantly higher energy to maintain homeostasis or cope with stress. This same temperature effect was observed in the gut microbiome diversity and function where fish reared at 18°C displayed higher heterogeneity between samples, were enriched with more abundant taxa, and had higher abundance of genes involved in adapting to the host environment. The study generally showed that rainbow trout can be grown to market size without significant reduction in growth rate, and other performance characteristics including gut microbiome if given a plant-based diet with a 50/50 mixture of animal and vegetable oil.

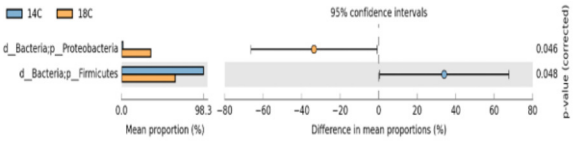

Figure 1: Extended bar plot showing the Top 2 most abundant bacteria phylum in fish gut samples grouped according to temperature reared (14°C & 18°C) (q<0.05).

A QUANTITATIVE SWOT ANALYSES OF KEY AQUACULTURE PLAYERS IN AFRICA

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Africa's contribution to world aquaculture production in 2018 is still insignificant (2,196,600 tons; ~ 2.7 %) albeit significantly increasing with larger-scale investments in Egypt (1,561,457 tons), Nigeria (291,233tons) and Uganda (103,737 tons) producing substantial quantities (91 %) of the total fish production from the region. The quantitative SWOT technique and the Multi-Attribute Decision-Making method (MADM) were used to determine and compare aquaculture performance in production, technology, market, policies and framework among Egypt, Nigeria, Uganda and South Africa. Factors such as the adoption of new technologies used in the formulation and production of aquafeed (i.e. extruded feed and sinking feed); adoption of best farm management practices; commercialization of aquaculture, growing demand-supply gap due to high per capita consumption of fish and government's prioritization of aquaculture industry development through the creation of enabling environment for private sector participation were key strengths and opportunities identified amongst the leading players. The quantified SWOT shows Egypt exhibited aquaculture development strengths and opportunities; Nigeria has aquaculture development opportunities but weak in competitive strengths; while Uganda and South Africa both possess low, competitive strengths and being faced with threats. Capitalizing on the available opportunities and critical success factors of the leading aquaculture players in Africa, this analysis highlights strategic actions that could boost the development of aquaculture in South Africa. The quantified SWOT analysis was used to determine the competitive position of the aquaculture sectors of the compared countries and can be used as a basis for aquaculture policies and roadmaps.

THE MANAGEMENT OF ALDABRA MARINE PROTECTED AREA (MPA) OF REPUBLIC OF SEYCHELLES

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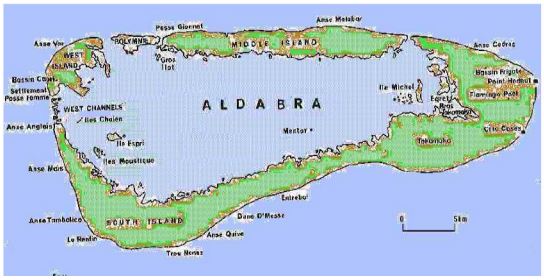
Marine protected area is any area of intertidal or subtidal terrain, together with its overlying water and associated flora, fauna, historical and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment. The term MPAs include marine parks, marine reserves, seagrass beds, shipwrecks, archeological sites, tidal lagoons, mudflats, saltmarshes, mangroves, rock platforms, seabed in deep water.

Aldabra MPA is located on a raised coral atoll in the Indian Ocean at 9^0 24' S, 46^0 22' E, southwest of Mahé, Seychelles. The MPA has a total area of 190km^2. The Aldabra environment and resources provide the Seychellous government valuable goods and services including the fisheries, tourism, seafood and employment. The MPA contains unique and important natural habitats that possess tourism quality and as well as evolutionary and ecological studies. It is recognized as the home for high population of giant tortoise The Aldabra MPA is managed by the Seychelles Island Foundation (SIF). The MPA is finance by various organizations including the UNDP and the GEF to support the sustainability of the area and to maintain its status as one of the UNESCO World Heritage Site. MPAs are controlled or managed by local, state, territorial, regional or national authorities. The designation of authorities differ substantially from nation to nation. Individual country has the legal responsibility for the designation and enacting legislation which governs their MPAs. Proper management of MPAs is very important in conserving and protecting the biodiversity of the ecosystem. This study reviews the management of Aldabra Marine Protected Area of republic of Seychelles.

Figure 1 Map of Seychelles Island

Figure 2 Map of Aldabra



GENDER DIMENSION, ACCESS TO AND CONTROL OVER LAND RESOURCE AMONG AQUACULTURE MARKETERS IN SOUTHWESTERN, NIGERIA

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Gender relations have received significant attention in literature on the Nigerian aquaculture sector, however focused has been on the fish producers, and not on other actors such as the fish marketers. Although, male and female have equal rights and benefits from developmental resources, little studies have shown gender involvement in the allocation of land resources among fish marketers in South West Nigeria. Therefore, this study was aimed to gather information on male and female access to land, control over land, and determinants of factors, that influence access to and control over land resources among fish marketers in Lagos and Oyo States, Nigeria.

Multistage sampling method was used to select fish marketers of catfish and tilapia in Lagos and Oyo States, by proportionate sampling of Agricultural Development Programme (ADP) zones in each states. Total number of respondents used for the study was 202. Stratified sampling method was used to select the respondents in each states. Primary data was collected through in-depth interviews, focus group discussions, and structured questionnaires. Data was collected on socioeconomic characteristics (primary occupation PO and secondary occupation SO), age, marital status, and education. Land access by gender, control over land resource, and factors influencing land access and control were determined between the states. Harvard Analytical Framework was used to determine gender access to and control over land resource in the two states. Statistical analyses used include descriptive statistics and probit regression analysis.

In Lagos State (LS), majority of males (61.5%) and females (55.6%) practice fish marketing (FM) as a secondary occupation, while in Oyo State (OS), all males and females practice FM as their primary occupation. A higher percentage of females (42.6%) were recorded to have access to land in LS, while males (76.9%) have control over land resource in LS; but in OY access to and control over land resource were dominated by males. Marital status (-0.272;-0.271) and age (-0.131, -0.144), education (-0.64,-0.063) are determinants of access to and control over land among fish marketers respectively . There were significant differences in access to and control over land resource between both states ($p<0.05$), but education was significantly different for both state at ($p<0.1$). Favorable regulatory structures, access to and control over" productive resource, and gender perspective in the allocation of land among aquaculture fish marketers, would prevent uneven accessibility of productive resources. Gender aware policies will encourage fair and impartial involvement of male and female in fish marketing, and in turn help in achieving Sustainable Development Goals (SDGs) on gender equality.

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

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A nine week experiment was conducted to test the effects of supplementation of inulin and mannan-oligosaccharides (MOS), single or combined, on the growth performance, feed utilization, fatty acid profiles, and gut morphology of Chamo strain Nile tilapia *Oreochromis niloticus* (L.) fry reared at sub-optimal temperature. Nile tilapia fry with initial weight of 1.623 ± 0.074g were used in this study. Four dietary treatments were formulated to incorporate prebiotics at 0 g of prebiotic/kg of fish feed (Diet-T_1), 5 g kg^{-1} inulin-supplemented diet (Diet-T_2), 6 g kg^{-1} MOS-supplemented diet (Diet-T_3), or combination of 2.5 g kg^{-1} inulin and 3 g kg^{-1} MOS-supplemented diet (Diet-T_4). Each diet was randomly assigned to three aquaria and hand-fed 8% of their body weight divided into three portions daily. The results showed that Nile tilapia fed diet Diet-T_4 had the highest final weight, weight gain and specific growth rate (SGR) than fish fed Diet-T_1, Diet-T_2 and Diet-T_3 although it was not affected significantly ($p > 0.05$). Fish fed Diet-T_4 also had lowest FCR than other feeding groups. Diet-T_4 and Diet-T_3 had highest content of polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA; 22:6 *n*-3) and eicosapentaenoic acid (EPA; 20:5 *n*-3) than Diet-T_1 and Diet-T_2. Gut morphology parameters revealed significant ($P < 0.05$) increase in villus length (VL) and goblet cell number (GCN) of fish fed Diet-T_4 and Diet-T_3. Overall, this study suggested that supplementing fish feed with MOS alone or with combination of inulin improved growth performance, intestinal morphology and fillet quality of Chamo strain Nile tilapia.

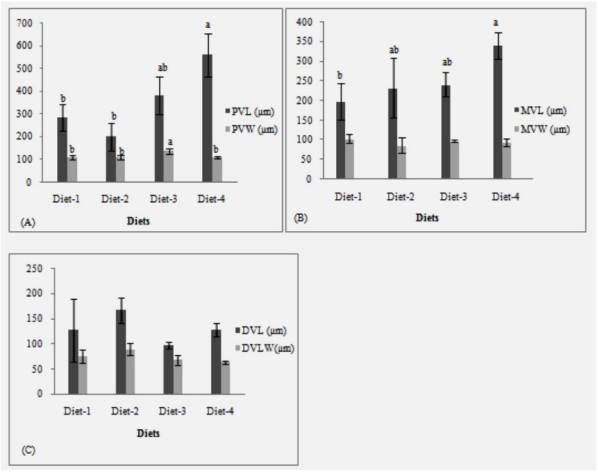

Figure 1. Graph showing proximal villi length (PVL) (A), middle villi length (MVL) (B), and distal villi length (DVL) of small intestine of Nile tilapia in µm

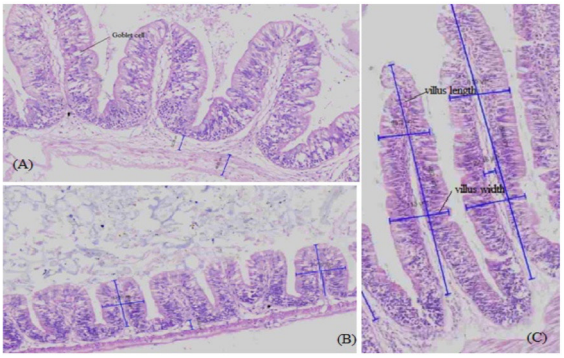

Figure 2. Gut morphology slide samples scanned by MoticEasyScan Pro 6 (USA) (arbitrarily chosen as examples): goblet cell (A), villi length and width (B& C)

AVIAN PREDATION ON LOW-SALINITY SHRIMP AQUACULTURE

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Pacific white shrimp (*Litopenaeus vannamei*) is the most commonly produced shrimp in the world and prominent in the seafood industry in the United States. Aquaculture producers in the United States raise >2,000 metric tons of shrimp each year using various low-salinity water (LSW) sources. Although many bird species frequent aquaculture facilities and are known or suspected of consuming shrimp, no studies have examined the impact these birds may have on final yield. Therefore, our objectives were to 1) assess the distribution and relative abundance of predatory birds on commercial shrimp farms in Alabama and Florida, 2) quantify the diet of these birds, 3) and estimate the total amount of shrimp consumed annually.

During May-October 2020, we conducted biweekly surveys to estimate the diversity and relative abundance of birds and then conducted collections of individuals observed actively foraging around shrimp production ponds at farms in Alabama and Florida. Collected birds were injected immediately with cold (<10° C) phosphate buffered saline to halt digestion and placed on ice. Necropsies were then performed to determine the diets of each bird. A total of 58 birds (7 species) were collected with most (n=34) being collected closer to the harvesting months of September and October. Of these 34 birds, 21 (61.8%) had consumed shrimp with Pied-billed Grebes (*Podilymbus podiceps*), Great Egrets (*Ardea alba*), and Double-crested Cormorants (*Phalacrocorax auritus*) consuming the most shrimp. Pied-billed Grebes consumed an average 1.67g dry weight shrimp/bird, Great Egrets consumed 1.85g, Double-crested Cormorants consumed 5.34g, Great Blue Herons consumed 1.32g, and Little Blue Herons consumed 0.47g (Figure 1). We found that only select avian predators consume shrimp and do so closer to harvest when shrimp are mature and pond waters are lowered suggesting that management actions to mitigate losses may be targeted to a few species and may be most effective immediately before shrimp are harvested.

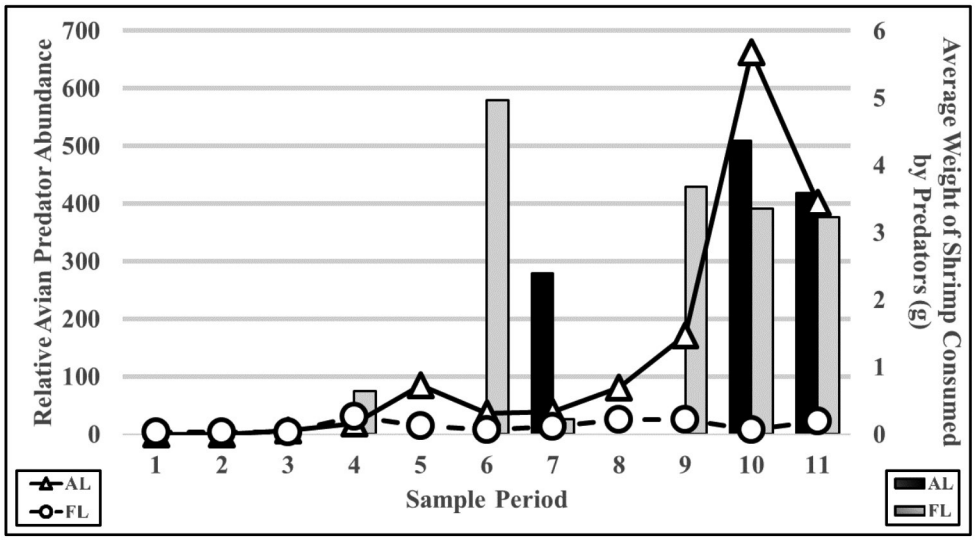

Figure 1: Relative Predator Abundance & Average Shrimp Consumption

ROE ENHANCEMENT OF THE PURPLE SEA URCHIN *Strongylocentrotus purpuratus* COLLECTED FROM BARREN GROUNDS AND FED PREPARED DIETS

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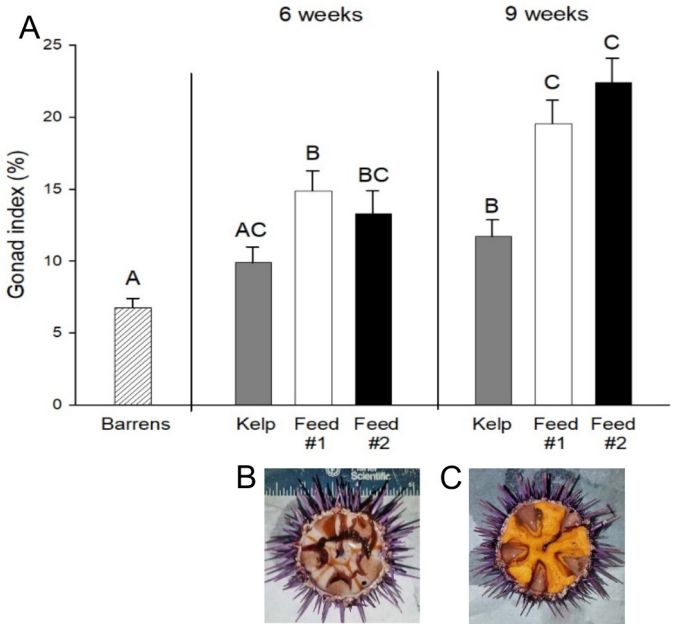
Over the last decade, an overabundance of purple urchins (*Strongylocentrotus purpuratus*) that exert destructive grazing pressure on essential kelp forest habitat has caused a rapid expansion of barren grounds along the west coast of North America. Although the harvesting of several urchin species has led to high-value fisheries worldwide, purple urchins presently have little to no commercial value, especially in a malnourished, barren condition. Harvesting purple urchins from barrens and enhancing their roe through aquaculture has the potential to transform these destructive grazers into high-quality seafood that also benefits ecological restoration of kelp forests. We collected purple urchins from barrens and fed them whole kelp or two different formulations of prepared feed in a recirculating aquaculture system. The gonad index (GI) of urchins fed the two prepared diets doubled in 6 wk, increased from a barren condition to a marketable yield (GI > 15%) in 9 wk, and was significantly greater than the GI of urchins fed kelp. Analyses of proximate constituents and amino acid composition of gonad tissue also revealed differences among the roe of urchins fed the three diets for 9 wk. In particular, one of the two prepared feeds resulted in a significantly greater amount of bitter-tasting amino acids in enhanced gonads compared to urchins fed whole kelp, while the other prepared formulation indicated a more natural flavor profile. Our results highlight an untapped potential to quickly produce a highly valued seafood product from seemingly low-value purple urchins. Echinoculture could thereby serve as a tool to stimulate urchin industries that also facilitates the restoration of kelp forests from urchin barrens.



DEVELOPMENT OF AN *Enterocytozoon hepatopenaei* (EHP) CHALLENGE MODEL IN SCREENING GENETIC LINES OF *Penaeus vannamei* SHRIMP

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Hepatopancreatic microsporidiosis (HPM) caused by a microsporidium, *Enterocytozoon hepatopenaei* (EHP) is currently one of the major pathogens of penaeid shrimp that is causing enormous losses in shrimp production in Asia. Recently, the disease has been reported from Venezuela as well in the western hemisphere. One of the limitations in developing HPM resistant line of shrimp is the lack of a robust and reproducible laboratory experimental challenge method that could be used for screening genetic lines of penaeid shrimp.

Herein, we report an *E. hepatopenaei* experimental challenge model using two Specific Pathogen Free (SPF) *Penaeus vannamei* shrimp lines originating from different geographic locations. The shrimp (weight range 6.0 to 7.0 g) from each population were tagged with a unique elastomer tag, maintained in the same tank before exposing to fecal strings from known EHP-infected shrimp as inoculum. Fecal strings were collected daily by siphoning from one 1000 L tank containing 60 EHP-infected shrimp. Histopathology and real-time PCR assays were conducted for confirmation of EHP infection in the challenged animals. The final survival was equivalent in both population (i.e. ~90%), but the prevalence of EHP varied between the two populations. SPF1 had significantly higher growth than SFF2 line (for both EHP-challenged and unchallenged treatments tanks).

Histopathology revealed typical EHP lesions in both challenged groups. Real-time PCR data showed prevalence of EHP at 77% and 52% in SPF 1 and SPF2, respectively. Considering the shrimp from SPF1 and SPF2 were maintained and experimentally challenged under the same tank environment, the difference in the severity of infection observed in these two groups is likely due to the inherent genetic background. The data suggest that experimental challenge using fecal string as an inoculum source would be a viable approach in screening for EHP resistance in penaeid shrimp and in developing therapeutics/ functional feed against EHP.

FLOATING NURSERY RACEWAY PROOF OF CONCEPT FOR PRODUCTION OF PHASE I FINGERLING LARGEMOUTH BASS (*Micropterus salmoides)*

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Intensive production of largemouth bass (LMB) requires production of fingerlings approximately one month old (phase I) for feed training. Intensive indoor systems are constrained by foods required by the fish. Dedicated nursery ponds can be managed for abundant food, but recovery of fish, weed problems, and damage during harvest are issues. Cannibalism may occur if fry of different sizes from different spawns are stocked together. In this study, a floating raceway was designed as a nursery for LMB giving them access to natural food from the pond, while being confined in the raceway.

Three floating nursery raceways with a volume of 1700 L, designed and constructed for production of phase I LMB were each placed in a separate 0.1-acre pond. Three 0.1-acre ponds were managed as a control treatment. All six ponds were fertilized with organic (chicken crumbles and soybean meal) and inorganic (18-46-0) fertilizer to produce an abundant plankton bloom as food for the LMB. Fish were not fed a prepared diet. An electric pump delivered a flow of plankton rich pond water at 57 L/min into each raceway. Each pond and raceway unit was stocked with 6,000 fry, seined, then drained at harvest.

Mean recovery of LMB fingerlings after 27 days was 98% from nursery ponds and 41% (range 1.6 to 77%) inside the floating raceway and 48% outside the raceway. Average weight of fish was 1.4 g in ponds, 0.7 g inside, and 1.7 g outside the raceway. Though growth trended higher in ponds variation within treatment was great, making it impossible to demonstrate statistical significance.

The floating nursery successfully produced phase I LMB, but escapement and food availability arose as issues that can be addressed. Harvesting fish in the floating nursery (photo) took less labor and was faster than harvesting the fish in a pond. The suspended floating raceway design used in this study has no docks or posts. It is easily constructed with cost for materials of approximately $500/unit.

GENETIC OVERVIEW TO REVEAL HOW THE CAPTIVE POPULATION WAS, IS, AND SHOULD BE MANAGED

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Genetic Overview (GO) is a compelling service to characterize populations in captivity that are usually managed for commercial purposes. Using a set of software tools and calculations the GO report describes the levels of diversity, consanguinity, and the genetic structure of the sampled population. These proprietary techniques of the Center of Aquaculture Technologies make use of all types of Single Nucleotide Polymorphisms (SNP) arrays: Low Density (LD), Medium Density (MD) and High Density (HD). These arrays are now available for a variety of aquatic species, including shrimp (*Litopenaeus vannamei*), oysters (*Crassostrea gigas* and *Crassostrea virginica*), and fish (salmon; (*Salmo salar),* Red snapper (*Lutjanus peru),* cobia (*Rachycentron canadum),* yellowtail (*Seriola rivoliana, Seriola lalandi),* halibut (*Hippoglossus hippoglossus),* sable fish (*Anoplopoma fimbria*), among others. The option of design and customization of genomic tools for non-traditional aquaculture species is also available.

The present work describes the parameters of GO used to characterize these cultured stocks and its biological meaning. To protect the confidentiality, synthetic (simulated) data is used for this purpose. Four fictitious breeding lines; AA, BB, CC, and DD are compared to show the meaning of diversity parameters such as the percent of polymorphic loci, the heterozygosity (observed and expected) and the Nei's diversity index. The extent of consanguinity is assessed by pairwise comparisons of the relatedness coefficient (r). Finally, the structure of the breeding lines based on their differentiation or similarity is explained using the interspecific variation coefficient (Fst), the Principal Coordinates Analysis (PCoA) and the Structure plots.

Some of the applications of this type of analysis will be further summarized. It is particularly powerful in monitoring genetic health over time in breeding programs based on mass selection but is broadly applicable to all programs. In general terms, the Genetic Overview is a snapshot of the current state of the population genetic characteristics. It is highly recommended at the beginning of the breeding programs of all types to understand the level of diversity and relatedness, and the history that the DNA reveals about previous management and origin of the lines, this helping to device sound breeding strategies. Adopting GO into the hatchery practice will help to evaluate the impact of selection and assess the genetic quality of the lines, guide the mating decisions to maximize diversity and decrease the rate of inbreeding/relatedness, and consequently provide an evaluation of the genetic management through the production process.

MARKET OPPORTUNITIES FOR U.S. AQUACULTURE PRODUCERS: THE CASE OF BRANZINO

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The US is the world's largest seafood importer by value, with an increasing share of imports. Despite numerous policy initiatives, production and growth in the US aquaculture sector is limited. In this paper the recent success of imported branzino is used to show that the market is not a constraint. Branzino is known as sea bass in Europe and is a portion sized white fleshed fish primarily farmed in the Mediterranean, with no obvious equivalents produced in the US. Since the turn of the century imports have grown from zero to almost 10,000 mt, a quantity that would have made it the 4th largest farmed fish species if produced in the US. Until 2013, Greece was the main supplier, while in recent years Turkey has been the most important source, indicating how any producer creating a market opportunity will face keen competition when succeeding.

From 2015 when the quantities became more significant, the species entered the large whitefish market. However, branzino has a significant price premium relatively to tilapia, the largest species in this market, indicating that the opportunity to create separate niches in the seafood market is limited.

VENTURA SHELLFISH ENTERPRISE: REALIZING OPPORTUNITIES FOR OFFSHORE AQUACULTURE IN CALIFORNIA

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Increasing the supply of safe, sustainably produced domestic seafood is a priority for the Department of Commerce and National Oceanic and Atmospheric Administration (NOAA). Globally, bivalve shellfish culture has been very successful in producing high value seafood with limited environmental impacts. However, the current complexities and costs associated with US aquaculture permitting represent significant barriers to industry expansion. Over the past six years, Ventura Port District and its volunteer partners have sought to establish the Ventura Shellfish Enterprise (VSE) project, a new offshore mussel farm in federal waters that would eliminate or reduce these permitting hurdles for private shellfish companies. The project is designed to accommodate turnkey participation by members of the Ventura working waterfront community via pre-permitted parcels for mussel culture. Working in collaboration with NOAA and other regulatory agencies, a number of tools have been developed that can assist private shellfish companies that may want to establish a farm in the Santa Barbara Channel or elsewhere in California.

Deliverables executed by the Ventura Port District, its volunteer partners, and consultant team to support this permitting effort include: a comprehensive permitting analysis; detailed permit applications; comprehensive siting analyses developed by NOAA's National Ocean Service to minimize conflicts among marine uses; engineering analyses and optimization studies to assure that the selected longline gear can endure 100-year storm events; a navigational risk assessment requested by the U.S. Coast Guard; a detailed Operations Plan including a biological assessment, draft mitigation plan, and draft monitoring/reporting plans; detailed economic analysis of the benefits of offshore aquaculture; and the project pro-forma. Project efforts include extensive public outreach and collaboration with NOAA and the Food and Drug Administration (FDA) to ensure compliance with the National Shellfish Sanitation Program (NSSP) and Seafood Sanitation Inspection Program (SSIP) guidelines, including an ongoing sentinel study.

This presentation will discuss the lessons learned during this process as Ventura Port District engaged in outreach with key stakeholders, such as commercial fishing interests and environmental NGOs, and addressed concerns raised by regulatory agencies. The presentation also will provide a broad overview of the tools developed during this process and how they can be utilized by private aquaculture companies; upgrades to the Ventura Harbor infrastructure to support aquaculture; and recent sentinel data confirming that the Santa Barbara Channel provides a superb growing area for offshore aquaculture.

AN INNOVATIVE WASTE-TO-FOOD RECIRCULATING AQUACULTURE SYSTEM FOR WASTEWATER REUSE AND ANTIBIOTIC-FREE AQUAFEED PRODUCTION

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Recirculating aquaculture system (RAS) plays a vital role in the sustainability and commercial viability of aquaculture that provides a significant protein source to human nutrition. Despite that RAS reduces water demand and allows for year-round intensive production, intensive aquaculture leads to common challenges such as wastewater/waste management, high feed cost, and most importantly, the widespread use of antibiotics and the increasing ineffectiveness of antibiotics against aqua species pathogens. Therefore, there is an urgent need for a cost-effective approach to overcome these challenges for sustainable aquaculture.

This study developed a novel RAS-polyhydroxybutyrate (PHB) system (called RAS-PHB) to address the challenges described above. The RAS-PHB system can produce PHB-rich biomass (single-cell proteins, SCPs) as healthy aquafeed from aquaculture wastewater/wastes. The PHB-rich SCPs are rich in protein and contain high PHB as a biocontrol agent and immunostimulant, eliminating the need for antibiotics and improving the survival and growth of aquatic species. The configurations of RAS-PHB system and conventional RAS are illustrated in Figure 1. Briefly, the sand filter in conventional RAS (Figure 1A) is replaced with a zeolite sorbent unit to recover nutrients (particularly ammonia). The biofilter is also substituted with a nonsterile bioreactor for cultivating PHB-producing SCPs using nutrients recovered from the sorbent unit and supplemental carbon sources like agro-industrial wastes. Finally, chitosan as a nontoxic, healthy biocoagulant was used to harvest PHB-rich SCPs as an aquafeed (Figure 1B). The composition of the PHB-rich SCPs produced from various wastes was analyzed and compared to conventional aquafeeds. A simple analysis was performed to compare the economic advantage of using the proposed RAS-PHB over the traditional RAS system.

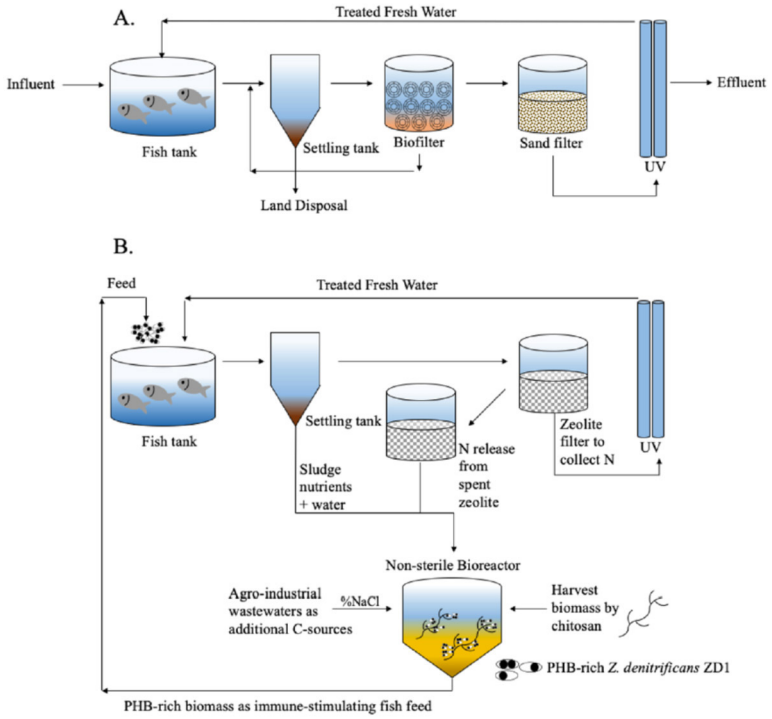

Figure 1. (A) Conventional Recirculating Aquaculture System (RAS)
(B) Proposed RAS-PHB system

FERTIGATION MANAGEMENT FOR SOILLESS CUCUMBER PRODUCTION USING AQUACULTURE EFFLUENT WITH AND WITHOUT SUPPLEMENTATION

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An experiment was conducted to determine optimum fertigation duration for cucumber grown using aquaculture effluent (AE) from Nile tilapia, with and without nutrient supplementation compared to conventional hydroponic (cHP) nutrient solution. A split plot design nested in a randomized complete block with four replications was used. Main-plot factor was fertigation duration (1, 2, 3, or 4 minutes) and subplot factor was nutrient solution type (cHP, supplemented AE, or non-supplemented AE). Fertigation was supplied every 30 during daylight hours. Analysis of variance showed that, plants grown in cHP solution had significantly ($p=0.0343$) lower (19.55 μmol [CO_2] m^{-2} s^{-1}) leaf photosynthetic capacity (A_{max}) than both supplemented and non-supplemented AE (21.13 and 20.78 μmol [CO_2] m^{-2} s^{-1} respectively). The effect of fertigation duration on A_{max} also depended on nutrient solution type (interaction effect; $p=0.0342$). Stomatal conductance decreased with increasing fertigation duration, significantly lowering at 4 minutes (432.9 mmol [H_2O] m^{-2} s^{-1}). The chlorophyll index (SPAD value) measured at 28 days after transplanting showed significantly ($p=0.0339$) lower value for non-supplemented AE (34.84) than cHP (36.45) and supplemented AE (36.02) but was not affected by fertigation duration or its interaction with nutrient solution type. There was no significant ($p>0.05$) effect of the factors or their interaction on yield recorded so far. By adjusting fertigation duration and achieving significantly similar yields imply reducing fertigation duration could increase water and nutrient use efficiency. Also, the comparative yield performance of non-supplemented TE with cHP and supplemented TE shows promising potential in using tilapia effluent for soilless cucumber production.

LAND BASED CULTURE OF OLIVE FLOUNDER *Paralichthys olivaceus*: OPPORTUNITY FOR INCREASING MARINE FISH AQUACULTURE PRODUCTION IN THE UNITED STATES

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On the east coast of the United States, the overexploitation of flatfishes has been increasingly apparent in recent years. With the decline in U.S. landings of flatfish species in the Atlantic states, more stringent management regimes have been created to rebuild these stocks. These flatfish fisheries have historically been of high economic importance to working waterfront communities. With reduced quotas and other management restrictions for flatfish species in these states, working waterfront communities have been heavily impacted. Reducing harvest quotas can stunt economic prosperity, as well as force fishers to find other sources of income. While such situations are not unique to fishing communities throughout the U.S., there is the potential for sustainable flounder aquaculture to be implemented in regions historically reliant upon wild flatfish landings. These working waterfronts stand to benefit from supplementing wild caught flatfish demand with a farm-raised alternative in an already established market.

The olive flounder, *Paralichthys olivaceus*, is a commonly cultured species in both Japan and Korea and is often referred to as *hirame*. This species has a low FCR, high growth rates for flatfish species, and can be grown at high stocking densities compared to other commonly cultured marine finfish species. At the University of Miami Experimental Hatchery (UMEH), broodstock olive flounder volitionally spawn regularly and produce high quality fertilized eggs. Recent efforts with this species have been focused on developing grow-out production economic models for this species in pilot-scale land-based seawater rearing systems while also significantly increasing seedstock supply through nursery production trials to service prospective farmers in the U.S. At the UMEH facility, seedstock production begins following a broodstock spawning with the collection and stocking of fertilized embryos into larval rearing tanks. The larval olive flounder are reared on rotifers, *Artemia*, and micro diets before being transferred to raceway tank nursery systems by day 45 post hatch. Juvenile fish in these raceways are typically stocked at densities of 2.1 - 4.4 kg/m^3 (0.6 - 1.1 kg/m^2), and final densities in these raceways range from 96 - 247 kg/m^3 (25 - 47 kg/m^2). During their time in these nursery systems, juvenile flounder are transitioned onto progressively larger dry feeds, with high survival and low FCRs over the course of this period. Results of recent seedstock production and nursery trials will be presented, as well as other aspects of this flounder project supported by the Atlantic States Marine Fisheries Commission (ASMFC) and the National Oceanic and Atmospheric Administration (NOAA). The consistent production of flounder at high densities in land-based aquaculture systems supports the fact that olive flounder is a prime species candidate for land-based aquaculture in the U.S. and offers an exciting opportunity for diversification in working waterfront communities of the U.S.

THE EFFECTS OF TWO DIETARY SYNBIOTICS ON GROWTH PERFORMANCES, HEMATOLOGICAL PARAMETERS, AND IMMUNE RESPONSES IN JAPANESE EEL, *Anguilla japonica* AND NILE TILAPIA *Oreochromis niloticus*

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Two experiments were conducted effects of two dietary symbiotic; *Bacillus subtilis* with mannan oligosaccharides and *Enterococcus faecium* with fructo-oligosaccharides were evaluated on growth performances, hematological parameters and immune responses in Japanese eel, *Anguilla japonica* and Nile Tilapia *Oreochromis niloticus* for 8 weeks. Exp.1: Six experimental diets consist: a basal diet (CON), an antibiotic diet containing CON with 4 g kg-1 of oxytetracycline (OTC), and 4 synbiotic diets; *Bacillus subtilis* at 10^7 or 10^8 CFU g^{-1} plus 4 g kg^{-1} mannan oligosaccharides (BS_7 and BS_8) and *Enterococcus faecium* at 10^6 and 10^7 CFU g^{-1} plus 4 g kg^{-1} fructo-oligosaccharides (EF_6 and EF_7). Eighteen tanks of fish averaging of 6.00 ± 0.07g (mean ± SD) were randomly assigned into six experimental diets in 3 replicates. Weight gain (WG) and specific growth rate (SGR) of fish fed BS_7 and BS_8 diets were significantly higher than those of fish fed CON and OTC diets (P<0.05), however, there were no significant differences among those of fish fed BS_7, BS_8, EF_6, and EF_7 diets. WG and SGR of those of fish EF_6 and EF_7 were significantly higher than those of fish fed CON diets (P> 0.05). Feed efficiency of fish fed BS_7, BS_8 and EF_6 diets were significantly higher than those of fish fed CON and OTC diets, however, there were no significant difference among fish fed BS_7, BS_8, EF_6, and EF_7; among those of fish fed EF_7 and CON; and among those of fish fed OTC and CON (P> 0.05). Protein efficiency ratio of fish fed BS_7, BS_8, EF_6, and EF_7 diets were significantly higher than those of fish fed CON and OTC diets (P < 0.05). Average myeloperoxidase activity of fish fed BS_7 diet were significantly higher than those of fish fed CON, OTC, BS_7, EF_6, and EF_7 diets (P < 0.05). After the challenge test by *Vibrio anguillarum*, average cumulative survival rates of fish fed BS_8 were significantly higher than those of fish fed CON diet, however, there were no significant differences among those of fish fed OTC, BS_7, BS_8, EF_6, and EF_7 diets (P > 0.05). Exp.2: Six experimental diets include a basal diet (CON), a positive control diet (OTC) with antibiotics (0.4% oxytetracycline of diet) and 4 synbiotic diets consisting the basal diet with B. subtilis (1×10^7 or 1×10^8 CFU g-1) with 0.4% of mannan oligosaccharide (BS_7M or BS_8M) and the basal diet with E. faecium (1×10^6 or 1×10^7 CFU g-1) with 0.4% of fructo-oligosaccharide (EF_6F or EF_7F). Eighteen tanks of fish averaging 4.8 ± 0.05 g (mean ± SD) were randomly assigned into six experimental diets with three replicates, and fish fed the experimental diets for eight weeks. Weight gain of fish fed OTC, BS8M, EF_6F and EF_7F diet were significantly higher than those of fish fed CON diet (P<0.05). Weight gain of fish fed EF_7F diet was significantly higher than those of fish fed BS_7M diet although there were no significant differences among fish fed OTC, BS_8M, EF_6F and EF_7F diet. Specific growth rate of fish fed OTC, BS_7M, BS_8M, EF_6F and EF_7F diet were significantly higher than those of fish fed CON diet (P<0.05). Specific growth rate of fish fed EF_7F diet was also significantly higher than those of fish fed BS_7M diet although there were no significantly differences among fish fed OTC, BS_8M, EF_6F and EF_7F diet. These results suggest that four synbiotics; BS_7, BS_8 (*Bacillus subtilis* at 10^7 or 10^8 CFU g-1 plus 4 g kg-1 mannan oligosaccharides) EF_6, EF_7 (*Enterococcus faecium* at 10^6 and 10^7 CFU g-1 plus 4 g kg-1 fructo-oligosaccharides) could be the potential feed additive and antibiotics replacer in Japanese eel and Nile tilapia feeding and farming.

EVALUATION OF DIETARY MICRO-ALGAE *Schizochytrium* sp. AS A FISH OIL REPLACER IN RAINBOW TROUT, *Oncorhynchus mykiss*

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Two experiments were conducted to evaluate the dietary micro-algae, *schizochytrium* sp. as a fish oil (FO) replacer in rainbow trout, *Oncorhynchus mykiss*.

Exp.1: Seven diets were formulated to replace FO at 0% (CON), 20% (T20), 40% (T40), 60% (T60), 80% (T80) and 100% (T100) by using *schizochytrium* sp., and 38% of commercial Micro algae (CMA) diet. Triplicate groups of 20 fish averaging 3 ± 0.4g (mean ± SD) were fed one of seven experimental diets. After the 8 weeks of feeding trial, weight gain (WG), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER) of fish fed T20 diet were significantly higher than those of fish fed the other diets (P<0.05). Fish fed CON had higher WG, SGR, FE, PER than those of fish fed T100 and CMA diets. Superoxide dismutase (SOD) activity of fish fed T20 and T40 diets were significantly higher than those of fish fed CON diet. Lysozyme activity of fish fed T20 diet were significantly higher than those of fish fed the other diets (P<0.05). For 10-days challenge test with pathogenic bacteria (*Lactococcus garvieae* $1x10^8$ CFU/ml), cumulative survival rate of fish fed T20 diet were significantly higher than those of fish fed CON, T80, T100 and CMA diets.

Exp.2: Apparent digestibility coefficients (ADCs) of dry matter, crude protein and crude lipid in various feed ingredients including *schizochytrium* sp. were determined for rainbow trout. The ingredients consisted of fishmeal, soybean meal, poultry by-product meal, *schizochytrium* sp. produced by CJ Inc. and commercial micro algae. A reference diet with 0.1% Cr_2O_3 as the inert marker was mixed with test ingredients in a 7:3 ratio to produce a series of test diets. Fish were fed their respective diets and fecal samples were collected by stripping. ADC of ingredients of protein and lipid for rainbow trout ranged from 86.7–98.0% and 24.6–55.3%, respectively. ADC for protein of fishmeal was significantly higher than those of *schizochytrium* sp. (P<0.05). However, *schizochytrium* sp. had significant higher ADC for protein than other ingredients (P<0.05). ADC of *schizochytrium* sp. were significantly higher than other ingredients (P<0.05).

Therefore, it could be concluded that *Schizochytrium* sp. can replace FO up to 80% without any negatively influence on growth, non-specific immune responses and disease resistance in rainbow trout. Also, *schizochytrium* sp. could be a feed ingredient as the lipid source in rainbow trout.

EGG QUALITY AND THE TIMING OF BROODSTOCK DIET SHIFTS IN SOUTHERN FLOUNDER *Paralichthys lethostigma*

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Egg and larval quality can be influenced by broodstock diet even when larvae are reared under uniform conditions and fed a high-quality diet. A study was conducted to determine how selected broodstock diets affect southern flounder (*Paralichthys lethostigma*) egg quality (fecundity, egg viability, hatching length, hatching rate) and to determine when broodstock diets should be changed to influence egg fatty acid content, which affects egg quality.

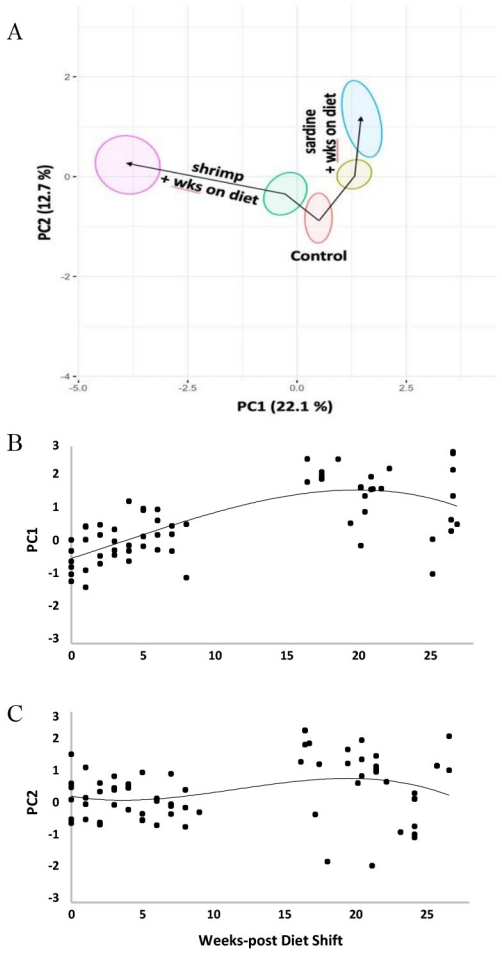
Southern flounder broodstock were fed one of four diets: (1) shrimp, squid, and mackerel (2:1:1 by wet weight) plus a nutritional supplement (Mazuri Gel) year round (SCT diet); (2) the same diet with the nutritional supplement given 1 month before spawning (MDC diet); (3) shrimp and sardines (1:1) until 16-26 weeks before spawning then shrimp only (shrimp diet) or (4) shrimp and sardines (1:1) until 16-26 weeks before spawning then sardines only (sardine diet).

After hormone injection, adults were strip-spawned, eggs incubated under uniform conditions, and egg quality was assessed. Fatty acid compositions of broodstock diets and eggs were analyzed by gas chromatography.

One-way analyses of variance revealed significant differences among treatments in larval hatching length ($P < 0.05$), with significant differences among all pairs of treatments except sardine vs. MDC ($P = 0.49$). No significant differences were observed for fecundity ($P = 0.08$), egg viability ($P = 0.63$) or hatching rate ($P = 0.06$).

To assess timing of dietary changes on egg composition, data for the shrimp and sardine diets were combined with data from a prior study that used the same methods. Principal components analysis of fatty acid profiles separately characterized the shrimp and sardine signatures in eggs. The shrimp signature increased continuously 2 to 8 weeks after the diet shift and stabilized by 16 weeks. The sardine signature was apparent only 16 weeks or more after the diet shift.

These results can be used to develop more efficient broodstock feeding regimes that improve egg quality.



(A) Principal components analysis of Southern Flounder eggs 0-26 weeks after diet was changed to shrimp or sardines. PC1 is the shrimp signature in eggs; PC2 is the sardine signature. Temporal change in (B) shrimp and (C) sardine signatures relative to time after diet shift.

THE EFFECTS OF SYSTEM TYPE, SALT FORMULATION, AND SUGAR ADDITIONS ON PACIFIC WHITE SHRIMP *(Litopenaeus vannamei)* PRODUCTION, DIGESTIVE ENZYME ACTIVITY, AND DISSOLVED MINERAL COMPOSITION

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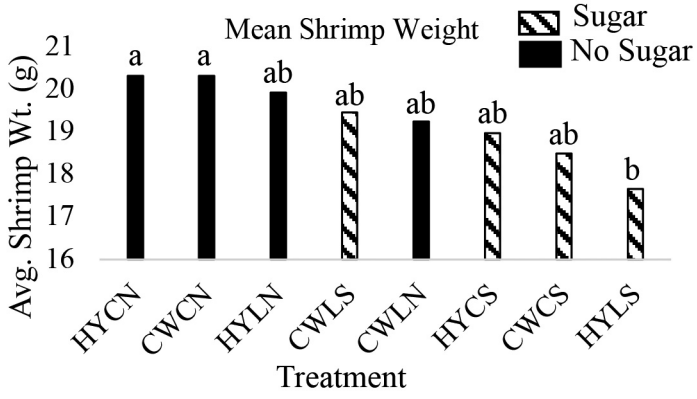
The use of a low-cost, homemade salt mixture may reduce operational costs for inland marine aquaculture producers. Clearwater (CW) RAS is an effective strategy for shrimp production but may have relatively high equipment and operating costs. The use of simpler hybrid systems (HY) may be advantageous as external biofilters maintain water quality, but solids filtration is minimal to help reduce costs. The addition of sugar in RAS may benefit shrimp growth by making suspended microbes richer in protein, which shrimp can then consume. However, sugar addition can deplete oxygen and generate excessive concentrations of solids. Additionally, increased digestive enzyme activity has been noted in shrimp raised in biofloc systems but has apparently not been studied in HY or CW systems. Measuring changes in mineral concentrations over time may indicate when specific elements need to be supplemented. This study examines the effects of low-cost salt, system type, and sugar addition on the production, water quality, digestive enzyme activity of *L. vannamei*, and dissolved mineral composition.

In this study, two levels of each experimental factor were used. The factors used were system type (CW vs HY), salt type (Least Cost, L vs Commercial, C), and sugar (presence or absence of sugar addition: S vs. N). There was a total of eight treatments: HY-L-S, HY-L-N, HY-C-S, HY-C-N, CW-L-S, CW-L-N, CW-C-S, and CW-C-N which had three replicates each and were randomly assigned to 1m^3 tanks. All tanks were stocked at 250 shrimp per m^3, salinity was maintained at 15 ppt, and sugar was added at 55% of the weight of the feed. DO, pH, temperature, and salinity were measured twice daily, and TAN, nitrite, nitrate, turbidity, total suspended solids, and volatile suspended solids were each measured weekly. At the end of the experiment, the hepatopancreas was removed from 6 shrimp from each tank for digestive enzyme analyses and solids from each tank were collected for mineral composition analyses. Shrimp production data (average individual weight of shrimp, total harvest weight, FCR, growth/week) were calculated at harvest.

Results from the experiment showed no significant differences in shrimp performance between HY and CW systems. The least cost salt showed similar shrimp performance as the commercial salt mixture. The use of sugar in both HY and CW systems with sugar addition showed a significant negative impact on mean shrimp weights compared to those with no sugar addition. Results from the water quality, mineral, and digestive enzyme analyses are pending. The shrimp production results of this study indicate that low-cost salt mixtures can be adopted successfully by producers and that system type does not seem to have much short-term impact on shrimp performance.



PRESENT STATUS OF SOFT SHELL CRAB (*Scylla olivacea*) FARMING AND CHALLENGE IN BANGLADESH

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Soft shell crab (*Scylla olivacea*) farming industry is established recently in Bangladesh seeing huge demand of international market. In quest for this, soft shell crab aquaculture present and future prospects assessment was accomplished with the aim of improving the life and livelihoods of poor and landless coastal families, vulnerable peoples due to climate changes, because they depend on the income from soft shell crab farming. Furthermore, Strength, weakness, opportunities and threats (SWOT) of this study is also analyzed. Our survey data clearly indicated that number of farm is increasing year by year (Figure 1). Farmers stock wild crablets which collected by catcher from the mangrove area of Sundarbon, associated tidal rivers, estuaries and canals. Mortality during culture remains unsolved. These farms also created significant manual labor employment for vulnerable peoples. About 1200 to 2800 kg/year and 5600 to 10,000 kg/year produce in small and larger farm. It is observed from survey that approximate 1200 kg waste is produced per year by a small farm. Volume and value of soft shell crab are also increasing (Figure 2 & 3). Very short value chain is observed in supply chain of soft shell crab. In cold storage, after cleaning and processing than keep in the freeze. These is 100% export oriented product. Frozen soft shell crabs are popular to USA, Australia, United Kingdom, China etc. leading the way as major exporting destinations. A lack of communication between soft shell industry and governmental organization has left a lot of unanswered questions for soft shell crab culture problems.

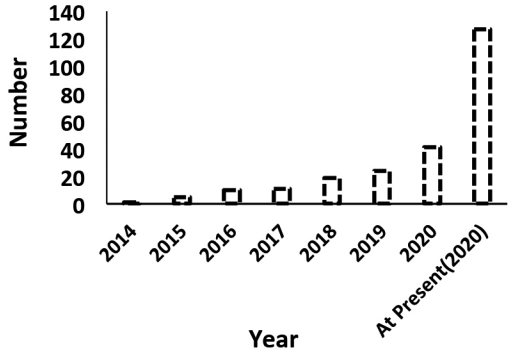

Figure - 1: Number of Soft Shell Farm Establish Each Year in the study area

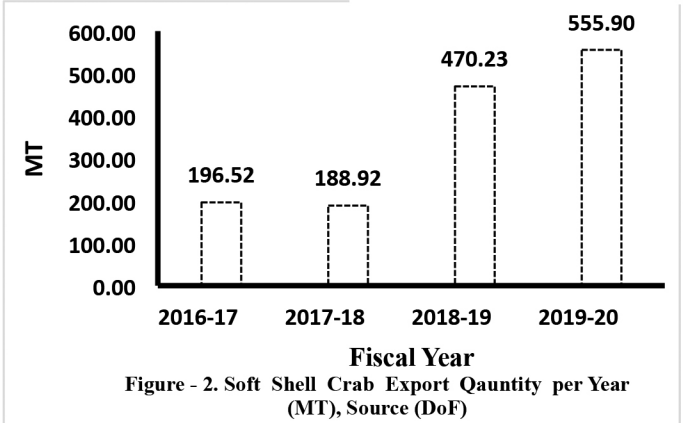

Figure - 2. Soft Shell Crab Export Qauntity per Year (MT), Source (DoF)

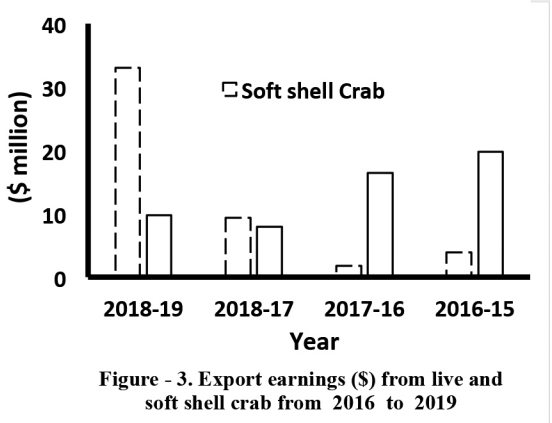

Figure - 3. Export earnings ($) from live and soft shell crab from 2016 to 2019

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

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

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

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

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

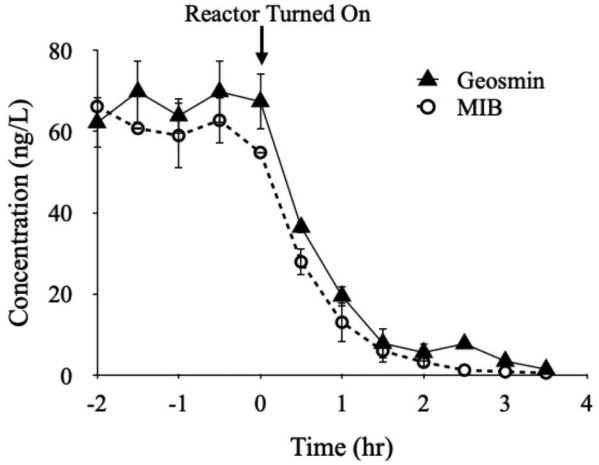

Fig. 1. Geosmin and MIB removal from an 18 m^3 depuration tank using eAOP®.

PROMOTING SPINACH CONSUMPTION AND SUSTAINABLE AGRICULTURAL PRACTICES IN SCHOOL USING AQUAPONICS

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The Center for Disease Control and Prevention (CDC) reports that in 2013 to 2014, over 20% of adolescents aged 12 to 19 years old were obese and also reported in their 2018 State Indicator Report on Fruits and Vegetables that only 2% of American adolescents meet the current vegetable recommendation. Information on food consumption patterns reveals that children and adolescents will consume more than the average of particular foods when they have participated in growing and/or preparing the food. School gardening is not a new concept for teaching children about food production and encouraging consumption of more fresh fruits and vegetables. However, teaching concepts of sustainable agriculture is an emerging concept and is embraced because it promotes conservation of natural resources. An example of sustainable agriculture is aquaponics which is a system that produces both fish and plants. Aquaponics and nutrition education in the classroom may be an effective intervention strategy to use with adolescents, which provides the opportunity for hands-on learning about nutrition, food safety, food production and sustainable agricultural practices and may in turn increase consumption of vegetables, particularly those grown in an aquaponics system. The goals of this project were to: 1) increase nutritional knowledge and consumption of leafy green vegetables; 2) enhance good handling practices and food safety during production and preparation; and 3) promote South Carolina agriculture and sustainable production practices.

MONITORING THE DEVELOPMENT AND MITIGATION OF SOYBEAN MEAL INDUCED ENTERITIS (SBMIE) IN TWO DIFFERENT STRAINS OF RAINBOW TROUT *Oncorhynchus mykiss* OVER A 30 -WEEK LONG TERM FEEDING TRIAL

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Plant protein sources have been largely studied for their potential in replacing fish meal (FM) as the main source of protein in aquafeeds. Specifically, soybean meal (SBM) has been widely used at high inclusion levels to improve sustainability and affordability of fish feeds. However, carnivorous species like rainbow trout (*Oncorhynchus mykiss*) are susceptible to feeds formulated with high inclusions of SBM, exhibiting reduced growth rates and distal intestinal inflammation or enteritis. Mitigation of SBM-induced enteritis (SBMIE) may be done by dietary supplementation of L-glutamine (Gln) because it regulates the development and function of the intestinal tract in fish.

The goal of this study was to evaluate the potential mechanisms by which Gln might exert a protective effect on reducing inflammation and restore barrier function in two different commercial strains (A and B) of rainbow trout over a long-term experimental period. A total of 2,250 fish (strain A= 1125 and strain B = 1125), initially weighing ~ 25.0 ± 1.0 g, were randomly distributed into 18 350-L tanks (125 fish/tank). Three experimental diets (isonitrogenous: 40% crude protein and isolipidic: 20% lipid) included a FM diet (control), a SBM diet (30% inclusion level) and a SBM-Gln diet (1.5% L-Alanyl-Gln) were fed to both strains at apparent satiation for 30 weeks. During the trial fish were sampled five times at 6, 12, 18, 24 and 30 weeks.

Growth performance in terms of weight gain only showed differences for strain A at 18 weeks, being significantly higher in fish fed the SBM diet. ($p < 0.05$). Histology analyses of distal intestine showed significant differences ($p < 0.05$) in terms of villi length and width at all dietary treatments, and inflammation was reduced in the SBM-Gln group in both strains (Fig. 1). Samples were collected from the distal intestine for gene expression analyses of the inflammatory markers: TNF-α, NF-κB, IL-8, IL-10; barrier function markers: MLCK, ZO-1, occludin; as well as brush border transporters: FABP2, and SLC1A5. Distal intestine samples were also collected to evaluate glutathione peroxidase enzymatic activity and glutamate quantification.

Overall, the data show a positive effect of Gln in SBMIE histology after 12 weeks of dietary exposure in both strains. Enzyme activity and gene expression analyses in the distal intestine will help elucidate the molecular mechanism underlying Gln driven SBMIE mitigation.

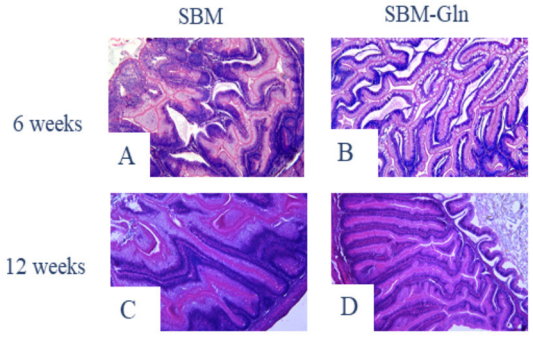

Fig. 1. Histopathology of distal intestine of trout A strain after 6 and 12 weeks. 200X magnification.

A SELF-REACTIVE WAVE ENERGY CONVERTER FOR OPEN OCEAN AQUACULTURE

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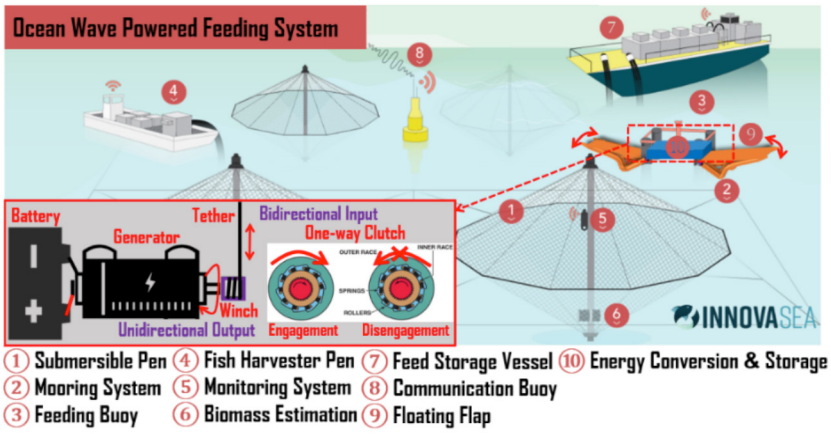
Offshore aquaculture farms have sprouted up all over the world because the ocean provides almost limitless space to raise much healthier fish. The main challenge for offshore farms is that they do not have access to centralized electrical grids and are also generally quite remote leading to high operation and maintenance cost diesel generators used on their farms. They need power for a whole host of operational activities such as feeding, monitoring, lighting, and heating and cooling for crew members living on barges.

E-Wave is co-designing a wave energy converter (WEC) with Innovasea and Virginia Tech through a US Department of Energy STTR project. Innovasea has provided aquaculture systems to offshore fish farms around the world. The first WEC is being designed to survive and operate in one of these farms owned by Open Blue. This farm is 13 km off the coast of Panama and needs a 35 kW WEC to power their operations. Their current system uses a boat that is equipped with a diesel generator that makes daily 14 hour visits to the farm site to power feeding systems. The diesel fuel cost alone to do this is $30,000 per month.

The WEC is designed to be integrated into existing infrastructure already on a farm. New mooring installations are expensive, by taking advantage of on-site infrastructure the WEC becomes more affordable and competitive. The existing infrastructure depends on the farm but the two types that are planned to be used are buoys and on-site barges. The WEC consists of two inclined flaps hinged to the structure below the water. The flaps oscillate as waves strike them. Note that our flap type of wave energy converter is totally different from an oscillating surge WEC, which only uses the surge waves in the horizontal direction. In our device we've optimized the shape of the flap to achieve absorption of *both heave and horizontal wave energy*.

Power is essential to the development and expansion of aquaculture. With worldwide attitudes and pressure mounting to make industries accountable and sustainable, where aquaculture gets their energy will become an important topic. Our WEC is meant to provide farms with an affordable, operational 24/7, on-site, and powerful energy generation system to energize the numerous systems an aquaculture farm must have to farm fish profitably and sustainably.



NUTRIENT AVAILABILITY FROM CLAM MEAL AND HEMP MEAL IN FLORIDA POMPANO *Trachinotus carolinus*

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There is a growing demand for alternative feed ingredients to replace traditional rendered fish meal (FM) and fish oil (FO). Fish feed manufacturers are relying on an increasingly diverse array of alternative feedstuffs of plant, animal, and microbial origins. These alternatives are complex and require further investigation to determine their nutritional value, productivity, and profitability. The transition from dependence on FM and FO cannot be accomplished without accurate information on the nutritional value of prospective alternatives. This requires both the compositional and availability of nutrients from these alternatives. Product availability, ease of use, relative safety for animals, and nutritional value relative to cost are the drivers that affect the extent to which underutilized and new ingredients are used.

The objective of this study was to determine
the availability of nutrients (proximate components, amino acids, fatty acids) to Florida pompano from clam meal and hemp meal. A basal diet meeting the known requirements for Florida pompano, and three test ingredients were evaluated as a completely randomized design, with four replicate tanks per diet. The three test ingredients clam meal, hemp meal, and soybean meal (Table 1) were substituted at 300 g/kg for 300 g/kg of the basal diet (30:70 w/w test ingredient: basal diet). The basal diet incorporated yttrium oxide (Y_2O_3) at 0.5% of the diet as an inert marker for determining apparent digestibility/availability coefficients.

Feed and pooled fecal samples were evaluated for N, energy, total lipid, organic matter, and Yttrium. Feed, and fecal samples were also evaluated for amino acid and fatty acid content. Apparent nutrient digestibility coefficients for each ingredient were calculated based on the ratios of nutrient and marker in feed and feces.

The overall goal was to produce descriptive statistics on nutrient availability for two novel feed ingredients, clam meal and hemp meal. Nutrient availability values for SBM are known and serve as a baseline for validation. In addition, comparing nutritional values in clam meal and hemp meal relative to SBM gives insight into their biological value relative to SBM in Florida pompano diets. These digestibility values can then be applied for future business and research applications.

Table 1. Ingredient and experimental diet composition (%).

	FM	CM	HM	SBM
Ingredient				
Crude Protein	65.3	76.9	51.5	49.8
Crude Fat	8.4	2.7	12.5	0.7
Crude Fiber	0.8	0.0	13.5	4.4
Experimental Diet				
Crude Protein	48.2	56.4	48.4	48.6
Σ Fatty Acids	5.8	5.1	7.3	5.5
Crude Fiber	5.2	3.4	6.8	5.6

FM, fish meal; CM, clam meal; HM, hemp meal; SBM, soybean meal.

FRESHWATER AQUACULTURE BUSINESS IN NIGERIA: PERSPECTIVE, PROCESS, AND PROSPECT FOR YOUTH EMPOWERMENT

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Aquaculture is the fastest-growing animal food-producing industry in the world. The annual supply from inland aquaculture increased from 38.6 million tons in 2011 to 51.4 tons in 2016. Freshwater aquaculture in Nigeria mirrored the same trend as the world, particularly in catfish farming. Despite the potentials for a successful aquaculture business in Nigeria, imported fish food is the primary source of animal protein to Nigerians because of a shortfall in domestic supplies. Currently, about 80% of the aquaculture business workforce in Nigeria is women, and the youth population with a 13.96% unemployment rate has low participation in aquaculture. For the aquaculture business to reach its full potentials in Nigeria, aquaculture operators must expand beyond the aged farmers to include the youth population, which are characterized as notable change agents for economic growth and development. Strategies to address the perspectives, recruit and retain, and the prospect to marry the existing workforce and resources with the anticipated growth should be implemented to empower the youth through freshwater aquaculture business in Nigeria.

ISOLATION AND *IN VITRO* CULTURE OF PRIMARY GERM CELLS DERIVED FROM OVARIAN TISSUES OF WHITE AND BLACK CRAPPIE

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Investigations were conducted to develop a general protocol for the isolation and *in vitro* culture of ovary-derived germ cells in Black crappie (*Pomoxis nigromaculatus*) and White crappie (*Pomoxis annularis)*. Ovarian tissues were obtained from one-year-old Black and White crappie. Five different digestive enzymes: 0.25% trypsin-EDTA, 0.05% trypsin-EDTA, 500U/mL collagenase type I, 500U/mL collagenase type IV, and TrypLE™ Express were evaluated for cell isolation. Cells isolated from ovarian tissues were cultured in two different concentrations (10%, 20%) of Fetal Bovine Serum (FBS) in the L-15 growth media. In addition, four incubation temperatures (15, 20, 25, and 30°C) were also evaluated to determine optimal culture conditions for these ovarian germ cells. Viable cells were obtained from all enzyme treatments. However, the number of viable cells obtained from the 0.25% trypsin and TrypLE™ Express treatments were significantly higher than the other treatments. No significant effects were observed in cell growth between the 10% and 20% FBS treatments. Cells isolated using TrypLE™ Express and 0.25% trypsin attained 80-90% confluency in 25 mm^2 cell culture flasks within five days of inoculation in 20, 25, and 30°C incubation regimes. At a 15°C incubation temperature, the isolated cells took ten days post-inoculation to reach 80-90% confluency. Upon microscopic inspection, cells incubated at 20 and 25°C appeared morphologically healthier than cells incubated at 30°C, where cell detachment from the substrate and irregular cell shape was observed. Based on these findings, we concluded that TrypLE™ Express and 0.25% trypsin were optimal for cell disassociation and isolation, while an incubation temperature of 20-25°C was conducive for cell culture in L-15 media supplemented with either 10 or 20% FBS.

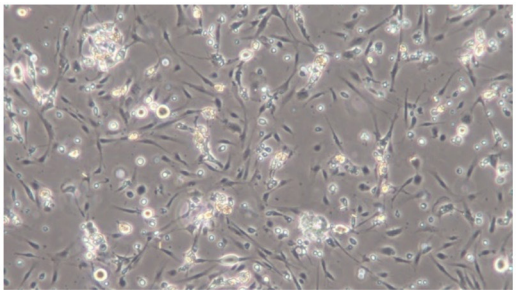

Figure 1. Primary cells 18 hours post-inoculation in the cell culture flask.

EFFECTS OF HARD AND SOFT PELLETS IN CONJUNCTION WITH PELLET SIZE ON PRODUCTION CHARACTERISTICS OF FLORIDA POMPANO *Trachinotus carolinus*

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Florida Pompano (*Trachinotus carolinus*) are on the path to commercialization in the US marine aquaculture industry. Florida Pompano are molluscivorous, and greater than 70% of their natural diet consists of hard-shelled gastropods and bivalves. They also eat crustaceans and other invertebrates. Florida Pompano possess a specialized feeding mechanism, the pharyngeal jaw, which forms a crushing surface used to grind their prey. In aquaculture, the hard-shelled prey are substituted with a prepared diet, an extruded pellet, and their feeding apparatus results in pellets fracturing and loss of fragments through their gill rakers. The main objective of this study was to examine if the uptake efficiency of Florida Pompano can be improved through feed manufacturing technology. Two experiments were conducted to address this: Experiment 1 (duration 294 days) examined whether the Feed Conversion Ratio (FCR) could be reduced by feeding smaller pellets than the fish would normally be fed to evaluate whether pellet size has an effect on the amount of fractured pellet wasted (collection method: 200 µm parabolic filter) during consumption. Experiment 2 (duration 90 days) addressed whether a soft extruded pellet (newly developed water stable semi-moist pellet) reduces the quantity of fractured pellet waste (collection method: large 200 µm sieve) relative to standard commercially produced hard extruded pellets.

No statistical difference was seen in fractured pellet waste between the standard pellet size and smaller pellet size treatments. Experiment 2 showed a statistical difference (Figure 1) in the morning (first feeding of the day) fractured pellet waste between the hard and soft pellet treatments, however, no statistical difference was seen in the late afternoon (last feeding of the day) fractured pellet waste. Our results suggest that pellet size does not reduce the amount of fractured pellet waste produced, while the use of soft versus hard pellets does reduce the amount of fractured waste.

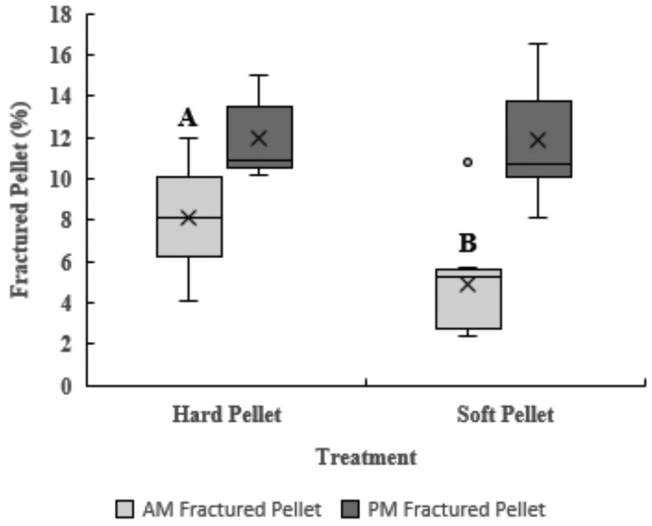

FIGURE 1: Fractured pellet waste percentages for AM (first feeding of the day) and PM (last feeding of the day) for hard and soft pellet treatment groups. Letters (A, B) indicate a statistical difference between hard and soft pellet treatments.

TRANSCRIPTOMIC ANALYSIS OF PRE-METAMORPHIC SOUTHERN FLOUNDER *(Paralichthys lethostigma)* LARVAE: APPLICATIONS FOR AQUACULTURE AND STOCK ENHANCEMENT

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The Texas Parks and Wildlife Department raises Southern Flounder (*Paralichthys lethostigma*) for stock enhancement on the Texas coast. Information on molecular markers is currently lacking and is needed to help improve the culture of Southern Flounder. Potential applications include screening of broodstock and selection for beneficial characteristics (i.e. increased tolerance of variable estuarine environments, normal pigmentation), identification of hormones or physical treatments to induce metamorphosis, and the appropriate temperature to produce female-biased fingerlings. Pre-metamorphic Southern Flounder larvae were used to generate 289,367,813 raw nucleotide reads using next generation RNA sequencing on an Illumina HighSeq 2000 platform of 21 libraries, prepared with RNA from Southern Flounder. A total of 329,198 short read sequences were produced and 96.48% were mapped to assemble a "De novo Southern Flounder pre-metamorphic larvae transcriptome". The quantitative measures indicated that 81.3% of the transcriptome assemblages were complete, 7.7 % were fragmented, and 11.0 % were missing. Gene identification, function, annotation, and differential gene expression were measured and showed statistical significance (at P value 0.001) of expressed genes**.** Identified **g**enes include sex determination, metamorphosis, pigmentation, salinity, and temperature tolerances; as well as antifreeze proteins and metabolic genes related to growth and stress responses. Preliminary results of the induction of metamorphosis on larvae using Thyroxines will be presented.

STATION PADRE: EXPLORING THE ENVIRONMENTAL AND GEOSPATIAL FEASIBILITY OF A PLATFORM-BASED OFFSHORE AQUACULTURE FARM IN THE SOUTHWEST GULF OF MEXICO

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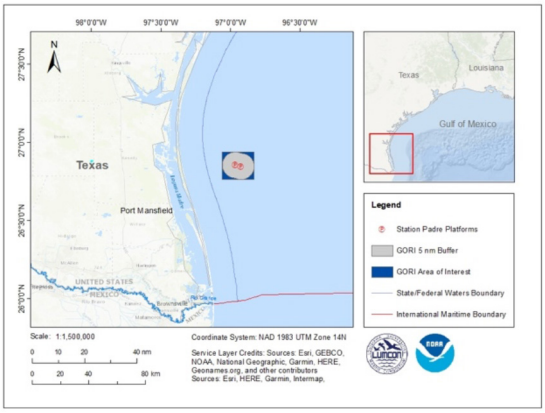
The continental shelf waters of the Gulf of Mexico are highly industrialized, with 1,800 oil and gas platforms across the region. The rising number of decommissioned platforms (about 175 yr^{-1}) has resulted in an expansive network of unused offshore infrastructure, which has the potential to be repurposed into multi-use MMEERSET (Marine Monitoring, Energy and Environmental Research, Science Education and Training) stations. In this project, our team worked to assess two platforms of interest near N. Padre Island, TX (site Station Padre) to determine the environmental and geospatial suitability of site development for offshore aquaculture farming.

A Nortek Signature 500 current profiler unit (ADCP) was deployed in 51 m of water from July 3rd – August 6th, 2020 to collect data on current direction, velocity and wave height. The significant wave height was between 0.35 - 1.40 m with maximum waves between 0.39 - 2.81 m during normal conditions. During the deployment, Hurricane Hanna passed over the study site, which saw a maximum wave height of 10.39 m and a significant wave height of 6.22 m, which exceeded 4 m for over 24 hours. The parameters measured are characteristic of highly exposed, open ocean conditions with a mean near surface current of 0.23 m s^{-1} and mean significant wave height of 0.89 m. Any aquaculture infrastructure installed on site will need to withstand this high energy environment and submersible infrastructure is highly recommended.

A regional geospatial analysis was conducted in ArcGIS using NOAA's AquaMapper geodatabase. Station Padre is located 35 miles NE of Port Mansfield, TX at a water depth of 155 ft. Temperature ranged from 18-29 °C, salinity maintained at 35 ppt, and dissolved oxygen > 4.7 mg l^{-1}. Mean current ranged from 0.05 - 0.2 sv with a maximum current of 0.75 sv and mean wave height of 1.3 m. Wave energy period, wind direction, light attenuation, nutrient profiles, and benthic composition were also assessed. Station Padre is located within a Military Operating Area, which covers an extensive portion (30,000 sq. km) of the northwest GOM. Additionally, platforms are located 80 and 215 m from a shipping fairway, which transects the Area of Interest. Shipwrecks, artificial reefs, and hard bottom habitat are located no closer than 6.6 km from the platforms. Site characterization revealed favorable conditions and it was determined that 77% of the Area of Interest represents Opportunity Area for future farm development.



USING GERMPLASM REPOSITORIES TO SUPPORT EASTERN OYSTER AQUACULTURE: TRACKING THE FLOW OF MATERIALS AND INFORMATION

Sarah Bodenstein*, Isabelina Nahmens, Brian R. Callam, William C. Walton, Jerome F. LaPeyre, Terrence R. Tiersch

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Oyster aquaculture is an integral part of the economy and culture of Louisiana. However, many growers in the region are experiencing oyster mortality events, especially in the summer months. While the exact causes of these events remain unclear, scientific tools such as physiological studies, genetics research, breeding programs, and sperm cryopreservation can be used to address such problems. Unfortunately, information from across these disciplines has not been used collectively, decreasing potential contributions of each tool and their combination. Germplasm repositories offer a solution. Repositories are collections of genetic material that benefit agriculture by storing physical genetic samples, such as sperm. They also store relevant information associated with the samples, such as biological or genetic data. Although successful cryopreservation protocols for freezing oyster sperm (and other aquatic organisms) exist, a comprehensive repository system does not yet exist for any aquatic species. To address this issue, this study used industrial engineering tools such as process flow mapping to integrate oyster cryopreservation protocols into a repository development pathway. The flow of informational components was also incorporated. The biological information of oyster populations from field studies and laboratory studies, sample information from individual oysters, and cryopreservation information were all integrated into the pathway (Figure 1). All cryopreserved sperm was stored in the USDA National Animal Germplasm Program (NAGP) repository and all associated information was recorded in the NAGP Animal-GRIN database. Additionally, process flow maps were paired with time study data gathered during oyster cryopreservation to generate simulations of the repository development process, which can serve as a model for other aquatic species.

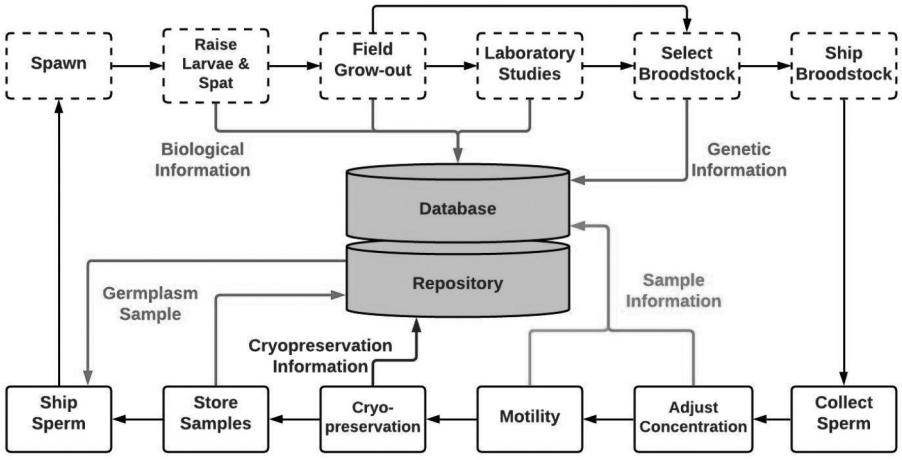

Figure 1. A process flow map outlining how information from different disciplines (such as biology and genetics) can be collected, integrated with a cryopreservation pathway, and stored alongside germplasm samples in a repository. Solid boxes indicate actions taken by a cryopreservation facility and dashed boxes indicate actions taken by farmers or hatchery owners.

EFFECT OF COHORT ON PHYSIOLOGICAL PARAMETERS OF EASTERN OYSTERS, *Crassostrea virginica,* BRED AT SEPARATE HATCHERIES IN THE GULF OF MEXICO

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Louisiana has a long history of oyster farming, however, many farmers in the region are now experiencing high oyster mortality. These mortality events could be caused by environmental stressors such as low salinity or high water temperature, pathogen infection (*Perkinsus marinus*), stress from gametogenesis, or other unexplained causes. To address these challenges the germplasm of oysters native to Louisiana could be stored in germplasm repositories. This would provide oyster hatcheries easy access to a wide variety of genetics for breeding programs without the burden of having to maintain multiple lines as live oysters. Germplasm from oysters bred at hatcheries could also be stored allowing for easy reconstitution of lines. However, the hatchery that breeds a particular oyster line could be an important factor when trying to predict performance in the field. During a 2019-2020 field study, mortality differences were observed among the same lines of oysters (the progeny of Calcasieu Lake, Vermillion Bay, and Sister Lake wild broodstock) bred at two different hatcheries. The two hatcheries were the Auburn University Shellfish Lab (AUSL) and the Louisiana Sea Grant Oyster Research Lab (LSU), and oysters bred at each hatchery constituted the two cohorts of the study.

To investigate potential differences between cohorts, diploid and triploid Sister Lake oysters from both cohorts were transported to the Animal & Food Sciences Department at LSU. There, the maximum oxygen consumption rates (VO2 max), absorption efficiencies, ammonia excretion rates, clearance rates, *P. marinus* infection intensities, and mortality rates of oysters were measured. Oysters from the AUSL cohort had higher VO2 max after withholding food for 7 days and higher ammonia excretion rates than oysters in the LSU cohort, across ploidy. Differences between how hatcheries spawned each cohort, such as the larger number of broodstock parents used to spawn AUSL oysters, could have contributed to the differences observed in physiological parameters. This points to the importance of tracking multiple factors such as pedigree and which hatchery an oyster originated from when developing repositories.

Table 1. The average VO2 max (mg O_2/ hr/ g of tissue) and ammonia excretion rate (µg ammonia/ hr/ g of tissue) plus or minus standard deviation for AUSL and LSU oysters. Superscript letters indicate significant differences between groups ($p < 0.05$).

Cohort	Ploidy	VO2 starved	Excretion Rate
AUSL	Diploid	$1.49^{a} \pm 0.43$	$21.88^{a} \pm 20.73$
AUSL	Triploid	$1.77^{a} \pm 0.49$	$12.21^{a} \pm 21.24$
LSU	Diploid	$1.39^{b} \pm 0.56$	$8.39^{b} \pm 15.10$
LSU	Triploid	$1.25^{b} \pm 0.37$	$8.13^{b} \pm 9.88$

A REGULATORY IMPACT ASSESSMENT OF ORNAMENTAL AQUACULTURE FARMS IN FLORIDA

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The ornamental aquaculture trade is a diverse sector of aquaculture and faces unique challenges which other commodity groups do not have to contend with. The United States is the world leader in imported ornamental species bringing in $65.3 million worth of imports in 2018. Conversely, the U.S. ranked 9th in terms of exported ornamental fish with only $11.4 million in 2018. A paucity of literature currently exists regarding the direct on-farm costs associated with regulatory compliance and the value of production lost due to regulations in other aquaculture sectors which led to an investigation of regulatory impacts on Florida ornamental farms. A survey was administered to ornamental stakeholders in Florida to better characterize these impacts. Direct on-farm regulatory costs totaled $5.2 million and the value of lost production due to regulations were $23.2 million on ornamental farms in Florida.

Results from an industry-wide census have shown that there is a high regulatory burden on ornamental farmers for some regulatory categories. The restriction of beneficial drugs and chemicals resulted in the largest regulatory costs found, totaling $2.1 million across the industry. Additionally, this category had the largest value of lost production due to regulations at $8.4 million industry wide. Producers also had to manage high losses due to predators, averaging a 24% annual loss in production per farm, and were limited in the control measures to reduce predation losses. Costs due to predator control regulations totaled more than $500 thousand and the value of lost production due to predator regulations was $6.5 million in the Florida ornamental industry. The restriction of valuable species such as *Scleropages formosus* and *Cichla ocellaris,* led to a reported value of lost production of $1.4 million. Larger farms were also able to limit the impact from regulations better than smaller farms by spreading their regulatory costs and value of lost production across larger sales volumes.

While the values of lost production were five times higher for ornamental producers, direct regulatory costs were three times less compared to the salmonid and sportfish/baitfish sectors. The unique characteristics of the ornamental aquaculture sector, coupled with the regulatory framework in Florida, may be contributing factors to the relatively lower regulatory costs. While this study only assessed the costs and value of lost production due to regulations, further research should be conducted to analyze the benefits of environmental and human health regulations which are vital to a sustainable industry. The objective of this research was to provide policy makers and regulators with the data they need in order to make informed regulatory decisions with regards to the ornamental aquaculture industry in Florida.

OVERCOMING THE TRAGEDY OF THE OYSTER: A GLOBAL REVIEW ON PRODUCTION AND GOVERNANCE

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Current understanding suggests that oyster reef area has declined substantially around the globe (Beck et al 2013). Attempts to govern oyster resources against the innovative harvester and market value have been challenged to achieve sustainable resource use since the earliest times of harvest. The implementation oyster governance and shifts in harvest practices signify changed human behavior to perceived scarcity events in order to maintain or enhance harvest to meet demand. To highlight these scarcity events throughout history, a conceptual model of human responses to oyster scarcity was developed, and is applied to specific oyster harvest regions from around the globe. The model provides a framework for comparing and contrasting the development of oyster production industries and associated governance response to perceived scarcity overtime. The apparent similarity of ancient and very modern trends in oyster production creates a seeming paradox. Seen in concert, the modern decline of wild oyster fisheries, concomitant to growing aquaculture paints a picture of widespread failure of modern governance to protect wild oyster resources. At the same time, the ancient historical records would suggest the possibility that oysters have been exploited and even overexploited for literally millennia. Yet oysters remain today, so past approaches to sustaining them must not have wholly failed. This retrospective look provides insight to inform future management developments given the modern challenge of recovering oyster reefs for their ecosystem services value in addition to retaining historic consumptive value.

AN ANALYSIS OF U.S. RESTAURANT MENUS TO DETERMINE THE IMPORTANCE OF PRODUCT ATTRIBUTES FOR FARMED OYSTERS

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A significant share of seafood consumption in the U.S. occurs in restaurants, making knowledge about important product attributes important for producers to succeed in the marketing of their product. Moreover, there are also indications that attribute preferences vary regionally and that prices vary with attributes. Of particular interest, while oyster aquaculture has already been successful in several regions of the U.S., it is being promoted by government agencies to increase overall production and consumption. For instance, Florida Department of Agriculture and Consumer Services (FDACS) has been interested in seeking ways to market Florida farmed, half shell oysters to fetch a premium when sold to restaurants. To understand the importance of attributes and qualities of a premium half shell oyster, we have conducted a preliminary analysis of restaurant menus around the U.S. We randomly selected 266 restaurants that sell oysters on the half shell across 12 major U.S. cities to analyze the attributes that are provided on the menu. We found that restaurants on the West Coast provide more information about the oyster being sold, while also fetching a higher average price per oyster. Additionally, restaurants that offered a higher number of West Coast oysters were more likely to provide more information about the oyster products. On the other hand, Gulf Coast oysters consistently had less information provided when compared to East Coast and West Coast oyster products. This study provides a starting point for further analyses that will survey restaurant owners to collect information about the preferred attributes for potential oyster products.

RESOURCE USE IN ECUADORIAN SHRIMP FARMING

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The survey included 101 shrimp farms for which the average production pond area was 141 ha per farm (range = 1 ha-1,579 ha), and average pond size was 6.59 ha. Mean stocking density was 22.5 postlarvae/m^2, crop duration typically was 70-100 days, and an average of 3.6 crops were produced annually. Feeds were used at all farms, and various amendments were applied to ponds. The number of farms using different amounts follows: liming materials, 90; fertilizers, 88; molasses, 80; zeolite, 43; products for disease control, 38; hydrogen peroxide, 37; probiotics, 34; piscicides, 28; disinfectants, 8. Most farms (87) applied daily water exchange at an average rate of 8.5% of pond volume per day (range = 0.7%-30%). Mechanical aeration was applied at 47 farms. Average annual production for the five provinces ranged from 3.67 to 11.95 t/ha/yr (average = 7.03 t/ha/yr). Resource use was estimated to be: total land, 0.54 ha/t shrimp; total water, 76,817 m^3/t shrimp; total energy, 61.2 GJ/t shrimp; wild fish included in feed, 0.65 t/t shrimp. Nearly 80% of water use was incurred through water exchange. The major direct energy use was for pumping water and aeration. About half of total energy use was embodied, and half of the embodied energy was in feed. The farms exhibited a wide range in the use of these resources, and resource use declined in response to greater intensification – especially land and water use.

ROLE OF AQUAULTURE IN WORLD PROTEIN PRODUCTION

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The global supply of animal-source protein for 2018 of an estimated 90,916 Kt was derived from the following sources: milk, 30,889 Kt; chickens and other poultry, 17,169 Kt; pigs, 9,948 Kt; eggs, 8,686 Kt; captured aquatic animals, 7,135 Kt; farmed aquatic animals, 6,815 Kt; cattle, 6,796 Kt; sheep and goats, 2, 257 Kt; other land animals,1,221 Kt; Aquatic animals provided 13,950 Kt or 15.3% of global animal-source protein. Aquaculture was the source of 7.5% of the global animal-source protein, and it was responsible for 48.9% of the aquatic protein. Fisheries and aquaculture are an important segment of the global protein supply, but much more important in some countries than indicated by the global assessment.

Because production of protein by the capture fishery has most probably reached its limit, the expected increase in future demand for aquatic-animal protein will have to come from aquaculture. Aquaculture production (live weight basis) reached 82,087 Kt in 2018, but it required an estimated 12,500-14,344 Kt wild fish for fish meal and fish oil included in aquatic animal feeds. The limit on wild fish from the reduction fishery appears to be about 18,000-22,000 Kt. Aquaculture must reduce its dependence on wild fish or it may not be able to meet the future demand for aquatic protein.

TRANSCRIPTOME AND FATTY ACID ANALYSIS OF FLORIDA POMPANO (*Trachinotus carolinus*) CULTURED AT DIFFERENT SALINITIES

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A major economic impediment to the aquaculture of marine finfish in land-based recirculating systems is maintaining the system salinity at a range for optimal growth. Florida Pompano (*Trachinotus carolinus*) are warm water, euryhaline, marine finfish shown to be a suitable candidate for low-salinity culture. Due to its popularity among sport and commercial fishers, high market value, and ability to readily consume pelleted feeds, the Florida Pompano has become a renewed target for commercialized aquaculture. Although the Florida Pompano has been successfully grown at various salinities, its optimal growth salinity has not yet been established. Studies have shown culturing at different salinities has an effect on digestion rates, feed utilization, and lipid biosynthesis. Identifying the optimal salinity for growth would result in decreased energy expenditure on maintaining homeostasis, since osmoregulation is a highly demanding process. This would allow for energy to be spent on the efficient use of nutrients.

Our study was designed to determine how Florida Pompano larviculture at various salinities affects fish health with transcriptomics (RNA-seq) and fatty acid analysis. RNA-seq was used to identify genes actively transcribed and expressed at the time of sampling. After hatching in a salinity of ~ 30 ppt, larvae were reared in 345 L tanks at one of three salinities (10, 20, 30 ppt) in triplicate. Larvae samples for RNA-seq and fatty acid analysis were collected every three days until weaning. Samples for fatty acid composition were analyzed using a gas chromatography-mass spectrometry (GC/MS). RNA was extracted from homogenized whole-body samples using the Qiagen RNeasy Mini kit. Total RNA was sequenced on the Illumina HiSeq 2500 System. Raw sequences were filtered for low-quality sequences, aligned to a draft Florida Pompano genome, and quantified. Each sampling day was compared across salinities to establish the differentially expressed genes (DEGs) between salinities. These DEGs were functionally annotated to explore the functions that were different between salinities.

We hypothesized that an upregulation of expression of osmoregulation genes would occur in fish not reared at their optimal salinity. These genes include those associated with ion exchange, extracellular matrix remodeling, and general stress. Exploring correlations between expressed genes and fatty acids may help highlight novel associations between un-annotated genes and fatty acid expression, which would provide targets for future research. By understanding the differential expression of fatty acid genes at specific salinities and developmental milestones, diets can be optimized to supply the lipids essential for all life stages. This study will help enable the optimization of Florida Pompano larviculture using information on the optimal salinity and fatty acid content for growth and facilitate more cost-effective rearing methods.

MAINE AQUACULTURE FINANCIAL BENCHMARKING AND RISK MANAGEMENT REPORTS – USING INFOGRAPHICS TO EXPAND REACH AND ENGAGEMENT

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The first-of-its-kind Maine Aquaculture Financial Benchmarking Report was completed in the summer of 2020. The report, written by Dr. Carole Engle and Dr. Jonathan van Senten, in partnership with the Maine Aquaculture Association, is designed to be used by growers as a method to compare their performance to industry averages, and by lenders and financiers to assess investment in aquaculture businesses. The report includes benchmarks that were rated as the most important by growers and financiers such as production, expenses, breakeven prices and yields, profitability, loan repayment, and efficiency. Each benchmark quantifies industry medians, averages, and variance around the means.

The Maine Aquaculture Association (MAA) worked with Science Crunchers, a Portuguese firm that specializes in translating scientific data into visual and written content, to create two-pagers for each species included in the report (oysters, mussels, scallops, and seaweed). The two pagers, which use a combination of text, images, and infographics, condensed 172 pages worth of material into a handful of pages. The materials were designed to provide a user-friendly approach for all levels of prior aquaculture knowledge and highlight the report using visuals (such as mussels growing on ropes) and infographics (visually presenting findings) to span topics from a general sector overview to the granular economic details of each species. The team additionally created a two-pager on integrated multitrophic aquaculture.

MAA also wrote a white paper that overviews aquaculture risk management and mitigation in Maine, including a breakdown by categories of risk and available resources, along with a section explaining aquaculture crop insurance and the current options available. Science Crunchers partnered with MAA on this project as well to provide a two-pager on risk management, including insurance options, and a beginner's guide to risk management decision tree. Both products rely on graphics, visual groupings, and brief text to compartmentalize and facilitate the steps that can be taken on a farm to improve risk management and mitigation.

The presentation will use the two-pagers to highlight the findings from the benchmarking report and illustrate its science communication. It will then detail the risk management two-pager and beginner's guide to risk management decision tree, and show how a more text-driven report can be transformed for increased engagement.

MAINE AQUACULTURE BUSINESS PLANNING TOOLS

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For many years, Maine aquaculture producers have primarily relied on back-of-the-envelope calculations to plan their businesses. As more businesses ramp up production, and a growing number of new entrants joins the sector, looking at a bank statement once per year is no longer going to cut it.

The Maine Aquaculture Association (MAA) has worked with growers, lenders, and researchers to create Maine-specific aquaculture business planning tools. The tools, which focus on business and production planning, are based on species (oysters, mussels, scallops, and seaweed), production methods, and growth rates provided by Maine researchers and the Maine aquaculture financial benchmarking studies. While tools from other regions and sectors may exist, growers in Maine needed a series of tools specific to the products they grow, the production methods they use, and the growth rates in Maine waters.

The tools were designed to, with minimal input from growers, create a simple profit and loss statement, analyze farm profitability, and plan out inventory as well as cash flow. At the request of growers, the tools can also track production, growth, and mortality rates on each site, metrics which can then be incorporated into updated farm projections. Lenders and financiers also reviewed the plans to ensure that they would be appropriate for growers seeking access to capital.

Over 30 meetings have occurred with growers to discuss these business planning tools along with other business planning resources regarding benchmarking, risk management, and busines strategy. The tools have received positive feedback and adoption from the Maine aquaculture sector, and notes are being compiled during meetings with growers to create an updated version in the summer of 2021.

SUBAQUATIC CLASSIFIER FOR REAL-TIME ACOUSTIC DETECTION OF MARINE MAMMALS

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The detection and identification of marine mammals is of vital importance to both regulatory bodies and commercial aquaculture industries. Knowing the locations and migration patterns of these animals can substantially improve the ability of fishing vessels to avoid them, or alert wildlife experts to animals that have become entangled more rapidly. Current methods of manually tagging marine mammals in order to track their movements by satellite are labor intensive and require some amount of luck to successfully tag the animals. Furthermore, acoustic data currently used to identify and locate marine life is processed in an inefficient manner not conducive to proactive or even reactive responses to the presence of marine life. Current processing of acoustic data involves a subaquatic recording device that is placed and then retrieved at a later date, and often the sound data is sent to third-parties for processing and identification of marine species of interest. Improvements in these processes of subaquatic acoustic detection and localization of marine mammals would have dramatic effects on aquaculture industries and species protection regulations.

To this end, we formulate a passive approach for analyzing subaquatic acoustic patterns in order to identify marine mammals by taxonomic family and further identify them by genus and species. Our approach utilizes spectrogram feature generation and Recurrent Neural Networks to classify the acoustic patterns of marine mammals in order to determine the species. This model is lightweight enough to fit on microcomputers, and has highly accurate performance of 91% at the species level and 97% at the taxonomic family level. Additionally, we offer a supplemental multilateration approach to sound source localization which can accurately determine the 3D coordinates of the marine mammal relative to the hydrophone network. As such, our model can be used to monitor the locations and frequencies of marine mammal activity for use by both industries and regulatory agencies in studying the impact of aquaculture projects on the behaviors of marine mammals. Our hope is to use the data from these systems for both real-time intervention in the interest of species protection and to develop an understanding to better predict future marine mammal behavior.

DETERMINANTS OF THE DEMAND FOR ASC LABELED SEAFOOD IN GERMANY

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Understanding consumer decision-making processes is a critical issue in today's highly competitive markets, as firms can only satisfy those needs, they are aware of. Product attributes play an important role from the viewpoint of both demand and supply of products. A growing number of studies in the food markets deal with consumer decisions with respect to the attribute "sustainability". In the seafood sector, the label of the Marine Stewardship Council (MSC) receives the highest attention. However, the counterpart for farmed seafood, the Aquaculture Stewardship Council (ASC) label just gets limited attention and there exists no study which provides estimates of price premiums as well as price and income elasticities.

The analysis is based on the GfK Consumer Scab Scanner Panel dataset on whitefish purchases of German Households, covering a sample period of five years from January 2012 to December 2016. First, a hedonic price study is applied to estimate price premiums for ASC labeled seafood in German retailing, Second, a multi-stage estimation procedure is used. In a probit regression, we figure out, which household characteristics influence the probability of buying ASC labeled seafood. Thereafter, a fixed effects panel regression selects all those determinants that influence the quantity demanded. Finally, we determine whether the price elasticity of demand depends on income and household size.

The results of the hedonic regression indicate that the ASC-premiums in Germany vary between species, from 14% for Trout products, to 7% for Pangasius and a discount of 0.3% for Tilapia products. The results of the probit regression show, that socioeconomic, do not have an significant impact on the purchase probability of whitefish products. The tendency to buy ASC labeled products from traditional brands increases with income. The results of the fixed effects regression indicate that the demand for ASC labeled whitefish in Germany is inelastic. Low absolute price elasticities indicate that price promotions at retail level will not lead to an increase in sales. Instead, other instruments like communication strategies would be more promising.

AQUAPONICS IN VICTORY GARDENS (AQUAPONICS VICTORY GARDEN). A SUSTAINABLE SOLUTION TO URBAN FOOD INSECURITY AND ECONOMIC INEQUITY. PROOF OF CONCEPT TEST RESULTS

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By breaking many of the established food chains, the COVID19 pandemic and subsequent recession demonstrated a weakness in the American food production and distribution systems. The result was a dramatic increase in food insecurity across metropolitan areas of the United States, particularly in food deserts. Though the recession and pandemic may now be abating, the food issues will leave a residual impact.

One of the solutions suggested to address the growing levels of urban food insecurity was to revive the World War II Victory Gardens that in 1945 produced 40% of the food in the nation from individual back yards. The challenge today is that the modern back yard is a postage stamp compared to the yards of the past, thus not providing enough space to produce the average 800 pounds (363.4 kg) of vegetables per family as was done back then. This paper describes a proof of concept study in Phoenix Arizona that tested if aquaponics can provide a solution to this dilemma by increasing home food production levels thus allowing the Victory Garden business model to be revived.

Using the "Deep Water Culture" aquaponics method discussed in Brooks, G. B. 2019 Aquaculture Magazine V45#4 – page 60-64, the results suggest it would be possible to produce the targeted amount of food (800lbs/ 363.4kg) within the 400 ft2/37.16m2 of backyard garden space commonly available at homes in the Phoenix Arizona Metro area. The results also suggest the potential for monetary savings in the home food and water budgets.

AQUAPONICS IN THE CITY OF PHOENIX ARIZONA 2021 FUTURE OF FOOD INITIATIVE

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Working with local food producers, grocery and retail, restaurants, caterers, distributors, and all facets of the food value chain, the "Feed Phoenix (Arizona) Initiative" helped to mitigate the economic and food insecurity stressors caused by the 2020 – 2021 Pandemic Recession for its residents. Building on the success of this earlier effort, the 2021 "Future of Food in Phoenix Initiative" continues and expands the programs started in 2020 including providing food for COVID-19 impacted individuals and families, while creating jobs, economic opportunities, and strengthening Phoenix's local food network, achieving the goals established in the 2025 Phoenix Food Action Plan; and creating a resilient food system.

Within the Future of Food in Phoenix initiative is a Backyard Food Production Pilot Project that will fund the installation and comparative study of backyard and community gardens using aquaponic plant and fish production, raised beds, and other water conserving growing methods as well as training in their use for 90 residents located in food deserts across the city. With a focus on polyculture and nutrition, the data projected to be gathered include pounds/kg of food produced per ft2/m2, pounds/kg of food produced per kwh of electricity, pounds/kg of food produced per gallon/liter of water used. This project will allow for the evaluation of production costs for the different methods as well as for the evaluation of different business models, savings opportunities and best practices for the sustainable production of food on residential properties within Phoenix's increasingly hot and water scarce environment.

EVALUATING SCREEN MESH SIZE AND GRID DEPTH ON FLOW RATE OF A FLOATING RACEWAY

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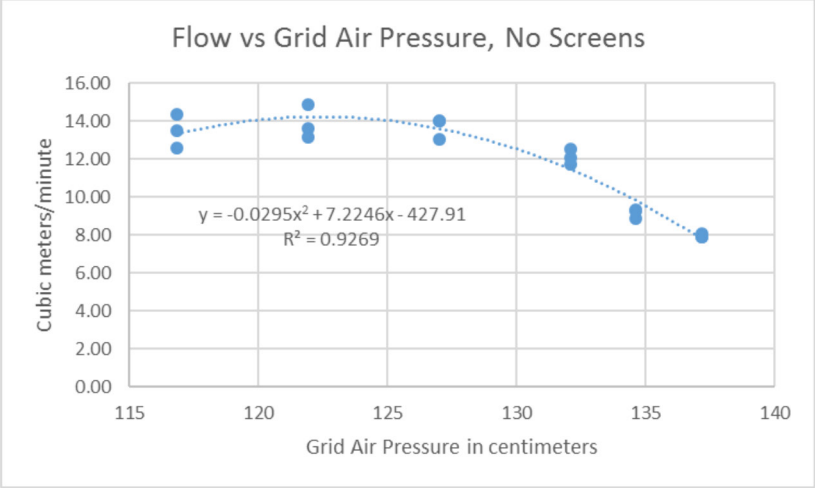
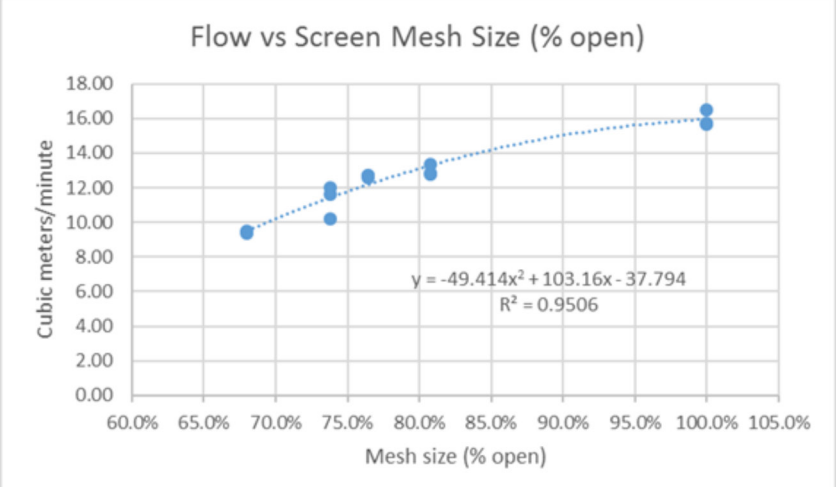
The rate at which water flows through a floating raceway is critical to its function. Flow rate supports water quality by transporting dissolved oxygen into the raceway and removing waste. Flow rate may vary with design of the water-moving device and the use of screens. Screens ensure fish retention,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

A Superior Raceway Model 11000 floating raceway was installed in a 0.8 ha pond. It was fitted with an adjustable airlift grid to vary grid depth, resulting in air pressure between 117 and 137 centimeters of water. Air was supplied by a 1 HP regenerative blower. Regression analysis of grid depth and respective flow rate reveals best fit with a second-degree polynomial and has a correlation of 92%. Average flow rate ranged from 7.97 m^3/min (137 cm water) to 13.87 m^3/min (122 cm water).

Screens (1.2m x 1.8 m) were placed at the inlet and outlet of the raceway. Screen mesh was rated by the percentage of open area: 100% (no screen), 80.8%, 76.4%, 73.8%, and 68%. Regression analysis of screen mesh and flow rate also fits a second-degree polynomial and has a correlation of 95%. Average flow rate ranged from 9.45 m^3/min (68% open) to 15.96 m^3/min (100% open).





ASSESSMENT OF BURBOT *Lota lota maculosa* IMMUNE RESPONSES FOLLOWING EXPERIMENTAL INFECTION WITH *Aeromonas SPP.*

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Burbot (*Lota lota maculosa*), also known as freshwater cod, have shown strong potential as a new aquaculture species in the western United States. Previous burbot research at the Cain Fish Health Lab (University of Idaho) has found that this fish species is relatively refractory to pathogens present in rainbow trout production systems, such as *F. psychrophilum* and *F. columnare,* potentially providing an opportunity for polyculture in existing salmonid production systems. However, it has been found that burbot are susceptible to infection with pathogenic *Aeromonas* sp, which cause Motile *Aeromonas* Septicemia (MAS) in many fish species. As such, two recent trials were conducted to assess the virulence of novel *Aeromonas* sp. isolates, including *A. hydrophila*. In Trial A, triplicate tanks of 25 fish per tank (mean weight of 40g) were challenged with a known virulent *Aeromonas* sp. isolate (A141), along with a newly recovered IR034 isolate at approximately 1 x 10^8 CFU mL^{-1} in a 100 μl IP-injection. Mock controls were also challenged using culture media (tryptic soy broth). Endpoint cumulative percent mortality (CPM) at 21 days post-challenge (dpc) was 49.3±22.0% for A141 and 4.0±0.0% for the less virulent IR034. A similar trial was conducted with 55.0 g burbot, incorporating another recent isolate, IR004 (8 x 10^7 CFU mL^{-1}), along with a previously reported (in warmwater species) virulent strain of *A. hydrophila* (ALG-15-097; 3 x 10^7 CFU mL^{-1}). Both isolates were found to be virulent, displaying a CPM of 85.3±6.1% for the IR004 and 97.3±4.6% for the ALG-15-097 at 21 dpc. Through the initial infection period (0h, 48h and 120h post-challenge) spleen and kidney samples were collected from three fish per tank for immune gene expression analyses to monitor changes from the onset of infection. Additionally, phagocytic capacity of head kidney-derived leukocytes was assessed using flow cytometry. Trial results indicate that burbot are susceptible an array of different *Aeromonas* sp. isolates, including *A. hydrophila*. Based on the challenge outcomes, both A141 and IR004 will be used as candidate isolates to develop a killed vaccine for MAS infections and examine the vaccination response in this unique fish species.

INVESTIGATION OF GROWTH PERFORMANCE AND GUT MICROBIOTA IN CULTURED BURBOT *Lota lota maculosa* FED PLANT-BASED DIETS

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At the University of Idaho, recent efforts have focused on the evaluation and selection of optimal diet formulations for burbot (*Lota lota maculosa*). Previous findings demonstrated that juvenile burbot perform well on marine-type commercial diets, whereas subadults grow equally well on a trout-like diet. As with many cultured fish species, utilizing plant-based proteins in aquafeed formulations is desirable to ultimately reduce the amount of required fishmeal. A 72d feeding trial was conducted to characterize the growth performance and associated intestinal microbiota associated with feeding burbot diets containing fishmeal replacement with soybean meal (SBM) and dried distillers gains with solubles (DDGS). As such, young-of the year burbot (8.2±0.1g; triplicate tanks) were cultured in aerated flow-through tanks at 13°C. With respect to dietary treatments, an Atlantic cod marine-type (fishmeal-based control; REF) diet was formulated to approximately 48% crude protein and 16% lipid and fishmeal protein content was replaced at 25% with inclusions of SBM and DDGS. In this trial, burbot were fed to apparent satiation to maximize feed intake and obtain accurate data metrics for consumption and conversion. Performance data collected at 36d and 72d were analyzed using ANOVA and post-hoc testing with Tukey's HSD (α priori=0.05). Growth results from the trial endpoint indicate comparable performance for the FM and SBM diets, while DDGS diet did not show favorable performance when compared to the SBM or FM diet. For feed conversion ratio (FCR; P=0.019), the burbot fed the REF diet showed the best conversion (0.80±0.01), which was better than the SBM (0.98±0.03) and DDGS (0.96±0.05) diets. Fecal material (trial start, 36d, and 72d) was collected for an assessment of the intestinal microbiota via 16S sequencing (V3 and V4 regions). Results indicate a difference in observed amplicon sequence variants (ASVs) across dietary treatments (P=0.030) and trial sampling timepoints (P=0.013), with the DDGS shifting constituents from 36d (17.9) to 72d (66.1; P=0.039). With respect to alpha diversity analyses, the REF diet displayed an increase in the Shannon index over time (P=0.031) and was found to be greater than the SBM group at 72d (P=0.034). The results from this feeding trial will be of interest to commercial coldwater aquaculture producers, as this study better defines a satiation feeding schedule for burbot and demonstrates the potential of incorporating plant-based protein ingredients into future burbot diet formulations.

CHANNEL CATFISH PRODUCTION IN THE PARTITIONED AQUACULTURE SYSTEM

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Over a six-year period, channel catfish (*Ictalurus punctatus)* were grown in Clemson University's Partitioned Aquaculture System (PAS). Catfish fingerlings (20 - 100 gm) were stocked as size-sorted cohorts within three individual cells in each of six 1/3-acre prototype PAS raceways. Fish were fed to satiation twice daily with commercial 32% protein floating feed, with typical growing seasons averaging 160 - 190 days. Each cohort average weight was determined at stocking and harvest, with cumulative feed consumption of each cohort recorded daily. In addition, morning and afternoon dissolved oxygen, pH, total ammonia nitrogen, secchi disk visibility and water temperature were recorded daily.

Equations predicting feed consumption vs fish weight (Figure 1) and FCR vs fish weight were derived from a range of estimated values presented in Lovell (1998). These equations were used to predict growth of stocked fingerlings vs time under optimum growing conditions (Figure 2). Observed PAS harvest weights averaged 89% of predicted fish weights. However, there was substantial variation from predicted weights, resulting in an R-value of 0.51. The six-year, pooled average FCR for PAS grown catfish was 1.6.

In an effort to improve correlation between observed and predicted harvest weights, Aranguren (2004) incorporated fish-feeding rate correction factors to account for variation in environmental factors. Adjusted theoretical models yielded a smaller SSE (squared sum of errors) than statistical regression models. Variation in seasonal water temperature accounted for 95% of the impact of environmental variables influencing both models.

Previously presented estimates of catfish feed uptake and FCR were used to provide projections of catfish growth in the PAS. Introduction of temperature correction factors can improve accuracy of such projections. However, additional factors driving significant variation between observed and predicted catfish growth remain unidentified.

EXPANDING AQUACULTURE TO REDUCE ECONOMIC DISPARITIES AFFECTING MINORITY AND LIMITED-RESOURCE FARMERS

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The United States Department of Agriculture (USDA) funds capacity building grants in order to strengthen teaching, research and extension programs in the food and agricultural sciences by building the institutional capacities of the 1890 Land-Grant Institutions. Although many of these grants explore ways of making aquaculture more accessible to limited-resource farmers, many of these farmers avoid aquaculture due to the high cost of entry in temperate U. S. states such as Kentucky, higher risks, and marketing difficulties, when compared to more traditional food products such as produce.

Despite the challenges associated with aquaculture, there are many avenues for beginning farmers to consider. Many of these farmers in Kentucky are refugees who have traditional consumption habits of live/fresh fish, which could lead to a significant marketing advantage. Tilapia aquaculture can be conducted in outdoor tanks, requiring modest investments, and the product could be sold in African and Hispanic communities which have shown a preference for live tilapia. Therefore, aquaculture might have a place on limited-resource farms, even as a tool of supplementary income and whole farm risk mitigation.

This project, funded by the USDA beginning farmer training program, investigated the marketing and sales of small scale aquaculture products in Kentucky. A survey was conducted in the summer of 2021 in order to determine available aquaculture products in the state, the seasonal availability, and the current market prices. The results showed that due to limited availability of locally grown aquaculture products in urban areas, direct-to-consumer sales in urban ethnic markets could be an avenue for minority and limited-resource farmers to enter into aquaculture.

THE VALUE OF EXTRUSION TECHNOLOGY IN FISH AND SHRIMP NUTRITION

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Although most commercial aquaculture feeds in the world are manufactured using extrusion, the impact of this technology on performance, efficiency and health of fish and shrimp is overlooked by several aquaculture disciplines. Raw material processors, educational institutions and nutritionists have limited access generally to affordable extrusion technology. The influence of pellet quality (e.g. bulk density, durability, water stability, oil absorption capacity, starch gelatinization) remains too often disregarded in fish and shrimp studies. The assessment of many experts is that extrusion stands at the frontier of science and art. Nowadays, it has become crucial to demystify, and make as widely available as possible, tools to evaluate the impact of extrusion on feed quality.

In this presentation, feed processing through extrusion is described comprehensively. The importance of feed formulation, grinding, mixing, extruding, drying, vacuum coating, cooling, and packaging are covered holistically. Extrusion technology is complex and encompasses principles of physics, chemistry, and animal science. To fully achieve its potential, expertise in engineering and nutrition are essential. Recent development on the roles of compression, thermal energy, melting, starch and protein functionalities on nutrient availability and animal performance is explained concisely.

Finally, aquaculture stakeholders are invited to appraise how extrusion can contribute to their success. Interacting with service/impact-oriented experts with proven-tracked records is essential to achieve this goal.

MONOSEX AND STERILE FISH PRODUCED USING GENOME EDITING

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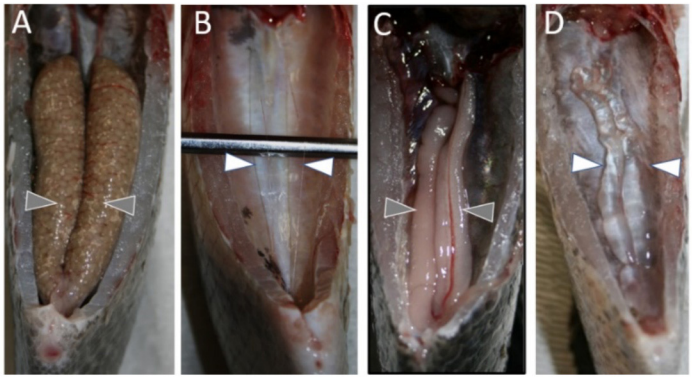
The ability to produce sterile progeny from broodstock for aquaculture has significant benefits to culture productivity and environmental sustainability. We describe the development of strategies to generate, breed and mass-produce infertile fish. Our solutions rely on precise genetic modifications to create broodstock lines that can be incorporated into breeding programs. These approaches were validated in tilapia but are transferrable to multiple species of fish. We expect that adoption of these technologies will result in broad economic and environmental benefits for aquaculture.

One strategy for mass producing sterile fish is designed to produce monosex, sterile populations in culture. In addition to the benefit of sterile fish, this allows the benefit of sexually dimorphic performance traits in culture. We first investigated gene mutations in two evolutionarily conserved pathways, one governing sex differentiation and the other sex competency. We created edits in genes necessary for spermiogenesis and steroid hormone synthesis causing male sterility and masculinization, respectively. Double gene edit combinations for these genes produced all-male sterile populations. Likewise, we created variants in genes whose inactivation caused females to develop atrophic ovaries arrested at a previtellogenic stage or string-like ovaries lacking oocytes. We further disrupted genes causing genetic males to sex reverse into females. Double gene edit combinations for these genes produced all-female, sterile populations.

Propagation of the double KO broodstock lines was achieved via germ cell transplantation from a juvenile donor into a germ cell free wild-type recipient embryo. In the resulting recipients, the induced edits had no effect as the genes targeted are not expressed in germ cells. With this approach, we generated fertile broodstock that successfully mass-produced sterile, monosex populations.

Fig 1 A-D. Dissected gonads of fertile and sterile Nile tilapia. Female (B) and male (D) with genome edited changes show string-like ovaries and translucid testes devoid of oocytes and spermatozoa, respectively. Age matched control female (A) and male (C) display mature gonads. Gray arrow heads point to gonads from fertile fish and white arrow heads point to the gonad from sterile fish.

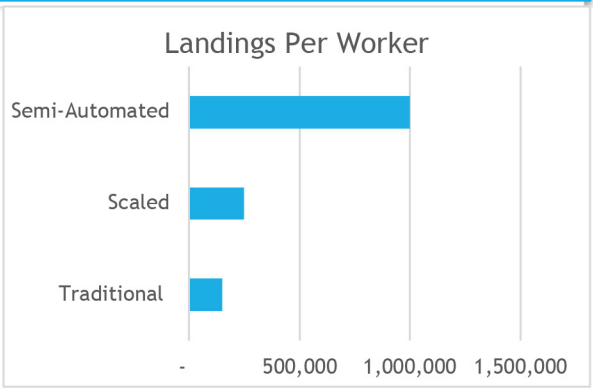


A SEMI-AUTOMATED OYSTER GROWING SYSTEM
ONE-MILLION LANDED OYSTERS PER WORKER YEAR PER AT A LOWER COST

Keith Butterfield * Aaron Pannel
Butterfield Shellfish 17 Haskell Avenue 04071, keith@butterfieldshellfish.com

This Semi-Automation Pilot Addressed Oyster Farming's Key Challenges

- **Equipment Loss and Damage**
  - Gear permanently attaches to the 24mm polypropylene rope, i**n use** for 4 years
  - Low maintenance on gear
- **Fouling Control**
  - Oyster and gear hygiene is fast
  - The ease of controlling biofoul permitted scaling landings per worker
- **High Staff Cost and Turnover**
  - Reduced by 75%.
  - Now attracting staff with a wider range of capability
- **Predator Abatement**
  - Flatworm and mud worm impact was essentially eliminated by the ability to keep gear much cleaner.
  - Bird/Animal predation was reduced.
- **Maximized Space**
  - Grading at the farm site with a small barge reduced land needs.
- **Variable Growth**
  - Oyster shape was more uniform due to shape of baskets and movement of basket.
  - Food source improved by increased waterflow from perpetually clean gear.



Results in New Zealand and Australia; Maine SeaGrant Study is pending

SeaGrant Study Premises (Jan 2022)
One million landings per worker
Cost per oyster reduction by >20¢
Documented Shell shape and strength
Fun, Safe, Easy Labor

COMPARING THE EFFECTS OF INORGANIC IRON (FESO4) AND ORGANIC IRON (CHELATED WITH METHIONINE) AT DIFFERENT CONCENTRATIONS IN PLANT-BASED DIETS ON PERFORMANCE OF CHANNEL CATFISH *Ictalurus punctatus*

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

A feeding trial with catfish fingerlings is being initiated. Twenty fish (5 grams initially) in 3 replicate recirculating tanks per diet will be fed twice daily to satiation for 10 weeks. Higher growth, iron bioavailability and better fish health is expected in fish fed diets fortified with organic iron than with inorganic iron. Results will be presented at the meeting.

Table 1. Composition of basal diet in trials with channel catfish with different amounts/sources of iron.

Ingredients	Amount (g/kg)
Soybean meal	410
Canola seed meal	130
Bone meal (poultry)	20
Wheat flour	220
Wheat gluten	40
Corn grain	135
Vitamin mix	5
Fe-Free Mineral mix	5
FeSO4	0
Fe-methionine	0
Soybean oil	15
Fish oil	15
L-Lysine-HCl	5

MECHANIZED OYSTER (*Crassostrea virginica)* SETTING USING THE SEA CRADLE

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Texas House Bill 1300 was passed in 2019 to support Texas oyster mariculture which can bring in between $70 and $90 million to the state. Traditional oyster culture methods include rack and bag and floating cages, however, the Lower Laguna Madre (LLM) is unique in contrast to the rest of Texas because of its shallow depths (average 3 ft). Therefore, traditional oyster farming is not viable in the Laguna .

An alternative method for the LLM uses concrete string but the string method is currently inefficient due to oysters forming clumps. To increase the efficiency of uniform setting, larvae are hypothesized to set evenly through rotational motion and further, more larvae will set on strings due to the agitation caused by the rotation. A mechanized system with constant rotation, called the Sea Cradle was conceptually designed in Solid Works (Figure 1) to test this hypothesis.

In the building process, a sub-assembly to evenly coat string with cement was made, which greatly alleviated the messy and inefficient coating done without the sub-assembly (Figure 3). A prototype for a 3 ft long aquarium was built and tested (Figure 2).

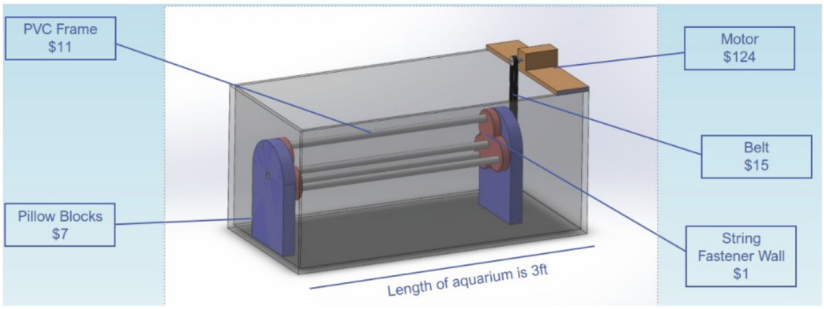

Figure 1 Sea Cradle conceptual design in SolidWorks

Figure 2 Final working prototype of the Sea Cradle

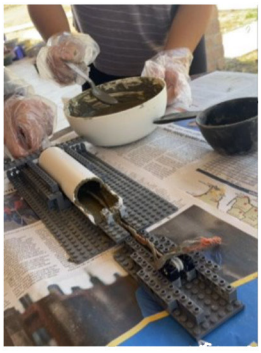

Figure 2 Cement coating

GROWTH EFFECTS OF SPRAY DRIED PLASMA IN DIETS FOR JUVENILE WHITE SHRIMP *Litopenaeus vannamei*

Joy Campbell*, Niti Chuchird, Watwarat Janjariyakul, Javier Polo, Yanbin Shen, and Joe Crenshaw

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Spray-dried porcine plasma (SDP) is a feed ingredient with a diverse mixture of functional proteins including albumen, globulin, transferrin, peptides, growth factors, and other components that improve diet digestibility, growth, feed efficiency, health, and survival in mammalian, avian, and aquaculture species. White Feces Syndrome is highly prevalent in SE Asia shrimp farms causing high mortality and reduced biomass yield. Soybean meal (SBM) is commonly used in shrimp feed to reduce reliance on fish meal but is less digestible by shrimp and may contribute to poor pond water quality. Poor pond water quality propagates the growth of *Vibrio parahaemolyticus,* which is the primary pathogen associated with White Feces Syndrome and causes damage to the hepatopancreas. Functional plasma proteins in shrimp feed may improve diet digestibility and provide better immunity to support shrimp health and growth performance. The study objectives were to determine the effect of 0.0, 1.5, 3.0, 4.5 or 6.0% SDP replacing SBM in pelleted shrimp diets fed to post-larvae 12 (PL12) white shrimp *Litopenaeus vannamei* on growth, survival, protein efficiency (PER) and feed efficiency (FCR) during PL rearing for 45 days under controlled water temperature (29 ± 1°C) and optimum water quality in fiberglass tanks (4 tanks per diet; 120 PL/m^2; 80 shrimp/tank).

Over the 45-day PL feeding study of shrimp under optimum water quality and temperature conditions, there was a linear ($P<0.01$) increase in total biomass, average shrimp body weight (BW), survival, feed per shrimp, and PER and a linear ($P<0.01$) reduction in FCR for shrimp fed increasing levels of SDP.

In conclusion, results suggest optimum growth, efficiency, and survival was achieved with a minimum of 4.5% SDP under lab conditions with high water quality.

TABLE 1: Growth performance, survival, and biomass increase.

Spray Dried Plasma, %	0.0	1.5	3.0	4.5	6.0	SEM	P Value Linear
Survival d 45, %	78.13	80.31	80.94	85.94	86.25	0.81	<.0001
Avg shrimp BW d 45, g	2.58	2.73	2.88	3.07	3.13	0.05	<.0001
Total biomass d 45, g	161.10	175.13	186.61	210.71	215.84	4.01	<.0001
Feed per shrimp d 45, g	4.00	4.20	4.17	4.37	4.35	0.04	<.0001
Feed conversion ratio	1.55	1.54	1.45	1.43	1.39	0.03	0.0003
Protein efficiency ratio	1.66	1.67	1.78	1.81	1.85	0.04	0.0004

MMUNOLOGICAL EFFECTS OF SPRAY DRIED PLASMA IN DIETS FOR JUVENILE WHITE SHRIMP *Litopenaeus vannamei* DURING LABORATORY CONDITIONS.

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White Feces Syndrome is highly prevalent in SE Asia shrimp farms causing high mortality and reduced biomass yield. Soybean meal (SBM) is commonly used in shrimp feed to reduce reliance on fish meal but is less digestible by shrimp and may contribute to poor pond water quality. Poor pond water quality propagates the growth of *Vibrio parahaemolyticus,* which is the primary pathogen associated with White Feces Syndrome and causes damage to the hepatopancreas. Evaluation of ingredients to improve immunity are needed. Spray-dried porcine plasma (SDP) is a feed ingredient composed of a mixture of functional proteins including albumen, globulin, transferrin, peptides, growth factors, and other components that improve diet digestibility, growth, feed efficiency, health, and survival in mammalian, avian, and aquaculture species. Spray-dried plasma proteins in shrimp feed may improve immunity to support shrimp health. The study objectives were to determine the effect of 0.0, 1.5, 3.0, 4.5 or 6.0% SDP replacing SBM in pelleted shrimp diets fed to post-larvae 12 (PL12) white shrimp *Litopenaeus vannamei* on immune responses after PL rearing for 45 days under controlled water temperature (29 ± 1°C) and optimum water quality in fiberglass tanks (4 tanks per diet; 120 PL/m^2; 80 shrimp/tank).

On day 45, immune parameters were measured on 20 shrimp per diet. Also 30 shrimp per tank were kept for an additional 4 days to undergo challenge with *Vibrio parahaemolyticus* (10^5 CFU/mL; 96 hr-LD_{50}) to evaluate post challenge survival. Five shrimp per tank were collected before challenge and 4 d after challenge for hepatopancreas histology and detection of infection.

At day 45, total hemocyte count (10^6 cells/mL), phagocytosis (%), phenoloxidase activity (units/min/mg protein) and superoxide dismutase (% inhibition) increased as level of SDP increased ($P < 0.01$). On d 4 after challenge, shrimp fed diets with 3.0, 4.5 or 6.0% SDP had lower ($P < 0.05$) mortality compared to 0 or 1.5% SDP and hepatopancreas histology revealed less cell damage without bacterial infection for shrimp fed diets with 3 to 6% SDP. However bacterial infection was observed in hepatopancreas cell of challenged shrimp fed diets with 0 or 1.5% SDP.

In conclusion, juvenile shrimp feed containing 3 to 6% SDP supported better immunity and under challenge with *Vibrio parahaemolyticus,* reduced mortality, hepatopancreas cell damage and bacterial infection. Spray dried plasma is a functional protein that can support better immunity and health of shrimp and is an excellent alternative to antibiotics, especially in countries that prohibit use of antibiotics in shrimp feed or have policies to reduce reliance on antibiotics.

1.5% SDP. TABLE 1: Immunological measurements.

Spray Dried Plasma, %	0.0	1.5	3.0	4.5	6.0	SEM	P Value Linear
Total Hemocyte Count	2.24	2.40	2.78	2.85	2.94	0.06	<.0001
Phenoloxidase activity	261.3	265.1	275.5	276.1	275.5	2.67	0.0003
Phagocytosis	62.67	63.50	73.84	74.67	75.17	0.80	<.0001
Superoxide dismutase	56.65	57.92	62.83	62.86	63.85	0.767	<.0001
Mortality, %	70.83	68.34	55.00	55.00	51.67	1.27	<.0001

EVALUATING THE EFFECTS OF DIETARY COTTONSEED OIL ON GROWTH PERFORMANCE OF JUVENILE RED DRUM *Sciaenops ocellatus* AND HYBRID STRIPED BASS *Morone chrysops* × *M. saxatilis*

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According to results from previous studies conducted in our laboratory pertaining to dietary cottonseed flour utilization, palatability appeared to be reduced in diets whose formulations had higher amounts of cottonseed flour, which also contributed higher levels of lipid. Thus, two 6-week feeding trials were conducted in order to evaluate the effects of cottonseed oil extracted from cottonseed flour on juvenile red drum and hybrid striped bass. The basal diets for both trials were composed of practical ingredients to provide 45.9% crude protein and 8.88% lipid primarily from menhaden oil. Experimental diets were prepared by adding cottonseed oil at 2 or 4% by weight in place of menhaden oil.

The three diets were each fed to triplicate aquaria of juvenile red drum (initial weight 1.9 g/fish ± 0.033) and hybrid striped bass (1.4 ± 0.038 g/fish) which were stocked as groups of 14 fish in 38-L aquaria which were operated as a brackish water (3.5 ppt) recirculating system. Fish were group-weighed weekly and fed twice daily based on a percent of body weight that approached apparent satiation. At the conclusion of the 6-week trials, there were no significant ($P>0.05$) differences in weight gain percentage of red drum fed the diets with increasing levels of cottonseed oil although the trend was decreasing (Fig. 1). No differences in feed efficiency ratio, survival, fillet yield, intraperitoneal fat ratio, or condition factor were observed for red drum. In contrast, hybrid striped bass exhibited a significant decrease in weight gain as cottonseed oil increased in the diet (Fig. 1) although feed efficiency, survival, intraperitoneal fat ratio, fillet yield, or condition factor were not affected. Thus, results of this study showed increasing levels of cottonseed oil in place of menhaden oil tended to reduce weight gain of red drum and hybrid striped bass but was much more pronounced for hybrid striped bass.

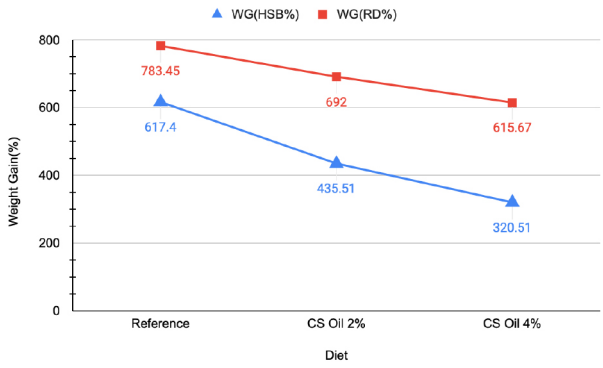

Fig. 1. Weight gain percentage of hybrid striped bass (blue) and red drum (red) fed incremental amounts of cottonseed oil

AQUACULTURE WITHOUT FRONTIERS: ROLE IN DEVELOPMENT, OPPORTUNITIES AND OBSERVATIONS

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Aquaculture without Frontiers (AwF) was started in May 2004 with the enthusiasm and dedication of some of the most recognized leaders in aquaculture throughout the world and members of the World Aquaculture Society. Through the years leadership has changed, management has changed, funding has changed and like all things, have also seen programs and directional changes. However, throughout all the changes, the key components have not changed: aquaculture is a great development tool, it can empower society's weakest contributors and it lends itself to primary, secondary and tertiary opportunities throughout the world.

Throughout the world and specifically in developing nations, there are a few identifiable trends observed: people are hungry, protein is deficient, and jobs are scarce. This all leads to food and health insecurity and quite possibly, political unrest. Aquaculture development can alleviate some of these social and economic stressors.

AwF has been involved in several development projects, many originally focused on small, independent aquaculture projects, and others have morphed into larger and more vibrant projects. These seed projects have led to spin off opportunities in several sectors including support industries, processing initiatives, sales and marketing, and even education.

By participating in several AwF supported development projects, it was eye opening to see other NGO supported projects at work, observing the cross over and inclusion potential for greater achievements in development work. Other observations included the health improvement of children and elders where aquaculture took hold, women getting more involved and supporting the development of the industry around them in order to participate, and the creation of buying and selling cooperatives for better management and fiscal responsibility for the groups and expansion into both supporting and secondary industries. Continued efforts, support, and involvement of multi skilled volunteers are needed for further development and potential achievements in economic, health and food security for many underserved communities.

POTENTIAL SOURCE OF MARINE FISH FOR EXHIBITS AND CONSERVATION – IDENTIFICATION OF EGGS AND LARVAE AT THE TEXAS STATE AQUARIUM

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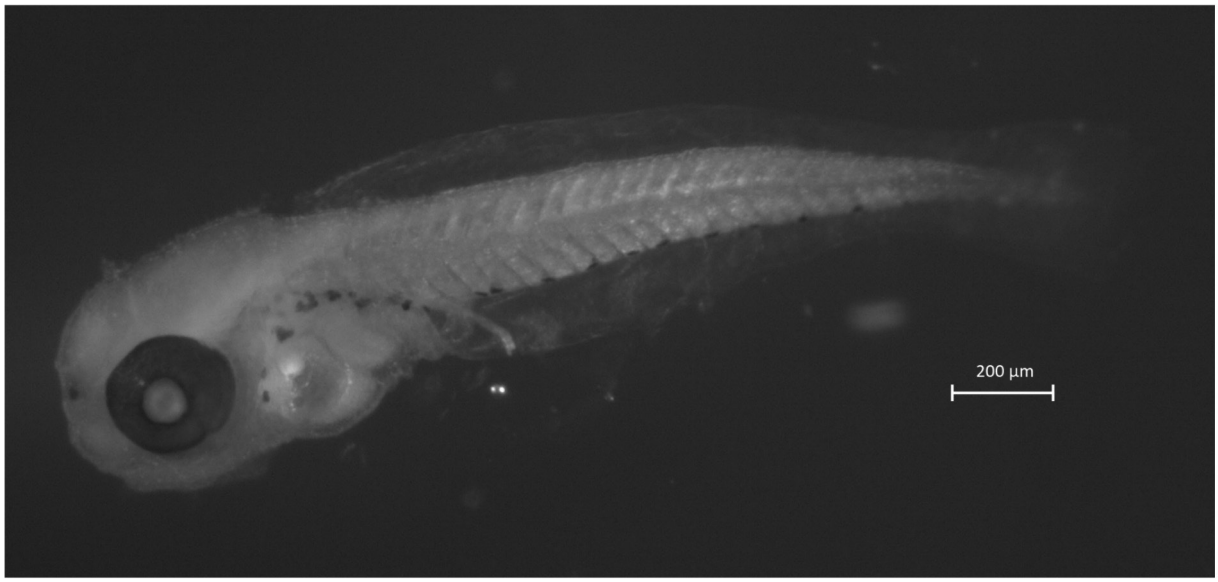
The global ornamental fish trade is valued at an estimated $348M per year. The marine aquarium trade is highly dependent on wild sources of reef fishes. Marine fish populations and reef ecosystems are threatened by overexploitation and harmful collection techniques. Aquariums can provide an alternative source of ornamental fishes through rearing of eggs and larvae from volitional spawning. Pelagic eggs and early-stage larvae in the Coral Reef exhibit at the Texas State Aquarium, Corpus Christi, TX were photographed, measured, and morphologically analyzed. Bicolor damselfish (*Stegastes partitus*) larvae from the Coral Reef exhibit were identified by characteristic pigmentation and DNA barcoding. Early-stage identification of eggs and larvae in mixed species tanks will provide a foundation for determining and implementing specialized larval culture techniques of target species. Further research will strengthen the capacity of the Texas State Aquarium and other public aquariums in supporting and providing outreach for sustainable aquaculture and fisheries.



EFFECTS OF POLYETHYLENE MICROPLASTIC-CONTAINING FEEDS ON THE METABOLITE PROFILES OF JUVENILE YELLOW PERCH *Perca flavescens*

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Microplastics (MPs) are defined as plastic particles with a diameter <5 mm, which may derive from the fragmentation or degradation of larger pieces of plastic from manufactured products in the environment. In recent years, MPs have been recognized as a global threat to aquatic ecosystems causing widespread concern about their potential toxic effects. In fact, due to their small size and persistence in the environment, MPs can be ingested and accumulate in the tissues of a variety of aquatic organisms, including various species of fish. While several studies have reported MP ingestion by marine fish, less is known about MP ingestion and its effects on freshwater fish such as yellow perch (*Perca flavescens*), an important species in the Great Lakes.

A 9-week feeding trial was conducted in a flow-through aquaculture system to evaluate the effects of feeds containing high-density polyethylene (HDPE) MPs on the liver metabolite profiles of juvenile yellow perch, using a Nuclear Magnetic Resonance (NMR)-based metabolomic approach.

Feeds containing three different HDPE MP levels (0, 2, and 8 g/100 g feed) with particle size ranging from 120 to 125 µm, were tested. Three tanks of fish were randomly assigned to each diet at a stocking density of 15 fish/tank (average body weight, 25 g/fish). The fish were fed at a feeding rate of 2 % body weight daily. Liver samples were collected at the end of the feeding trial to evaluate possible differences in metabolite profiles among the dietary treatments (n=12/treatment). Polar extracts were analyzed by NMR spectroscopy and multivariate statistical analysis was applied to the processed NMR spectra (Fig. 1). Significant differences in metabolite profiles were detected between fish fed the HDPE MP-containing feeds and the control diet, with more pronounced effects induced by the highest HDPE MP levels (8 g/100 g feed) compared with the low-level HDPE MP diet (2 g/100 g feed).

Our study indicates that chronic ingestion of HDPE MP particles (size = 120-125 µm) through feeding induced alterations of hepatic metabolic profiles in juvenile yellow perch, and disturbed bile acid metabolism.

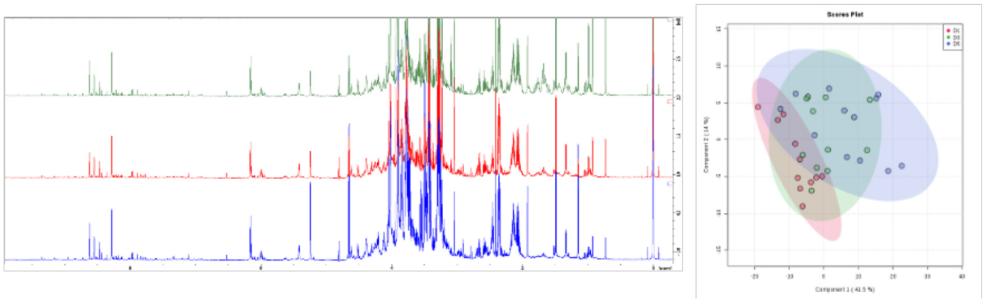

Figure 1. ^{1}H-NMR spectra of liver tissue extracts (polar extracts) and PLS-DA scores plot from juvenile yellow perch fed diets containing HDPE MP at different levels (D1=0, D3=2, D5=8 g/100g feed).

COMPARISON OF GROWTH AND MORTALITY OF THE EASTERN OYSTER *Crassostrea virginica* BY BROODSTOCK SOURCE, GEAR TYPE AND STOCKING DENSITY IN MATAGORDA BAY, TEXAS

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Oyster aquaculture in the U.S. has rapidly expanded into a prominent component of the seafood industry. Due to recent passage of enabling legislation, Texas is now poised to become a major contributor. The objective of this research was to characterize growth and mortality of oysters: 1) from different seed/bay sources 2) using different gear-types, and 3) at different stocking densities. Broodstock oysters were obtained from two Texas bays (Copano Bay and Matagorda Bay) and spawned at two hatcheries in Alabama (L3 and Dauphin Island Shellfish Laboratory, respectively). A research farm was established in Palacios, Texas consisting of 29 OysterGro™ (OG) 2-slot floating cage pontoons and 6 Adjustable Longline Systems (ALS) capable of holding 30 growing containers on each line. When seed oysters from both sources were capable of being retained in a 14-mm growing bay, oysters were subdivided into the following treatments and the experiment was initiated. The OG pontoons were stocked at densities of 150, 200 and 250 oysters per bag. There were 4 replicates of 3 densities from 2 broodstock sources for a total of 24 OG growing bags. In the ALS system, oysters were stocked at densities of 60, 75 and 90 oysters per growing container. This yielded 10 replicates of 3 densities from 2 broodstock sources for a total of 60 ALS replicates.

Monthly samples of 10 oysters from each ALS and OG growing containers were taken with replacement. Length (L), width (W), height (H), and whole wet weight (WWW) of each sample were recorded to yield a growth curve and determine mortality. 20 oysters from each replicate were collected for L, W, H and WWW analysis. From these 20 samples, 10 were collected for determination of condition index (CI) and meat tissue analysis. The results from this study will serve as baseline information for incipient farmers to the Texas industry.

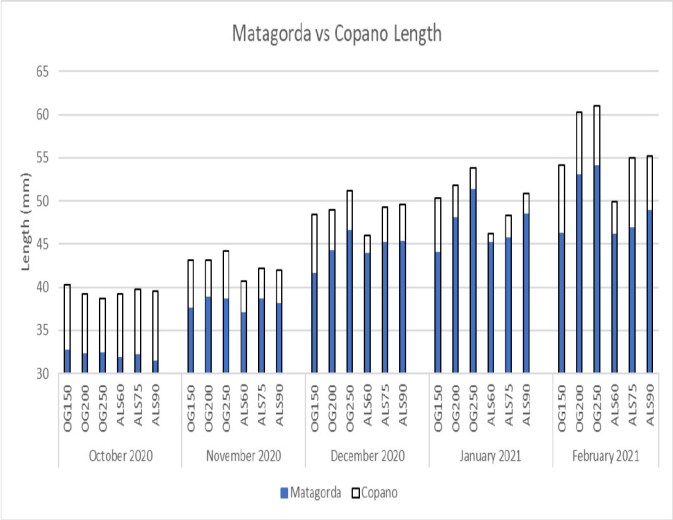

Fig. 1: Growth in length (mm) of oysters, by treatment, over the first four months. Each treatment is labelled by gear-type (OG or ALS) and density of that gear-type. Note: Copano oysters spawned 1 month before Matagorda.

ADOPTION OF PRODUCTIVITY-ENHANCING TECHNOLOGIES IN THE U.S. CATFISH INDUSTRY

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The U.S. catfish industry has undergone significant technological progress in an attempt to achieve cost efficiencies. Producers have been increasingly adopting improved production practices such as split ponds and intensively aerated ponds in recent years. Subsequently, there has been increased adoption of complementary technologies such as fixed-paddlewheel aeration, automated oxygen monitors (AOM), and hybrid catfish in the foodfish sector. Similarly, fingerling producers are relying on an oral vaccine against Enteric Septicemia of Catfish (ESC). This study monitored the progress of the adoption of alternative and complementary technologies in the US catfish industry. A 2020 multi-state in-person survey in Alabama, Arkansas, and Mississippi (n=68), revealed increased adoption of intensively aerated ponds (6,315 ha) and split ponds (1,176 ha). Adoption of such intensive practices has also led to greater use of complementary production technologies. As a result, the average aeration rate in the tristate region has increased to 7.8 kW/ha with 96% of catfish farms adopting automated oxygen monitors. About 54% of the water surface area in the tristate region was in hybrid catfish production. Eighty-three percent of the fingerling farms and 67% of the fingerling production area were vaccinating against ESC at the time of the survey. The increasing adoption of productivity-enhancing technologies in the US catfish industry explains the recent increases in foodfish yield (43%). Monitoring the progress of adoption of alternative and complementary technologies will guide researchers and Extension personnel involved in the refinement and dissemination of recommendations for these technologies.

Table 1. Adoption of productivity-enhancing technologies in the U.S. catfish industry, 2013 and 2020.

Regions	Total area under			Average	
	Intensive aeration (ha)	Split ponds (ha)	Hybrid catfish (ha)	Aeration rate* (kW/ha)	Foodfish yield Kg/ha
Alabama	1,588	13	1,635	7.4	6,847
Arkansas	254	115	551	6.9	6,608
Mississippi	4,472	1,048	9,830	8.3	8,176
Tristate in 2020	6,315	1,176	12,016	7.8	7,621*
Tristate in 2013	475	670	~5,000	5.3	5,850*

*weighted averages

EFFECT OF PHYTASE ON GROWTH PERFORMANCE, MINERAL COMPOSITION, AS WELL AS PHOSPHORUS AND NITROGEN EXCRETION OF JUVENILE RED DRUM (*Sciaenops ocellatus*)

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A 9-week feeding trial was conducted to evaluate the effects of dietary phytase on phosphorus (P) utilization of juvenile red drum (*Sciaenops ocellatus*). Two control diets were designed to contain 40% crude protein primarily contributed by menhaden fishmeal and plant-protein feedstuffs. One diet without extra monocalcium P supplementation was the negative control diet and contained 0.56% available P (0.77% of total P). The positive control diet was supplemented with monocalcium P to achieve the previously established minimum dietary P requirement (0.7% of dry diet). Another four experimental diets were formulated by adding phytase (Quantum Blue Phytase, AB Vista) at 1000, 2000, 4000, or 8000 FTU/kg to the negative control diet. A total of 480 fish (initial weight 4.99 ± 0.09 g/fish) were assigned as groups of 20 fish to 24, 110-L glass aquaria connected as a closed-recirculating system maintained at 7 ppt salinity. Each dietary treatment was randomly assigned to quadruplicate aquaria. Fish were fed twice daily at representative percentage of body weight that approached apparent satiation and was adjusted weekly after group weighing. In addition, at the end of the trial, P and nitrogen (N) excretion of fish after feeding was quantified at different time intervals as well as disappearance of N and P from different sections of the gastrointestinal tract.

After the 9-week feeding period, there were no significant ($P< 0.05$) differences in survival, hepatosomatic index, or intraperitoneal fat ratio among fish fed with various experimental diets. However, compared to fish fed the negative control diet, those fed all other diets had significantly increased weight gain and feed efficiency (Table. 1). In addition, fish fed the diet supplemented with 8000 FTU/kg of phytase had significantly higher weight gain and muscle yield than fish fed the positive control diet. More results will be supplied after remaining samples are analyzed.

Table. 1 Weight gain and feed efficiency of red drum fed negative and positive control diets and the negative control diet supplemented with different amounts of phytase

	Negative	Positive	1000	2000	4000	8000	PSE	*P*
Weight gain (%)	891^{c}	1064^{b}	1123^{ab}	1089^{ab}	1203^{ab}	1239^{a}	55.7	0.0055
Feed efficiency	0.83^{b}	0.91^{a}	0.95^{a}	0.92^{a}	0.94^{a}	0.95^{a}	0.028	0.0457

Means followed by different lower-case letters within the same row indicate significant difference

CONSUMER'S WILLINGNESS TO PAY FOR ATTRIBUTES OF LOCALITY AND SUSTAINABILITY OF SOUTH CAROLINA AQUACULTURE PRODUCTS

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Evaluating the effect of attributes used in seafood labelling schemes has relied on empirical evidence from consumers' preferences. Attributes of interest in the case of this study are locality and sustainability, whether the seafood is a product of South Carolina, or any other state and whether the products display ecolabels from the Marine Stewardship Council (MSC) or Aquaculture Stewardship Council (ASC).

Residents of South Carolina (N=1,947) were surveyed on their preferences and perceptions towards South Carolina aquaculture products in order to estimate their willingness to pay (WTP) for locally and sustainably produce. Table 1 shows the explanatory variables that affect a respondent's likelihood that they are willing to pay a premium for local aquaculture products.

Consumers who have previously purchased aquaculture products, regardless of the source, on average are 7.5% more likely to be willing to pay a premium for aquaculture products from South Carolina, while older consumers are less likely willing to pay a premium for local aquaculture products. Using the results of this logit model, we included only main attributes of source and method in the WTP estimates.

We estimated consumers' WTP a premium for local oysters, clams and shrimp per pound and if labeling (Aquaculture Stewardship Council (ASC)) affect consumers' WTP. Table 2 shows that across all models, local shellfish products are valued higher than non-local products, and in the case of oysters, locally grown oysters (lbs.) are valued higher than non-local wild-caught oysters.

Table 1. Logit model estimates and marginal effects of respondent's willingness to pay a premium for South Carolina aquaculture products

Variable	Estimate	Std. Error	Marginal Effects
(Intercept)	0.154	0.232	
Aquaculture Products	0.343***	0.052	0.075
Location	-0.081	0.11	-0.018
Age (years)	-0.008**	0.031	-0.001
Years of Residency (years)	0.009*	0.005	0.002
Household Income ($)	1.79e-06*	1.03e-06	3.93e-07
Education	-0.033	0.037	-0.007

*** p < 0.01, ** p < 0.05, * p < .10

Table 2. Results of mixed logit model estimations for WTP for South Carolina clam, oyster, and shrimp

	Clam			Oyster			Shrimp		
Variable	Coefficients	SE	WTP	Coefficients	SE	WTP	Coefficients	SE	WTP
Price	-0.656***	0.061		-0.37***	0.036		-0.514***	0.043	
South Carolina	1.571***	0.133	2.40	1.27***	0.11	3.43	1.298***	0.11	2.53
ASC	-0.076	0.11	-0.12	0.117	0.094	0.32	-0.125	0.102	-0.24
Buyno	-4.67***	0.631		-2.71***	0.341		-3.25***	0.339	
Log - likelihood	-1864.7			-1959.44			-1892.28		
AIC	3745.41			3934.88			3800.55		
Observations	7,695			7,440			7,464		

***p < 0.01, **p < 0.05, *p < 0.1

COMPARISON OF STOCKING TIME AND DENSITY ON GULF KILLIFISH GROWTH IN FRESHWATER PONDS

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The Gulf Killifish (*Fundulus grandis*) is a popular baitfish and a candidate for commercial-scale production. For inland production, ponds may need to be stocked at different times and densities to meet market demands. Therefore, in Experiment 1, juveniles were stocked into freshwater ponds at a density of 25,000 fish/ha, with two cohorts (mean total length (TL) = 3.92; 3.52 cm) stocked 1 month apart and evaluated for 12 weeks. Cohort 1 had a lower overall survival (40%) than cohort 2 (79%); with no difference between mean final TL (8.46; 8.00 cm); reaching a market size of 6 cm in 22 days and 32 days, respectively. In Experiment 2, juveniles (mean TL= 3.16; 3.23 cm) were stocked into freshwater ponds at two densities (25,000 fish/ha and 50,000 fish/ha). Mean final TL was greater in the lower stocking density (8.68 cm) than the higher stocking density (8.20 cm); with no differences in survival between treatments (75; 72%) respectively. Lower stocking and higher stocking densities reached a market size of 6 cm in 29 and 31 days, respectively. These results suggest that multiple cohorts can be stocked and grown to market size in one season at a stocking density of 50,000 fish/ha.

TABLE 1. Mean ± SE stocking and harvesting variables in ponds stocked with juvenile Gulf Killifish, in June 2017 and July 2017 at a density of 25,000 fish/ha (Expt 1) and two stocking densities in (Expt 2.)

Variable		Expt 1: Cohort		Expt 2: Stocking density (fish/ha)	
		1 6/28/17- 9/18/17	2 7/31/17- 10/16/17	25,000	50,000
Initial	Weight (g)	0.78 ± 0.03 *	0.52 ± 0.02	0.40 ± 0.01 *	0.36 ± 0.01
	TL (cm)	3.92 ± 0.04 *	3.52 ± 0.04	3.16 ± 0.03	3.23 ± 0.02 *
	K	1.19 ± 0.04 *	1.06 ± 0.01	1.20 ± 0.02 *	1.01 ± 0.01
Final	Weight (g)	8.63 ± 0.13 *	7.17 ± 0.11	9.86 ± 0.13 *	8.13 ± 0.11
	TL (cm)	8.33 ± 0.04 *	8.14 ± 0.04	8.68 ± 0.1 *	8.20 ± 0.0
	K	1.47 ± 0.01 *	1.30 ± 0.01	1.49 ± 0.0 *	1.45 ± 0.0
Growth$^{\Delta}$ (cm)		7.75 ± 0.72	6.64 ± 0.34	8.98 ± 0.32 *	7.66 ± 0.15
Δ K		0.28 ± 0.06	0.25 ± 0.02	0.28 ± 0.05	0.43 ± 0.05 *
Survival (%)		40 ± 16	79 ± 3 *	75 ± 2	73 ± 4

TABLE 3. Previous and current studies evaluating culture of Gulf Killifish, in earthen ponds.

Reference	Salinity (ppt)	Density (fish/ha)	Stocking size (g)	Market size	Days to market size	Survival (%)
Tatum & Helton 1977	8-18	125,000	0.25	5.6 cm	38	88
Tatum et al. 1978	11-16	250,000	0.2	5.6 cm	52	82
Trimble et al. 1981	8-11	250,000	0.4	5.7 cm	49	93
	8-11	370,000	0.4	5.7 cm	49	93
Waas & Strawn 1982	6-14	200,000	0.5	6.4 cm	70	86
Perschbacher & Strawn 1983	12-22	150,000	0.2-0.5	3 g	66	70
	2-9	330,000	0.2	3 g	91	87
Burger et al. 2018	0.5	26,500	0.47	3 g	28	53
Experiment 1 (Cohort 1)	0.5	25,000	0.78	6 cm	22	40
(Cohort 2)	0.5	25,000	0.52	6 cm	32	79
Experiment 2	0.5	25,000	0.40	6 cm	29	75
	0.5	50,000	0.36	6 cm	31	72

ANAEROBIC DIGESTION OF SLUDGE FROM RECIRCULATING AQUACULTURE SYSTEMS: IMPACT OF SOLIDS CONCENTRATION ON METHANE PRODUCTION

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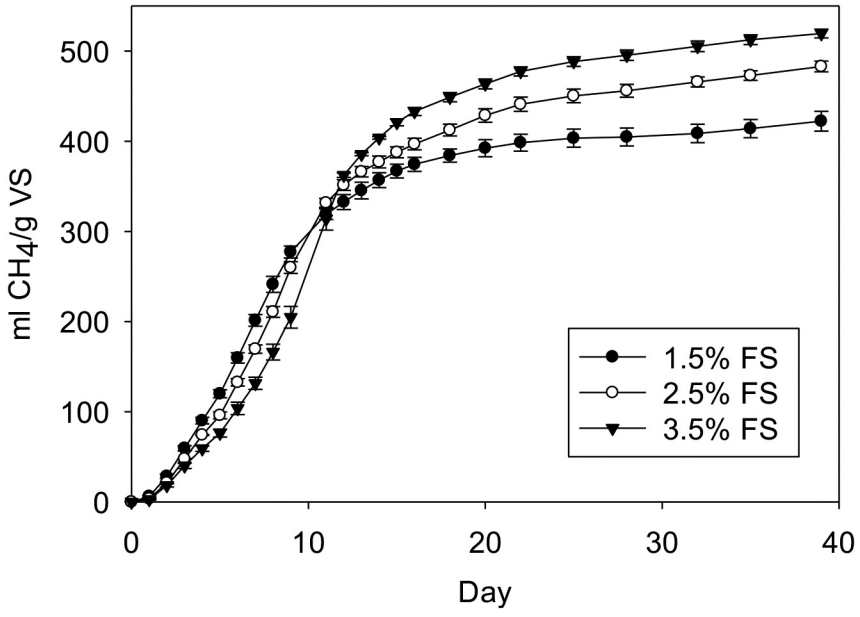
Recirculating aquaculture systems (RAS) are efficient at solid waste capture and collection, though the concentrated waste stream still requires disposal, often at a high cost to the farmer. One potential treatment option may be anaerobic digestion, a microbial process occurring in the absence of oxygen, which reduces organic matter. Anaerobic digestion also produces a mixture of methane (CH_4) and carbon dioxide gases, collectively known as biogas. Turning waste into value, the biogas can then be captured and used to generate electricity. Increasing the amount of organic matter in the feedstock can result in higher biogas production, but it may also lead to process failure due to excess acid production. In this study, the effect of varying total solids (TS) concentration from fish sludge (FS) on biogas production was evaluated using a biochemical methane potential test. The three treatments consisted of different initial TS concentrations (1.5% FS, 2.5% FS, and 3.5% FS) from a mixture of salmon and trout sludge generated at the Freshwater Institute RAS facility. Methane production was measured, quantified, and normalized on a volatile solids (VS) basis. The three treatments were significantly different, with the 1.5% FS, 2.5% FS, and 3.5% FS producing 422 ± 11 mL CH_4/g VS, 483 ± 6 mL CH_4/g VS, and 519 ± 5 mL CH_4/g VS, respectively. The 1.5% FS treatment reached peak CH_4 production on Day 7 (78.2 mL/day), while the 3.5% FS treatment had a peak CH_4 production on Day 11 of 96 mL/day. This study demonstrated that RAS sludge is a valuable feedstock for anaerobic digestion, even at higher organic loads.



SALINITY AFFECTS GROWTH OF PACIFIC WHITELEG SHRIMP AND THREE HALOPHYTIC PLANTS IN MARINE AQUAPONIC SYSTEMS

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In the present study, we evaluated the growth performance of whiteleg shrimp (*Litopenaeus vannamei*) and three halophyte plants, red orache (*Atriplex hortensis*), okahijiki (*Salsola komarovii*), and minutina (*Plantago coronopus*) in marine aquaponic systems with three salinity treatments (10, 15, and 20 ppt) for 4 weeks. Shrimp performed better in 15 and 20 ppt than those raised in 10 ppt. The results of final weight, WGR, SGR, and FCR for 10 ppt were 1.82±0.16 g, 79.24±6.09 %, 2.08±0.12 %, and 1.67±0.13, respectively; those in 15 ppt were 2.00±0.10 g, 89.88±2.18 %, 2.29±0.04 %, and 1.47±0.04, respectively; and those in 20 ppt were 1.99±0.07 g, 93.93±5.39 %, 2.40±0.10 %, and 1.38±0.08, respectively. On the other hand, plants had a totally opposite trend than the result of shrimp. The growth performance and nutrient content in the three halophytic plants decreased with the increasing salinity. Although plant growth was affected by the salinity, there was no significant difference between 10 and 15 ppt treatments. Given that, 15 ppt was suggested as the optimal salinity for whiteleg shrimp and the three halophytes in an indoor marine aquaponics.

IMPACTS OF COVID-19 ON U.S. MOLLUSK AQUACULTURE

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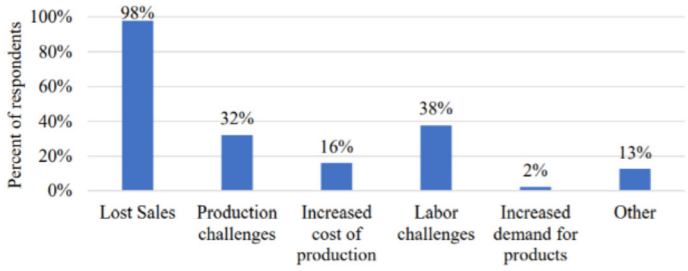
The coronavirus (COVID-19) disease pandemic has disrupted the lives and livelihoods of everyone on our planet. As governments around the globe implemented measures to protect the health and safety of their populations, there were multiple ripple effects that reverberated throughout all sectors of our economy. The aquaculture and aquaponics sectors were also amongst those affected by the impacts of COVID-19. The implementation of "social distancing" policies, restaurant dine-in prohibitions, and stay-at-home orders across the U.S. has translated into a disruption of market channels for aquaculture, aquaponics, and allied businesses. As a result of the lost market channels, businesses have experienced a loss of revenue, interruptions in cash flow, challenges with production, challenges with labor, and more. To address the challenges faced by the industry, a study was launched to assess the impacts of the coronavirus disease (COVID-19) on U.S. aquaculture, aquaponics, and allied businesses. This study was a collaborative effort between Virginia Tech, The Ohio State University, and Mississippi State University Extension, prompted by contacts with producers raising their concerns and attempting to identify resources that could aid their businesses.

Data for this study was collected through a survey, which was developed in Qualtrics so that it could be rapidly distributed online and through social media to aquaculture, aquaponics, and allied businesses around the U.S.. The National Aquaculture Association (NAA), USDA NIFA Cooperative Extension, National Sea Grant Extension, multiple industry associations, and other stakeholders assisted in distribution of the survey. Given the urgency to gather exploratory information on how U.S. aquaculture was being affected, a non-probability, self-selection method was used. The survey was be distributed quarterly throughout 2020, to capture the evolving effects of coronavirus disease (COVID-19) on the industry.

This presentation will cover the findings regarding the impacts of COVID-19 on U.S. mollusk aquaculture businesses.

Q7. Has your farm or business experienced any of the following as a result of the coronavirus disease (COVID-19) in 2020? Please select all that apply.
(n = 183)

- Lost sales : 98%
- Production challenges (not related to labor) : 32%
- Increased cost of production : 16%
- Labor challenges : 38%
- Increased demand for products : 2%
- Other : 13%



AQUACULTURE EDUCATION WEBINAR SERIES ADDS VALUE AND EXTENDS REACH

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As COVID-19 took hold around the world, the United States Aquaculture Society webinar committee was hard at work bringing valuable information and advice to our members and beyond. Using the 2017 USAS member survey as a starting point, the committee located experts from various fields of aquaculture and education that were willing to share their research and knowledge virtually. Partnerships with WAS, the National Aquaculture Association (NAA) and the Alabama Cooperative Extension System (ACES) helped shape the success and extend our reach.

The 2020 joint USAS/NAA/ACES webinar series was indeed a success, with eight webinars conducted and more to come in 2021. The total number of unique registrants for the webinars was 1,961 people from 79 countries, but several people attended multiple webinars. Table 1 provides details on the registration, live viewing and recording views for the calendar year 2020. These webinars and views of the subsequent recordings provided more than 2,500 hours of continuing education for participants and viewers around the world and will continue to add value for years to come. Attend this presentation to learn more about the value and impact of this effort.

Table 1. Details on the registration, live viewing and recording views of 2020 USAS webinars.

Date	Webinar	Registered	Attended Live	Aqua Ed YouTube Recording	USAS YouTube Views	Total Views
3/20/2020	Aquatic Weed Control	*	57	413	132	602
4/10/2020	Aquatic Animal Nutrition	*	213	666	950	1829
5/15/2020	Aquaculture Genetics Basics	839	298 +47 livestream	1973		2318
7/10/2020	Perceptions of Marine Aquaculture	592	597	530		1127
8/21/2020	Seafood Consumption Patterns	305	115	482	49	646
9/4/2020	Impacts of COVID on Aquaculture	308	125	520	62	707
10/2/2020	Aquaponics Economics	309	116	422	204	742
11/20/2020	Aeration Theory and Practice	743	228	1207	196	1631
	Total	**3096**	**1796**	**6213**	**1593**	**9602**

The videos are housed on YouTube on the USAS Channel as well as the Aquaculture Education and More YouTube channel and linked to the partner v

* Registration numbers lost during computer glitch.

EVALUATION OF POST LARVAL *Litopenaeus vannamei* HATCHERY PRODUCTION USING WATER QUALITY AND HEALTH STIMULATING PROBIOTICS

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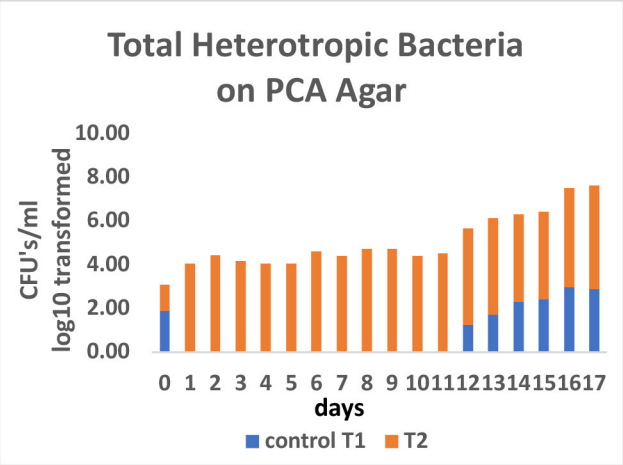
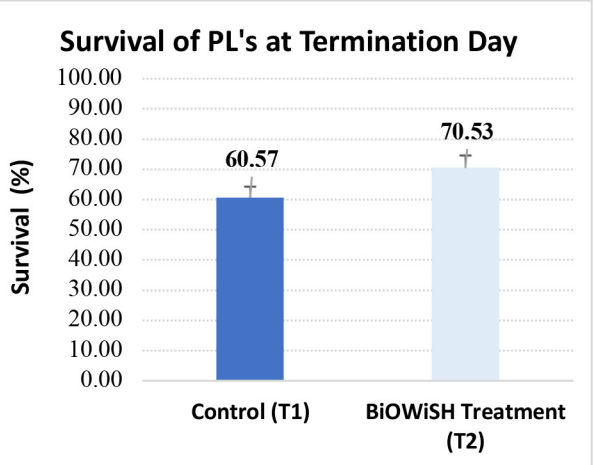
A hatchery trial was carried out at ShrimpVet hatchery in Ninh Thuan province, Vietnam whereby a probiotic protocol was compared to a non-probiotic protocol. In one treatment activated probiotics designed to improve water quality were applied daily, and the feed was coated with a probiotic designed to colonize on the shrimp gut. No probiotics were used in the other treatment.

The hatchery practiced strict biosecurity. Each treatment had three replicate 8 m^3 tanks filled to 7 m^3. Seawater used during the trial was filtered, settled, disinfected with chlorine, and disinfected by UV radiation before filling the tanks and used for water exchange. Water exchange in both treatments was 10 to 15 percent of tank volume daily. Water quality parameters analyzed on a daily basis included ammonia, nitrite, alkalinity, pH, dissolved oxygen, and temperature. Water samples were taken for bacterial analysis on PCA agar.

On harvest day post larvae from both treatments were analyzed for overall health based on FAO procedures which scores hepatopancreas lipid vacuoles, hepatopancreas color, hepatopancreas condition, intestinal content, muscle to gut ratio, and size variation. Post larvae from both treatments were sent to ShrimpVet laboratory in Ho Chi Minh City and subjected to a *Vibrio parahaemolyticus* AHPND (EMS) disease challenge.

Hatchery survival was improved by 17% when probiotics were applied. Post larvae health as defined by FAO assessment scoring was improved by 40% when probiotics were applied. Resistance to AHPND (EMS) challenge was improved by 8% when probiotics were applied. Probiotics increased shrimp hatchery sustainability and improved the quality and disease resistance of post larvae.





THE ROLE OF AQUACULTURE IN THE CONSERVATION OF AMPHIBIANS AT THE SAN MARCOS AQUATIC RESOURCES CENTER, UNITED STATES FISH AND WILDLIFE SERVICE

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Aquaculture has become an increasingly important tool for meeting the needs of a growing human population in a sustainable manner. However, aquaculture also plays a critical role in the conservation of at-risk species. Captive assurance colonies and captive breeding colonies of amphibians have been established by universities, nonprofits, zoological institutions, and conservation agencies around the world. The International Union for Conservation of Nature and Natural Resources (IUCN) listed captive programs among the top response priorities relevant to amphibian conservation globally. The San Marcos Aquatic Resources Center (SMARC), a United States Fish and Wildlife Service facility, is an aquaculture facility dedicated to the support of federally listed species. The mission of the SMARC is to provide support for, and undertake research on, endangered, threatened, and species at risk. The SMARC maintains captive assurance colonies for multiple amphibian species, including the Texas blind salamander (*Eurycea rathbuni*), San Marcos salamander (*Eurycea nana*), Barton Springs salamander (*Eurycea sosorum*), and the Houston toad (*Anaxyrus* [*Bufo*] *houstonensis*). Aquaculture-related activities for these species are inherent to this mission.

Figure 1. Barton Springs Salamander offspring developing at the SMARC

USE OF A KAOLINIC CLAY IN DIETS OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN LOW SALINITY WATER

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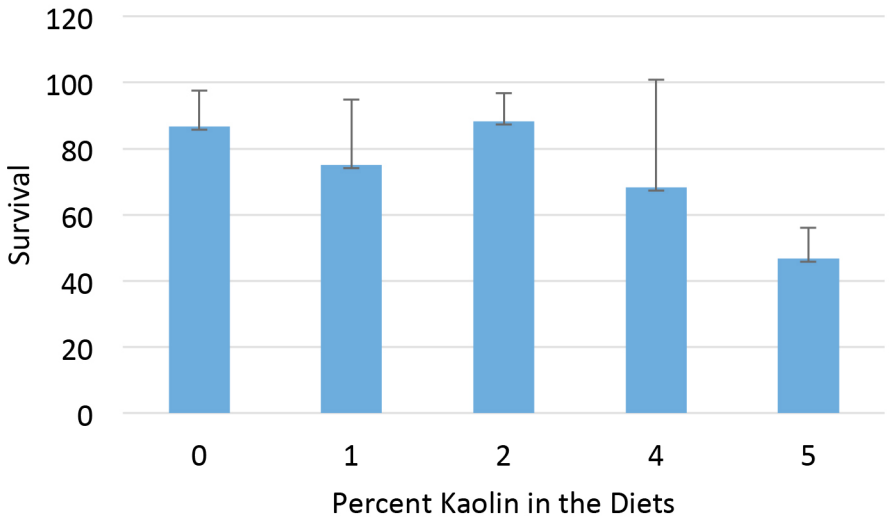
Kaolin clays have been used in a variety of industries, including diets for animals. Kaolin clays have antimicrobial properties and there is limited information on the benefits in the diet of aquatic species. The use of clay minerals in shrimp has demonstrated increased retention time in the gut and improved growth and feed efficiency. A 6-week study was conducted to determine if Akuapro®, a kaolinic clay, added to the diets would increase growth, feed conversion, and immunological parameters in the Pacific white shrimp *Litopenaeus vannamei*. Twenty shrimp were stocked into 20 75-L aquaria that were part of a recirculation system consisting of a sand filter, biofilter and sump (3900 L total system volume). Salinity was maintained at 2.3 g/L using reconstituted seawater. Shrimp were fed one of five isocaloric, isonitrogenous diets (35% CP) containing 0, 1, 2, 4 or 5% clay added to the diet. No significant differences in shrimp growth, specific growth rate, or survival occurred with shrimp fed any of the diets. However, apparent survival was lowest in shrimp fed the 5% diet. Osmolality and immune parameters are being measured and will be reported.

Figure 1. Survival rates for Pacific white shrimp fed diets containing 0, 1, 2, 4, or 5% clay added to the diet.



AUTOMATED BIOFOULING CONTROL OF AQUACULTURE OYSTER CAGES AND CROP

Olagoke Daramola*, Vishwamithra Sunkara, Megan Gima, Sanjib Sarkar, Christopher Webb, and Gero Nootz

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The Aquaculture industry provides essential food to the growing world population, especially in the supply of protein to low-income and food deficient countries[1]. Biofouling is a major problem in aquacultural production as both the target culture species and/or infrastructure are exposed to a diverse array of fouling organisms. This problem has been partially mitigated over the years by manually lifting or flipping infrastructure like oyster cages periodically above the waterline though it is labor-intensive and costly. This is to expose the crop as well as the farm equipment in the air and sun to minimize biofouling. Automated oyster cage operation would help in the reduction of manual labor and increase productivity. It will address these challenges by providing remote control that can vertically move farm structures based on user input or predefined rules which can be based on any combination of time of the day, wave height, air temperature, humidity, wind, precipitation, and other weather conditions. Such flexibility allows optimizing the desiccation cycles for maximum farm production. An automated control unit has been developed that is comprised of low-cost air pumps, solenoid valves, solar cells, microcontrollers, and other electronics, as shown in Figure 1. The pumps allow automatic desiccation of the oyster cage by moving air into the side floats and bottom floats. This process lifts the cage above the water line into the desiccation position. The valves allow the air inside the floats to escape and water to re-enter the floats to sink the cage. As bottom floats are filled with water, top floats hold the cage right below the water surface in the feeding position. The side floats stabilize the cage during the transitions between feeding and desiccation position. The sinking and raising of the system are controlled via an IoT (Internet of Things) microcontroller, i.e., the cage is connected to the internet for remote operation. All electronics are powered by solar energy. The performance of the system is monitored via telemetry, which includes the Wi-Fi strength, cage state, pumps, and valves states, and battery voltage level. The telemetry also includes local environmental parameters like temperature and humidity inside the control unit. This study currently advances towards the development of a cellular data link to a master cage at the remote farm site and a local mesh network between the rest of the oyster cages. This will allow the farmer to adjust the desiccation times to the oysters' growth stage and react to weather events based on the local conditions and forecasts from internet-based weather services. Future research will focus on the refinement and ruggedization of the system and the optimization of the desiccation cycle

FAO (2020). The state of World Fisheries and Aquaculture 2020. Sustainability in action. Rome
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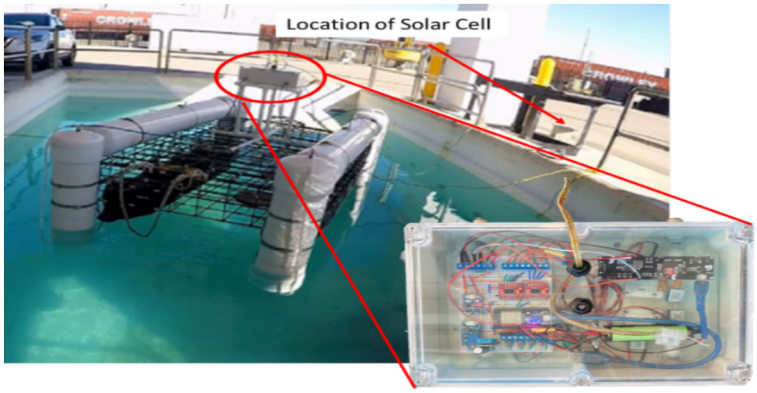

Figure 1 Testing of the Oyster cage system at the Marine Research Center pool, USM, Gulfport, MS, USA

A WELFARE ANALYSIS OF NORWAY'S EXPORT PROMOTION PROGRAM FOR WHITEFISH

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Farm groups have a long history of supporting generic advertising and other activities designed to strengthen the demand for their products in domestic and foreign markets (Forker and Ward, 1993). A large empirical literature has developed on the costs and benefits of these programs (for examples see Piggott et al. 1995, Kaiser et al. (2005), and Kinnucan and Cai (2011); for a review, see Williams et al. (2018)). This study contributes to the literature of generic advertising in two ways. First, using Norway's export promotion program for whitefish as a case study we show that the benefit-cost ratio (BCR) from the producer perspective, defined as the increase in producer surplus associated with the advertising divided by the decrease in producer surplus associated with the levy used to finance the advertising, is invariant to the supply elasticity. This result is implied by Kinnucan and Myrland's (2000) analysis of the optimal levy and is demonstrated in empirical studies by Alston et al. (1998, 2005). Nonetheless, it has been largely ignored in the BCR literature. An important implication of the invariance property is that the BCR can be approximated using strictly demand-side information. If the goal is to obtain an estimate of the producer benefits of the advertising in relation to producer costs, estimates of the own-price and advertising elasticities of demand suffice; there is no need to estimate the supply side of the market. Second, we show that the BCR from the consumer perspective, defined as the increase in consumer surplus associated with the advertising divided by the decrease in consumer surplus associated with the levy, is identical to the BCR from the producer perspective, which is new. Our study considers Norwegian whitefish as a case study.

Norway recently increased the levy used to fund its export promotion program for whitefish from 0.50% to 0.75%. Study results suggest the intensified program is welfare increasing. The net social gain, defined as difference between the increase in economic surplus associated with the increased advertising and the decrease in economic surplus associated with the increased levy, is estimated at between $81 million and $174 million per year. The associated benefit-cost ratio (BCR), defined as the ratio of the aforementioned changes in economic surplus, is between 16 and 34 (Table 1). The BCR is invariant to the supply elasticity. The invariance property is useful as it implies the BCR can be estimated using strictly demand-side information; there is no need to estimate the supply side of the market. This result is generalizable to any advertising program funded by a levy on industry output or sales.

Table 1. Benefit-cost ratios associated with the expanded promotion program

Supply	Foreign Consumer			Domestic Producer		
Elasticity	ΔCS_A	$-\Delta CS_T$	BCR	ΔPS_A	$-\Delta PS_T$	BCR
	---------- Demand elasticity = - 0.416 ----------					
0	0	0	undefined	179	5.33	33.5
0.5	98	2.90	33.8	81	2.41	33.6
1.0	127	3.75	33.8	52	1.56	33.6
1.5	140	4.15	33.8	39	1.15	33.6
2.0	149	4.39	33.8	31	0.91	33.6
	---------- Demand elasticity = - 0.863 ----------					
0	0	0	Undefined	86	5.33	16.1
0.5	32	1.95	16.3	54	3.37	16.2
1.0	46	2.85	16.3	40	2.46	16.2
1.5	55	3.37	16.3	31	1.94	16.2
2.0	60	3.71	16.3	26	1.60	16.2

DIFFERENT CARBON SOURCES AFFECT MORPHOLOGY AND PLANKTONIC COMPOSITIONS OF BIOFLOCS

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Biofoc technology system (BFT) is a microbial-based fish culture system that works by elevating the carbon nitrogen ratios in the culture unit. The biofloc composition, water quality and performance of aquatic animals, however, can sometimes depend on the added carbon source. Therefore, it is most likely that the carbon sources influence the microbial community in BFT. This study seeks to establish the effect of carbon sources on the structure and planktonic compositions of bioflocs.

A 21-day experiment was conducted in the wet laboratory of Universiti Putra Malaysia, using 50 L of water housed in 100 L capacity glass aquarium. Each of the 9 aquaria received 20 g of grinded fish feed (42% crude protein) at three days interval to have a similtude of aquaculture wastewater. Three different carbon sources, sucrose, glycerol and rice bran were experimented and added at carbon/nitrogen ratio of 15 in each tank with the experiment triplicated. The water quality was monitored daily and after 21 days, 50 ml of the biofloc water in each tank was collected for morphological examination, phytoplankton and zooplankton analyses using standard procedures.

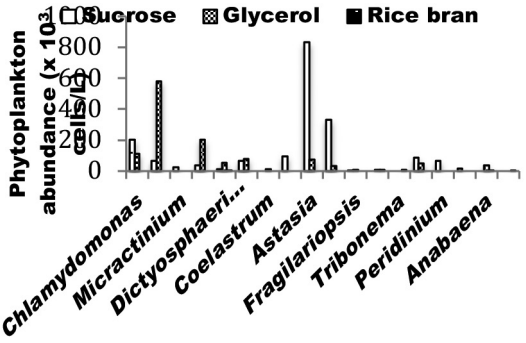

Figure 1: Phytoplankton composition of the bioflocs

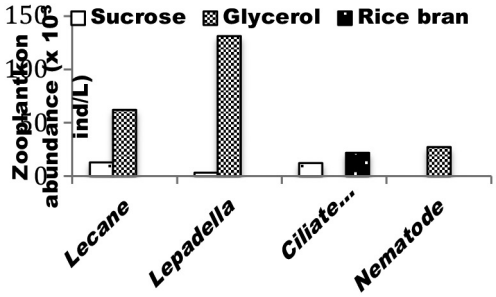

Figure 2: Zooplankton composition of the bioflocs

The colours of the bioflocs from the different tanks were different, glycerol biofloc was greenish, sucrose biofloc tended towards reddish while rice bran biofloc was brownish in colour. The morphostructure of the bioflocs from different carbon sources as observed under microscope were different, though all the bioflocs had irregular agglomerates. The bioflocs in glycerol treatment were well dispersed. Sucrose and rice bran had aggregated structure, and it is more compacted in rice bran. The planktonic composition was also different among the different carbon sources. Phytoplankton from 18 genera and 5 phyla were observed (Figure 1). These include; Cholorophyta (*Chlamydomonas*, *Palmella*, *Micractinium*, *Oedogonium*, *Dictyosphaerium*, *Coelastrum* and *Scenedesmus*), Euglenophyta (*Astasia*), Ochrophyta (*Gonyostomum*, *Fragilariopsis*, *Amphora* and *Tribonema*), Charophyta (*Coleochaete*), Dinoflagellata (*Peridinium* and *Ceratium*) and Cyanobacteria (*Anabaena* and *Gomphosphaeria*). The phytoplankton abundance in the sucrose and glycerol treatments were higher compared to rice bran treatment. The glycerol treatment was dominated by Cholorophyta while sucrose was dominated by mixtures of Euglenophyta, Ochrophyta and Chlorophyta. Rice bran treatment had the least diverse and abundant phytoplankton with only four genera (*Anabaena*, *Gomphosphaeria*, T*ribonema* and *Dictosphaerium*). The sucrose treatment had in total of 13 phytoplankton genera while glycerol had 12 genera. The *Dictosphaerium* (Chlorophyta) was found in all treatments.

In general four groups of zooplankton were observed in the biofloc treatments which include; rotifers (*Lecane* and *Lepadella*), protozoa (*Ciliate*) and nematode (Figure 2). The glycerol treatment had most abundant zooplankton from rotifers, it also had some nematodes. The sucrose treatment had both rotifers and protozoa, but rice bran treatment had only protozoa.

The results in this study indicated that carbon sources affect both the morphological structure and microbial composition of the bioflocs.

COMMERCIAL DEVELOPMENT OF RAS-SPECIFIC DIETS: EVALUATING EFFECTS ON WATER QUALITY AND ATLANTIC SALMON *Salmo salar* PERFORMANCE

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Increased investment in land-based, recirculating aquaculture systems (RAS) is driving demand for specially formulated diets that are compatible with water quality and waste discharge in addition to fish performance. For example, RAS-specific diets must produce intact and settleable fecal material that is less prone to disintegration and release of fine particles and dissolved nutrients. Specialty diets for the production of market-size Atlantic salmon in RAS are in particular demand, as commercial development of this aquaculture sector has grown rapidly over the last decade.

To support this industry need, the Freshwater Institute recently partnered with commercial feed supplier Cargill Inc. to evaluate four diets fed to post-smolt Atlantic salmon (1.77 kg initial weight) within twelve replicated water reuse systems. A 2x2 factorial design was employed to evaluate diets that: i) included or excluded a proprietary binder-like ingredient (B), and ii) were formulated with either land animal (LAP) or fishmeal-based (FM) proteins (N=3). Water quality, with focus on total suspended solids (TSS), was comprehensively assessed throughout the 6-month trial, and fish performance metrics were evaluated bimonthly.

A highly significant difference in TSS concentration was detected (P= 0.000) in the culture water of reuse systems relative to inclusion of the binder-like ingredient (Fig. 1). Trends for increased solids settleability as measured by TSS levels in the cone-bottom and overtopping flows of settling devices suggested improved fecal stability for diets containing the binder. Significantly lower fine particle (2-30 µm) counts were also associated with these diets.

A difference in growth as measured by mean salmon weight was detected between treatments (P= 0.000) at each sampling interval, where fishmeal-based diets resulted in faster growth than diets formulated with land animal proteins. Statistical differences in survival and fish health metrics were not found. Overall, this trial provided data-driven proof-of-concept for the development of a RAS-specific diet for post-smolt Atlantic salmon growing towards market-size.

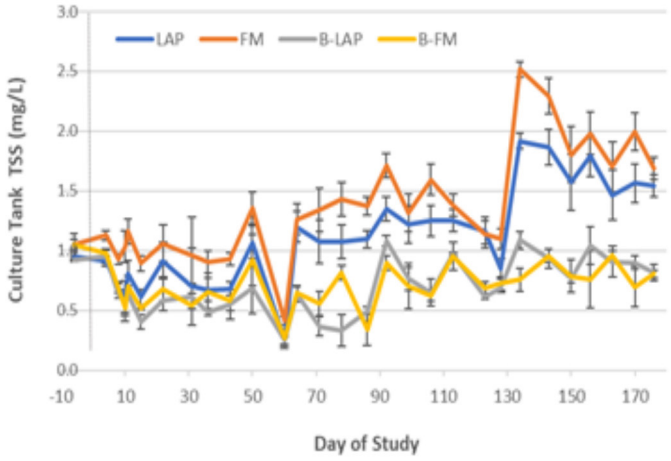

Fig. 1. Culture tank TSS levels associated with each diet.

PASSIVE ACOUSTIC FEEDERS AS A TOOL TO ASSESS FEED RESPONSE AND GROWTH IN SHRIMP POND PRODUCTION

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Shrimp production has been one of the most important sectors of aquaculture for the last few decades for both its market value and acceptance. As production systems move towards increasing intensification, animal nutrition takes a central role as an important growth, environment and health factor. It is well reported that shrimp are opportunistic grazers that eat a wide variety of organisms, yet diets with low or no inclusion of fish meal are often still regarded as of lower quality and a potential cause of less than ideal growth and production. As the majority of shrimp feeding protocols in typical production setups rely in a combination of feed trays and predetermined feed plans, direct assess of shrimp appetite and feed preferences can be complicated. However, for the last decade, development of passive acoustic monitoring has allowed a much more direct measurement of shrimp feed intake by capture and integration of clicking sounds produced by shrimp while eating. Tying this to a automated feeding systems has allowed the development of on demand feeding for shrimp. Hence, this technology is a potential tool to help understand feed preferences when the feeding protocol is based on real time demand for feed rather than predetermined quantities. Building on previous research, the goal of this trial was to use passive feedback acoustic feeders as a tool to evaluate if shrimp prefer commercial diets with different protein sources when given the option to eat as much as requested. This 13-wk trial was performed in 16, 0.1 ha outdoors ponds, stocked at 30 shrimp/m^2 and equipped with the AQ1 acoustic feeding system. All ponds were fed the same predetermined protocol during the first month after which acoustic systems were initiated and four treatments were assigned with a 35% crude protein commercial diet with different protein sources: all-plant, 8% poultry meal (PM), 8% fish meal (FM) and 12% FM. Results for this trial are summarized in Table 1. We did not find any differences in statistical differences in any of the main production parameters. Results of this study indicate that shrimp did not clearly favor a particular diet. Hence suggesting that when well balanced commercial feeds and feed quantity are not a limiting factor, shrimp growth was not highly impacted.

Table 1 - Pacific White Shrimp response to four commercial diets with varying protein sources

Treatment	g/week	Final mean weight (g)	Feed Input (Kg/ha)	Percent Survival	Yield (kg/ha)	FCR
All Plant	1.64	21.02	7898	91.66	5355	1.54
8% PM	1.67	21.54	8084	88.03	5725	1.48
8% FM[1]	1.72	22.59	7596	92.95	6276	1.21
12% FM	1.64	21.49	7631	80.92	5227	1.50
P-value	0.770	0.7604	0.492	0.798	0.607	0.608
PSE[2]	0.06	0.73	17.46	8.13	540.5	0.14

[1]n=3
[2]PSE: Pooled Standard Error

A COMPARISON OF GROWTH AND TAURINE RETENTION BETWEEN PLANT AND ANIMAL PROTEIN-BASED DIETS IN JUVENILE WHITE SEABASS *Atractoscion nobilis*

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A 70-day trial was conducted to evaluate the effect of dietary protein matrix (soybean meal or poultry byproduct meal-PBM) on taurine efficacy and retention in juvenile white seabass *Atractoscion nobilis*. Diets were formulated to contain 40% protein and 12% lipids with taurine supplementation ranging from 0 – 1.37% taurine. Fish were fed 4-8% body weight/day over the course of the trial. Growth performance metrics and taurine retention in fish were measured and compared using a linear regression model with diet type as covariates (growth parameters) or a quadratic model with diet type as covariates (taurine retention and deposition). Fish fed diets with soy proteins had lower percent gain, survival, feed fed/fish, and thermal-unit growth coefficients (TGC) at similar levels of taurine inclusion when compared to diets with primarily PBM as the protein source. Taurine level was positively correlated with mean final weight, weight gain, percent gain, TGC of fish regardless of diet, and negatively correlated with FCR regardless of the diet. A taurine requirement based on whole-body deposition was calculated to be 1.18%, 1.21%, and 1.03% for all diets, PBM diets, and soy diets, respectively (Figure 1). The 95% confidence intervals for the taurine requirement estimates all overlap, so there is no statistical difference in the estimates. These results demonstrate that the protein source did not influence the taurine requirement and suggest that taurine deficiencies are not the driver of lower TGC or survival in white seabass fed diets with soy, and that other factors warrant investigation.

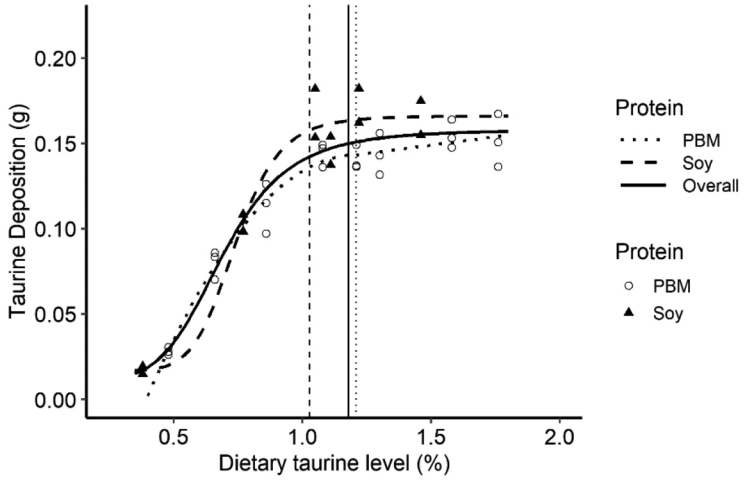

Figure 1. Taurine deposition and fitted models used to calculate the requirement estimates. Solid lines represent the predicted values of the most parsimonious model for each series (solid line = all diets, dotted line = PBM diets, dashed line = soy diets). Vertical dashed lines represent the requirement estimate for each diet series and all diets combined.

A COMPARISON OF THE TECHNICAL EFFICIENCY OF AQUACULTURE STEWARDSHIP COUNCIL SHRIMP FARMS TO NON-CERTIFIED FARMS

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Aquaculture will play an increasingly important role in the global seafood supply as fisheries harvests have plateaued. Shrimp are a highly valuable aquaculture commodity are produced largely for global trade. The Aquaculture Stewardship Council's shrimp certification standard is meant to serve as a market-based tool that rewards the better actors in the industry for improved performance in areas like technical efficiency, social responsibility, and traceability. The goal of this study was to compare production methodology and efficiency of farms certified to the ASC shrimp standard to farms from recent field surveys in the same geographical areas. Certified farms were statistically larger on average (four times larger in Latin America and 10 times larger in Asia). Farms in Asia operate at higher production intensities but no differences were seen due to certification status. No differences were seen in the FCR of farms in Asia, but ASC farms in Latin America had the higher average FCRs than non-certified farms (1.79 vs. 1.33). ASC farms in Asia used drastically less water exchange than other types of farms, and were more energy efficient than other farms as well. These findings were used to make recommendations for the ASC standard and certification standards in general, including a greater emphasis on requirements for limits on efficiency-based metrics beyond simply reporting the outcome of the calculation.

QUANTITATIVE LYSINE REQUIREMENT FOR JUVENILE FLORIDA POMPANO *Trachinotus carolinus* FED PLANT-BASED DIETS

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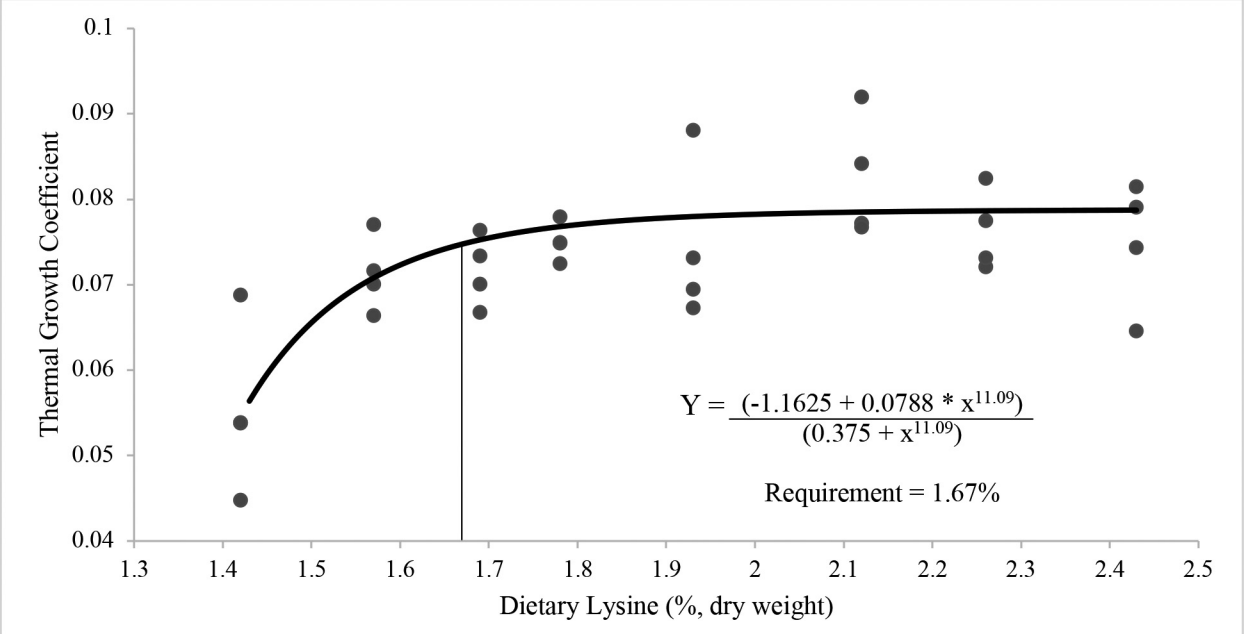
Florida pompano, *Trachninotus carolinus* is positioned to be an important fish species in US mariculture. If commercial production is to be developed sustainably and with reduced feed costs, it is critical to maximize the level of plant-based proteins in the diet. Thus, good information is needed first on limiting amino acids, which would include lysine. This work was conducted using eight diets formulated with graded levels of lysine (1.42-2.42% dry weight) and fed to juvenile pompano (mean initial weight 13.07 ± 0.46). Dose responses and significant effects were observed in final weight (FW), percent weight gain (PWG), thermal growth coefficient (TGC) and feed conversion ratio (FCR). Significant differences in mean fish whole body amino acids were observed in aspartic acid, glutamic acid, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Regression models, such as broken-quadratic model (BQM), 4-parameter saturation kinetic model (4-SKM), 5-parameter saturation kinetic model (5-SKM), one-slope broken line model (BKL.1) and two-slope broken line model (BKL.2) from R were fitted against different parameters to determine the dietary lysine requirement. Thermal growth coefficient was the parameter utilized to fit a 4-SKM model and determine the quantitative lysine requirement. Based on these results, a minimum lysine requirement of 1.67% dry weight is recommended for practical feed formulations.

Figure 2. Thermal growth coefficient of juvenile Florida pompano to graded levels of lysine. The dietary lysine requirement was fitted by the 4-parameter saturation kinetic model.



THE UNITED STATES' ORNAMENTAL FISH TRADE DURING THE COVID-19 PANDEMIC

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Global trade in ornamental fish has expanded over time. The USA is one of the top traders in live ornamental fish export and import. This study has analyzed the United States' performance in ornamental fish trade during the COVID-19, compared to the long-term trends. Besides, it has measured the United States' comparative advantage in the ornamental fish trade. Using the Vollrath (1991) approach, it has quantified relative export advantage (RXA), import specialization index (RMA), and relative trade advantage (RTA) of the United States. The study revealed that global trade in ornamental fish has significantly reduced in 2020 due to COVID-19. In 2020, the value of international trade in ornamental fish was US$159.5 million, based on export statistics, or US$194.0 million, based on import statistics. Forty-seven countries/ territories exported live ornamental fish to 63 countries/ territories of the world in 2020. Global exports increased steadily from US$183.7 million in 2001 to a peak of US$379.4 million in 2011, then declined to US$326.2 million in 2019. Global imports increased steadily from US$227.6 million in 2001 to a peak of US$389.4 million in 2006 and gradually declined to US$ US$304.5 million in 2019. In 2020, compared to 2019, global exports (US$159.5 million) was 49% lower, and global import (US$ 194.0 million) declined by 36%. More than 93% of the global export was by ten countries. In 2020, top 10 exporters were: Japan (having 29.1% of global export), Spain (19.0%), Czech Republic (%), United Kingdom (12.4%), Netherlands (11.2%), Germany (8.7%), USA (4.2%), Philippines (1.7%), Colombia (1.6%), and Sweden (1.2%). On the other hand, the top 10 importers in 2020 were the USA (34.6% of global import), United Kingdom (9.2%), Japan (9.1%), Germany (8.3%), Netherlands (6.7%), Hong Kong (4.8%), Canada (3.0%), Spain (2.9%), Australia (2.8%), and Italy (2.5%). In 2020, compared to 2019, the United States' total export (US$6.7 million) was 33% lower, but total import (US$67.2 million) was 7% higher. In 2020, the USA was the 7th largest exporter with a market share of 4.2%. It had exported ornamental fish to 48 countries and territories of the world. Major export destinations were Canada (36.4%), Hong Kong (9.6%), Mexico (6.5%), Japan (5.6%), Singapore (5.0%), and Germany (4.8%). Major sources of import were Singapore (15.4%), Indonesia (15.1%), Thailand (14.8%), Sri Lanka (10.5%), Japan (8.5%), and the Philippines (8.4%). A major challenge in the ornamental fish trade is fish health concerns and stringent regulation related to fish trade. The pandemic has increased the risks and biosafety concerns. The United States exporters also face steep competition in price. Attractive fish with an assurance of high-quality certified ornamental fish export comes to the benefit of the United States' exporters.

EXPEDITING VIRAL DISCOVERY IN SHRIMP AQUACULTURE BY COMBINING CONVENTIONAL HISTOPATHOLOGY AND GENOMICS

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Infectious diseases are a threat to sustainability of shrimp aquaculture worldwide. The growth and profitability of shrimp industry has been punctuated with disease outbreak periodically. In the vast majority of cases, the etiologic agent of a disease is discovered only after the disease has spread beyond its initial geographical origin to across countries and even continents. Routinely the etiologic agent is identified by conducting an experimental bioassay using the suspected pathogen, histopathology, pathogen isolation, and genomic characterization. There is a need to expedite pathogen discovery in order to prevent worldwide spread of shrimp diseases.

We demonstrated the feasibility of combining conventional histopathology and next generation sequencing (NGS) to expedite pathogen discovery using known archived histology tissue blocks of White Spot Syndrome Virus (WSSV), and Taura syndrome virus (TSV), infected *Penaeus vannamei* shrimp (01 year old block for WSSV and 15 year old for TSV). Thin (5 µm) sections of Davidson´s-fixed paraffin embedded (DFPE) archived shrimp tissues were stained with Hematoxylin and Eosin stain to confirm WSSV and TSV pathognomonic lesions. Parallel tissue sections were taken for isolation of nucleic acid, detecting WSSV and TSV by real-time PCR/ RT-PCR before taking the nucleic acids for NGS. For WSSV, DNA was also isolated from frozen tissue samples of the isolate corresponding to the archived histology block samples and NGS were carried out using the isolated DNA. NGS generated millions of short (~150 bases) sequence reads for both WSSV and TSV infected samples and the sequences were taken for further analysis. Upon subtracting the host genome sequence from the sequence pool, WSSV and TSV genome were reconstructed *de novo*. For WSSV, mutations (substitutions, deletions and insertions) detected in the *de novo* assembled genome sequence compared to the reference sequence in the NCBI database were confirmed by amplifying the corresponding regions from the original set of nucleic acid and sequencing the amplicon by Sanger sequencing method. The complete genomes of WSSV and TSV were reconstructed from ~2-year-old and 15 year-old archived tissue blocks. Phylogenetic analysis performed using TSV sequence derived from histology block showed that the *de novo* assembled genome clustered with homologous sequence deposited in the GenBank databases. This confirmed the authenticity of the genome sequence generated by NGS analysis from histology tissue blocks.

Until now, the use of nucleic acids derived from DFPE shrimp samples has not been tested for downstream high-throughput genomic analysis. The findings reported here demonstrate the utility of archived tissue samples for virus discovery and evolutionary studies involving viral disease in crustaceans. This opens an avenue to expedite pathogen discovery and enhance developing diagnostic tools to prevent the spread of viral diseases worldwide.

INVESTIGATION INTO THE PATHOGENESIS OF BLUE CATFISH ALLOHERPESVIRUS (BCAHV)

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Blue catfish alloherpesvirus (BCAHV) is a strain of Ictalurid herpesvirus 1 (IcHV1) genetically similar (94%) to channel catfish virus (CCV). Limited information on the pathogenesis of this virus combined with its potential to cause damages at the production facilities warranted a detailed study of BCAHV. To determine the host-specificity and potential host range of BCAHV, the virus was inoculated onto fish cell lines belonging to families Ictaluridae, Cyprinidae, Centrarchidae, and Clariidae. The virus replication and exhibition of cytopathic effects (CPEs) were restricted to cell lines from family Ictaluridae indicating the host preference of BCAHV. Subsequently, fish challenge trials were carried out to evaluate intrinsic and extrinsic factors influencing BCAHV infection. Mortality due to BCAHV infection was significantly high in blue catfish and low in channel catfish further specifying the host preference of the virus (Figure 1). Clinical symptoms of BCAHV infection included ascites and exophthalmia. Histopathological alterations of the BCAHV infected fish revealed prominent splenitis with severe erythrophagia. Host susceptibility to BCAHV differed with age and fish at 60 days post hatch were more susceptible. Temperature had a significant role in the activation and pathogenesis of BCAHV. Fish exposed to BCAHV at a sustained high temperature had significantly low mortality suggesting the likelihood of virus inactivation. In another fish challenge simulating crowding, mortality was found to be significantly higher in densely stocked tanks, indicating the role of horizontal transmission in BCAHV pathogenesis. The heightened pathogenicity of BCAHV towards blue and hybrid catfish as observed in this study points to the potential of this virus to cause significant concern in catfish production.

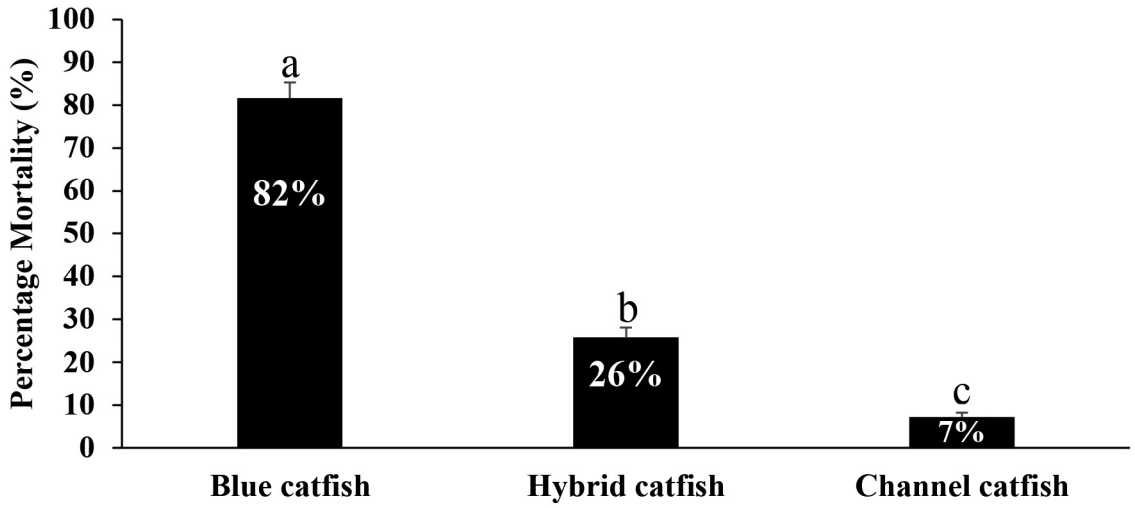

Figure 1. Susceptibility of blue, channel, and hybrid catfish to BCAHV. Data represent average mortality ± SEM (standard error mean). Values with different letters indicate statistically significant means ($p \leq 0.05$).

EXPRESSION OF THE SREB FAMILY OF G-PROTEIN COUPLED RECEPTORS ACROSS FISH EVOLUTION

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Super-conserved Receptor Expressed in Brain (SREB) is a highly conserved family of G-protein coupled receptors implicated in diabetes and several neurological disorders, and consists of three members (*sreb1*, *sreb2*, and *sreb3*). Previous genomic analyses revealed a novel fourth member (*sreb3b*) specific to euteleost fish. However, *sreb3b* expression is not yet characterized, and few studies have measured these receptors across multiple tissues in fish, including the gonads where SREBs are important in reproductive functions. The objective of this study was to measure SREB expression in gonads, and broadly characterize *sreb3b* throughout fish evolution. To this end, diverse fish species were used in qPCR, including Florida gar (*Lepisosteus platyrhincus*), zebrafish (*Danio rerio*), sailfin molly (*Poecilia latipinna*), African turquoise killifish (*Nothobranchius furzeri*), and green-spotted puffer (*Dichotomyctere nigroviridis*). Killifish ovaries were used for *in situ* hybridizations to determine *sreb3b* spatial patterns. Overall, ancestral gar showed high *sreb* expression in ovaries, while more derived species exhibited elevated levels in testes. However, *sreb1* was elevated in testes for all species. Across organs, *sreb3b* was highest in neural tissues, exhibiting patterns most similar to *sreb2*, while *sreb1* and *sreb3* were elevated in both neural and gonadal tissues. *In situ* hybridizations identified *sreb3b* in oocytes and follicle cells, indicating possible roles in both ovarian development and as a stored transcript for embryonic growth. Expression patterns indicate that SREB roles may not be conserved across fishes. Future studies should focus on the roles of the SREB family in gonads, and *sreb3b* functions in the brain.

EXPANDING AQUACULTURE INVESTMENT IN OREGON USING A NOVEL GEOSPATIAL AND FINANCIAL PLANNING TOOL

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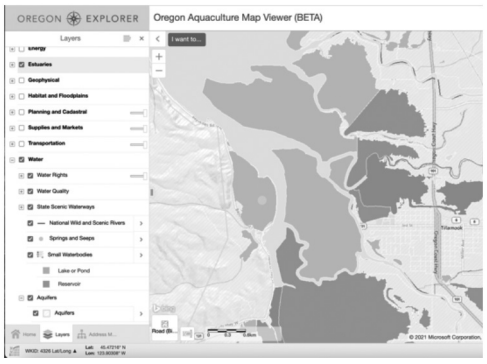
The Oregon Aquaculture Association (OAA), Business Oregon, Oregon State University (OSU), and Oregon Sea Grant (OSG) are currently expanding data and species profiles for their Oregon Aquaculture Explorer Platform. This Platform includes a geospatial and financial planning tool (Oregon Aquaculture Financial Planning Tool) that allows potential entrepreneurs and investors to explore spatial resources and develop plans for aquaculture business opportunities.

Currently, the tool has been built and peer reviewed for three inland aquaculture species (specifically, tilapia and sturgeon in recirculating systems, and hybrid striped bass in ponds). We are now in the process of beta testing and expanding the tool to include coastal shellfish and seaweed aquaculture products. The functionality of the tool will remain the same for the new species including using a GIS platform. The tool allows the user to select a site (choosing on a map or entering an address), develop an initial resource site report, and then determine likely costs for inputs including energy, water, feed, seed, and labor. Each of these costs can be refined based on the user's estimates. A unique attribute of the platform is directly linking spatial data as inputs into the financial models. The tool allows the user to run multiple scenarios based on alternative production levels, costs, output prices, and resource constraints. Start-up costs, including construction and materials, are also considered. The tool provides a downloadable Excel file for the user to conduct more detailed and site specific analysis.

Although the initial phase pf the project has focused on inland species and systems, phase two will focus on marine species and coastal and estuarine aquaculture. With the exception of oyster culture, estuarine and nearshore aquaculture is limited in Oregon. New research will focus on investment opportunities and challenges for novel and emerging systems and species such as red seaweed (dulse), abalone, and scallops.

The goal of this presentation is to provide an introduction to and overview of the Oregon Aquaculture Explorer Platform and tool, and to elicit feedback and conversation around potential refinements, species, and concerns. While the tool has undergone peer review for the inland species, the coastal species are new, and additional considerations will be necessary before the full release of the tool.



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

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

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

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

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

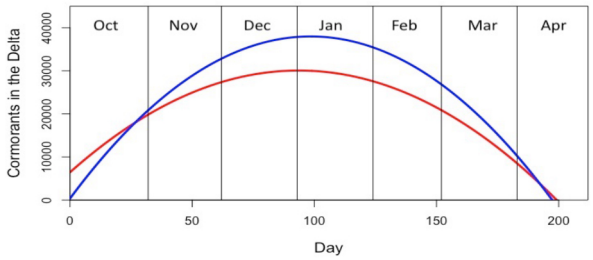

Figure 1. The estimated abundance trend for cormorants residing in the Mississippi Delta during winters 2016-2017 (red line) and 2017-2018 (blue line) calculated from aerial surveys of night roosts.

MICROBIAL COMMUNITY, STRUCTURE, AND FUNCTION IN A RECIRCULATING EASTERN OYSTER, *Crassostrea virginica,* HATCHERY PRODUCTION SYSTEM USING ARTIFICAL SEA WATER

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The oyster fishery in the Gulf of Mexico has been a global leader in oyster production. However, degrading water quality, storm events, the Deepwater Horizon oil spill, and extended opening of the Bonnet Carré Spillway, have diminished wild oyster populations. Aquaculture has the potential to supplement wild populations and support a commercial oyster aquaculture industry. The industry relies on hatchery production of single-set oyster seed which can be unreliable due the variability of the natural seawater (NSW) used for hatchery production. The use of artificial seawater (ASW) has the potential to enhance aquaculture production for the Eastern oyster, *Crassostrea virginica*, by expanding production capability into inland areas, avoiding fluctuating environmental conditions, and reducing risks from anthropogenic pollutants. NSW, however, contains complex communities of microorganisms that play a critical role in maintaining ecological processes that affect oyster development, growth, and survival. Characterization of microbial communities in ASW through molecular sequencing will lead to improved aquaculture operations.

Samples were collected from recirculating ASW, oyster production systems located at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center, in Ocean Springs, Mississippi, USA during the 2018 - 2020 hatchery seasons. Samples were filtered through Sterivex-GP 0.22uM filter (Millipore-Sigma) and genomic DNA was extracted using the FastDNA® SPIN kit (MP Biomedical Inc.), and ~2 ng/μL of sample was used at the Integrated Microbiome Resource (IMR) facility at Dalhousie University (Halifax, Nova Scotia, Canada) for 16S rRNA gene amplification and sequencing, targeting the V6–V8 variable regions. Samples were quantified, and analyzed on an Illumina MiSeq platform, generating 300 bp, paired-end sequences. For bioinformatics analyses, we have used Quantitative Insights into Microbial Ecology (QIIME2). QIIME2-DADA2 pipeline was used for quality control and the generation of amplicon sequence variants (ASV), which were clustered into OTUs and used for taxonomic identification and phylogenetic assignment against the ribosomal RNA database reference (SILVA). Bacterial plating, nutrient analysis and larval performance was examined to identify potential correlations between bacterial community composition and larval health and condition.

Preliminary data show differences in microbial diversity at different locations within the hatchery system and through time. Further analyses will determine how microbial communities change as water inventory ages and throughout larval culture cycles. This data will improve understanding of the role of microorganisms in artificial saltwater oyster hatchery systems and have potential to inform methods to establish bacterial communities to sustain oyster larval health and development.

EVALUATION AND BUSINESS PLAN FOR PINFISH *Lagodon rhomboides* PRODUCTION UTILIZING A RECIRCULATING AQUACULTURE SYSTEM IN FLORIDA

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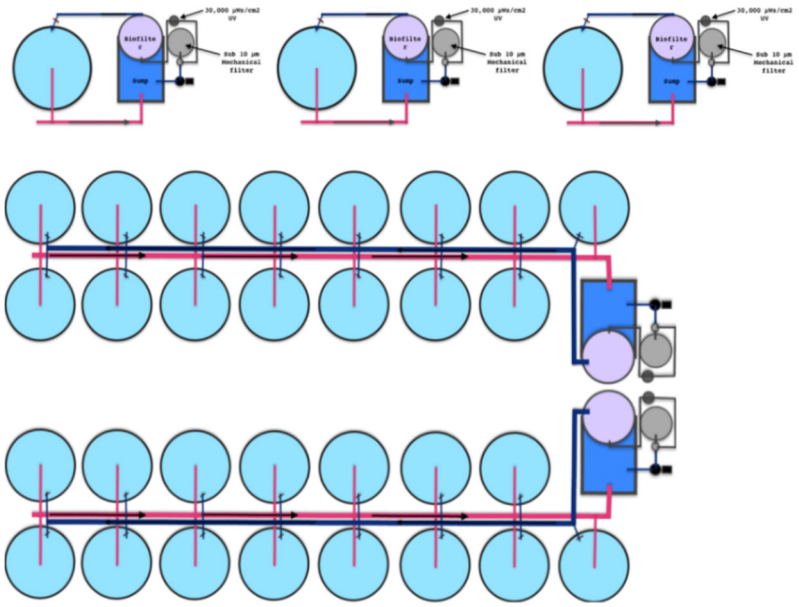
Pinfish (*Lagodon rhomboides*) are a common baitfish in the southeastern United States and have been shown to be a preferred bait species in Florida specifically. Currently, all pinfish supplied to bait stands in Florida are wild-caught, which has led to high variability in the quantity and quality of bait provided by bait stands. This deficiency causes a demand from marine recreational anglers for a consistent supply of pinfish and produces an opportunity for an aquaculture market to develop. Aquaculture production could supplement pinfish supply while providing the consistent quality that is demanded by anglers and retailers. This evaluation was conducted to determine if pinfish production using a recirculating aquaculture system could be financially viable in Florida.

Current research supports using recirculating aquaculture systems for pinfish production, which led to the system that was developed for this evaluation. A business plan was designed to determine the methods in which a future pinfish production could operate and become financially viable. The business plan serves as a guide for future marine baitfish producers, especially in Florida. The proposed production methods, the managerial structure of the business, marketing potential, and production budgets for the future farm were considered in this assessment. The goal of this evaluation was to present a potential method for pinfish production that could prove to be financially viable if such a marine baitfish market were to develop.

Image 1: Schematic design for a future pinfish production using a recirculating aquaculture system



ECOLOGICAL MANAGEMENT OF NUISANCE ALGAL BLOOMS AND AQUATIC WEEDS IN STREAMS AND PONDS

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Habitat degradation, excessive nutrients, reduced streamflow and water scarcity create an environment conducive to undesirable algal blooms and nuisance aquatic weeds in streams and ponds. Nuisance algal blooms and aquatic weeds are often removed with herbicides or chemical treatments. The application of chemical treatments not only removes target species but also removes beneficial bacteria and algal species, along with disrupting the existing microbial ecosystem which assimilates nutrients and wastes. An alternative method to chemical treatments is to enhance the natural microbial communities to process nutrients and waste.

The Water Cleanser (Marine Easy Clean, Aust.) provides an alternate means of eliminating algal blooms, including Cyanobacteria and filamentous algal mats. The Water Cleanser is a wax block infused with carbonaceous and trace elements along with millions of microscopic capillaries which provide habitat for beneficial bacteria. The development of a biofilm of beneficial bacteria and other microbiota, increases the process of organic waste decomposition.

Methods of utilizing blocks to eliminate Cyanobacteria and filamentous algal blooms along with aquatic weeds were developed for streams and ponds. Blocks were evaluated in different high and low flow streams at numerous sites. The blocks were also evaluated in three one-acre ponds with Comanche Springs pupfish with duplicate ponds with blocks and a control pond receiving no treatment. Ponds were monitored for water quality, dissolved oxygen, fish health and abundance. Blocks were also evaluated in channel catfish ponds.

The blocks were effective in eliminating Cyanobacteria and filamentous algal blooms in ponds and streams. Cyanobacteria, filamentous algal blooms and *Chara* sp. were eliminated within one to two weeks with the use of blocks in streams. Untreated parts of the stream continued to have nuisance algal blooms. Blocks were used in pupfish ponds with existing Cyanobacterial mats, filamentous algae and *Chara* sp. Besides reducing Cyanobacteria, filamentous algae and *Chara* sp., zones of submerged aquatic plants were also eliminated. Dissolved oxygen concentrations were less variable in treated ponds than in the pond with no blocks. When adequate numbers of blocks were used in channel catfish ponds, there was improved water quality and the elimination of algal mats and selected weeds. Overall, the blocks were effective in algae, Cyanobacteria and selected aquatic plant removal when the appropriate number and placement of blocks were employed. Proper use of the blocks resulted in improved water quality, health and propagation in the both channel catfish and pupfish.

PROGRESS IN CATFISH XENOGENESIS

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Xenogenesis has been accomplished previously in ictalurid catfish. However, spawning rates and fry production were inadequate for large-scale application to produce channel catfish, *Ictalurus punctatus,* female X blue catfish, *I. furcatus*, male hybrid catfish.

In the current study, xenogenic channel catfish and white catfish, *Ameiurus catus*, harboring gametes from channel catfish and blue catfish were utilized to produce pure species and hybrid progeny. Various factors allowed significant increases in spawning rates and fry production.

ANALYSES OF TEXTURE AND SENSORY TRAITS, CARCASS TRAITS, AND FILLET COLOR IN DIFFERENT GENETIC TYPES OF FARMED CATFISH: GENETIC APPROACHES TO ENHANCE CATFISH FILLETS

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Channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ × blue catfish (*Icatlurus furcatus*) ♂) aquaculture dominates the US aquaculture industry. Significant research has been devoted to genetics of various performance traits, but not towards improving meat quality, an important consumer attribute. Multiple studies were conducted using channel catfish, blue catfish, imported catfish and the hybrid catfish to evaluate their carcass, texture, sensory, and color traits of catfish fillets.

Channel catfish, blue catfish, and hybrid catfish at three size classes: small (<0.68 kilograms), medium (0.68-0.92 kilograms) and large (>0.92 kilograms) were compared and with increasing size, hardness, chewiness, gumminess, and toughness attributes increased in channel catfish and hybrid catfish ($p<0.001$). This phenomenon was not observed in blue catfish. When medium hybrid catfish and channel catfish were compared, the channel catfish had higher means for all texture traits, and was more tough and fibrous than the hybrid catfish fillets ($p<0.05$). Hybrid catfish were observed to have a mushier fillet ($p <0.05$)

Commercial catfish fillets were compared to imported Vietnamese swai fillets (*Pangasianodon hypophthalmus*), and control swai fillets were tougher, harder, and chewier than domestically farmed catfish ($p<0.05$). With a treatment of sodium phosphate, these differences between US catfish and Vietnamese swai were nullified, and the resulting fillet was more mushy and less fibrous than controls of both fillet products.

Comparison of texture, sensory, and color traits of catfish fillets from 7 channel catfish strains revealed several strain and sex effects ($P<0.05$). Strain differences were observed for fillet %, redness of the fillet, hardness, cohesion, and all sensory traits evaluated except flavor.

Combining ability of channel catfish dams and blue catfish sires in their hybrid progeny indicated that additive genetic variance was found in channel catfish dams for hardness, chewiness, and gumminess. Specific combining ability estimates revealed dominance and epistasis interactions for fillet yield, resilience, springiness, and yellowness of the fillets.

A heritability study of channel catfish revealed additive genetic variance for fillet yield and redness of the fillet. Direct selection for sensory and texture traits was not feasible, however, heritability estimates revealed the potential to implement a genetic enhancement protocol for increasing fillet % and decreasing redness of channel catfish fillets. Increasing fillet % from the same sized channel catfish and decreasing redness in fillets would prove to be beneficial for the catfish industry and increase profits to channel catfish farmers and processors due to the detrimental effects red fillets have in the catfish market. Redness had a low but potentially significant genetic correlation with yellowness of the fillet, and selection against redness would also potentially decrease yellowness in channel catfish fillets.

What is a desirable fillet, hard, mushy, neither? A small survey of graduate students indicated some people like hard, some mushy and some in between. This complicates breeding goals.

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

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The effects of CRISPR/Cas9 knockout of the myostatin (MSTN) gene and *mc4r* gene in channel catfish, *Ictalurus punctatus*, was investigated. Growth was generally higher in MSTN mutants when compared to controls at all life stages and in both pond and tank environments. Heterozygous F1 mutants were 218% larger than controls at the stocker stage in ponds. Mean expression level of MSTN was 2.90 times higher in controls than in MSTN F1 progeny ($p=0.009$). When challenged with *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC), MSTN mutants performed equally or better than controls.

Growth was generally higher in MC4R mutants when compared to controls at all life stages and in pond and tank environments. There were no significant differences in body weight between MC4R F1 progeny and controls generated in 2018, although there was a positive relationship between zygosity and growth, with F1 homozygous/bi-allelic mutants reaching market size 30% faster than F1 heterozygotes in earthen ponds ($p=0.022$).

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

EFFECT OF NANO-FE ON GROWTH PERFORMANCE AND IMMUNE FUNCTIONS OF STELLATE STURGEON *Acipenser stellatus*

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The present study aimed to investigate the effects of iron nanoparticles (Fe-NPS) on growth performance, liver histopathology and some immune parameters of stellate sturgeon (*Acipenser stellatus*) juvenile. A total of 144 fish (182.09 ± 9.05g) were fed diets containing graded levels of Fe-NPs (0, 25, 50 and 100 mg kg $^{-1}$ diet) as T_0, T_{25}, T_{50} and T_{100} respectively for 8 weeks. No significant differences were observed ingrowth performance parameters, lysozyme activity, albumin and liver enzymes concentration between treatments ($P>0.05$). The T_{50} group showed a significant difference in the white blood cells, neutrophils, total protein, respiratory burst activity, & total immunoglobulin level ($P<0.05$) with the less negative effect on the liver. These results indicated that Fe-NPs at level 50 mg Kg $^{-1}$ will improve the welfare of *A.stellatus* juvenile.

Sturgeon culture is an important industry to provide meat and caviar. Preparing the food for fish is very important because at least half of production costs belong to food. Iron is one of the most essential micronutrients in terms of its effect on the functioning of the immune system and defense against various infections. Positive effects of iron NPs have been shown on growth rate, and immunity responses in *Labeo rohita*, *Clarias gariepinus*, *Oreochromis niloticus*, etc. To our knowledge, there is no information about the effects of Fe-NPS in stellate sturgeon.

A total of 144 fish (182.09 ± 9.05g) were fed diets containing graded levels of Fe-NPs (0, 25, 50 and 100 mg kg $^{-1}$ diet) as T_0, T_{25}, T_{50} and T_{100} respectively for 8 weeks. Finally, growth parameters, blood and immune factors, alteration in liver tissue were examined and data were analyzed using SPSS software.

Results of growth performance and blood & immune parameters are presented in Table 1 and 2 respectively.

Liver cells in all treatments were completely seamless, without any cellular disorder and abnormalities. The structure of the liver sinusoids was normal, without any bleeding.

Supplementation of dietary Nano-Fe appears to be most positive for improving immune parameters of stellate sturgeon juvenile.

TRANSCRIPTOMIC ANALYSIS OF PROTEIN-CODING AND NON-CODING GENES OF STARVATION-INDUCED ATROPHIED MUSCLE IN NILE TILAPIA *Oreochromis niloticus*

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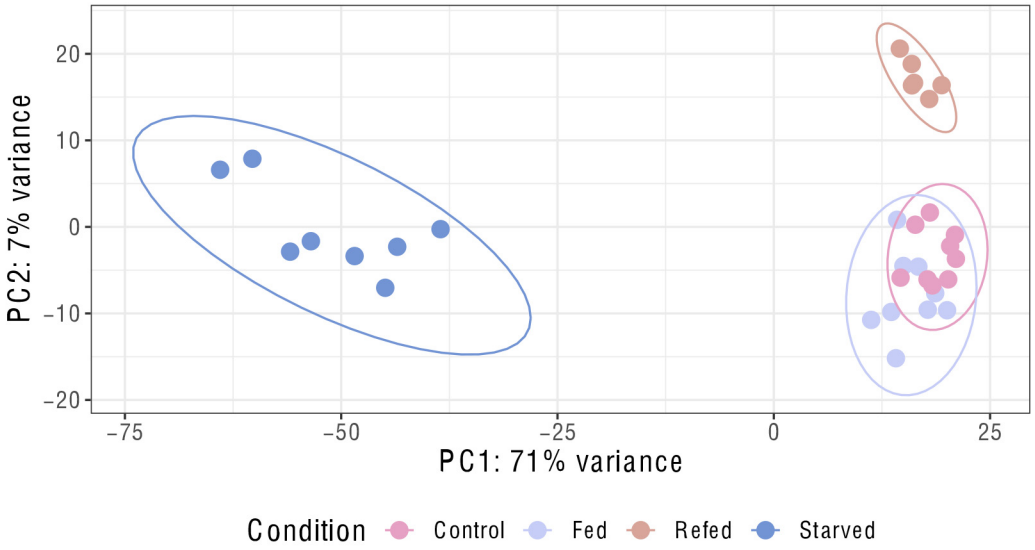
Muscle yield is a trait of environmental and economic importance in aquaculture, affecting resource use efficiency and farmer profits. Muscle tissue atrophies when proteins degrade faster than they synthesize. Often, protein synthesis rates remain stable while protein degradation rates vary. Thus, breeding programs can improve genetic gain by selecting for reduced protein degradation through biomarkers. While previous studies have demonstrated protein-coding genes and regulatory non-coding genes play a role in muscle atrophy, the mechanisms in Nile Tilapia are not fully understood. Here, we identify coding and non-coding genes involved in starvation-induced muscle atrophy in Nile Tilapia, investigate the interplay among the genes through the generation of a gene co-expression network, and present biomarker candidate genes. Using RNA sequencing, we compared the expression patterns of mRNAs, long non-coding RNAs (lncRNAs), and microRNAs (miRNAs) of atrophying skeletal muscle from fish under starved, refed, and fed control conditions (n = 32).

Results highlighted that a total of 4,639 mRNAs, 161 lncRNAs, and 104 miRNAs were differentially expressed (FC ±3, FDR < 0.01). Among the up-regulated cellular processes include the proteosome complex ($p < 0.01$) and processes involved in muscle structure development were down-regulated ($p < 0.01$). Results of the gene co-expression network will be discussed.

FIGURE 1. Principal components analysis showing the clustering of skeletal muscle data.



COST DRIVERS AND PROFITABILITY OF U.S. POND, RACEWAY, AND RAS AQUACULTURE

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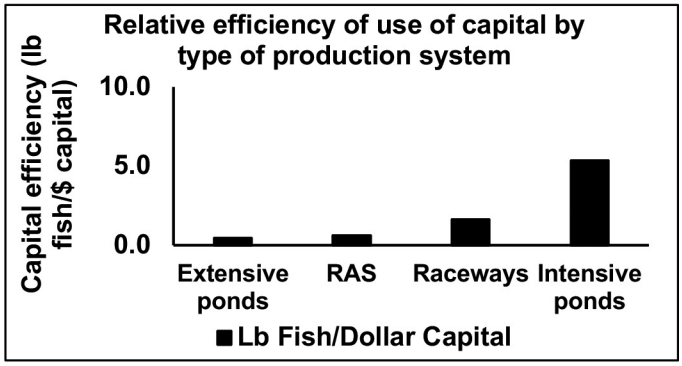
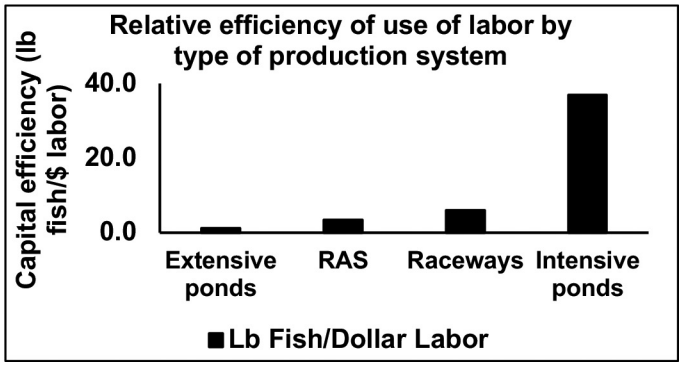
U.S. aquaculture has grown at a slower rate than the rest of the world in spite of abundant resources to support its growth. There has not been a comprehensive attempt to examine cost structures, productivity, and associated efficiencies across species, production systems, and scales. Comprehensive enterprise budgets (58) were developed for various management strategies/scales of production for catfish, baitfish/sportfish, and largemouth bass foodfish in ponds, trout production in raceways, and recirculating aquaculture system (RAS) production of Atlantic salmon, trout, and tilapia in the U.S. Results showed long-term profitability only for pond production of catfish, trout, largemouth bass, and baitfish/sportfish. None of the RAS showed profits, but larger-scale RAS showed fewer losses (in $/kg of fish produced) than smaller-scale RAS. Economies of scale were found for all species/systems analyzed. Feed, capital, labor, management, energy, and fingerling costs (in some scenarios) were identified as the major factors contributing to overall costs of production, but the order of importance varied with the species/system/scale. For those species/systems for which variable costs tended to be the greatest type of costs, improvements in feed conversion ratio and labor productivity (kg of fish produced per $ of annual labor cost) have the greatest potential to reduce per-unit costs of production. For RAS, improving productivity of both labor and capital (in terms of kg of fish produced per $ of annualized capital costs) may be as important as production scale to achieve profitability. Labor and capital use productivity were much greater on the well-established and generally profitable catfish and trout businesses than in the RAS scenarios analyzed. Results should be of value to lenders, investors, those who make decisions on research funding priorities, and policy-makers.





RESOURCE USE EFFICIENCY IN U.S. AQUACULTURE: FARM-LEVEL COMPARISONS ACROSS FISH SPECIES AND PRODUCTION SYSTEMS

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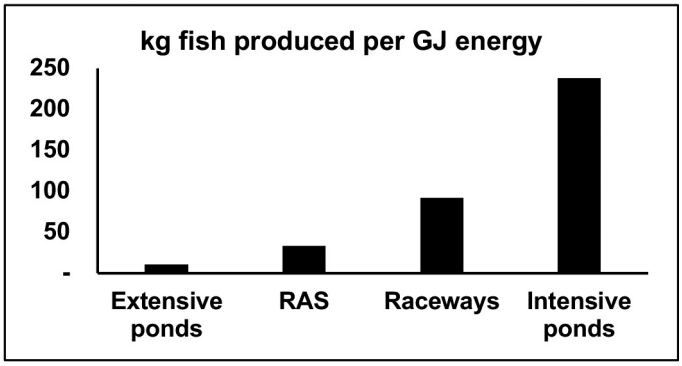
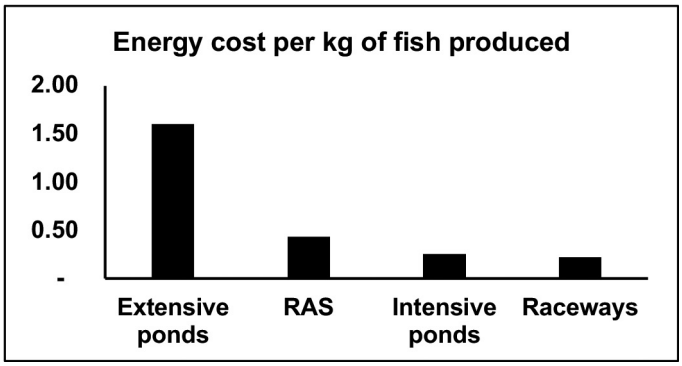
Understanding farm-level efficiencies of resource use is critical to comparisons of the sustainability of aquaculture production systems. This study developed a set of practical resource-use efficiency metrics to calculate and compare resource-use efficiency with resource-cost efficiency across major species and production systems in U.S. aquaculture. Results showed that no one production system used all resources most efficiently. Intensive pond production of catfish *Ictalurus punctatus* demonstrated the greatest efficiency in the use of water, energy, labor, management, and capital resources, while RAS production was most efficient in terms of land and feed use. Among the wide array of pond scenarios examined, more intensive scenarios generally were more efficient in terms of several metrics, but economic sustainability also depends upon business models that effectively meet differing demand requirements of customers. Thus, less intensive production systems were economically sustainable in areas with relatively abundant land and water resources available at lower cost. Labor efficiencies varied widely across scenarios analyzed. Given increasing concerns related to the availability of labor for aquaculture farming in the U.S., greater attention to the efficiency of labor on farms is warranted. The metrics used were aligned with common farm management tools (e.g. enterprise budgets) that allow for ease of use by farms and researchers to assess effects on comparative resource-use efficiencies of new farming practices and technologies under development.





ECONOMIC TRADE-OFFS AND RISK BETWEEN TRADITIONAL BOTTOM AND CONTAINER CULTURE OF OYSTERS ON MARYLAND FARMS

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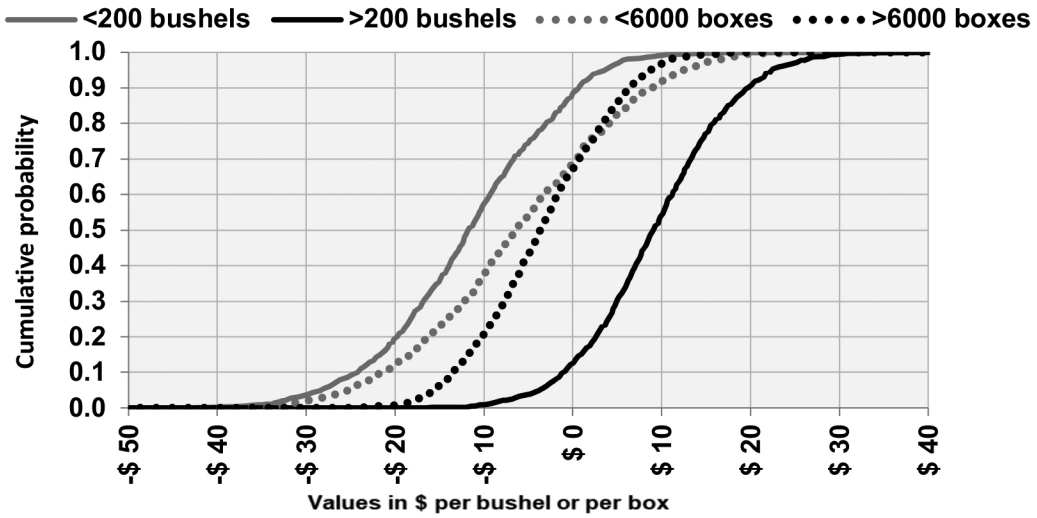
The traditional method of raising oysters in Maryland is to plant spat-on-shell directly on the seafloor area of the lease. Container culture, however, has grown rapidly in Maryland. There are clear economic trade-offs between the two production methods, but these trade-offs have not been evaluated with farm-level data. Maryland oyster farmers were interviewed in 2017 and 2018 to measure on-farm production and marketing costs for traditional bottom culture and container culture. Comparisons were made of: total initial investment (facilities, infrastructure, equipment), annual costs (major costs, cost structures, variable and fixed costs, and scale effects), profitability, marketing costs, and risk.

Cost structures differed substantially between the two production methods. For example, fuel was the greatest expense for traditional bottom culture farms whereas labor was the greatest expense for container culture farms. Producers using traditional bottom culture methods tended to use primarily unpaid owner/family labor. Container culture required greater investment capital and marketing costs with overall greater startup costs than did traditional bottom culture. Per farm, the most profitable scenario was that of the larger container culture farms, followed by the larger traditional bottom culture farms. Substantial profitability risk was found across production method/scales, but was generally greater on smaller-scale and container culture farms. Results demonstrate the importance of basing economic analyses on detailed farm-level data to capture the farm-to-farm variability and ensure that recommendations to producers are realistic.



CAN ECOLABEL CERTIFICATION FOR RESTAURANTS INFLUENCE CONSUMER CHOICES AWAY FROM HOME?

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With increasing concerns over the environmental impacts of the production process for seafood, many consumers are willing to pay premiums for ecolabels to support sustainable fisheries. Most studies of consumer preferences for ecolabels are conducted in the retail setting with limited attention given to sustainable seafood consumption away from home. However, seafood consumption away from home at restaurants takes a significant share in the overall seafood consumption market. Using a national online survey, this paper investigates consumer preferences and willingness to pay for eco-labeled seafood restaurants. The results show there is a positive WTP for eco-labeled seafood restaurants, indicating that consumer preferences for seafood ecolabels seem to be consistent over the consumption settings. Age, education, race, preference for eco-label certification, preference for the rating of restaurants, preference for the restaurant type, and knowledge of ecolabels are variables significantly affect the amount of WTP for eco-labeled seafood restaurants. The results of this study provide deep insight into the ways to further foster the sustainable seafood movement by enforcing the importance of restaurants as a significant role in the supply market.

IN-POND RACEWAY SYSTEM: PERFORMANCE AND PROFITABILITY

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In-pond raceways systems (IPRS) can have higher fish yields than conventionally managed catfish ponds (8,000 kg/ha) with IPRS units having annual fish production greater than 15,000 kg/ha. A four-year project at Auburn University was initiated with the objective of evaluating the performance and economic efficiency of fish produced in IPRS units. For this four-year trial, a floating IPRS unit was placed into each of four 0.4 ha ponds. In year 1, Ponds B1 and B2 had 64 m^3 IPRS units place into them, and ponds B3 and B4 had 45 m^3 units. Each pond had a total of 3 HP of aeration: a 1.5 HP blower for the IPRS unit, plus a 1.5 HP blower for the in-pond water mover-destratification unit. Hybrid catfish were grown during Year 1 using a large raceway ("grow-out") with 3.0 HP aerator and in year 2 Channel catfish were grown using 4.0 HP aerator. In Year 3 the previous year configuration was used and added a smaller raceway ("stocker generator") next to the grow-out raceways (also 2.0 HP was added in each pond). Once grow-out units were harvested, the stockers would be harvested and placed into the vacated, adjacent grow-out unit for growth to foodsize fish. For Year 4, a tilapia cage was placed into 2 of the 4 ponds (B2 and B4) and were used to reduce blue-green algae populations and large daily dissolved oxygen variations. Five HP of aeration was kept (maintaining the dissolved oxygen above 3 mg L-1). Fish were fed a 32% CP commercial diet twice daily. Water quality parameters had acceptable ranges for all years. Total production (all years) equaled or surpassed production yields from conventional catfish pond production systems. Growth performance results from years 1-4 are summarized in Table 1.

Hybrid catfish (from Year 1) performed better than Channel catfish (from Year 2). Hybrid catfish (stocker + grow-out) had yields ranging from 15,969 to 16,275 kg/ha (Year 3). In Year 4, tilapia (2,175kg/ha) increased total production by 11%, with no additional feeding (total yields ranged from 17,990 to19,506 kg/ha. Enterprise budget fixed and variable costs were developed using actual investment and production data. Costs of production varied among raceways and were influenced by survival and FCR. Accounting enterprise budgets (net return calculated minus the value of non-cash inputs) are closer to what adopters of this technology would actually encounter and showed positive net returns for hybrid catfish in years 1, 3 and 4. Full four-year trial results and enterprise budgets will be presented at the Aquaculture America meeting.

Table 1. Average growth performance of grow-out and stocker generation of hybrid catfish (*Ictalurus punctatus,* ♀ *x blue catfish, I. furcatus*, ♂) and Channel Catfish (*Ictalurus punctatus*) produced in IPRS during years 1, 2, 3 and 4.

	Year 1 – Hybrid catfish		Year 2- Channel Catfish		Year 3 – Hybrid catfish				Year 4 – Hybrid catfish			
	Grow -out				Grow -out		Stocker		Grow -out		Stocker	
Ponds (0.4 ha)	B1 or B2	B3 or B4	B1 or B2	B3 or B4	B1 or B2	B3 or B4	B1 or B2	B3 or B4	B1 or B2	B3 or B4	B1 or B2	B3 or B4
Production cycle (days)	270	270	217	217	228	228	143	143	254	254	142	142
Number of fish stocked	11,058	7,952	10,754	10,457	8,685	8,597	14,079	14,441	8,269	9,164	9,751	9,697
Mean weight at stocking (g)	41.6	42.0	44.3	45.5	31.1	31.5	27.2	30.9	293	292	28.8	28.8
Mean weight at harvest (g)	732	763	574	757	613	612	162	183	854	658	183	184
Total harvested (kg)	6,489	5,483	3,924	3,665	4,824	4,831	1,563	1,679	6,203	5,621	1,599	1,574
Standing crop (kg/ha)	16,222	13,708	9,089	9,161	12,061	12,078	3,908	4,197	15,508	14,054	3,998	3,936
Survival (%)	81	91	88	81	87	94	74	70	87	94	89	88
FCR	1.62	1.55	2.10	2.09	1.71	1.67	1.38	1.37	1.73	1.67	1.51	1.67

MUSTARD 'GREEN WAVE' *Brassica juncea* AND GARDEN ORACHE 'COPPER PLUME' *Atriplex hortensis* PERFORMANCE IN BRACKISH WATER AQUAPONICS

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Inland, brackish water recirculating aquaculture systems (RAS) face unique challenges with effluent disposal due to high salt content. Water reuse is a research focus and brackish water aquaponics can facilitate nutrient removal from system water, particularly nitrogenous compounds that build up over time in RAS. Mustard is a member of the Brassicaceae family and garden orache belongs to the Amaranthaceae family. Both families contain several halophytic species in addition to showing some tolerance to saline/sodic growing conditions. Garden orache is a halophyte and is therefore adapted to growing in saline/sodic environments; however, mustard is a glycophyte and although there are limited studies testing the plant's salt tolerance, there are no known studies using brackish water hydroponic or aquaponic systems. Continuing suitability studies examined *Brassica juncea* 'Green Wave' and *Atriplex hortensis* 'Copper Plume' for their tolerance to and performance in brackish water conditions.

Trials were conducted in a controlled environment under LED lighting programmed for a 12-hour photoperiod. Randomized, replicated treatments in 18 L aerated tanks included 0, 5, 10, 15 and 20 ppt. salinity. Seeds were germinated in perlite and fresh water, and then transplanted to grow grips upon true leaf emergence. Acclimation to salt was accomplished with five biweekly increases in salinity, with the final salinity increase achieved at stocking. Four plants were stocked in each 18 L tank and each tank was fertilized using 50 mL Fox Farm Grow Big Hydroponic fertilizer. Trials were harvested when growth was impeded due to limited space, at which time growth metrics were measured. Water quality parameters were measured twice weekly (temperature, dissolved oxygen (DO), pH and salinity), with weekly monitoring of total ammonia nitrogen (TAN), nitrite-nitrogen and phosphate. Potassium and nitrate-nitrogen were also monitored weekly for the mustard trial. Initial and final water samples were analyzed for an array of elements and dissolved nutrients.

Mustard had 100% survival through 15 ppt. and 66.7% survival at 20 ppt. Orache had 91.7% survival at 0 ppt. and 100% survival at all other salinities. Mustard growth was reduced as salinity increased, with a 51.6% growth reduction in height and a 75% reduction in biomass wet weight from zero to 15 ppt. Orache growth increased with salinity, peaking at 10 ppt. Orache had a 33.3% increase in height and a 246.4% increase in biomass wet weight from 0 to 15 ppt. chlorophyll content index (CCI) levels for both plants peaked at 15 ppt. Nitrate and potassium uptake in mustard decreased as salinity increased, phosphate absorption for both plants was similar regardless of salinity. Foliage on both plants remained healthy at all salinities. Future trials will examine tolerance of both plants to reused shrimp water in addition to full grow-outs in an aquaponics system.

2021 GLOBAL REVIEW OF TILAPIA PRODUCTION AND MARKETS

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On a global scale, tilapia production has continued a steady increase in recent years up until 2020. Due to the global covid pandemic and subsequent recession, production dropped severely in China and subsequently in other major producing countries. This was in part caused by hatchery and farm workers not being available to work due to lockdowns and transportation restrictions and in part by farmers and processors not risking production or processing with unknown demand. In fact, consumption dropped rapidly in many countries where tilapia was popular in the restaurant trade. Demand for live and fresh whole fish and fresh fillets dropped globally, starting with Chinese New Year and then dropping around the world as restaurants, cruise ships, and schools stopped purchases of fresh and frozen fish of all types.

However, it must be noted that even though tilapia has become an international commodity with millions of tons of fish traded internationally, most tilapia consumption still occurs in the domestic markets of producing countries. Often, tilapia farmers and their neighbors are the single biggest consumers. This floor of demand held up as producers shifted sales into local markets consuming tilapia in place of imports no longer available.

As the pandemic continued, the major importing countries reported increasing grocery sales of fresh and frozen fillets to be prepared for home consumption. The mild flavor and smell of tilapia may have encouraged more consumers to select tilapia over fish with a reputation for leaving strong “cooked fish smells”. For the US markets, this led to recoveries in the imports of fresh fillets from Latin America and frozen fillets from Asian producers. Imported tilapia from China to the US was still constricted due to the imposition of trade tariffs, but Chinese tilapia was somewhat replaced by imports from Indonesia and Latin America into US markets.

India had been showing a rapid increase in tilapia production prior to 2020, but this was stalled in 2020 as both domestic demand and export markets decreased. Ecuador was reported to have reduced tilapia production, switching some production from tilapia back to shrimp. Egypt reported stable production with increasing production from the huge Egyptian-Chinese joint venture replacing some decreased production from smaller farms due to the pandemic. Brazil reported several new tilapia ventures supported by some of Brazil’s agri-business giants but as none had come on-line by 2020, we expect that production probably decreased due to the severity of the covid pandemic in Brazil.

Overall, the best estimates suggest that global production may have decreased slightly, but was essentially stable. There was severe market disruption in the early months of 2020 but recovery in the second half of the year. The impacts going into 2021 are unclear as new restrictions related to the delta and other variants of the covid virus are still being felt globally.

SEVEN YEARS OF AQUACULTURE DEVELOPMENT IN MYANMAR

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Myanmar has the tenth or eleventh largest national aquaculture industry on a biomass basis. However, the bulk of that is provided by polyculture of carps in extensive aquaculture conducted in large (10ha+) ponds. With the economic and democratic opening up starting in 2013 several development and aid groups began to work with the industry to introduce updated technology and training. The US Soybean Export Council, Aquaculture Without Frontiers, US-AID, JICA, KOIKA, AUSAID, WorldFish and Chinese and European countries, were among the first to devote resources. In addition to providing basic culture techniques and post-harvest handling support, the aid groups were also introducing new equipment, feed ingredients and processing improvements. Another area of intense interest was introduction of selectively bred shrimp, tilapia, sea bass and other species produced in more intensive systems that would garner higher prices compared to the ubiquitous carps.

USAID, the EU-GIZ, and FAO each supported multiple projects that contributed to aquaculture development. Capacity building for aquaculture education at the university level was a key component. Building collaborations between academia and industry was an associated activity which has proven to be crucial. Support to upgrade fish, shrimp, *Artemia*, *Macrobrachium*, and *Scylla* crab hatcheries was also a priority. Several activities were devoted to helping small farmers to better understand the basics of water quality and pond management, fish and shrimp nutrition and feed management, harvest and post-harvest techniques were also taught on a broad basis.

Legislative approval and publication of a National Aquaculture Development Plan with associated documents was a key improvement. Training in more sustainable farming practices and the role of NGO's and how to achieve certifications was another key topic of instruction. Phone apps, training manuals, workshops and online instruction during covid restrictions were all important tools for building the capacity of farmers to produce more seafood products in a more sustainable manner.

OXYGEN DECISIONS IMPACTING RAS SUSTAINABILITY

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As the sizes of land-based RAS fish farms grow into the tens of thousands of annual fish production, the options for sourcing oxygen are increasing. Decisions about the quality and source of oxygen will have a material impact on the economic sustainability of the farm.

There is a general lack of knowledge by the oxygen source decision makers, RAS designers or farm owners, about the decisions that need to be made to achieve the optimal capital investment requirement, risk profile, and life-cycle cost of oxygen supply.

This presentation intends to inform RAS fish farm owners and designers which decisions are meaningful to the farm bottom line. Technical, reliability, commercial, and economic comparisons among each onsite generated oxygen technology available today and imported liquid oxygen will be presented. By being better informed of the ramifications of each oxygen decision required, educated decisions will ensure optimum and sustainable aquaculture operation results – AKA maximize profitability.

EFFECT OF WOODCHIPS, BIO-BEADS, AND EXPANDED CLAY PELLETS ON DENITRIFICATION EFFICIENCY IN REUSED SHRIMP AQUACULTURE WATER

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Nitrate is a key factor in limiting water reuse, particularly in marine recirculating systems, where the cost of salt makes water replacement a significant expense. Denitrification is a microbial process by which nitrate is reduced to dinitrogen gas and can be used to reduce nitrate levels in aquaculture systems. The denitrification process can be unstable and difficult to utilize in indoor shrimp production operations that are often limited in space and capital to buy and operate dedicated denitrification filters. This study examines the effectiveness of low cost, simple denitrification filters and media in discharged shrimp aquaculture effluent.

To test the different media, four treatments were used, each with three 159 L tanks that were randomly assigned to a treatment. The treatments included plastic bio-media (BM), expanded clay pellets (EC), woodchips (WC), and a control (CO) using no media. To hold the media, each tank had a simple denitrification reactor constructed of 10.2 cm diameter PVC pipe. The water used in the experiment came from an established *L. vannamei* production system with a salinity of 17 ppt and an initial nitrate concentration of 39.3 mg/l. The retention time of the reactors was maintained at 2h to maximize contact time and maintain internal DO around 1 mg/l. Carbon in the form of 10g of sucrose was added daily to provide an exogenous electron donor critical for heterotrophic microbes. Phosphate, alkalinity, nitrite, and TAN were measured twice per week, while temperature, DO, salinity, pH, and ORP were recorded twice a day. Nitrate was sampled at the beginning of the study and end.

All four treatments, including the control showed some signs of denitrification. The project ended after 22 days when signs of denitrification (reduction in phosphate and increase in alkalinity) ceased in the WC treatment. WC showed the highest nitrate reduction of 31.6 mg/l on average, BM underwent an average reduction of 21.5 mg/l, EC reduced the nitrate by 18.2 mg/l, and CO reduced the nitrate by 9.0 mg/l. While the control had the lowest nitrate reduction levels, some denitrification did occur, indicating that it may be possible for small-scale inland shrimp farmers to denitrify their water without the use of media. Future studies will focus on denitrification without the use of media, as well as further exploring the use of wood chips.

DEVELOPMENT AND POTENTIAL OF A NEW AQUACULTURE INDUSTRY FOR TEXAS – OYSTER FARMING

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In 2018, the U.S. commercial oyster farming industry had a value of $219 M and was practiced in all coastal states, with the exception of Texas. In May of 2019, enabling legislation was passed to establish regulatory authority within Texas Parks and Wildlife which culminated in adoption of official rules and regulations governing commercial oyster farming. This presentation provides information on the current status of the industry including historical aspects of development, comments on potential, and future prospects.

NORWEGIAN SALMON EXPORTERS: AGGLOMERATION AND TRADE PERFORMANCE

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It is well-known that clustering of economic activities may increase productivity at the firm-level, *e.g.*, through knowledge-spillover externalities, a more dynamic labor market, and access to suppliers, services and traders in the value chain. As a knowledge intensive and export oriented industry, salmon producers and exporters have the potential to benefit from agglomeration externalities. While such externalities have been demonstrated at the production level of Norwegian aquaculture, there has been less focus on agglomeration effects downstream from the producer. This is surprising as transportation, logistics and marketing are activities that often are associated with network externalities and external economies of scale.

In this paper, we investigate the interaction between agglomeration and trade performance in the Norwegian salmon aquaculture industry. This particular industry serves as a good case since it is highly export oriented. During the period 2004-2018, about 90 % of the Norwegian salmon production was exported to more than 70 countries. We utilize a unique set of transaction level custom data (period 2004-2018) that is linked to export firm information; *i.a.*, localization of exporters' main office and average wage level of the employees. We include a variable for clustering in a standard gravity model (i.e., controlling for distance, average income level, and the economic size of the destination country), and estimate its impact on achieved export price, different margins of trade, and wage level (as a proxy for productivity).

MAGNESIUM OR MAGNESIUM TO CALCIUM RATIO? EFFECTS OF DIFFERENT LEVELS IN LOW SALINITY WATER ON GROWTH AND SURVIVAL OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Magnesium (Mg^{2+}) is the most abundant intracellular divalent cation with a central role in many cellular processes such as activation of a large number of enzymes, hormonal signaling, protein synthesis, cell division, etc., while calcium (Ca^{2+}) is generally the most abundant cation found within the body of crustaceans. These elements also comprise a major portion of their exoskeleton, and greatly affect molting frequency, hemolymph osmolality and carapace mineralization. Scientists argue that shrimp uptake of Mg^{2+} may be influenced by the concentration of Ca^{2+} and vise-versa due to their chemical similarities by having the same electron configuration in the outermost shell. Inland low salinity aquifers such as those of west Alabama possess concentrations of Mg^{2+} and Ca^{2+} that vary widely. This culture water is quite deficient in Mg^{2+} while excess levels of Ca^{2+} have been recorded on most farms (Mg:Ca ratios of 0.5 - 1.0 or lower are quite common).

The present study was conducted as an incomplete 3x3 factorial design with three Mg^{2+} (101, 56, 28 mg/L) and Ca^{2+} (35, 105, 210 mg/L) levels at 3 g/L salinity (while keeping the remaining ionic concentrations constant) to determine the effect of different Mg^{2+} and Ca^{2+} combinations on the growth, survival and physiology of shrimp. However, due to a limitation of the research system, the treatment with the lowest Mg^{2+} and highest Ca^{2+} (28 and 210 mg/L, respectively) was dropped. A low-cost salt mixture (LCSM) was used (comprised of Na, K, Ca and Mg concentrations of 298, 9, 17, and 39 mg/L, respectively in a 1-g/L solution, which is closely comparable to the major cations in 1-g/L dilute seawater) to formulate waters with different Mg^{2+} and Ca^{2+} levels. Three growth trials (initial weight of 0.15 ± 0.02, 0.30 ± 0.02, 0.90 ± 0.02 g, respectively) were carried out in 150 L polyethylene tanks (stocking density = 20 shrimp/tank), each equipped with a miniature fluidized bed bio-filter. Shrimp were fed four times per day using a standard ration for 21-days in each trial and growth performance, survival, hemolymph osmolality, osmoregulatory capacity, ionic concentration in hemolymph and whole body of shrimp were measured at the end of each experiment. Results indicate treatments with 101 mg/L Mg^{2+} had significantly higher growth and survival irrespective to the level of Ca^{2+} in water, indicating no significant interaction between Ca^{2+} and Mg^{2+} or no significant effect of Ca^{2+} on growth and survival of shrimp.

EFFECTS OF FERMENTTED CORN PROTEIN CONCENTRATE TO REPLACE FISHMEAL IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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One of the latest corn-based ingredients available in the market is a fermented corn protein concentrate (FCPC), which is expected to perform well in shrimp diets due to its nutritional profile and the probiotic properties of fermented products. The current study was conducted to evaluate the efficacy FCPC as a replacement for fish meal (FM) in practical diets of pacific white shrimps, *Litopenaeus vannamei*. Graded levels of FCPC (0, 4, 8, 11, 13, and 15 g/kg) were used to replace fishmeal (16, 12, 8, 4, 2, and 0 g fishmeal/kg) in the diet, which was evaluated over a 8 weeks growth trial (initial weight 0.17±0.01 g; n=3). At the conclusion, no significant differences were detected in growth, FCR, survival, or hematological parameters of shrimp (P-value>0.05). Results reveals the efficacy of FCPC to replace 100% fishmeal at an inclusion level as high as 15%, without compromising the performances of shrimp. Significant increase (p-value<0.001) in total hemocyte count (THC) and astaxanthin level in shrimp noted in respond to the inclusion level of FCPC (based on regression analysis) assumed to be due to the probiotic properties of fermented corn extractives in product and due to the availability of bio-convertible carotenoids in FCPC, respectively (Table 1).

Table 1: Response of juvenile shrimp (0.17 ± 0.01 g) fed with diets contained different levels of fermented corn protein concentrate (FCPC) over a 8-weeks experimental period. Values represented the mean of three replicates and the p-value is based on one-way ANOVA.

Diet	FCPC0	FCPC4	FCPC8	FCPC11	FCPC13	FCPC15	PSD	p-value
Final weight (g)	4.37	4.67	4.00	4.53	3.83	4.07	0.20	0.118
Weight Gain (g)	4.20	4.47	3.80	4.37	3.70	3.93	0.21	0.176
Weight Gain (%)	2497	2597	2244	2531	2118	2293	123	0.153
FCR	2.57	2.40	2.80	2.43	2.93	2.70	0.12	0.121
Survival	97.7	89.0	93.0	93.3	91.3	87.0	2.07	0.157
Total hemocyte count (THC)	23.0	38.0	33.9	48.5	46.7	52.8	14.21	0.559
Total astaxanthin in shrimp whole-body (µg/g)	1.8^{a}	-	4.2^{b}	-	-	6.5^{c}	0.19	<0.001

Values with different superscripts within the same row are significantly different based on Tukey Pairwise Comparisons; FCR = Feed conversion ratio; PSD = Pooled standard deviation

AQUACULTURE PERFORMANCE INDICATORS

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Currently, there are no consistently collected data documenting performance of aquaculture industries in the US and globally in terms of economic, social and environmental success. We draw on theory and practical knowledge to develop an extension to the Fishery Performance Indicators (FPIs) called the Aquaculture Performance Indicators (APIs). The Fishery Performance Indicators have been successfully used in a broad range of applications to compare global fisheries in environmental, economic and community performance, analyze species-specific complexes, and evaluate the effectiveness of investment and reforms. The APIs build upon the success of the FPIs to measure performance of aquaculture industries in economic, environmental, and social dimensions and to test hypotheses on inputs that lead to success or failure of a given aquaculture sector.

The APIs use 88 performance metrics that can be aggregated into three key sustainability indicators: environmental health, economic and community (Table 1). The metrics are also designed to be readily available, accurate, quantifiable, and relevant. Sixty-six input metrics are also scored which enable analysis of factors enabling or hindering achievement of environmental, economic and community outcomes. Input metrics reflect aquaculture management practices and broader enabling factors such as the quality of the management system, property rights system, and market conditions.

Data on more than 30 aquaculture industries around the world have been collected. This paper will introduce the novel API tool and summarize the preliminary findings of the initial case studies.

Table 1. Aquaculture Performance Indicators and respective output dimensions.

Indicator	Dimension
Environmental Health	Feed-related Impacts
	Water use and Effluents
	Impacts to Wildlife
	Environmental Compliance
	Certification
Economic	Production Performance
	Production Assets
	Risk
	Product Form
	Trade
	Supply Chain Performance
	Post-Harvest Assets
Community	Managerial Returns
	Labor Returns
	Health & Sanitation
	Community Services
	Local Ownership
	Local Labor
	Career

SALVEMOSNUESTRALAGUNAMADRE, A NEW MODEL TO POSITION AQUACULTURE AS A KEY INSTRUMENT TO DEVELOP VULNERABLE POPULATIONS IN COASTAL AREAS

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The Laguna Madre, located in the state of Tamaulipas, is the largest body of water in México. Its area surpasses 225 thousand hectares and has a coastline of more than 225 km. The Laguna Madre fisheries production exceeds 44,000 tons and is the source of livelihood for more than 10,000 families.

Over the last few years, the lagoon has fallen into a progressive process of environmental and fisheries resources deterioration that threatens to seriously damage its biodiversity. For this matter the State's Fisheries and Aquaculture Secretary of Tamaulipas has launched the initiative #SalvemosNuestraLagunaMadre. Its key objective is to increase the quality of life of the numerous fishing communities in the area. The initiative seeks to manage, protect and restore the ecosystem in the lagoon, and achieve sustainable fisheries and aquaculture development.

The initiative seeks to promote balanced progress based in the three fundamental aspects of sustainability: Social, Economic and Environment development. To implement this initiative, it will require the combined efforts of all stakeholder, including fishermen, aquaculture producers, government agencies, NGOs, and funding agencies; between others.

#SalvemosNuestraLagunaMadre is one of the largest initiatives for environmental conservation, fisheries and aquaculture development in Mexico, with the potential to become a model that could be applied anywhere in the world. The objectives and scope of the initiative will be described.

AQUACULTURE WITHOUT FRONTIERS: PAST, PRESENT, & FUTURE

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The World Aquaculture Society (WAS) played a major part in the formation of a non-governmental aquaculture organization (NGO) to assist in the alleviation of poverty in developing countries when Aquaculture without Frontiers (AwF) was launched at the WAS meeting in Hawaii in March 2004.

The organization is now established in USA, Australia and Latin America and whilst it has made some great achievements there is an exciting new future that needs to be explored. The organization is a great opportunity for people to engage whether you are young and starting out in aquaculture or older and looking for something to engage in during retirement.

The team will update the audience and then will have an open discussion with the audience

DIFFERENCES IN WATER CHEMISTRY BETWEEN HATCHERY AND RIVERINE CONDITIONS IMPACT NUTRIENT ABSORPTION BY JUVENILE LAKE STURGEON

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Background:
Extirpated populations of lake sturgeon (*Acipenser fulvescens*) are being reintroduced throughout their range using conservation aquaculture. The differences in environmental factors between the hatchery and the river are predicted to influence growth and survival of stocked juveniles. Furthermore, naturally recruited fish are hypothesized to differ physiologically from hatchery-reared fish due to differences in their developmental environments. Dietary components are known to differ between the feeding strategies employed over the animal's lifespan and ontogenetic changes to digestive processes create survival bottlenecks that occur at feeding transitions associated with life stage. Previous studies indicate that development of lake sturgeon larvae prior to exogenous feeding is determined by water chemistry, but ion uptake is driven by nutritional absorption after the onset of feeding. In juvenile fish, different compositions of ions available for branchial absorption are hypothesized to have an impact on nutrient absorption by the intestine. In this study, growth and nutritional status were compared in juvenile lake sturgeon exposed to either hatchery or riverine conditions.

Methods:
Young-of-year lake sturgeon were provided by the USFWS Warm Springs National Fish Hatchery and exposed for 3 weeks to either dechlorinated tap water or water collected from the Coosa River in two identical temperature-controlled, recirculating tank systems. Anatomical measurements, tissue samples, and digesta were collected at 0, 1, 10, and 21 days from fish (n=5) exposed to each water type. Specific growth rate, condition factor, hepatosomatic index, and survival were quantified. The feed conversion ratio was calculated on a per tank basis to indicate any treatment effects on gastrointestinal absorption rates, and diet utilization was examined by analysis of proximate composition of the collected intestinal contents, allowing for determination of the nutritional status of juvenile lake sturgeon fed standard commercial feeds (*i.e.* Rangen, bloodworms).

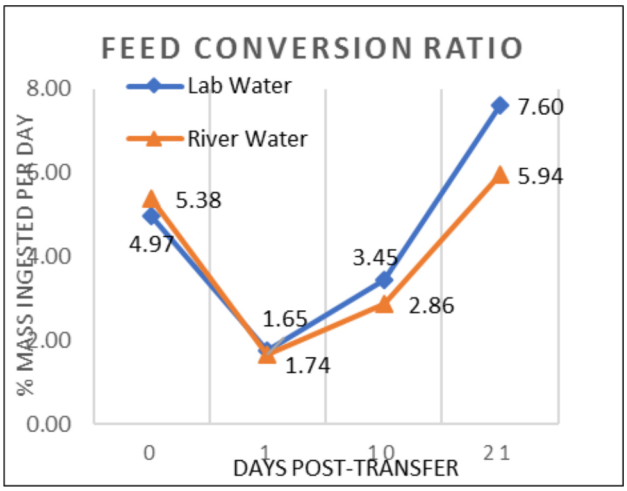

Figure 1. FCR of juvenile *A. fulvescens* exposed for 21 days to water treatments differing in ion availability

Results:
The body indexes of sampled fish paired with the feeding data provide a complete picture of the diet utilization of stocking-size lake sturgeon. No differences were observed in growth, condition, SGR, HSI, or gross intestinal anatomy, over the 21-day exposure period. However, treatment differences were observed for feed conversion ratio, with more efficient FCR in river water-exposed fish at 10 and 21 days post-transfer (Fig. 1). Additionally, the proximate composition of intestinal contents 6 h post-feeding indicated significant differences in nutritional absorption, suggesting that environmental ion availability influences intestinal function.

Conclusions:
This experiment investigated how environmental ion availability influences growth and nutrient absorption of juvenile lake sturgeon. Changes to absorption and nutrient allocation in young-of-year lake sturgeon at stocking may have impacts on their regulatory physiology and impact the survival of fingerlings. Our results indicate that following transfer to Coosa River water, lake sturgeon experienced similar growth but displayed more efficient nutrient use than fish held in laboratory conditions. Analysis of collected gut contents indicates functional changes in nutrient absorption. These results can be utilized to improve procedures to successfully rear and release this protected species into their natural habitat which is imperative to reestablishing the Coosa River lake sturgeon population.

DECOUPLED AQUAPONICS SYSTEM WITH *Salicornia neei* AND *Litopenaeus vannamei* IN BFT: EFFECTS OF CLARIFICATION OF WATER ON PLANT PRODUCTIVITY

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The halophyte *Salicornia neei* has potential for commercialization and it can be cultivated in aquaponics with waters of marine shrimp farming (Fierro-Sañudo et al., 2020). The use of aquaponics as part of intensive production of the shrimp *Litopenaeus vannamei* in Biofloc Technology System (BFT) requires integration cultivation modules and plants tolerant to high levels of total suspended solids (TSS) and saline waters (Rakocy, 2012).

The present work evaluated the performance of *S. neei* in a decoupled aquaponics system in deep water tanks with non-clarified waters (NC), waters clarified by flotation (SF) and by decantation/sedimentation (SD) from a BFT superintensive cultivation of *L. vannamei*. For each treatment, aquaponics system was set up on three tanks (replicates) with and active volume of 280 L, aeration in the bottom and twenty *S. neei* plants fixed in a floating raft with 0.15 m^2 area. Aquaponics systems received waters from a 237 m^2 raceway stocked with 450 shrimp m^{-3} along 71 days of a BFT cultivation cycle. The aerial biomass productivity of *S.neei* were quantified and analyzed with repeated measures ANOVAs.

TABLE 1: Average TSS contents in water in different treatments NC, SF and SD:

Variable	NC	SF	SD
TSS (mg L^{-1})	645.89 ± 53.16a	532.34 ± 51.33b	491.56 ± 54.19b

TABLE 2: Aerial biomass productivity of *S. neei* in differents treatments NC, SF and SD:

Treatments	*S. neei* productivity (kg m^{-2} 30 $days^{-1}$)
NC	5.12 ± 0.52b
SF	5.82 ± 0.54bc
SD	4.27 ± 0.51c

RUSSIAN ECONOMICS OF TROUT AND SALMON AQUACULTURE

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There are two basic salmonid farming methods in Russia:
- in cages (salmon in the Barents Sea and trout in fresh lakes in the North-West of the country),
- in a recirculating aquaculture system (RAS), which can be situated near the consumer.

Comparison of the economics of the two methods:
RAS:
Capital expenditure (CAPEX) on a farm with the shortest payback period, producing 1000 tons of fish, is $16 million. Annual revenue is $7 million. EBIT margin is 35-45%, and the payback period is 4-6 years.

Cage aquaculture:
CAPEX for a 1000-ton farm is $2.5 million. Only farms producing upwards of 5000 tons pay off due to the need for coastal service infrastructure, which is not necessary with RAS. Another difference from RAS is that the fish take twice as long to grow to marketable size, which means less revenue for such a project. Annual revenue is $3.5 million (achieved only with several farms. With one farm, revenue is received once every few years, depending on the growth cycle). EBIT margin is 30%, and the payback period is 3-4 years.

It should be noted that RAS has numerous additional advantages over cage aquaculture despite the latter's low capital intensity:

- Possibility of creating optimum conditions for maximum fish growth (water temperature, oxygen, feed)
- Monitoring fish disease
- Lower feed conversion ratio - no more than 1.2x per cycle
- Full growth cycle - 17-27 months, which is 1.5-2 times shorter than in cage aquaculture
- Saving water, land, and energy resources
- Fewer regulations and permits - like a typical construction project

CHARACTERIZATION OF THE VIRGINIA SEAFOOD SUPPLY CHAIN

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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, providing jobs for over 6,000 Virginians who work on the water. 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 characterization of the Virginia seafood supply chain is part of an ongoing study that will assess the economic benefits of the Virginia seafood industry to the Commonwealth's overall economy.

Commercial fisheries and aquaculture integrate the production level of the Virginia seafood supply chain. As the nation's third-largest producer of marine products with total landings of 393,065,090 pounds in 2019, Virginia commercial watermen annually harvest enough seafood to produce over 123 million meals. The production is only outpaced by Alaska and Louisiana. The dockside value to commercial fisheries alone was $184,270,303. Virginia also ranks as the largest seafood production state on the East Coast. Aquaculture also plays an essential role in the Virginia seafood industry. In 2018, the total sales from 191 farms were $112.6 million, making Virginia rank 4th in the U.S. aquaculture sales. The continued growth of the shellfish aquaculture industry in Virginia has added significant value to the State's seafood marketplace. Virginia's watermen-farmers provide consumers with a growing quantity of hard clams and oysters representing over $94.3 million dockside value, compared to $41.5 million from 2013. Oysters alone supported 70% of all aquaculture operations and 55% of sales valued at $62.4 million. Sales of food fish were $15.4 million, an increase of 35% from 2013.

Both fisheries and aquaculture production interact with processors and wholesaler-distributors in Virginia. Among the distributors identified, 82.8% transport fresh seafood, 64.6% live, and 55.6% frozen. 27.3% of those entities distribute non-Virginian species in trays or vacuum packs. Processed seafood is also found. 23.2% of the entities distribute prepared or partially prepared products with some value-added, 21.2% canned products, 12.1% pasteurized, 10.1% breaded or portioned, and 7.1% IQF - Individually Quick Frozen. All seafood distributors use motorized vehicles, while 30.3% use air freight, 13.1% marine cargo, and 9.1% export seafood in different market forms. Virginia seafood exports totaled $47.3million in 2017 to 20 countries. The entire industry provided approximately 11,000 full and part-time jobs for Virginians. Ninety percent of the fish and shellfish are harvested, processed, and shipped within 24 hours to domestic and international markets. With approximately 8.6 million inhabitants, Virginia consumed 138.5 million pounds of seafood annually, or 35.24% of the total landings in the State. 64.76% of the production volume is distributed to other states or countries.

ASSESSING THE EFFECTS OF WATER TEMPERATURE ON PRECOCIOUS MATURATION IN ATLANTIC SALMON *Salmo salar* POST-SMOLTS

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Early maturation of Atlantic salmon has, and continues to be, a major source of economic loss for farmers, as precociously developed fish often exhibit decreased growth and feed conversion efficiency, reduced product quality, and increased susceptibility to opportunistic microorganisms. The initiation of sexual maturation can be difficult to prevent, given the numerous factors, such as photoperiod, temperature, fish size, growth rate, nutritional status, and genetics, that can influence the onset of puberty. Early sexual maturation has been particularly problematic in land-based, closed containment recirculation aquaculture system (RAS) facilities raising Atlantic salmon, with reported early maturation ranging from 2% to 100% in salmon populations raised at seven surveyed RAS facilities. With the massive investment in land-based salmon farms within the past several years, the issue of early maturation appears to be largely ignored; however, it will likely be of profound economic significance for land-based salmon producers unless reliable remedial methods are identified and utilized.

Among other variables influencing maturation, water temperature appears to be a critical factor, and could explain why RAS-raised salmon, typically cultured at higher temperatures than those in sea cages, exhibit comparatively higher rates of early maturation. In particular, smolts and post-smolts experiencing an increase in water temperature while in land-based RAS appear to be at increased risk for early maturation prior to sea transfer. To further understand this problem, we sought to investigate whether there is a size threshold after which post-smolts will sexually mature in response to an increase in water temperature. To this end, we incubated and hatched mixed-sex diploid eyed eggs and reared the fry / parr in flow-through freshwater at 12 ^{o}C up to 40 g in weight, at which point 50% of the salmon received an S_0 winter photoperiod (i.e., 6 weeks LD12:12) to induce smoltification. All fish were then transferred and comingled in 15 0.5 m^3 flow-through tanks, to be raised to 400 g final size. Treatment groups (with n=3 replication) included: i) 12 ^{o}C for the entire study duration; and ii-v) temperature elevation to 14 ^{o}C at 100 g, 150 g, 250 g, and 350 g, respectively. At the time of abstract submission, the study is ongoing with anticipated final data collection in July 2021. At study termination, all fish will be humanely euthanized, and maturation will be assessed within each treatment group via gonadosomatic index quantification, to determine i) if there is evidence of a size-based threshold related to maturation in response to temperature elevation, and ii) whether receiving an S_0 winter photoperiod or not impacts maturation within each treatment group. Final study results will be presented at Aquaculture America 2021.

SOUTH CAROLINA'S FIRST OYSTER MARICULTURE INDUSTRY OUTLOOK ASSESSMENT

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The shellfish mariculture industry in South Carolina, specifically off-bottom oyster production, has been steadily growing in recent years. With 139,178 single mariculture oysters produced in 2014, growing to over 1.19 million in 2019 (an increase of 758%). Figure 1 shows the yearly trend from 2014-2019 (SCDNR, 2021).

A team of researchers and extension specialists from Clemson University and the South Carolina Sea Grant Consortium conducted the state's first ever Oyster Mariculture Industry Outlook Assessment, similar to other states' efforts (e.g. VIMS). This included implementation of surveys with shellfish mariculture producers throughout the state of South Carolina. Producers were asked questions about their 2019 production year, including how many oysters they produced, how much they sold, average price received, production costs, how much seed they bought, whether seed was sourced in-state or out-of-state, how many people they employed, and their perceived barriers and challenges to sustainable industry growth in South Carolina. Revenue and expense data were then entered into IMPLAN to estimate the total economic contribution of the off-bottom oyster mariculture industry in South Carolina. In order to increase accuracy of model results, the IMPLAN sector related to aquaculture (Sector 14) was adjusted based on producer surveys results related to production costs across different expense categories to better reflect production costs associated with average oyster mariculture operations in South Carolina. The model was also designed to capture additional supply chain economic contributions (producer investment and restaurant sales of locally produced oysters). Preliminary results are shown in Table 1 below, indicating that the total economic contribution of South Carolina's off-bottom oyster mariculture industry, including production, investment, and restaurant sales is estimated at over $8.6 million in 2019.

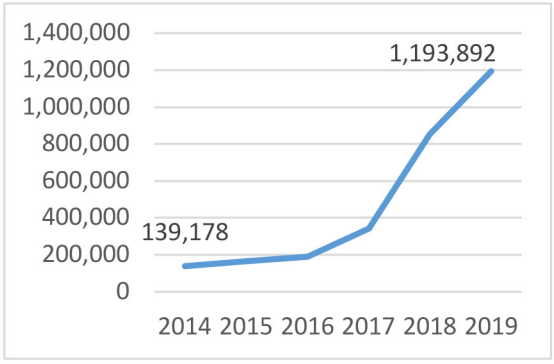

Figure 1: Mariculture Oysters Produced in SC, 2014-2020

Table 1: Estimated total economic contribution of South Carolina's off-bottom oyster mariculture industry; Preliminary Results

Impact Type	Employment	Labor Income	Value Added	Output
Direct Effect	104	$1,474,940	$2,900,382	$5,294,046
Indirect Effect	12	$555,906	$876,387	$1,816,286
Induced Effect	11	$458,447	$846,191	$1,523,539
Total Effect	126	$2,489,293	$4,622,962	$8,633,871

STUDYING THE PRODUCTION PERFORMANCE OF DECOUPLED AQUAPONICS SYSTEM: A CRITICAL REVIEW

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The concept of decoupled aquaponics system (DAPS) is very unique as it separates the recirculated aquaculture system (RAS) and hydroponic (HP) units with having the inherent advantages of plant and fish production 1 (Fig.1). The output efficiencies from the DAPS are also being upgraded compared to the traditional aquaponics system (TAPS) as it uses very minimum input resources 1. For example, the growth performance of plant species under the DAPS could be improved by 39% in comparison to the pure hydroponic control nutrient solution 2. In the case of fruit yield, DAPS could provide a 36% higher fruit yield as compared to the TAPS 3. Moreover, the PH and fertilizer management in DAPS were more effective as compared to the TAPS3. The fish production, however, from the TAPS and DAPS was comparable 3. In general, DAPS requires more water than the TAPS but it allows DAPS to better control over their water quality parameters, which leads improving their vegetable yield 4. Overall, the major benefits of DAPS include 5: a) highest efficiency configuration; b) low water use; c) less crop and animal risk; d) compact design in a small footprint; e) less plumbing; f) predictable, and consistent operation.

Reference:
[1]Goddek, S. *et al.* 2019; [2]Jijakli, H., Delaide, B. & Gott, J. (2016); [3]Monsees, H., Kloas, W. & Wuertz, S. (2017); [4]Gibbons, G. M. (2020); [5]Aquaculture Systems Technologies. (2020).

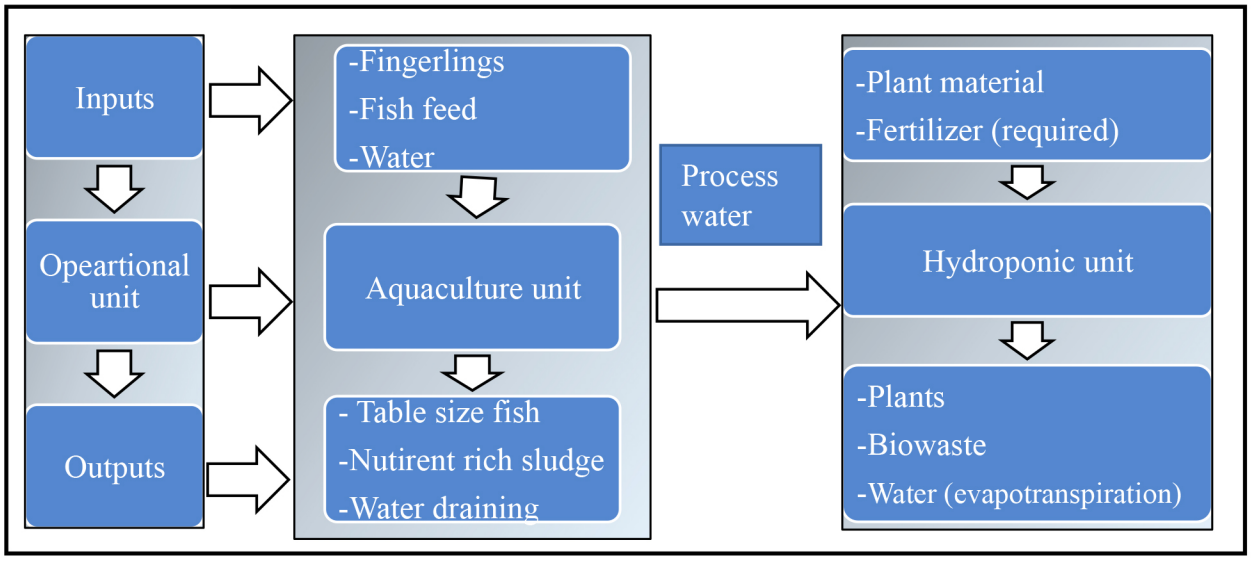

Figure 1. The basic layout of DAPS (two-loop)

Reference: [1]Goddek, S. *et al.* 2019; [2]Jijakli, H., Delaide, B. & Gott, J. (2016); [3]Monsees, H., Kloas, W. & Wuertz, S. (2017); [4]Gibbons, G. M. (2020); [5]Aquaculture Systems Technologies. (2020).

SHELLFISH HATCHERY PRODUCTION ANALYSIS: A CASE STUDY AT HORN POINT LABORATORY OYSTER RESEARCH HATCHERY

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Shellfish hatcheries have become an increasingly important component of aquaculture production in the United States. Although the industry has been advancing technologically over time to stabilize production and supply, detailed production analysis has rarely been reported. Understandably, commercial hatcheries may not want to openly share production deficiencies in order to preserve their reputation. Additionally, many commercial hatcheries may not keep copious amounts of records over time, which are necessary to track and analyze production trends.

To help fill the data gap and initiate a broader discussion on production trends, we report on long-term production trends (2011-2020) at Horn Point Laboratory's oyster hatchery, which included persistent production failure during the 2019 season. During the 2019 season, larval assays were conducted to determine drivers of production failure; however, no clear culprits were identified. Production metrics of interest where production yield (millions eyed-larvae produced) and production rate (days to reach the eyed larval stage). A variety of factors stretching across the entire production process (i.e. broodstock selection, conditioning, spawning, larval culture) were considered as possible predictors of production metrics. Furthermore, water quality in the Choptank River, the source of water for the hatchery, were used as input during analysis. Machine learning was used to train models of production yield ($R^2 = 0.54$) and hindcast the specific conditions when the hatchery's production was most efficient.

We identified several important factors that predicted production yield and production rate. Many of these factors the hatchery staff can manipulate or represent a stage in the production process, which improved, could lead to greater production efficiency. Collectively, we conclude that more research, data sharing, and cross-institution collaboration are needed to understand production variability within and among shellfish hatcheries to maintain high levels of consistent shellfish aquaculture production.

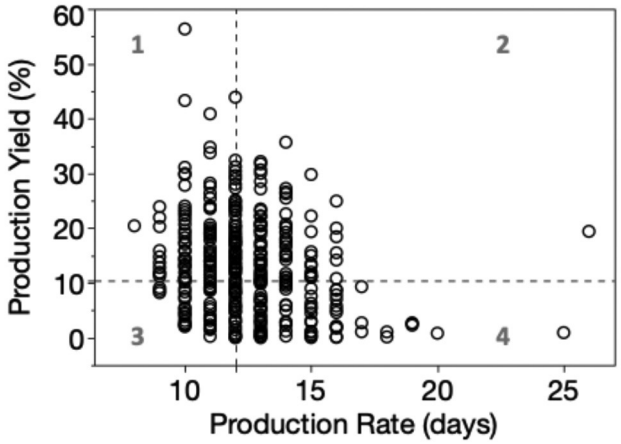

Figure 1. mean production yield over production rate at Horn Point Laboratory Oyster Hatchery divided among four quadrants separated by median values (dashed lines);

OYSTER AQUACULTURE WEB/PHONE APP FOR ENTERPRISE BUDGET DEMO

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This tool is being developed for stakeholders interested in started an off-bottom oyster commercial farm and is web or phone based since not everyone has access to or knows how to use excel spreadsheets.

The oyster farm enterprise budget is updated regularly by Extension personnel to reflect the cost of starting an operation and annual cost of production. Projected costs can be changed to estimate the associated cost for your level of production as well as the projected profit or loss. Growers should model these budgets and use the values that best reflect their operation.

This calculator will provide an estimate based on an oyster farm using six-bag floating cages with a stocking density of 1,000 oysters per cage. Note that it based on the oysters reaching market size in one year which is not the case in all areas. The gross profit estimate is earnings before interest, taxes, depreciation, and amortization. These estimates should be used as guide for planning purposes only and not for business forecasts.

LEVERAGING OPEN FABRICATION FOR COMMUNITY DEVELOPMENT AND AQUATIC GERMPLASM CONSERVATION

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Germplasm conservation is driven by the need to preserve genetic diversity in natural populations, and to back up lines of genetically modified and improved organisms for current and future applications. Advances in plants and terrestrial animals used for food have been significant for several decades. In contrast, germplasm preservation of aquatic species important for aquaculture, fisheries, conservation, and biomedical research has advanced at a slower pace. The large variation of reproductive mechanisms and the number of species involved requires considerable efforts to prevent loss of genetics in these organisms. Although diverse, aquatic germplasm preservation requires tools and hardware that can be similar in function but adapted to particular characteristics of each user and species. Open fabrication makes possible custom production of hardware (e.g., tools and devices) in ways that traditional proprietary development cannot.

The shared information for open fabrication (e.g. design files) should be suitable for transfer in electronic format. This can be initiated by single users, or groups of users with common or similar problems. These can include researchers, or innovators that design particular solutions. These solutions can then be distributed to early adopters, who will provide insights on the usability of the proposed solution, in real-life situations, and provide feedback to the innovator group. This will result in a diversified set of options, that can be integrated into the design cycle. After the design and usability steps proceed, the technology can be put in the hands of users. For example, repository developers, germplasm centers, or hatchery managers that want to maintain the genetics of broodstock. At this point the possibility of transferring electronic files such as CAD/CAM (computer aided design and manufacturing), provides a fast and cost-effective system and allows users to become makers, that is fabricate their own tools or devices, for example through 3-D printing. Furthermore, these makers can eventually become developers and design their own technology. These changes, from acquiring technology to fabricate and developing their own hardware can be greatly enhanced by the formation of communities that allow the exchange of ideas, tips, and troubleshooting in the way that old sewing and carpenter groups use to do, but a much faster digital-era speed (Figure 1).

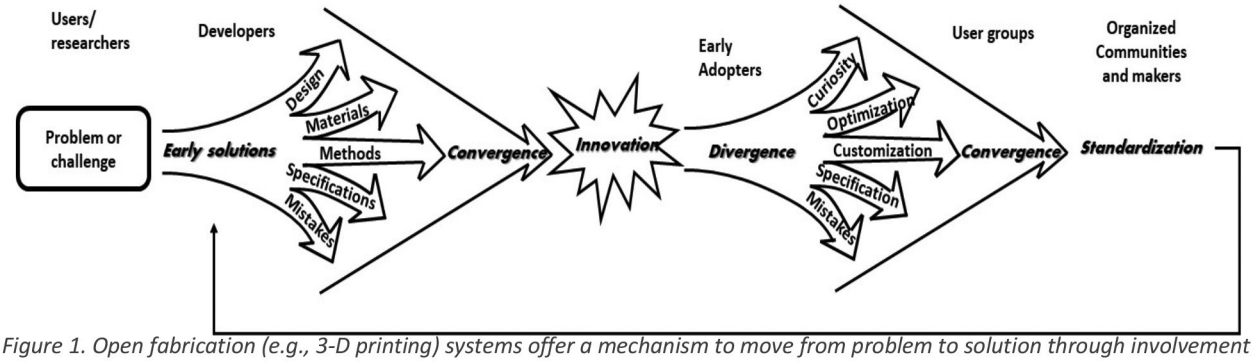

Figure 1. Open fabrication (e.g., 3-D printing) systems offer a mechanism to move from problem to solution through involvement of multiple users and associated community development.

EVALUATION OF IONIC COPPER FOR CONTROL OF ICH *Ichthyophthirius multifiliis* AT A COMMERIAL CATFISH FARM

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The ciliate parasite *Ichthyophthirius multifiliis*, commonly known as ich, infects all species of freshwater fish and causes high rates of mortality and severe economic losses in commercial aquaculture. Historical methods of control have proven hazardous to people (malachite green was found to be cancerous and subsequently banned) and to fish, where the dose of formalin needed to kill ich is often fatal to the fish as well. There is a dire need for better and safer alternatives to prevent and cure ich within fish breeding operations.

In March 2021, Haring Farms (Franklin Parish, LA) received a delivery of hybrid catfish fingerlings intended for commercial production within a new pond, and upon unloading noticed some dead carp in the delivery vessel. The grower submitted samples of the catfish for analysis by MSU's Aquatic Research & Diagnostic Lab, which confirmed the specimens were positive for ich.

At the recommendation of local ag advisors, the grower promptly began treating alternately with copper sulfate pentahydrate (35 lbs/acre, 0.2 mg/L as copper), copper sulfate + citric acid, and formalin (5.4 ppm), a formaldehyde-based product commonly used to treat for this infection. All the fish in the contaminated pond died (see figure) and the same process repeated in 3 more ponds, resulting in 100% fish mortality in 4 of the farm's 12 ponds, totaling more than $400,000 in direct losses and more than $1 million in lost opportunity cost.

A newly registered form of acid-stabilized liquid ionic copper was then trialed to treat the remaining 8 ponds known to be infected with the parasite, yet before many fish had begun to die. The dosing protocol, abbreviated as 3+3+1, consisted of treatments using 3 ppm, 3 ppm and 1 ppm as product, equivalent to 0.18 + 0.18 + 0.06 mg/L as ionic copper, applied over the course of 7 days. Subsequent lab analysis confirmed eradication of the ich from all 8 remaining ponds without any further loss of fish. The same 3+3+1 protocol was then applied to the original 4 infected ponds, with sentinel fish placed in cages to monitor efficacy and by-kill. By 2 weeks later, the original 4 ponds were free of ich and none of the sentinel fish had died (see table).

Our conclusions are that acid-stabilized liquid ionic copper offers an efficient and precise delivery method, is economical, disperses well without mechanical mixing, does not settle out, and is safer for fish than biocides currently employed.

RE-FRAMING WASTE AS A VALUABLE RESOURCE: A METABOLIC APPROACH TO VALUE ADDED PRODUCT VIA CYANOBACTERIAL CULTIVATION

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Aquaculture has increased faster than any other food sectors over the last few decades due to its potential as a provider of sustainable and healthy protein source for future populations. This growth has also led to an increase in the waste generation from fish farming, which is one of the major challenges that this sector is facing currently. Therefore, an environmentally friendly, sustainable waste management practice is essential for the growth of aquaculture. Photosynthetic microorganisms such as cyanobacteria that can uptake the harmful nitrogen and phosphorus nutrients from wastewater are an excellent remediation choice to deal with this issue. The major advantage of using cyanobacteria is that the biomass produced during the bioremediation of aquaculture wastewater can be used to produce economic commodities such as sugar, biofuels, and other bio derived products. Several cyanobacterial species accumulate carbohydrate in their biomass during nutrient limiting conditions which can further in several bioprocessing applications. The presented research studies the potential of a fast growing cyanobacterial species *Synechococcus elongatus* UTEX 2973 as a bioremediation agent for aquaculture wastewater and a raw material for sugar production. The growth and carbohydrate content of this particular strain during bioremediation of aquaculture wastewater was investigated to further use that biomass for sugar production. To complement the experiments, stoichiometric metabolic modeling of this strain was performed to predict the trend in the growth of the species and carbohydrate accumulation in biomass under different environmental conditions (by varying nutrients). Furthermore, the metabolic model of this strain was used to predict optimal nutrient levels in aquaculture wastewater for carbohydrate accumulation. Hence, by combining two important bioprocesses, this study addresses one of the most discussed issues of recent times, turning wastes into resources.

ECONOMIC IMPACT ASSESSMENT OF THE US CATFISH INDUSTRY

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The catfish industry is the largest US aquaculture sector and a major economic contributor to the rural economies of the Southern states of Alabama, Arkansas, and Mississippi. It is vital to accurately portray the economic contribution of this industry that includes supply chain actors such as hatcheries, feed mills, farms, and processing facilities. Economic contribution of the catfish industry in the tristate region was estimated employing the input-output (I-O) modeling approach, and the IMPLAN database and software (Impact Analysis for Planning MIG, Inc.). An analysis-by-parts approach was employed because the IMPLAN database does not disaggregate a catfish sector from other animal livestock industries with expenditure patterns that differ substantially from those of catfish. All major supply chain actors of the catfish industry viz., feed mills, hatcheries, foodfish farms, and processing plants were surveyed to obtain their expenditure patterns and output in terms of sales. The survey data consisted of 68 farms (hatcheries and foodfish combined), five feed mills, and nine processing plants in the tristate region. Standard enterprise budgeting techniques were used to convert the sales and expenditure values to coefficients to be imported to the IMPLAN model to estimate industry contributions for 2019. The direct output from the catfish industry ($1.1 billion) generated a total economic impact of $1.9 billion. The industry directly employed 4,298 people and created an additional 4,877 jobs in the tristate economy for a total employment effect of 9,175 jobs. Catfish industry spending created an indirect economic effect of $553 million in other secondary sectors, that supplied production inputs and services. The induced economic effect generated from spending by employees within the catfish industry and secondary sectors amounted to $255 million. Some of the key sectors influenced by the catfish industry are grain farming, banking and financial institutions, truck transportation services, electricity generation, equipment and machinery manufacturing, etc. The industry also generated $78 million in local, state, and federal taxes. Results of this study provide critical insights for policy-makers and others into the contribution of the US farm-raised catfish industry to local and regional economies as well as its diverse industry interconnections.

Table 1. The economic impact of the catfish industry in the tristate region, 2019.

Impact	Employment	Labor Income	Value Added	Output
Direct	4,298	$192,678,334	$216,124,324	$1,102,136,449
Indirect	3,074	$151,613,473	$231,590,932	$552,545,343
Induced	1,803	$73,028,887	$138,988,383	$254,688,108
Total	9,175	$417,320,694	$586,703,639	$1,909,369,900

PRODUCTION ECONOMIC RELATIONSHIPS IN INTENSIVE CATFISH PRODUCTION SYSTEMS

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The US catfish industry is evolving by adopting intensive farming practices such as intensively aerated pond and split-pond systems. The functional relationship between fish yield and key production inputs in these intensive systems was separately analyzed employing commercial catfish production data from over 143 pond observations. Production functions such as Cobb-Douglas, translog, and modified translog production functions were employed to define functional relationships between key input and fish yield in these two intensive catfish production systems. Choice of appropriate models was made after considering the measures of fitness (R^2, adjusted R^2, root mean square error, Akaike Information Criterion, and Bayesian Information Criterion) as well as minimizing econometric issues such as heteroskedasticity and multicollinearity. A Cobb-Douglas production function recognized the size of fingerlings at stocking, aeration rate, survival, and feeding rate as statistically significant variables influencing fish production in intensively aerated pond systems. A modified-translog function identified variables such as initial fingerling stocking biomass, feed conversion ratio, feeding rate, and pond size as important variables influencing production in split-pond systems. Although the latter model had weak heteroskedasticity (P= 0.05), such occurrences are common given the high variance in input usage in commercial-farm data. Nevertheless, the high predictive power of this modified-translog function provides meaningful estimates of the functional production relationship. The quantity of feed used in the ponds was found as an important variable in both models. Both production functions indicated further room for improvement in use of inputs to increase production. The study provides insights into input elasticities, magnitude, and direction of effect of key production inputs on fish production in intensive catfish production systems.

Table 4. Cobb-Douglas production function explaining the relationship of various production variables influencing catfish production in intensively aerated ponds (N= 52 commercial pond observations).

Production variables	Coefficient	Standard Error	P-value
Stocking size	0.175	0.054	0.002
Aeration rate	0.283	0.079	0.001
Survival	0.767	0.095	0.000
Harvest size	0.119	0.106	0.270
Feeding rate	0.566	0.087	0.000
Pond size (dummy for smaller pond <2.5 ha)	-0.079	0.071	0.278
Intercept	1.863	1.152	0.113

REGULATIONS IN THE US AQUACULTURE: PERSPECTIVES FROM INLAND AQUACULTURE

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Despite being the largest seafood market in the world, the US aquaculture sector has registered relatively sluggish or even negative growth in recent decades. Mounting evidence attributes this to the increasing complexity in the US regulatory environment. Excessive regulations not only hinder the growth of the aquaculture sector but also exert an economic burden on fish farms. The cost of regulations extends beyond the often-negligible permits and license fees. A large proportion is constituted by implicit costs such as the value of time spent on paperwork, cost of management changes, and economic value of lost opportunities. Over 1,300 regulations are found to affect US aquaculture and are categorized as environmental regulations, food safety, fish health, interstate transport, legal and labor standards, and culture of commercially harvested species (Fig 1). Among environmental regulations, the Migratory Bird Treaty Act has become the biggest concern for many inland aquaculture sectors such as catfish. Although the federal government has been making revisions to the existing depredation permits, its benefits to the farmers are yet to be discovered. Other regulations such as the Clean Water Act have caused certain aquaculture sectors such as trout to incur huge investment costs on wastewater management. Many of these regulatory costs are fixed costs that could only be spread over greater volumes of production, a difficult task for small-scale producers. In times when the US aquaculture sector is facing greater competition from the cheaper seafood imports, the huge disparity in regulatory costs relative to the Asian seafood trading partners hurts the US aquaculture in its effort to reduce the widening US seafood trade deficit.

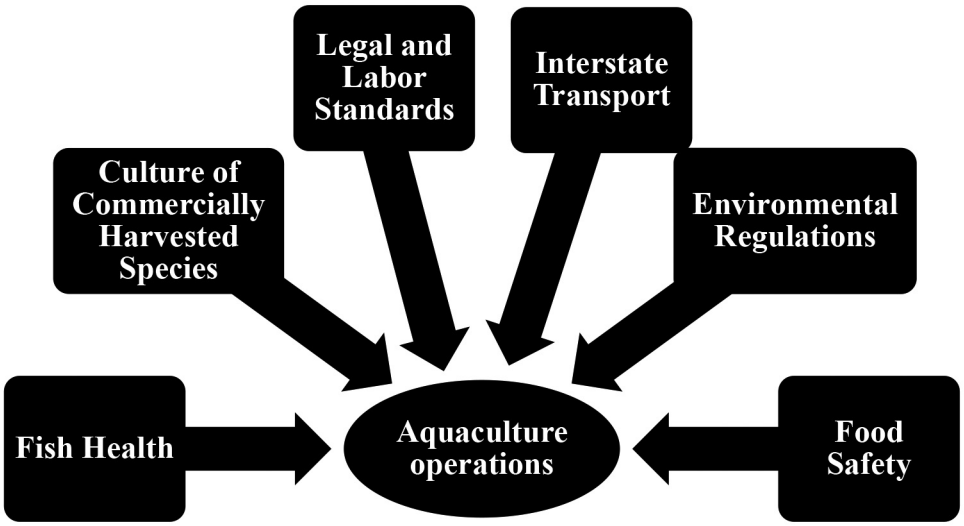

Figure 1. Classification of regulations affecting aquaculture farms in the US.

EFFECTS OF HYDROPONIC SYSTEM TYPE ON GROWTH AND NUTRIENT UPTAKE IN LETTUCE IRRIGATED WITH AQUACULTURE EFFLUENT

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Aquaponics, which is the combination of hydroponics and aquaculture, is a rapidly developing technology that has potential to increase food production in urban and urbanizing areas. An experiment was conducted at Auburn University to determine the effects of hydroponic system type on mature size and micronutrient uptake in lettuce (*Lactuca sativa* L. 'Rex') irrigated with aquaculture effluent. The experiment consisted of three treatments and three replicates. Each replicate contained 48 individual plants. Treatments were: 1) substrate trough culture with a perlite substrate; 2) nutrient film technique; and 3) deep water culture. Aquaculture effluent was supplied from a biofloc-type recirculating aquaculture system producing Nile tilapia (*Oreochromis niloticus* L.). Nitrate concentrations in aquaculture effluent ranged from 270 to 350 mg L^{-1} during the experiment while pH and electrical conductivity remained relatively constant from 6.0 – 6.4 and from 1.2 to 1.45 mS cm^{-1}, respectively. There were no significant differences in head fresh weight after 28 days. Chlorophyll content (SPAD index) was highest for the substrate troughs and lowest in nutrient film technique. There were slight differences in foliar nitrogen concentrations between hydroponic system type. Micronutrient concentration was significantly increased in substrate culture and deep water culture compared to nutrient film technique. The pH between repetitions varied slightly due to fluctuations in the tilapia aquaculture environment. Aquaculture effluent from the biofloc filter was not screened when filling the reservoir tanks. Micronutrient supplementation may not be necessary in aquaponics as long as particulate matter is allowed to interact with plant root systems. Further research should focus on identifying and quantifying plant growth promoting bacteria in aquaponic systems to further analyze nutrient acquisition relationships.

EFFECT OF SEASONALITY FOR OPTIMIZATION OF STEM CELL EXTRACTION FROM MATURE BLUE CATFISH (*Ictalurus furcatus*) TO PRODUCE ALLOXENOGENIC CATFISH

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Xenogenesis is an innovative tool for hybrid catfish (♀ channel catfish, *Ictalurus punctatus* × ♂ blue catfish, *I. furcatus*) seed production. The xenogeneic process can be accomplished by transplanting diploid primordial germ cells (PGCs), spermatogonial stem cells (SSCs) or oogonial stem cells (OSCs), derived from a donor diploid fish into a sterile recipient, which then enables recipient fish to produce donor-derived gametes. Usually, stem cells are collected from immature fish. However, there is a potential to collect donor-derived cells from mature fish during certain times of the year depending upon seasonal temperature fluctuations. Therefore, the current study was carried out with the objective of evaluating seasonal variations in germ cell count and sex steroid hormone profiles in mature blue catfish. Mature males and females (weight >1 kg and total length >50 cm) were sampled each month for an entire year. At each time sampling, gonadal weight (± 0.05 g), gonado-somatic index (GSI), and stem cell counts, including total number of live and dead SSCs and total number of live and dead OSCs in fish were determined. In addition, a blood (~1 mL) sample was collected from each fish to quantify the level of sex steroid hormones, testosterone (T), 11-ketotestosterone (11-KT), and 17β-estradiol using ELISA.

The highest quantity of live type A SSCs ($P < 0.0001$) in male blue catfish was recorded in April (7.053×10^4 cells), which then gradually decreased until November, where the lowest production was reported (0.19×10^4 cells). Mean GSI of blue catfish males ranged from 0.067% to 0.318%, which was significantly higher in November to March compared to levels reported in July to September. The highest quantity of live OSCs ($P < 0.0001$) in female blue catfish was observed in April (9.6×10^2 cells), which gradually decreased to zero over the months of May to July. No OSCs were observed during the months of August to February, as ovaries were full of immature oocytes. GSI levels of females remained consistent in the range of 11.3% - 6.7% from April to March, which showed no significant differences over time. Hormonal analyses are in progress and results will be presented.

In conclusion, we confirm the efficacy of using mature blue catfish males as donor species during the months of April, May, and June, which will be an added advantage during the xenogeneic process. However, it is less effective to use mature blue catfish females as donors due to their low OSC counts.

MAXIMIZING TARO (*Colocasia esculenta* L.) CORM PRODUCTION IN AQUAPONICS THORUGH MANIPULATION OF WATER QUALITY LATE IN THE VEGETATIVE GROWTH STAGE

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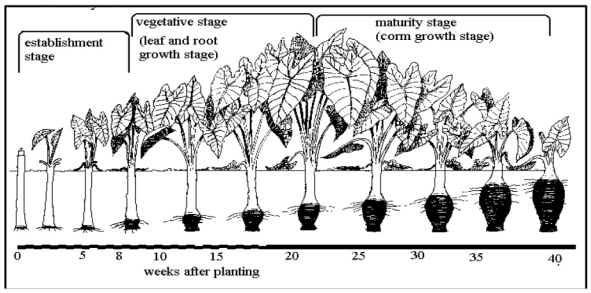
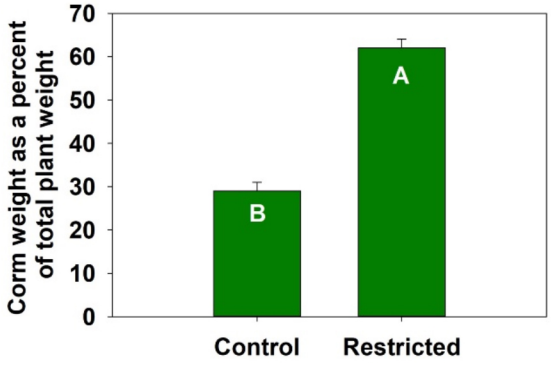
Aquaponics has been widely adopted in Hawaiian communities as a culturally relevant method of home food production. Taro (Kalo in Hawaiian) is a traditional Hawaiian staple crop that does well vegetatively in aquaponics, but does not produce corms of commercially acceptable size. As part of a larger community driven project (malamaaquaponics.org), we tested the hypotheses that low kalo corm yields were due to excessive water nitrogen levels late in vegetative development that hinders corm development. The kalo cultivar 'Maui Lehua' was planted in a randomized complete design with six replications in specially designed dual- tub systems that allowed for the application of two treatments: 1) fish effluent supplied throughout 8 months of plant development (control) and 2) Fish effluent restricted from the system at 4 months and fresh water supplied for the remaining 4 months of development (restricted). The ratio of corm to total biomass is a key indicator of plant maturity. Control plants produced significantly more biomass than restricted plants. Restricted plants had significantly more biomass partitioned to the corm (62% of total biomass) relative to control plants (22% of total biomass). This suggests that we were partially successful in transitioning photosynthate partitioning to the corm. However, individual weights of the primary corm were statistically similar between treatments (376-406g·plant-1). This is still low compared to recorded yields in terrestrial systems (>1kg). Modifications to the system are being made to address observed deficiencies in potassium and iron in plants of both treatments, and total time to harvest will be increased.





DEVELOPING TECHNIQUES FOR THE CULTIVATION OF THE HERBIVOROUS MONKEYFACE PRICKLEBACK *Cebidichthys violaceous* AS A SUSTAINABLE ALTERNATIVE TO UNAGI

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Monkeyface pricklebacks *Cebidichthys violaceous* are eel-like fishes in the family Stichaeidae that live in rocky intertidal and shallow subtidal habitats from Oregon to northern Baja California. The herbivorous nature of this species along with other life history characteristics, make them an attractive new species for aquaculture development in California. Their richly flavored flesh gives them potential for developing a new market as a sustainable product, and as an alternative to freshwater eels traditionally used for unagi. Our study is currently assessing their reproductive performance in captivity, as well as dietary studies to maximize growth.

Our reproductive study assesses monkeyface pricklebacks' potential for reproduction in captivity as well as conditioning of brood stock. To test spawning hypotheses, we constructed an array of 9 tanks at the MLML aquaculture center. Each environment has been implemented with different "nesting habitats". Males and female fish are placed in groups of n=8 individuals per tank and n=9 replicate tanks per treatment. By manipulating underlying substrate in these habitats and having enough of each "nest type" for every female, we can test whether Monkeyface pricklebacks have certain habitat criteria for reproduction and nesting. Sex determination in monkeyface pricklebacks is unknown. Ultrasound and Passive Integrated Transponders (PIT) tagging individuals for sex determination allowed for individual tracking in our experiment. After the conclusion of the reproduction experiments, we will examine cranial morphometrics to determine whether the size of the supraorbital crest and other features are sexually dimorphic traits. This allows us and others in the aquaculture industry to distinguish males and females when an ultrasound is not available.

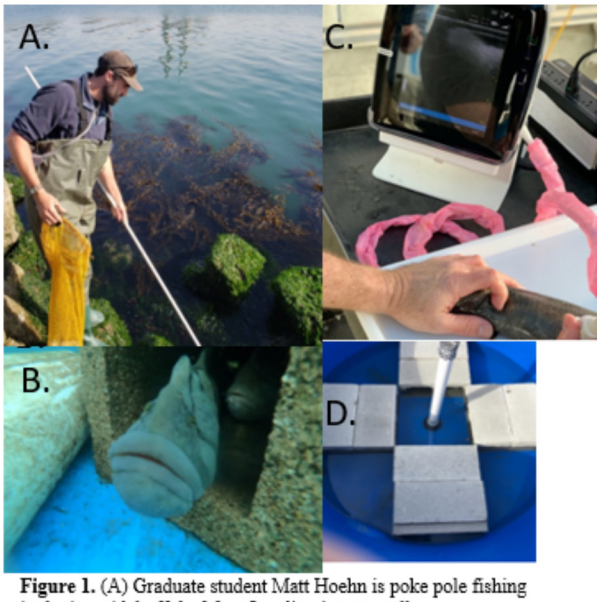

Figure 1. (A) Graduate student Matt Hoehn is poke pole fishing in the intertidal off the Moss Landing jetty to collect Monkeyface pricklebacks (*Cebidichthys violaceus*) for the project. (B) Images of Monkeyface pricklebacks in tanks at the aquaculture facility at MLML. (C) Erick Sturm from NOAA Southwest Fisheries uses a portable ultrasound to identify this Monkeyface prickleback as a female based on the presence of ovarian tissue (seen as a granular solid mass in the body cavity). (D) Photograph of the inside of a tank showing the replicate spawning "caves".

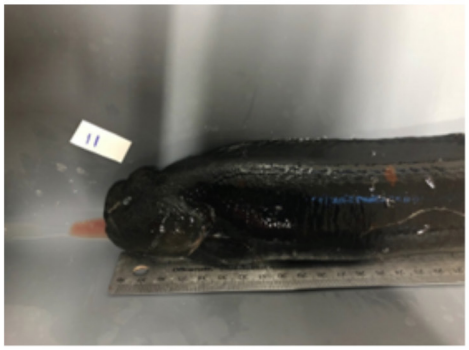

Fig. 2. Example image of a Monkeyface Prickleback that will be used for morphometric analysis of cranial features.

DEVELOPING CAPACITY FOR GENETIC IMPROVEMENT OF EASTERN OYSTER IN TEXAS

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In 2019, the state of Texas passed into law a bill making commercial aquaculture of oysters legal in state waters for the first time. Initiation and expansion of the oyster aquaculture industry in Texas, with 367 miles of coastline, is a considerable opportunity to increase the production of farmed seafood in the state. One unique characteristic of Texas oysters is the presence of two distinct genetic groups found naturally along the northern and southern parts of the Texas coast, respectively. These populations naturally co-occur in a "transition zone" spanning several bay systems that are increasingly exploited in the oyster fishery and are also well-suited for oyster aquaculture. This presentation will highlight current progress towards establishing selective breeding programs for Texas oysters, including developments in hatchery capacity as well as future research directions.

BALANCING DIETARY LIPID AND CHOLESTEROL TO INCREASE FILLET OMEGA-3 DEPOSITION IN RAINBOW TROUT FED A SOY-BASED DIET

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A 12-week feeding trial was conducted to evaluate the effects of cholesterol supplementation to different ratios of soybean oil (SO) and linseed oil (LO) on growth performance, fillet fatty acid composition, and muscle gene expression in rainbow trout, *Oncorhynchus mykiss*. Twelve isonitrogenous (48%, crude protein), isolipidic (21%, crude lipid) and isocaloric (22.5 MJ/kg, energy) experimental diets were produced: Two control diets (fishmeal/fish oil based (FM/FO) and plant protein/fish oil based (PM/FO)) and ten experimental diets with graded ratios of SO and LO (100/0, 75/25, 50/50, 25/75 and 0/100), with or without added cholesterol (C). Rainbow trout (initial body weight :18.8 ± 0.3g) of the USDA-UI strain crossed with Donaldson strain, selected for improved growth on an all-plant protein diet, were stocked into 145-L tanks at 25 fish per tank.

Compared with fish fed other diets, the weight gain of fish fed diet FM/FO, PM/FO or L100+C was greatest ($P \leq 0.05$). Different ratios of SO/LO did not affect trout weight gain; however, the addition of cholesterol to the LO100 diet (LO100+C) significantly increased fish weight gain. The interaction between the two main factors (cholesterol and SO/LO ratio) significantly impacted feed intake ($P \leq 0.05$); but had no significant effects on growth performance or feed utilization ($P > 0.05$). A hypocholesterolemic effect was observed in plasma of fish fed plant-based diets without cholesterol supplementation compared with the fish fed control treatments and diets supplemented with cholesterol. Plasma cholesterol of fish fed L100+C diet was significantly higher than fish fed SO100 and SO75/LO25 diets. Regarding fatty acid profile, the interaction of the two main factors (cholesterol x SO/LO ratio) significantly increased DHA content in fish fillet ($P \leq 0.05$). Eicosapentaenoic acid (EPA) content in fish fillet was also affected ($P \leq 0.05$). Supplementation of cholesterol did not affect the expression of liver and muscle genes involved in fatty acid elongation, desaturation, and β-oxidation ($P > 0.05$); but there was a non-significant trend toward increased gene expression for elongase and desaturase with cholesterol supplementation when linseed oil was provided above 50%.

In conclusion, results of the present study demonstrated that: 1) A plant-based diet without added cholesterol resulted in growth reduction and plasma hypocholesterolemia in juvenile rainbow trout, and 2) Fish fed linseed oil, as 100% of dietary lipid, with cholesterol supplementation had significantly higher weight gain and feed intake compared with other plant-based diets. Furthermore, the observation that cholesterol supplementation showed increased EPA and DHA levels in fish fillet when linseed oil was provided above 50% supports further research, over an entire production period, to evaluate the long-term effects at harvest.

OPTIMIZING THE FATTY ACID PROFILE OF NOVEL TERRESTRIAL OIL BLENDS IN LOW FISHMEAL DIETS OF RAINBOW TROUT (*Oncorhynchus mykiss*) YIELDS COMPARABLE FISH GROWTH, FATTY ACID COMPOSITION AND HEALTH RELATIVE TO FISH OIL

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Identifying and effectively utilizing suitable, novel, terrestrial oil sources either alone or as blends to replace fish oil (FO) is a prerequisite for improving the sustainability of global aquaculture. Therefore, the present study evaluates several novel terrestrial oil blends (TOBs), optimized for their fatty acid profile, as alternatives to FO in low fishmeal diets fed to rainbow trout (*Oncorhynchus mykiss*) and describes the subsequent effects on fish growth, fatty acid composition and health. Insect oil (IO), genetically modified canola oil (CO), palm oil (PO) and linseed oil (LO) were used for the formulation of three TOBs *viz.,* TOB-1 (30%IO+36%CO+34%LO), TOB-2 (40%PO+20%CO+40%LO) and TOB-3 (50%TOB-1+50%TOB-2). Formulas TOB-1 and TOB-2 considered the total fatty acid profile based upon the general FO fatty acid profile, published fatty acid research for rainbow trout, and the fatty acid requirements of rainbow trout. A low fishmeal based basal diet containing 44% crude protein was formulated, and FO, TOB-1, TOB-2 and TOB-3 were incorporated in the basal diet to prepare the experimental diet groups Control, TOB-1, TOB-2 and TOB-3, respectively. All experimental diets were fed to triplicate groups of rainbow trout juveniles (7.9 g) for 9 weeks. Growth performances in TOBs fed groups were equal to the FO-based control group. Fish fed the TOB-3 diet consumed more feed followed by the control and TOB-1 diet groups. Significantly lower feed intake was observed in the TOB-2 diet group. Feed conversion ratio and protein efficiency ratio were not significantly influenced by dietary oil sources. Fish fed the control group showed significantly higher hepatic lipid content compared to TOB groups, followed by TOB-2, TOB-3, and TOB-1, which was significantly lower in hepatic lipid content. Fatty acid composition of muscle and liver reflected that of the diets. Maximum values for n3 LC-PUFAs (EPA and DHA), lauric acid (C12:0) and C18:3n-3 were observed in the FO, TOB-1 and TOB-2 groups, respectively. Except for C12:0, muscle saturated fatty acid contents were significantly lower in TOBs-based diet compared to the FO-based control diet fish. As expected, muscle C12:0 content was significantly higher in the TOB-1 group followed by the TOB-3 group. TOB-2 and control groups had significantly lower content of C12:0. The fillet total n-3 LC-PUFA was significantly higher in fish fed the control diet group followed by TOB-3 and TOB-2 groups, TOB-1 showed significantly lower content of total n-3 LC- PUFA. Hepatic delta-5 desaturase (*Δ5fad*), delta-6 desaturase (*Δ 6fad*) and fatty acid elongase-2 (*Elovl-2*) gene expressions were significantly influenced by dietary oil sources, where TOB-based groups showed higher *Δ6fad* and *Elovl-2* expressions. Measured innate immunity and antioxidant markers were not affected by TOBs. Finally, we concluded that TOBs could serve as a substitute for FO in rainbow trout feed without negatively impacting growth and health performance. Moreover, fillets total n-3 LC-PUFA of TOBs fed fish also satisfies the suggested dietary requirement of total n-3 LC-PUFA relative to the suggested daily serving for humans.

A COMPREHENSIVE GUIDE FOR CREATING AND MAINTAINING A MID-SCALE AQUAPONIC SYSTEM

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The field of aquaponics has grown significantly in recent years as a viable alternative to traditional agricultural and aquacultural practices. Through the coupling of farming and aquaculture, total water consumption is drastically reduced, food security increases, and minimal waste is produced. Additionally, the relationship between crops and fish is mutually beneficial. Fish produce ammonia, which quickly becomes lethal for them. Our system sends ammonia-rich water to a sump tank to be converted to nitrites and then nitrates by nitrifying bacteria. Those nitrates are then filtered out of the water by the plant roots and denitrified water is sent back to the fish tank.

The use of large-scale aquaponic systems for commercial purposes is still widely unexplored as an option due to uncertainties regarding growth and yield of both fish and crops. Our USDA founded project "Bluewater: A Smart Circular Economy for Integrated Organic Hydroponic Aquaponic Farming to Empower an Underrepresented Workforce", aims to develop a mid-scale aquaponic system by students at Texas State University. Joining forces with the Biology, Agriculture, and Engineering departments, we designed a zero-waste automated mid-scale system that will allow us to design experiments for the optimization of ornamental fish culture and crop production. Furthermore, the incorporation of automation has been looked at as a tool in making aquaponic operations more accessible. For example, using sensors to signal intervention when system conditions are approaching dangerous levels. The long-term goal is to produce protocols that can be implemented at larger scale aquaponic systems. This study is being conducted in a greenhouse at Texas State University and will serve as a guide on how to construct and maintain multiple aquaponic systems.

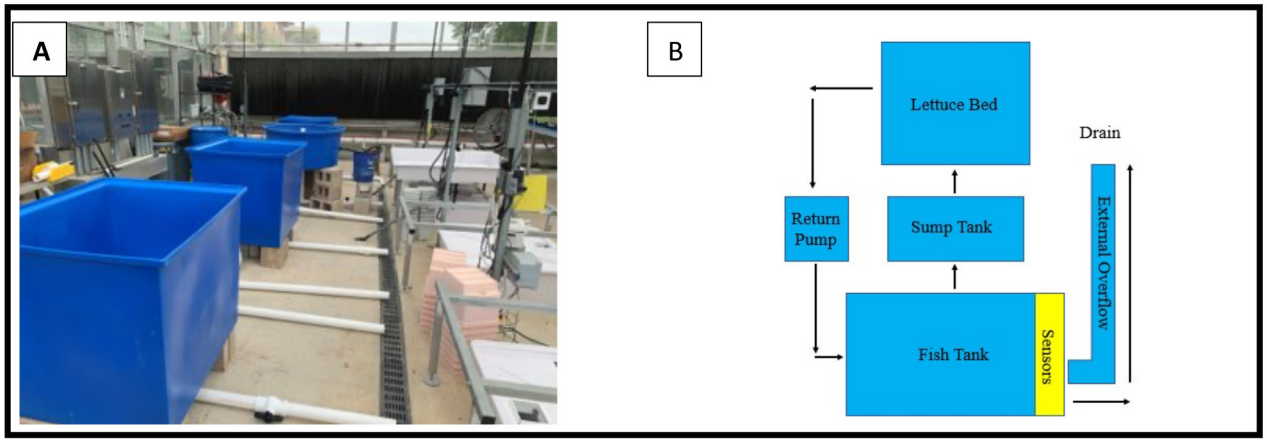

Fig 1. A) Picture of the four aquaponic systems at TXST. B) Design of a single 500L aquaponic system. Waterflow in the system is denoted by black arrows. Sensors will work to detect temperature, ammonia, nitrate, pH, and dissolved oxygen (DO) levels.

THE MAINE OYSTER TRAIL

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Prior to the COVID-19 pandemic, Maine's oyster producers relied on restaurants as their primary market. The closure of restaurants across the U.S. last spring threw oyster farmers for a loop, as the half-shell market dried up virtually overnight. This shock forced growers to quickly pivot and come up with creative solutions to sell oysters and sustain their business. Within weeks, oyster farmers had begun to make serious investments in pandemic-proof business strategies like local seafood home delivery, seafood markets, and direct sales off the farm. As more and more farms started to use social media to advertise local oyster sales, farm tours, and pop-up shucking events throughout the summer, the need for a centralized location to help local consumers find oysters became urgent.

The Maine Oyster Trail fills this need by providing an interactive, digital tourism tool that connects users to Maine oyster farms, their products, and Maine businesses that offer authentic Maine oyster experiences. Visitors from near and far use the Trail's customizable trip planner to find activities that match their preferences. The Trail's digital oyster passport allows users to check-in at locations along the Trail and receive free prizes for completing various challenges. The Maine Oyster Trail launched in June, 2021 and features Maine 52 oyster farms, 15 raw bars and mobile shuck trucks, and 8 boat tour and kayak outfits that offer oyster tours along the coast of Maine. By marketing the Maine oyster experience, the Trail builds market demand not only for Maine oysters, but also for a new kind of visitor experience that we like to call "aquatourism."

While this project is focused in Maine, the Trail model can be replicated in other states and regions and can serve as an important example of how a best-in-class food trail can diversify the aquaculture industry and ultimately make it more resilient in the face of changing market conditions.

NUTRITIONAL CONTRIBUTION OF FERMENTED SOYBEAN MEAL AS A FEED REPLACEMENT FOR THE PACIFIC WHITE SHRIMP (*Penaeus vannamei*)

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Shrimp farmers have been using fermented soybean meal (FSBM) as a partial replacement for commercial feeds for the pacific white shrimp. However, there are no reports published regarding the use of FSBM as feed replacement. Hence, two 6-week growth trials were conducted in clear water (20 shrimp/tank) and outdoor green water (35 shrimp/tank) systems to determine the biological value. FSBM was produced by first mixing a solution consisted of 0.3g of Lactobacillus and Saccharomyces, 0.15g phytase enzyme, 9g of molasses, and 100ml seawater which was fermented for ~24hours. This was then top coated on pelleted soybean meal (SBM) at ~30% (w/w) of the fermented solution, then was left to dry ferment for ~24 hours. FSBM was then used as a replacement for a commercial feed on an equal protein basis and 12 treatments using varying levels of feed, F-SBM and a partial replacement of the feed with FSBM were developed. Moreover, feed intake was evaluated using both acoustic monitoring and traditional feed consumption as a validation of shrimp (10/tank) intake of the FSBM over a 30 min period. Although it was confirmed that shrimp utilized the FSBM, the acoustic feeding activity and food consumption were significantly higher when fed commercial diet (P-value < 0.05). There was a linear response of shrimp growth with the increase of feed amount (F), however it was noticed that there was no increase of the shrimp growth with the increase of FSBM (Figure 1). Data indicates that the use of FSBM as a single protein source supports only 1/3 of the growth regardless of the amount offered to the shrimp. This study indicates that there is a limited contribution of the FSBM as a single source protein. ented soybean meal, feed replacement, feed consumption, *Penaeus vannamei*.

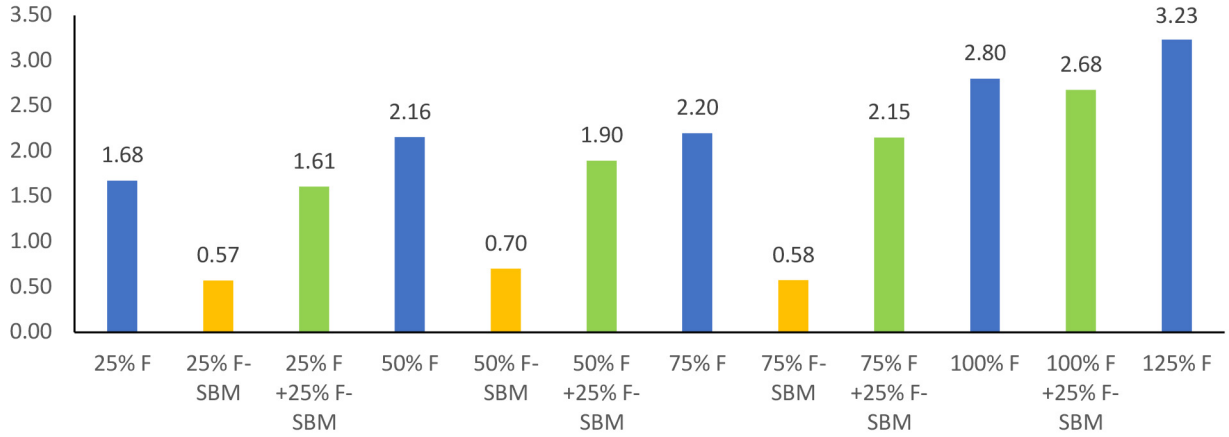

Fig. 1: Weight gain (g) of the shrimp produced; commercial feed is referred as (F) and fermented soybean meal as (FSBM)

RELATIONSHIP BETWEEN AEROBIC SCOPE AND THERMAL TOLERANCE OF WHITE-LEGGED SHRIMP (*Penaeus vannamei*) IN LOW-SALINITY CULTURE SYSTEMS

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Aquaculture of *Penaeus vannamei* in low-salinity ponds is a growing industry in the southeastern United States. A major challenge facing this industry is a phenomenon called late-term mortality. This is thought to be driven by thermal stress at the end of the growing season when water temperatures can reach or even exceed 36 ºC in shrimp production ponds. In this study, we test whether thermal tolerance decreases with increasing shrimp age/size, and whether aerobic scope (AS) is a useful concept for understanding the physiological basis of thermal tolerance in shrimp. Aerobic scope represents the excess capacity of an organism to deliver oxygen in support of activity, growth, and reproduction, and exhibits a unimodal relationship with temperature. In theory, the temperature at which AS = 0 represents the upper thermal limit of an organism where it is no longer physically capable of meeting its energetic needs. We exposed two size-classes (2.2 ± 0.8 and 25.8 ± 2.4 g) of shrimp to increasing temperature at a rate of 1 °C/h from 20 – 42 °C. At each temperature, we used intermittent respirometry to estimate resting metabolic rate (RMR) and the electron transport system (ETS) assay to estimate maximum metabolic rate (MMR). We directly measured thermal tolerance in terms of critical thermal maximum (CTM): the temperature at which shrimp lost equilibrium and were no longer able to right themselves. Preliminary results show that shrimp RMR rose with increasing temperature as MMR declined, resulting in a decrease in aerobic scope (MMR – RMR) with increasing temperature. Aerobic scope declined to zero at ~38°C, corresponding to the observed CTM. In addition, aerobic scope at sublethal temperatures was significantly higher for the small size-class compared to the large size-class. Reductions in aerobic scope appear to be a good predictor of lethal stress and may explain differences in thermal tolerance between small and large size-classes.

REFINING CULTURE METHODS FOR THE CYCLOPOID COPEPOD *Oithona colcarva*

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Marine finfish can be challenging to culture with many species heavily reliant on small, nutritious live prey for their first feed. Newly hatched copepod nauplii are an exemplary live feed due to their small size (~45 µm), high nutritional value, and quick escape responses thought to elicit predatory feeding behaviors in larval fishes. The cyclopoid copepod *Oithona colcarva* is a candidate for aquaculture due to its small size and tolerance to fluctuating culture conditions. Experiments were conducted to evaluate initial culture protocols used for *O. colcarva* production. Culture parameters including diet, stocking density, and photoperiod were assessed to quantify effects on naupliar production.

Initial diet experiments compared different combinations of live microalgae using the species *Tisochrysis lutea*, *Chaetoceros muelleri*, and *Tetraselmis chuii* fed at 400,000 cells/mL. Naupliar production was the highest when adult copepods were fed a 1:1 carbon equivalence ratio of *T. lutea* and *T. chuii*. This diet became the control in the following diet experiment examining how algal concentrate blends effect naupliar production. The live microalgae control diet outperformed the algal concentrate diets but further investigations are necessary before ruling out algae concentrates as potential feeds for *O. colcarva*.

In the stocking density experiments, adults were stocked at 8, 12, 16, and 24/mL and there was no significant difference in nauplii production among treatments. Photoperiod experiments yielded comparable nauplii production between 6-hr light : 18-hr dark and 12-hr light : 12-hr dark. Nauplii production under the 18-hr light : 6-hr dark photoperiod was significantly lower than both other treatments (*Figure 1*). Therefore, natural diurnal cycles should suffice for culture conditions. Results from these experiments were translated to commercial scale production in a greenhouse. Commercial scale (200L) production tanks produced an average of 2.56 million nauplii/day for 10 consecutive days. Results from these experiments have contributed to commercial production protocols for *O. colcarva,* a promising live feed for aquaculture.

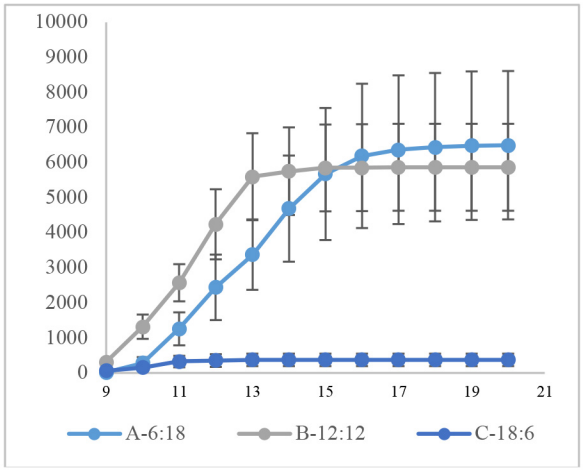

Figure 1: Cumulative nauplii and egg production over time for photoperiod experiment.

USE OF MARINE PROBIOTICS DURING THE NURSERY REARING OF WHITE SHRIMP POSTLARVAE IN A COMERCIAL FARM OF SONORA MEXICO

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The white shrimp aquaculture in extensive and semi-intensive systems is a highly profitable activity in the northwest of Mexico. However, the presence of different diseases in recent years has generated severe economic losses, affecting the supply chain. Therefore, it is important to explore production alternatives with an economic, social and environmental sustainability approach. Among the technologies incorporated, we found high-density units or nursery systems with minimal water exchange, constant aeration and use of microbial consortium as well as nutrient sources (probiotics and BFT biofloc technology). During the nursery period, the shrimp postlarvae develop, grow and improve their immune response prior to the stocking on earthen ponds; making them more robust for growout phase and shortening the development time during the initial phase.

The present study was carried out at a commercial shrimp farm located in southern Sonora, Mexico, and aimed to evaluate the performance of an experimental probiotic in a nursery system of white shrimp postlarvae (*Penaeus vannamei*). The trial was carried out in a raceway pond covered with liner and greenhouse dome, with an area of 1,440 m^2, 1.20 m of depth and a water volume of 1,728 m^3; which was stocked with an initial population of 4,545,000 shrimp PL (PL12, average weight of 3.5 mg). During 26 days the shrimp postlarvae were fed every 2 hours at 10% average body weight (ABW) until obtaining a suitable size (approx. 500 mg) for their transfer and stocking into the earthen ponds.

The preparation of the probiotic was developed with lyophilized bacteria of the genus *Bacillus sp*. (strains 13L, 36R, 42), which were obtained from marine environments of the coast of Sonora. This group of bacteria were preliminary evaluated in laboratory with *in vitro* and *in vivo* tests showing good results. For the preparation and activation of the probiotic it was added molasses, bran, urea, yeasts and constant aeration; the process was carried out in 3 phases of 18-24 h of incubation each, and in volumes of 10, 100 and 1,000 L. The last scaling phase was used for the daily application: 150 mL/kg for feed (mixing before feeding) and 200 L directly to culture water. To determine the effect of the consortium on the shrimp nursery system, daily biometrics, digestive tracts analysis, pigmentation and behavior activity were performed, as well as monitoring physicochemical water parameters (dissolved oxygen, temperature, pH, salinity, nitrite, nitrate, ammonia).

The results are showed in Table 1. The shrimp postlarvae reared in the nursery system with the experimental probiotic consortium and normal management, showed a good survival rate, growth and health, with proper development and weight gain. However, water quality was compromised during the final phase due to high temperature (above 35°C) and shrimp biomass. The use and application of the experimental probiotic in feed and water resulted as a good management strategy during the PL culture, however more studies and field trials in other gowout systems are recommended.

Initial average weight (mg)	Final average weight (mg)	Population (PL´s)	Total Biomass (kg)	Survival (%)	FCR	
3.5	555.19	4,545,000	2,382,040.5	94.4	1.09	
Oxygen (mg/L)	Salinity (g/L)	Temperature (°C)	pH	Nitrite (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)
4.03	41.9	33.4	7.2	0.184	4.65	2.33

Table 1. Performance of experimental probiotic in nursery system, shrimp zootechnical information and water quality parameters.

DESIGNING A GRADER FOR *Penaeus vannamei* NURSERY

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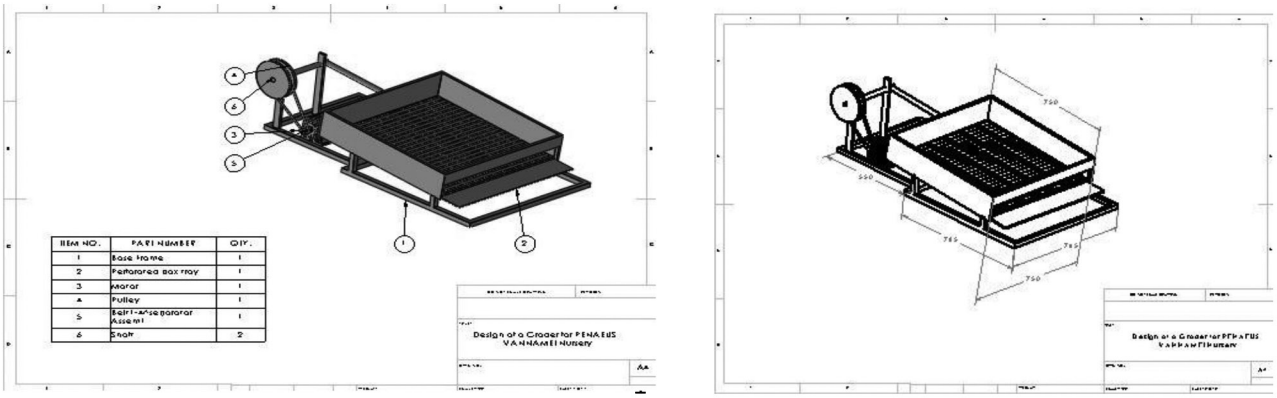
Traditionally the shrimp farmers used to stock pl (post larvae) of Penaeus vannamei shrimps in to grow out ponds directly but they faced many problems such as, running mortality syndrome, retarded growth, high FCR, increased production cost and lower profit. So, a new system of farming was introduced wherein a new phase is introduced in between hatchery and grow out i.e. called a nursery system or nursery phase. The main advantage of nursery phase is to remove the weaker post larvae and transfer the stronger ones to attain a compensatory growth in grow out ponds. In nursery systems pl is used to grow up to 0.5 to 2 g and then transferred to grow out ponds. But during the transfer of seeds due to the **"**heterogeneous growth**"** of the shrimp seeds it is quite difficult to attain the compensatory growth at an expected phase. So a grader was designed to separate the uniform size seeds which weigh above 0.5 g from small size seeds from L.vannamei nursery systems. During testing of the designed grader, it showed more than 75% efficiency in the separation of seeds that weighed 0.5 g and above. Thus, the results showed significant difference between the seeds that were graded and non-graded.

In nursery farming, the common process involves the grading of the shrimp seeds of required size to transfer it to the grow out ponds. The machine has a dimension of which is suitable for all type of nursery systems. The various components were selected after appropriate design of those components. After assembling the parts, a test was carried out to grade the appropriate size shrimp seeds with respect to the time taken to complete the grading operation and to find out the efficiency of the machine. Results showed that the machine has an efficiency of 75% and 25% for quantity retained in the mesh plate and quantity collected down respectively when 2 kg of shrimp seeds are loaded and the grading process completed within 7 minutes. So, a Farmer can transfer the shrimp seeds using this portable grading machine effectively. The portable grading machine has been found to be effective and efficient for the purpose of grading the shrimp seeds of weight 0.5 g from small size seeds. The designed machine has been made simple so that it could be understood and operated by even an unskilled person. Most materials used for the fabrication of the machine are locally available and affordable making the machine very cheap to fabricate and to maintain. Furthermore, it saves energy and time as it operates electrically.

Fig 1. The overall view of the grading machine



DEVELOPMENT OF A SYSTEMATIC APPROACH TO DIAGNOSTIC ULTRASOUND IN NILE TILAPIA *Oreochromis niloticus*

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Ultrasound imaging uses high-frequency sound waves (2 to > 55 MHz) to visualize internal anatomy for diagnostic procedures. Ultrasonography has been used in aquaculture and fisheries since the early 1980s; however, its widespread use and potential contribution is limited by high variability in equipment, diverse fish morphology, and inadequate reporting of control settings used. The goal of this study was to develop systematic fish handling and ultrasound scanning procedures for viewing internal anatomy and reproductive organs in Nile Tilapia. The objectives were to (1) assess fish handling and probe positioning procedures; (2) manipulate and evaluate primary ultrasound control settings (frequency, focus, depth, gain) at three external anatomy landmarks; and, (3) develop systematic, reliable, reproducible fish handling and ultrasound procedures.

Adult Nile Tilapia (24 females: 24 males) were scanned using the EVO II Ultrasound Scanner (E.I Medical Imaging, CO) equipped with a (6-14 MHz) multiple-frequency range waterproof probe in lateral, ventral, and dorsal recumbence. Probe positioning and settings were developed on external anatomy landmarks (Figure 1). Cross-sectional ultrasound images on the transverse plan (Figure 1-B), and the gross morphology of frozen fish were recorded (Figure 1-C). Fish positioning, probe placement at external landmarks, control settings, and data obtained from the frozen cross-section were used to inform image interpretation and development of a systematic ultrasonography approach to generate new data for reproduction research on Nile Tilapia and to create a diagnostic ultrasound user-guide for tilapia hatcheries.

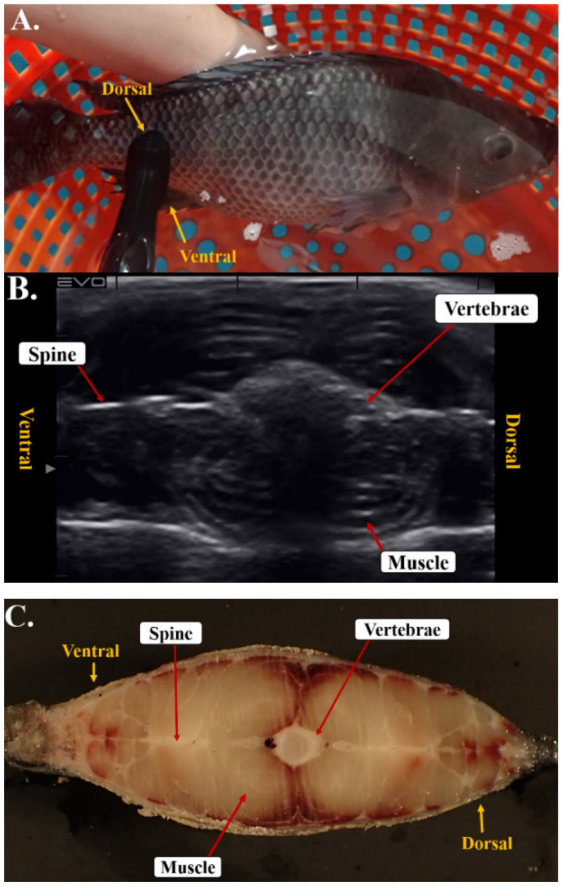

FIGURE 1. Nile Tilapia placed in ventral recumbency (1-A, upright-swimming position) and the corresponding ultrasound (B) and frozen-cross-sectional images (C). The ultrasound image (B) was generated using a frequency of 8 MHz, depth of 4 cm, Gain of 44 dB, and Focus of 3.2 cm).

CAN DIETARY MANIPULATION AMELIORATE EXTREME HEATWAVE STRESS IN FISH? THE CASE OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Global extreme climatic events impact all living organisms, including fish. This study aimed to evaluate the growth, hemato-physiological, and immune-antioxidant stress responses as indicators for the acclimatization ability of European seabass (*Dicentrarchus labrax*) fed with four different dietary supplements, with subsequent ambient extreme heatwave (32 °C) exposure.

Fish were fed with diets supplemented with vitamin C and E, propolis, phycocyanin, β-glucan, along with a control diet for 56 days, followed by 18 days of extreme ambient warm exposure. Results indicated that fish growth performance and survival were higher in fish fed with propolis followed by vitamins C, E and phycocyanin supplemented diets (Table 1).

During extreme warm exposure, red blood cells (RBC), hematocrit, hemoglobin, respiratory burst, and serum lysozyme activities were significantly higher in fish fed propolis, vitamins C, E and phycocyanin supplemented diets ($p<0.05$). On day 18 of extreme warm stress, blood glucose and lactate levels were significantly higher in control and β-glucan fed fish compared to other diets. On days 9 and 18, erythrocytic cellular abnormalities (ECA) and nuclear abnormalities (ENA) were significantly increased in control and β-glucan diets ($p<0.05$). Serum antioxidant enzymes' [superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione reductase (GR)] activities were comparatively lower in fish fed propolis and vitamins C and E supplemented diets during days 9 and 18 of the extreme warm stress. Most of the repeatedly measured parameters indicated that diets supplemented with propolis, vitamins C & E, and phycocyanin provide improved acclimation potential during ambient extreme warm exposure (32 °C).

Table 1. Growth performance of juvenile *D. labrax* fed with different diets for 56 days. Values (mean ± SD) with different lowercase letters within the same row indicate significant differences ($p < 0.05$).

Performance parameter	Diets				
	Control	Vit. C & E	Propolis	Phycocyanin	β-glucan
Initial weight (g)	8.93 ± 1.46a	8.81 ± 1.50a	8.65 ± 1.16a	9.12 ± 1.44a	9.05 ± 1.23a
Final weight (g)	25 ± 0.32c	27.16 ± 0.73ab	28.03 ± 0.35a	27.73 ± 0.83a	25.45 ± 0.68bc
Feed intake (g fish^{-1} 56-days^{-1})	17.17 ± 0.54a	16.30 ± 0.56a	17.16 ± 0.23a	16.42 ± 0.19a	16.21 ± 0.77a
Weight gain (WG, %)	201.78 ± 4.52ab	219.13 ± 4.98a	229.05 ± 4.00a	209.13 ± 5.81ab	199.88 ± 4.01b
Specific growth rate (% day^{-1})	3.05 ± 0.11bc	3.24 ± 0.08ab	3.37 ± 0.93a	3.13 ± 0.09abc	3.02 ± 0.08c
Protein efficiency ratio (PER, %)	1.87 ± 0.10c	2.25 ± 0.12b	2.27 ± 0.10a	2.21 ± 0.10ab	2.02 ± 0.02bc
Survival rate (SUR, %)	93.88 ± 1.33b	98.89 ± 1.02a	100.00 ± 0.00a	99.27 ± 0.52a	97.27 ± 1.01a

AQUAPONIC SYSTEM WITH *Macrobrachium rosenbergii* (De Man, 1984) AND *Lactuca sativa* L. IN DIFFERENTS RATIO: WATER QUALITY, NITROGEN COMPOSTS AND PERFORMANCE

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The aquaponic system is a recirculation aquaculture system were having an integrated production vegetable without soil with aquatic organism, fish or shrimps. This system has many great effects because the second culture will use the water, the nitrogen composts and phosphor compost from the first culture like energy supply, controlling the water system intake. Moreover, reuse water inside system and production integrated of two organisms, when analyses in future, have effect over costs, improve profitability of system production. The present study was conducted to determined what ration of prawn x lettuce is better to water quality, nitrogen composts and performance of prawn. The experiment was 40 days used 50 L tanks to prawns with density 2 larvae per liters, had 4 treatments and 3 repeats: 1 - control RAS – Recirculation Aquaculture System; 2 - ratio 2 lettuces to 1 prawn; 3 – ratio 4 lettuces to 1 prawn; and 4 – ratio 8 lettuces to 1 prawn. During the experiment the animals were fed twice a day with a specific commercial feed (PB 45%), the feeds were made by haul and the amount of feed offered was calculated as 15% of the biomass of each tank and readjusted for each biometry.

Table 1. Growth response (mean ± standard deviation) of shrimp reared for 40 days at different storage densities of lettuces in a recirculation system using constructed grow bed.

	Unit	Treatments				*F*	*P*
		50 lettuces	25 lettuces	13 lettuces	RAS		
Food Consumers	g	57 ± 1.00 a	63.83 ± 2.93 a	63.67 ± 10.07 a	68.23 ± 6.94 a	4.92	0.05
FCR		0.003 ± 0.001a	0.003 ± 0.001 a	0.003 ± 0.001 a	0.003 ± 0.001 a	0.49	0.5
Total Biomass	g	19.89 ± 6.30 a	20.97 ± 3.08 a	22.72 ± 6.87 a	20.78 ± 3.62 a	0.13	0.726
Mean Mass	g	0.25 ± 0.10 a	0.28 ± 0.07 a	0.29 ± 0.09 a	0.26 ± 0.03 a	0.08	0.784
Survival	%	81 ± 6.24 a	76.67 ± 8.33 a	78 ± 1.73 a	79 ± 5.20 a	0.11	0.75
Productivity	kg/ha	395.84 ± 125.43 a	417.32 ± 61.35 a	452.31 ± 136.83 a	413.65 ± 72.02 a	0.13	0.727

Table 2. Growth response of lettuce plants using nutrient rich shrimp tank water in different stocking densities.

	Unit	Treatments			*F*	*P*
		50 lettuces	25 lettuces	13 lettuces		
Number of leaves		9.28 ± 2.77 a	10.47 ± 3.29 a	8.83 ± 1.83 a	1.69	0.196
Length of stalk	cm/plant	5.51 ± 2.84 a	5.91 ± 2.28 a	6.97 ± 1.9 a	2.35	0.127
Length of roots	cm/plant	10.46 ± 4.40 a	12.17 ± 3.64 a	11.38 ± 1.80 a	3.9	0.05
Leaf mass	g/plant	9.17 ± 4.49 a	14.83 ± 7.66 b	10.82 ± 4.59 ab	17.27	< 0.001
Survival	%	82.67 ± 20.53 a	80 ± 17.44 a	15.39 ± 13.33 b	21.76	0.002
Yield	g/m²	0.14 ± 0.08 a	0.11 ± 0.07 a	0.01 ± 0.01 b	8.5	0.023

TEXAS AQUACULTURE UPDATE

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Texas has a long history in aquaculture and is a national leader in a shrimp, redfish and striped bass production. Through successes and challenges, Texas is a relevant case study for aquaculture in general in the United States and remains a land of opportunity and untapped potential. The Texas Aquaculture Association remains an advocate and unified voice for Texas producers. The TAA has been an active supporter and advocate during the quest for disaster assistance through ELAP for food fish losses resulting from winter storm Uri.

POLYCULTURE OF RED SWAMP CRAWFISH *Procambarus clarkii* AND PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN LOW SALINITY WATER

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Polyculture of Pacific white shrimp (*Litopenaeus vannamei*) and red swamp crawfish (*Procambarus clarkii*) is a new production strategy being explored by commercial shrimp farmers in west Alabama. Crawfish are being produced on a pilot-scale in earthen ponds and a split pond on one commercial shrimp farm. Traditional crawfish production relies on pond primary productivity to fuel the crop growth and reproduction, while low salinity shrimp culture involves annual stocking and supplemental feeding. The excess nutrients generated during shrimp production may be beneficial to crawfish growth. The main area of concern is that crawfish are known to feed on small fish, insects, detritus and plant material. This feeding strategy raises the question of whether crawfish would feed on juvenile shrimp, causing a negative impact on shrimp survival and production. Two controlled experiments were set up using two different systems to investigate the polyculture potential for these two species. The first experiment consisted of a 24-tank system (75 L per aquaria; 3900 L total system volume) with water recirculated through a sand filter, biofilter, and sump. The salinity was maintained at approximately 2.3 g/L using reconstituted seawater. The second experiment used a green water on-levee flow-through tank system (twelve 800-L tanks) at Greene Prairie Aquafarm in west Alabama. The shrimp production pond that supplied water to this system had a salinity of 2 g/L. In this green water system, pond water was pumped into the tanks (4.9 L/min) and drained back into a shrimp production pond. In both trials, three treatments were used to evaluate the impact of crawfish presence on shrimp survival. Eight replicates of each treatment were stocked (0.53 g mean initial weight) in the aquarium system. Following 21 days of culture, shrimp were harvested and evaluated for survival and growth. The green water system was stocked (0.15 g mean initial weight) using four replicates of similar treatments. The first experiment indicated crawfish presence had a significant negative effect on shrimp survival (Treatment A= 91.9%, Treatment B = 58.8%, Treatment C = 40.6%) (P<0.001). However, the second experiment showed no significant differences among treatments for survival or growth. These results indicate that although crawfish will prey on shrimp in a clear water system, the need or ability to prey on shrimp did not occur in a green water system.

Figure 1. Treatment assignment for two polyculture trials with Pacific white shrimp and red swamp crawfish.

Treatments	Aquarium System	Green Water System
A	20 shrimp, shrimp feed ration	35 shrimp, shrimp feed ration
B	20 shrimp, 1 crawfish, shrimp and crawfish feed ration	35 shrimp, 4 crawfish, shrimp and crawfish feed ration
C	20 Shrimp, 1 crawfish, shrimp feed ration	35 Shrimp, 4 crawfish, shrimp feed ration

PRELIMINARY INVESTIGATION OF CORRELATIONS AMONG GROWTH RATE, DIV1 RESISTANCE AND STRESS TOLERANCE OF THE PACIFIC WHITE SHRIMP, *Penaeus vannamei*

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In shrimp genetic improvement programs, fast growth, specific disease resistance and general robustness are the top three breeding goals for consideration. To properly design strategies for selective breeding, it is necessary to investigate if and how these traits are genetically correlated. The Pacific white shrimp, *Penaeus vannamei,* is the dominant penaeid species cultured, accounting for about two-third of the global production. Decapod iridescent virus 1 (DIV1) is an emerging shrimp viral disease, which is known to affect all stages of the Pacific white shrimp, crayfish and giant freshwater prawn and has caused tremendous economic loss. As an euryhaline species, *P. vannamei* can survive within a wide range of salinity, the optimal level for juvenile growth is between 15 to 25 ppt. Hyper-saline stress test can be used to evaluate the general robustness of shrimp.

In the study, 35 full-sib families of Specific-pathogen-free (SPF) *P. vannamei* were systemically assessed in an eight-week growth trial, DIV1 lab challenge test and hyper-saline stress tests. Results showed that, for average initial weight of 2.5g (ranging from 1.0 to 4.0g) juvenile shrimp, the average daily gain (ADG) of shrimp families varied from 0.38 to 0.53 g/day, and large between-family variations were detected for both DIV1 challenge test survival rates (SR%_DIV1, from 8.0% to 68%), and Hyper-saline stress test survival rates (SR%_HS, from 22% to 62%). Phenotypic correlations between ADG and SR%_DIV1, SR%_HS were 0.0672, -0.1026 respectively, both were not significantly different from zero (Figure). Phenotypic correlation between SR%_DIV1 and SR%_HS was -0.3004. Further analyses revealed that such slightly negative correlation could result from the initial sizes of juvenile shrimp. The larger juveniles seemed to be more tolerant to salinity stress while the smaller juveniles tended to survive better when infected by DIV1. Based on the results, a balanced index selection scheme is recommended for the selective breeding of *P. vannamei*.

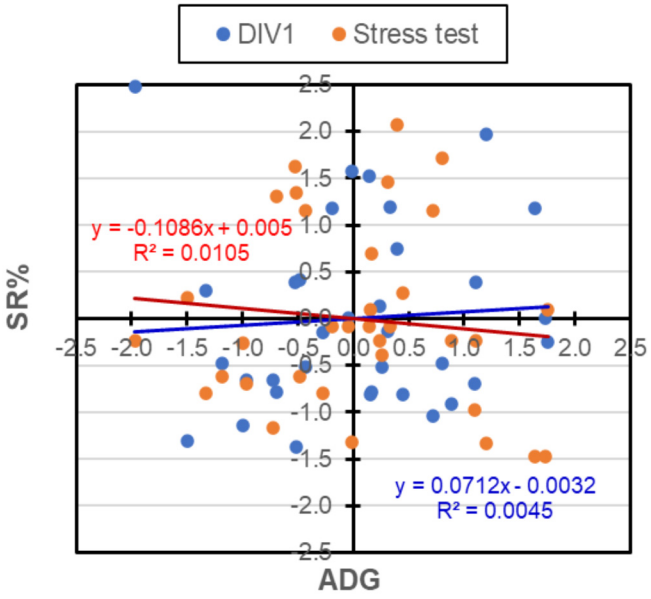

Fig. Correlations of growth rate (ADG) and DIV1 resistance (SR%_DIV1), Hyper-saline stress tolerance (SR%_HS) among 35 *P. vannamei* families.

COMPARATIVE TRANSCRIPTOMICS REVEALS DOMESTICATION-ASSOCIATED FEATURES OF ATLANTIC SALMON LIPID METABOLISM

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Domestication of animals imposes strong targeted selection for desired traits but can also result in unintended selection due to new domestic environments. Atlantic salmon (*Salmo salmar*) was domesticated in the 1970s and has subsequently been selected for faster growth in systematic breeding programmes. More recently, salmon aquaculture has replaced fish oils (FOs) with vegetable oils (VOs) in feed, radically changing the levels of essential long-chain polyunsaturated fatty acids (LC-PUFAs). The aim of our study was to study the impact of domestication on metabolism and explore the hypothesis that the shift to VO diets has unintentionally selected for a domestication-specific lipid metabolism. We conducted a 96-day feeding trial of domesticated and wild salmon fed diets based on FOs, VOs or phospholipids, and compared transcriptomes and fatty acids in tissues involved in lipid absorption (pyloric caeca) and lipid turnover and synthesis (liver). Domesticated salmon had faster growth (Fig.1) and higher gene expression in glucose and lipid metabolism compared to wild fish, possibly linked to differences in regulation of circadian rhythm pathways. Only the domesticated salmon increased expression of LC-PUFA synthesis genes when given Vos (Fig. 2). This transcriptome response difference was mirrored at the physiological level, with domesticated salmon having higher LC-PUFA levels but lower 18:3n-3 and 18:2n-6 levels. In line with this, the VO diet decreased growth rate in wild but not domesticated salmon. Our study revealed a clear impact of domestication on transcriptomic regulation linked to metabolism and suggests that unintentional selection in the domestic environment has resulted in evolution of stronger compensatory mechanisms to a diet low in LC-PUFAs.

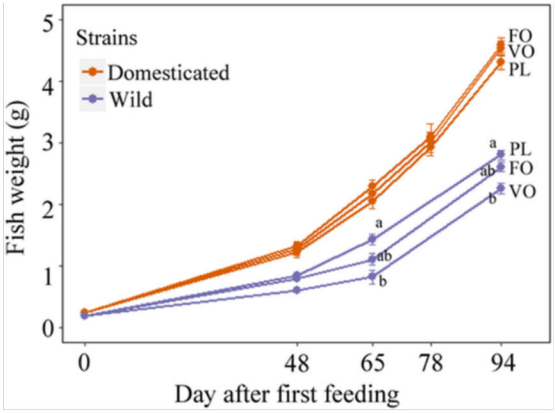

Fig.1 Growth performance of domesticated and wild salmon fed diets high in fish oil (FO), vegetable oil (VO) or phospholipids (PL) during early stages of development.

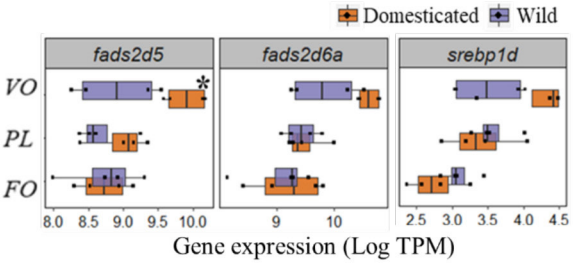

Fig.2 Expression of key genes involved in LC-PUFA synthesis in liver of wild and domesticated salmon fed either fish oil (FO), vegetable oil (VO) or phospholipid (PL) diets. An asterisk indicates differential expressed genes (DEGs, fdr < 0.05 and |log2FC| > 1) between domesticated and wild salmon.

THE EFFECTS OF CARBON/NITROGEN RATIO ON SUSPENDED GROWTH DENITRIFICATION IN REUSED BRACKISH AQUACULTURE WATER

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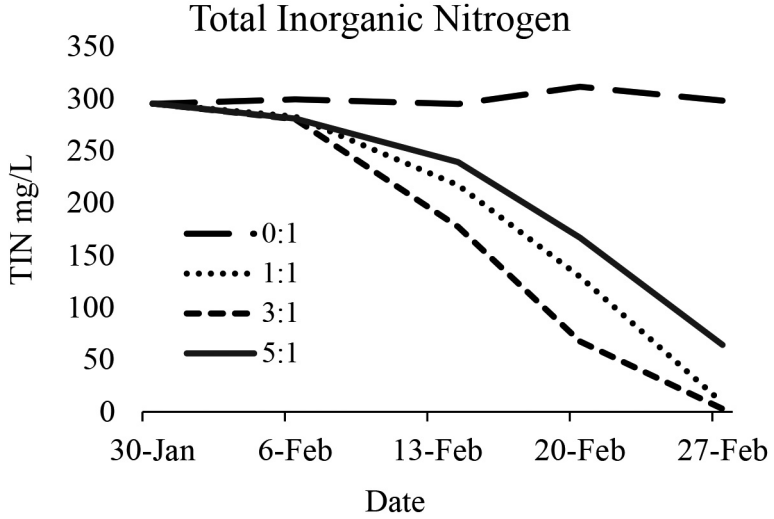
Nitrate accumulation is a key limiting factor in water reuse for RAS, as high concentrations result in negative impacts on aquatic animal production. Traditionally nitrate is removed by water discharge from the system, potentially causing negative environmental impacts and increasing production cost. This is especially true for inland brackish and marine aquaculture, where salt can be a significant expense. Another means of nitrate removal is denitrification, a method in which nitrate is ultimately reduced to dinitrogen gas, which is then harmlessly released into the atmosphere. Among the more common methods of denitrification are fixed film bioreactors which utilize media to provide a substrate for bacterial growth. However, the media and bioreactors can be expensive and take up valuable space within the production facility. The purpose of this study was to evaluate a suspended growth (biofloc)-based approach to denitrification using ethanol to achieve different C/N ratios.

Four different C/N treatments (5:1, 3:1, 1:1, no added carbon) were tested in triplicate using 150 L tanks filled with reused water from a commercial *L. vannamei* biofloc production system. The initial nitrate concentration was 296 mg NO_3-N/L which comprised all but about 0.3 mg/L of the total inorganic nitrogen (TIN), and salinity was 15 g/L. Carbon in the form of 95% ethanol (provided by Beam Suntory, Inc.) was added daily based on the previous week's TIN results. TAN, nitrite, nitrate, phosphate, alkalinity, sulfide, TSS/VSS, and turbidity were each measured weekly, and DO, pH, salinity, ORP, and temperature were determined daily.

The study ended after 27 days when total denitrification had been observed in some tanks. Both the 3:1 and 1:1 treatments showed significantly higher rates of denitrification than the 5:1 treatment. The 3:1 treatment showed total nitrate reduction of 294 mg/L and the 1:1 treatment showed a total reduction of 287 mg/L. The 5:1 treatment reduced nitrate by 280 mg/L but showed significant nitrite accumulation of 49 mg/L, and only reduced TIN by 232 mg/L. These results show the potential of suspended growth denitrification without the use of external bioreactors and indicate the optimal C:N ratio may be between 3:1 and 1:1.



GENETIC AND NUTRITIONAL APPROACHES TO ENHANCE THE EFFICIENCY OF SOYBEAN MEAL UTILIZATION IN RAINBOW TROUT

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The USDA ARS and U*of*I have developed several rainbow trout (*Oncorhynchus mykiss*) lines that show higher growth rates when fed an all-plant protein diet than non-selected lines of trout fed the plant protein diet or a fishmeal-based diet. So far, no commercial breeding programs has yet started to improve feed utilization efficiency in fish, mainly because of the difficulty in accurately measuring individual feed intake of fish reared in groups. Using these selected lines we proposed to test if feed conversion ratio (FCR) and body weight variations during successive periods of feed deprivation (FD) and re-feeding (RF) are correlated using a compensatory feeding regime. The goal of this study was to determine if indirect selection for the above traits could be used to develop alternative criteria to improve feed intake of the plant-based fed selected strain.

We began with1600 fish (av. Int. wt. 30 g) from 12 families of the selected line, fed the plant protein diet (50% soy, protein: 41% and lipid: 20%). First, fish were tagged individually and reared in a common environment, they were then placed on feeding challenge regime for 4 months (one month each challenge either FD or RF), and performance were recorded. Thereafter, fish were separated into four groups *(1331 fish)* based on individual performance during FD and RF challenge study. FCR was recorded for 3 months in all four groups followed by measuring the stability of response to the FD and RF periods (2nd feeding challenge) which was the same as the 1st one. Out of 1331 fish, 143 fish exhibited a similar pattern of weight loss and gain, confirming the stability. As expected, the most efficient group was the FD-/RF+ (FCR=0.99) and the least efficient groups with FCR of ~1.4 were FD-/RF- and FD+/RF- (Figure1A). Studying the gut microbiome of all four groups revealed that despite the variations dictated by FD and RF, the bacterial community of FD-/RF+ group (best performers) shows more stability than the FD+/RF- (worst performers) throughout the feeding challenges (Figure 1B and 1C).

Conclusively, FCR improved more than 15% in best group (FD+/RF-) compared to the FD-/RF+ group which means utilizing these fish selected for improved feed efficiency will reduce soybean consumption and greatly decrease the feed cost for sustainable aquafeed industry.

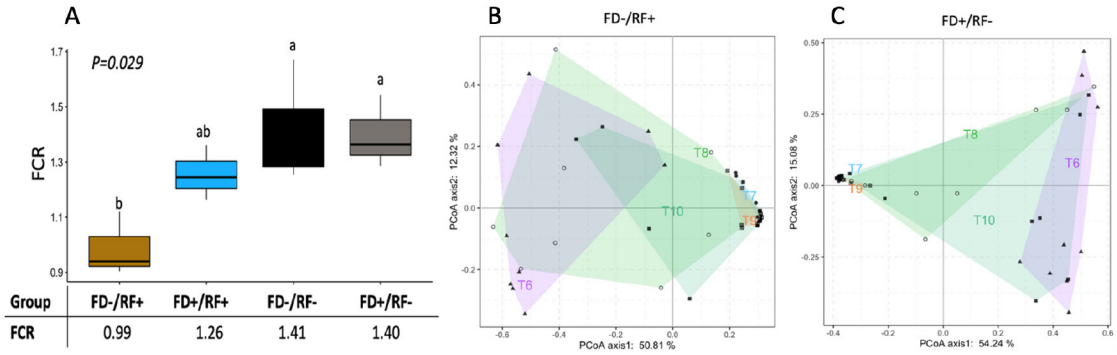

Figure 1: (A) Feed conversion ratio of rainbow trout based on their performance during the feeding challenge. (B and C) Microbiome variations of the best and worst performers during the second feeding challenge; T6: initial sampling, T7: 1st FD, T8: 1st RF, T9: 2nd FD, T10: 2nd RF

THE UNDERLYING MOLECULAR MECHANISM FOR UTILIZING THE SOY PROTEIN DURING COMPENSATORY FEEDING REGIME IN RAINBOW TROUT

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Aquaculture Research Institute at University *of* Idaho in collaboration with USDA has genetically selected the several lines of rainbow trout (*Oncorhynchus mykiss*) that show higher growth rates when fed all plant protein diet (PPD) than non-selected lines of trout fed a fishmeal-based diet. Aim of the study was to test if feed intake and body weight variations during successive periods of feed deprivation (FD) and re-feeding (RF) are correlated using compensatory feeding regime and has examined the expression of several classes of genes involved in growth, proteolysis and electron transport chain throughout this feeding challenge.

We have used 1600 fish from 12 families of the selected trout lines, fed PPD (50% soy). Fish were tagged individually, reared in common environment, went through the first feeding challenge, alternate months of FD and RF for 4 months, and performance was recorded. Thereafter, fish were separated into four groups *(1331 fish)* based on individual performance during FD and RF challenge studies. Feed Intake was recorded for 3 months in all four groups followed by measuring the stability of response to the FD and RF periods (second feeding challenge) which was the same as the first one. Muscle and liver samples were collected after each month of the challenge (FD and RF) and gene expression patterns were studied during the FD and RD periods via qRT-PCR.

The expression pattern of studied genes in a feed depravation and refeeding period is briefly shown in Figure 1. We examined the expression of three major categories of genes since weight gain and weight loss variations are correlated with growth, protein turn over and energy consumption in the body. Genes known to be involved in growth are upregulated in the liver and muscle of FD-/RF+ group (best performers) during FD and an opposite pattern is expected during the re-feeding time. However, more details are still under investigation.

Conclusively our study provides the molecular basis for efficient utilization of soy-based diet in trout, and it helps us to move toward the high inclusion of soy protein in salmonids industry.

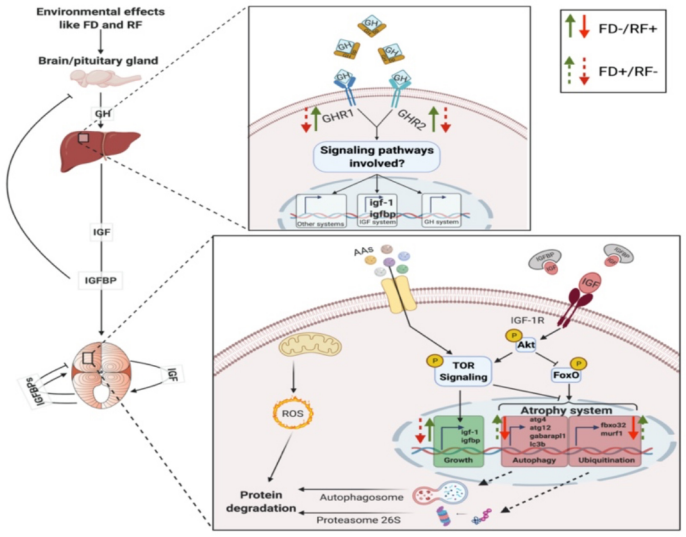

Figure 1: Schematic for gene expression pattern of several genes involved in growth and protein turnover in liver and muscle during a feed deprivation period. Genes involved in growth such as Growth Hormone Receptor 1 and 2 (GHR1 and GHR2) showed a higher expression in the FD-/RF+ group relative to the FD+/RF-. On the other hand, genes involved in proteolysis such as Autophagy and Ubiquitination-related genes are down regulated in FD-/RF+ in comparison to FD+/RF- .

ADSORPTION OF AQUACULTURE WASTEWATER CONTAMINANTS ON N-DOPED BIOCHARS

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Aquaculture industry has witnessed a tremendous growth globally in the last 50 years having a production of over 50 million tons in the present times. Consequently, this growth has environmental impacts as well. Aquaculture wastes contain nitrogenous compounds (ammonia, nitrite, and nitrate) as result of uneaten fish feed, fish respiration and excretion. However, these nitrogenous substances are necessary for the growth of aquatic plants, bacteria, and algae. An excessive concentration of these nutrients results in eutrophication. Thus, it is necessary to remove the ammonia and nitrate wastes from aquaculture streams. The goal of this research is to use a low-cost adsorbent to treat aquaculture nitrogenous wastes. Pine bark biochar is doped with a nitrogen containing precursor to alter the surface chemistry. The modified adsorbent is characterized in detail. The obtained properties of the adsorbent are used to design a continuous fixed-bed adsorption column to remove ammonia and nitrate from model aquacultural streams.

TOOLS OF THE TRADE: USE OF A MOBILE LABORATORY FOR IDENTIFYING FISH DISEASES ON FARMS

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Extension personnel use a variety of tools to help diagnose aquatic animal diseases, water quality problems and aquatic weed identification. Diagnosing aquatic animal diseases requires the use of water chemistry test kits, streaking various agar plates to determine if bacteria are the cause of a disease, as well as molecular techniques, all of which are standard equipment in fish disease diagnostic laboratories. Consequently, most Extension offices depend on producers to bring animals, water, and plants to the office for diagnostics. Unfortunately, the person that delivers the samples is often just a farm worker and does not have the intimate details of the problem. To circumvent this, we retrofitted a small recreational vehicle (RV) that would contain everything except the ability to run molecular diagnostics. This tool enables the Extension personnel to go to the farm and the pond that is a concern to the producer. On-site diagnostics ensures that moribund or sick fish are collected, water samples are fresh, and the ability of the diagnostician to actually see the behavior of the fish. This presentation will discuss the retrofitting of the RV and discuss the popularity of use. This RV was a valuable tool used frequently during the Covid-19 pandemic.

EFFECT OF WATER DEPTH ON *Litopenaeus vannamei* PRODUCTION AND WATER QUALITY

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In recent years, researchers and several commercial operators have proposed using relatively shallow tanks to rear shrimp. Such systems can be stacked several tanks high, thereby conserving floor space in a building. However, there is a risk that aeration may be less efficient in shallow tanks. Furthermore, shrimp are more concentrated in shallow tanks and therefore nutrients may likewise become more concentrated. The purpose of this project was to compare various water depths with regard to shrimp production and water quality impacts.

A research trial was conducted using eight, 18 m^2 HDPE-lined tanks equipped with Venturi-style aeration systems. The tanks were stocked at 160 shrimp/m^2 (mean body weight 0.2 g) and randomly assigned to one of three treatments dictated by water depth: 40 cm, 60 cm, and 80 cm; each treatment had two replicate tanks. During the experiment, the postlarvae were fed at 8% biomass of feed 4 times per day. The DO, pH, salinity, and temperature were monitored daily and ammonia, nitrite, nitrate, alkalinity, and solids concentration were measured weekly. At the end of the trial shrimp FCR, growth rate, and survival were calculated. All data were expressed as the mean ± SD. Significant differences among different treatments were analysed using a SPSS 17.0. After 40 days, water quality parameters measured during the experiment are shown in Table 1, and shrimp production data are presented in Table 2.

The project demonstrates that DO and nitrate concentrations were significantly impacted by water depth, but no noticeable differences in shrimp production were found. This indicates that perhaps with special consideration to water quality, shallow tanks with Venturi-driven aeration may be a feasible option for shrimp production.

Table. 1 Water quality data at 40 days *($p < 0.05$)*

Water depth (cm)	D.O (mg/L)	Salinity (psu)	pH	TAN (mg/L)	NO2 (mg/L)	NO3 (mg/L)	Alkalinity	SS(cm)
40	6.4±0.0^a	5.3±0.3	8.1±0.0	0.2±0.0	1.2±0.3	89.2±10.3^a	114.4±0.9	3.5±1.0^a
60	6.6±0.0^b	4.8±0.5	8.1±0.0	0.2±0.0	1.3±0.2	72.5±0.0^a	113.1±0.6	2.1±0.6^b
80	6.5±0.2^b	4.8±0.1	8.1±0.1	0.1±0.0	1.0±0.2	51.5±0.8^b	113.2±0.2	2.3±1.1^b

Table 2. Shrimp growth, survival rate, and FCR.

Water depth (cm)	Growth rate (%)	Survival rate (%)	FCR
40	69.98±3.5	76.8±14.2	0.98±0.03
60	72.18±1.8	70.0±12.7	1.00±0.35
80	72.92±6.2	65.0±5.6	0.87±0.01

USING COMPARATIVE GENOMICS TO ENHANCE THE AQUACULTURE OF THE FLORIDA POMPANO: COMPARING THREE *Trachinotus* SPECIES

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The Florida Pompano (*Trachinotus carolinus*) is a warm water, marine teleost fish species belonging to the family Carangidae. It can be found in the Atlantic Ocean off the coast of Massachusetts, U.S. down to Brazil, but is most commonly found off the east and west coasts of Florida, U.S. In Florida, it is regarded as a prized catch to sport and commercial fishers alike due to its great taste and texture. Demand exceeds supply resulting in a high value at market. Interest in the commercial aquaculture of this species began in the 1960s, but there were difficulties in preventing high juvenile mortality. With recent advances in nutrition and recirculating systems, there is renewed interest in bringing this species into commercial aquaculture. Research has shown the Florida Pompano to be a good candidate for large-scale aquaculture due to its ability to be sustained on a pelleted diet, be reared at low salinities, and breed in captivity. However, an economical and technological challenge in expanding domestic production is the supply of high quality seedstock (juveniles for grow-out) optimized for the production environment. It is possible to identify traits of interest to select for in a genetics-based selective breeding program to create a high quality seedstock with molecular and computational tools.

Using a representative draft genome that we have previously assembled and annotated, we are conducting a comparative genetic analysis of the Florida Pompano genome to two closely related *Trachinotus* species, Permit (*T. falcatus*) and Palometa (*T. goodei*). These two species were chosen as they inhabit a similar geographic range to the Florida Pompano yet drastically differ in size compared to the Florida Pompano. When fully grown, the average Florida Pompano reaches 60 cm and 3.6 kg. However, Permit grow twice in length (122cm) and ten times in weight (36 kg), while Palometa only grow to 51 cm and 0.5 kg. By gaining access to genetic information within the Permit and Palometa genomes, we will be able to determine genes and variable DNA regions that are potentially associated with the different rates of growth.

The Permit and Palometa genomes were sequenced on the Illumina HiSeq 2500 System. These two genomes were assembled using our Florida Pompano draft genome as a reference. A bioinformatics workflow was developed to annotate and compare the three genomes. Reads per gene were analyzed focusing on known genes linked to growth in finfish. The overall research goal of this study is to identify growth-related genes that can ultimately inform a genetics-based selective breeding program for enhanced seedstock, and thereby increase production and profits for farmers.

NUTRITIONAL PERFORMANCE OF JUVENILE RED DRUM *Sciaenops ocellatus* FED VARIOUS FISH, SHRIMP, AND SQUID DIETS

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Aquaculture continues to provide increased seafood production for human consumption. As production increases, it will become more important to identify and produce the most effective diets for grow-out. A 12 week feeding trial with juvenile red drum was conducted to determine performance of variations of the "gold standard" diet of fish, shrimp, and squid to identify if there is a better combination of these components for growth and health, which can provide information to develop fishmeal replacement diets (FMRD) that can closely mimic the performance of natural diets in fish growth. A 24-tank recirculating system was stocked with 25 fingerlings (27.45-gram average) in each tank. Eight diets containing fish, shrimp, and squid, and each single and double combination of these components, as well as a commercial fishmeal-based-diet were randomly assigned (3 tanks per diet) and fed isocalorically for the duration of the feeding trial based on biomass of each tank. Sampling occurred every 3 weeks, where 3 fish per tank were randomly selected and humanely euthanized, with livers, intestines, and muscle samples taken. Feeding amounts were adjusted based on new biomasses of each tank to ensure fish were fed isocalorically.

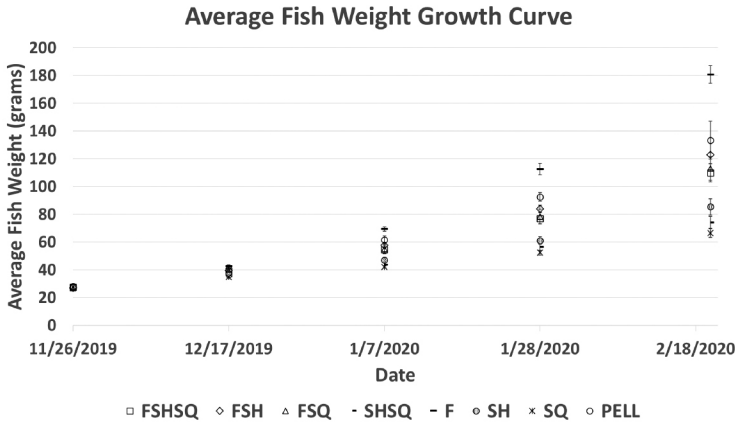

Figure 1. Growth curves (avg. weight ± SD) over the course of a 12-week feeding trial with juvenile red drum fed different natural diets (Fish + Shrimp + Squid, Fish + Shrimp, Fish + Squid, Shrimp + Squid, Fish, Shrimp, Squid, or commercial fishmeal based pellets).

EVALUATION OF VARIOUS APPROACHES TO MANAGEMENT OF BIOFOULING OF OYSTER CAGES IN COPANO BAY, TEXAS

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Management of biofouling is the largest operational cost factor associated with oyster aquaculture due to the high cost of labor required. Biofouling can be a detriment to oyster growth and health as it limits water flow through the aquaculture baskets. To determine the appropriate management practices for removal of biofouling from oyster baskets in Texas bays, this study investigated: 1) the method of removing biofouling (desiccation vs. pressure spraying); and 2) the time interval between removal of biofouling (7, 14, or 21 days). Images taken of each basket prior to treatment were analyzed using ArcGIS ArcMap v. 10.8 to determine the extent of coverage. The strongest significant difference on biofouling coverage was between one and three week cages, and the second most between two and three week cages (Table 1). One and two week cages were also significantly different, however less so. There was not a significant difference between removal method on the percentage of biofouling coverage.

Survival, growth (i.e., length, height, width) and a condition index are currently being evaluated to evaluate overall impact of biofouling. Subsequent recommendations regarding methods of biofouling control will contribute to the development of best management practices specific to Texas oyster farming and ultimately result in improved sustainability of the farming industry.

Table 1. Statistical analysis p values of time interval and removal method. The results were obtained using a general linear hypothesis and Helmert contrasts.

Time Interval	Pr(>|t|)	*Removal Method*	Pr(>|t|)
one week-two week = 0	0.00353	pressure spray – desiccation = 0	0.0855
two week-three week = 0	2.45e-08		
one week-three week = 0	< 2e-16		

OPPORTUNITES AND CHALLENGES IN DEVELOPING A GERMPLASM REPOSITORY PATHWAY FOR THE SEA HARE, *Aplysia*, GENERALIZABLE TO OTHER MARINE INVERTEBRATES

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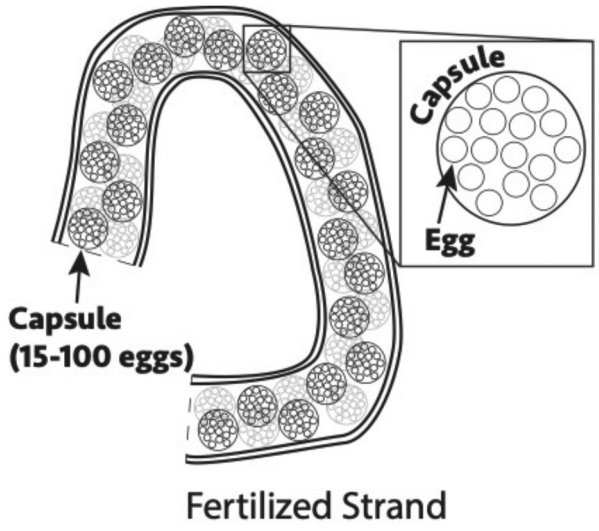
The safeguarding of economically relevant agricultural species has been driven by storing, evaluating, and distributing genetic resources as cryopreserved germplasm maintained in repositories. However, the shift to cryopreservation has been slow within the broader scientific community, especially for fish and invertebrates. To advance within aquatic species, it will be advantageous to develop generalizable pathways that can provide a foundation for repository development and a means for addressing cross-taxa challenges. Aquatic biomedical model organisms and imperiled species are examples of groups with great needs to begin broadening the scope of germplasm repositories. The California sea hare, *Aplysia californica*, is a biomedical model largely used to examine neural development, behavior, and aging. There are many challenges associated with maintaining *Aplysia*, for example, their production of copious mucus and toxic ink expulsion, which require flow-through aquarium systems or extensive filtration. In addition, culture of multiple algal species is required for feeding, and inbreeding is considered to have detrimental effects on development in this and many other species. Repository storage of frozen material will provide opportunities for the research community to preserve genetic diversity and to create and maintain mutant and transgenic lines. Repository development itself presents challenges, and there are specific problems related to tissue types and developmental stages. A relevant example is the encapsulation of multiple embryos within capsules and semi-rigid strands produced by many gastropods (Figure 1). In collaboration with the National Resource for *Aplysia* (NRA, University of Miami), our goal is to develop a generalizable cryopreservation pathway, including quality management and economics, that can be applied to this species, with the intention of extending the pathway to other aquatic invertebrates such as oysters and imperiled corals.

FIGURE 1. *Aplysia* enclose a variable number of eggs (15-100) within capsules that are helically organized within a semi-rigid strand. Typical cryopreservation focuses on freezing of single cells (e.g., sperm) but becomes more challenging as the number of cells and internal compartments increase. Organisms that lay eggs enclosed within a strand present new challenges as the embryonic and larval stages are multicellular and the capsule and strand inhibit penetration of cryoprotective molecules.



ARTIFICIAL INTELLIGENCE AND AQUACULTURE: HOW DIGITAL TOOLS ARE SHAPING THE FUTURE OF THE INDUSTRY

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Aquaculture growth has been accelerated by new technologies that enable farmers to operate more efficiently. There is currently a shift in the industry to smart technology that uses artificial intelligence and advanced data analytics to predict and provide insights into a wide array of farm operations. This concept, commonly known as precision aquaculture, aims to improve the ability of farmers to monitor, control and document all factors affecting fish production and fine tune operational decisions to achieve better efficiencies and higher profits. Despite finfish aquaculture having the lowest feed conversion ratios among agriculture species – there are still improvements that can be made if decisions are based on knowledge from data rather than subjective experiences.

One of the best applications of this technology centers around feeding. Feeding is central to all farm operations and the concept of data-supported feeding is to consider all parameters that impact not only when to feed, but how much and at what rate. Feed is the highest operating cost so small efficiency improvements happening daily can result in significant financial savings over the long-term. Biomass cameras that can sample hundreds of fish in a few hours to provide high accuracy estimates can help operators better determine how much feed is required. Underwater camera networks with fish satiation algorithms can assess feed demand on a second-by-second basis from a variety of behavioral indices and automated pellet detection and alert operators when to slow down or stop feeding.

A data-supported feeding program or farm management system would be able to assess all available data instantly and provide direction to any operator on the recommended feeding times and rates. These emerging technologies are currently being developed and validated at commercial farms and put into regular operation. While many of these developments are focused on the production of Atlantic Salmon, the diversity of farmed species continues to grow, especially in the open ocean and warm water environments. The progress that is made in the salmon industry will pave the way for the culturing of new species but only if best practices are digitized and the lessons learned through trial and error with salmon are applied to new species. The adoption of precision aquaculture is an important step for the successful growth of existing and future finfish aquaculture.

EFFECTS OF SALINITY AND WATER DEPTH ON PREFORMANCE OF *Litopenaeus Vannamei* AND WATER QUALITY IN RAS

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With the increase in demand for seafood as well as fresh locally grown products, inland and indoor shrimp production operations have gained popularity in the US and other world markets. Indoor production methods have relatively high startup and operating costs. Two substantial expenses facing indoor production are artificial salt and land or property costs. In recent years, low-cost salt mixes and denitrification methods have been developed to reduce salt cost and reuse water. Low salinities have been used to grow *L. vannamei*, in some cases as low as 5 ppt or less, however; results have been inconsistent. To maximize the use of space in indoor facilities, several researchers and commercial ventures have explored using shallow tanks, which can be stacked vertically. However, concerns have been raised as the smaller volume may concentrate toxic nutrients such as ammonia. In addition, the shallow water may not facilitate adequate oxygen diffusion as aeration contact time will be reduced. The purpose of this study was to examine the effects of varying salinities and water depths/volumes on *L. vannamei* performance and water quality over the course of a production cycle.

Six treatments were randomly assigned to 24 tanks providing four replicates per treatment; the experiment included three salinity levels and two water depths: deep (D) and shallow (S). The salinities used for the experiment were 5,10, and 15 ppt. and the volume/depth of water were $1m^3$/100cm depth (D) and $0.5m^3$/50cm depth (S). The treatments were as follows: 5ppt-50cm (5-S), 5ppt-100cm (5-D), 10ppt-50cm (10-S), 10ppt-100cm (10-D), 15ppt-50cm (15-S), 15ppt-100cm (15-D). Each flat-bottom tank was $1m^2$ in bottom area and had an 18.9 L settling chamber and an 18.9 L biofilter, both receiving water from a 1135 LPH pump. Tanks were salted using a low-cost salt mixture to the respective salinities. 250 shrimp were stocked into each tank at an average weight of 1.9g producing stocking densities of 250 shrimp/m^3 in the 100 cm treatments and 500 shrimp/m^3 in the 50 cm treatments.

Preliminary results suggest some differences in water quality. Lower oxygen concentration and lower pH levels in shallow tanks have been consistently observed. Although the study is currently ongoing, we suspect that these important differences in water quality will result in substantially different shrimp production levels between treatments. Such a finding would have important implications for commercial producers who are considering the use of shallow tanks. These producers will have to weigh the costs of added water quality maintenance against the benefits that come from maximizing the space in indoor facilities.

EFFECT OF UNDERSTOCKING DENSITY OF CHANNEL CATFISH FINGERLINGS IN INTENSIVELY AERATED MULTIPLE-BATCH PRODUCTION

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Multiple-batch production is the most widely practiced method of raising channel catfish. Producers are increasingly adopting intensified production practices in multiple-batch systems by increasing stocking density and aeration rates as a means to improve cost efficiencies. Proven stocking recommendations are required for the efficient implementation of recent developments in multiple-batch production. Twelve 0.4-ha ponds were understocked with 17,484, 20,612, and 26,124 fingerlings/ha (mean weight = 40 g/fish) over equal weights of carryover fish (0.46 kg/fish @ 4,589 kg/ha). Fish were fed once daily to apparent satiation with a 28% protein floating feed and aerated with a single 7.4-kW electric paddlewheel aerator. Density-dependent significant differences were absent for gross, net, daily-net yields, marketable yields (≥0.54 kg), growth (g/d), and survival. Sub-marketable yield (<0.54 kg) and feeding rate increased significantly with increased understocking density. Economic analysis revealed increased breakeven prices and diminished net returns with increased stocking density when sub-marketable fish were not considered as revenue. These differences in production costs and profits among the three treatments became minimal when sub-marketable fish were included as revenue. All three density treatments attained positive annual net cash flows. This study validates channel catfish understocking densities of 17,000-26,000 fish/ha to improve cost efficiency in intensively aerated, multiple-batch production systems.

ECONOMICS OF U.S. CATFISH FARMING PRACTICES: PROFITABILITY, ECONOMIES OF SIZE, AND LIQUIDITY

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Evolution of the US catfish industry, forged by dynamic market forces, has resulted in the development of an array of farming practices. This comparative economic study was developed with data from 325 ponds on 38 commercial catfish farms (Alabama, Arkansas, and Mississippi) and provides concurrent estimates of the cost of production, profitability, and indicators of farm liquidity for the most common catfish growout strategies. Detailed enterprise budgets were developed for nine different catfish production strategies and analyzed on three different farm scales (32 ha, 124 ha, and 592 ha). Profitability differed across catfish farming practices. The split-pond system using hybrid catfish was the least-cost production strategy ($1.97/kg), followed by the multiple-batch system employing channel catfish with increased aeration rates, and intensively aerated ponds using hybrid catfish. Long-term profitability of catfish farming practices increased with increases in farm size. While seven of the nine production strategies discussed here were profitable in the long-run on medium (124-ha) and large (592-ha) catfish farms, only split and intensively aerated ponds (hybrid catfish and multiple-batch production with channel catfish with increased aeration rates) were profitable in the long-run on small farms (32-ha). Low-intensity strategies involving channel catfish in single- or multiple-batch systems were not profitable on any scale. High-yielding strategies resulted in an increased cash flow coverage ratio and decreased debt-servicing ratios, indicating lower liquidity risk. Economies of scale were evident throughout the analysis but stemmed from two different effects: 1) intensification of production in individual ponds; and 2) larger farm size. Changes in cost structures and economic conditions have changed the degree of profitability of farming practices. A number of previously profitable practices are no longer profitable. Newer, split pond and intensively aerated farming practices were profitable but require greater attention to cost efficiencies.

HEPATIC PROTEOMICS REVEALS THE EFFECTS OF COMPENSATORY FEEDING REGIME ON FEED EFFICIENCY IN RAINBOW TROUT

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University of Idaho Aquaculture Research Institute and the USDA Agriculture Research Service (ARS/UI strain) have developed rainbow trout families that exhibit high growth rates when fed all plant protein. Our selected rainbow trout strain is a unique model to identify genetic and physiological parameters associated with sustainable plant protein utilization in fish. However, we need to identify the best lines among the selected families of rainbow trout, which can utilize to plant protein (soy-based ingredients) more efficiently. The overall goal of our study was to develop an indirect benchmark to select the families of rainbow trout to enhance the feed efficiency of soybean-based diets and also investigate the underlying mechanism using hepatic proteomics to divulge the proteins involved in the compensatory feeding regime. We tested if feed intake and body weight variations are correlated using compensatory feeding regimes with rainbow trout for genetic improvement of soybean meal based diets (SBMD) utilization efficiency using indirect selection, and to develop alternative criteria to improve feed intake of SBMD in fish. Twelve families (1600 fish, av. int. wt. 30 g) of selected lines of rainbow trout were used in the experiment. Selected lines of trout fed plant protein (50% soy) diet (protein: 41% and lipid: 21%). Firstly, fish were tagged individually and reared in a common environment, fish were on *1st feeding challenge* for 4 months (one month each challenge either feed deprivation, FD or refeeding, RF), and growth performance was recorded. Thereafter, fish were separated into four groups (1331 fish) based on individual performance during FD and RF challenge studies. Feed efficiency was recoded for 3 months in all four groups followed by measuring the stability of response to the FD and RF periods (*2nd feeding challenge* for 4 months) which was the same as the 1st feeding challenge. During the 2nd feeding challenge, liver samples were collected from each group at each sampling point, total five sampling points (initial, 1st FD, 1st RF, 2nd FD and 2nd RF).

Proteins from liver samples were measured by reverse phase liquid chromatography using an Agilent 1260 Infinity Binary liquid chromatography. Spectral files for each sample were analyzed using Spectrum Mill Software (Version B.04.01.141). Peptides were searched against the Uniprot Reference Proteome ID#UP000193380 for Rainbow Trout (Species ID: ONCMY; 46, 447 proteins; downloaded May 2021). In terms of bioinformatic analysis, peptides were sequenced and identified by Spectrum Mill at the MS/MS level, quantification at the MS1 level was performed using the DDA workflow in Skyline 20.2 (MacCoss Lab Software) with a score of 0.9, retention time window of 5 mins, and 5 missed cleavages with transition settings for TOF. Data were sorted and manually consolidated using Excel. Metaboanalyst 5.0 was used to replace missing values with 1/5 of the limit of detection (LOD), normalized using median, pareto scaling, and log transform, and then to conduct principle component analyses (PCA), ANOVA (Fisher's LSD post-hoc test with Benjamini-Hochberg FDR), and partial least squares discriminant analyses (PLSDA). Feed conversion ratio were significant different among four groups. Results revealed that hepatic proteins were significantly affected by sampling point and by groups. Data will be presented. Conclusively, cost of fish production decreased by enhancing the utilization of soybean meal via improving the feed efficiency in rainbow trout.

MODELLING AND FORECASTING ATLANTIC SALMON *Salmo salar* PRICES

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Atlantic Salmon prices are highly volatile, contributing to the financial and economic risk of participating in the salmon market. More knowledge regarding the underlying price processes will help the market participants better handle price risk. Modelling prices is however a challenging task, due to characteristics such as non-stationarity and potential structural breaks over time. In addition, a wide range of variables could play a role in price formation, with the importance of different drivers varying over time.

The extent of empirical work regarding determinants of the prices of Atlantic Salmon is limited, and especially so when it comes to forecasting. Our paper adds to the literature by exploring a wide range of potential price drivers, using a method that to the best of our knowledge has not previously been applied to salmon prices. The Bayesian approach of Dynamic Model Averaging (DMA) is an online prediction method developed by Raftery, Kárný, and Ettler (2010) that allows variation both in parameters and the set of explanatory variables. The model combines Bayesian Model Averaging (BMA), hidden Markov models and state space modelling, and is a recursive approach that allows the "best" model to vary over time. Kalman filtering increases robustness against non-stationarity and structural breaks, and DMA can incorporate a large number of variables and datapoints while at the same time have a parsimonious representation due to the use of forgetting factors for both the Markov chain models and the state space models.

The period investigated is 2007:05 through 2020:08. The Fish Pool Index (FPI) is used as a proxy for the market spot price of Atlantic Salmon, and a wide range of variables are included as drivers, such as biomass, smolt release, feed rates on the production side, and the stock prices for a selection of major farmed Atlantic Salmon production companies to incorporate information from exchange traded salmon assets. We provide posterior inclusion probabilities graphs that show the importance of different price drivers over time and discuss their importance in the context of major market changes.

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THE MONITORING AND EVALUATION OF STOCK HEALTH AND WELFARE IN AQUACULTURE USING SUBMERSIBLE REMOTELY OPERATED VEHICLES

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With the total annual aquaculture production contributing to 45% of the world's aquatic product consumption, the importance of aquaculture on the global food market cannot be overstated. As with any food resource, sustainability and animal welfare is a top priority. Keeping infrastructure and equipment in proper operational conditions and ensuring compliance with hygiene and health standards can have critical challenges.

The use of submersible remotely operated vehicles (ROVs) is changing how fisheries are monitoring and maintaining their farms. With an ROV, operators can get eyes underwater quickly to easily inspect and monitor both the farm and the stock.

There are numerous ways in which an ROV can work to monitor the health and welfare of fish on farms. Net health is absolutely imperative to the overall health of farms and stock. With an ROV users can quickly and accurately inspect nets without needing to schedule costly divers. With consistent inspection operators can ensure that their pens are structurally sound and catch minor issues early.

Observing fish behaviour is important as farmers can spot unusual behaviour quickly. By catching odd behavioural patterns early, operators can nip issues in the bud quickly to minimize negative effects.Feeding processes can also be observed and assessed using an ROV. Monitoring feeding processes allows operators to confirm that their processes are as effective as they can possibly be.Retrieving morts quickly allows operators to get effective insight as to how and why fish are being lost and at what life stage. Sampling tools allow users to retrieve water and sediment samplers to ensure quality parameters are being met.

In short, the use of ROVs provides operators with an efficient, safe and effective way to ensure stock health and wellness.

WHAT MAKES CHANNEL CATFISH TO TOLERATE HIGH WATER HARDNESS: AN INSIGHT INTO TISSUE RE-MODELLING

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Channel catfish (*Ictalurus punctatus*) is one of the most important farmed fish in the United States. Among various water quality parameters affecting the catfish survival and performance, increase in water borne hardness is one of the major concerns. Despite various reports on the effect of water hardness on growth performance of commercial important fish; the maximum tolerance range of channel catfish to elevated hardness is also not yet reported. Additionally, the mitigating effects of elevated water hardness on channel catfish in relation to increased environmental ammonia and increased salinity have also not been extensively reported on. To provide primary information on the tolerance of this species to elevated hardness, a 96 h-LC_{50} toxicity test was conducted. Following a range finding test, the 96 h-LC_{50} value was found to be 4940 mg/L (C.I. 4970-5223 mg/L). This maximum tolerance value in catfish is many times higher than other commercially important fish including salmonids, largemouth bass, striped bass. To get a better insight in determining the reasons for catfish's ability to tolerate a very high hardness and the possible interactions with other water quality parameters, a microscopic examination into the tissue (gills, liver, intestine and skin) morphological modification (remodeling) was performed in function of step-wise increment in water hardness (ranging from 200 mg up to 4000 mg/L) following a 15 days trial and at the three different levels [Control (100 mg/L), 494 mg/L, and 1250 mg/L] following a 60 day growth trial and 21 day challenge trial. Fish were exposed to 10 and 25% of the 96h-LC_{50} for two months, following the two months they were exposed to elevated environmental ammonia and elevated salinity for 21 days. Following both the two months and 21-day trials, tissue, water, and blood samples were taken for analysis. Various growth parameters were calculated following the two-month trial. It was found that there were differences in the growth parameters between the three dosage groups. There were also differences in the plasma ammonia levels, ammonia and urea excretion rates, oxygen consumption, and calcium concentrations within the various tissue and blood samples from the two trials.

At relatively lower concentration (200-1000 mg/L), primary and secondary lamellae appeared normal. Remarkable alterations were evident at relatively elevated hardness 1500 – 3000 mg/L wherein interlamellar cell mass (ILCM) were momentously developed. This reorganization is an attempt to reduce the surface area presumably protecting against the water borne hardness. Likewise, at higher hardness, liver, and intestine displayed compensatory adjustment, enabling catfish to tolerate high level of hardness.

OPEN HARDWARE TO FACILITATE DEVELOPMENT OF GERMPLASM REPOSITORIES FOR AQUATIC BIOMEDICAL MODELS

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Development of efficient and reliable germplasm repositories is critical for preservation of genetic resources of aquatic model organisms that are vital to advancing biomedical research. However, the most significant problem in repository development is the lack of reproducibility, posing great risks of losing valuable lines developed from billions of dollars research investment. Open hardware allows users to gain access to technologies through open-sharing mechanisms and enable individual contributions for improvement to facilitate community-scale standardization that would rarely be achieved through proprietary technologies. Our work recognizes 14 categories of open hardware for a cryopreservation processing pathway, and 6 categories for a corresponding quality management pathway to address the two impediments to establishing repositories among resource centers and research communities (Figure 1). Although cryopreservation protocols have been established through basic biological research, low quality samples are often produced that cannot be revived because of a lack of affordable, standardized, and reliable hardware to process samples along a production pathway. In addition, there are currently no cost-effective hardware options to enable quality management, including quality assessment for accurate evaluation, quality assurance mechanisms for prevention of defects, and quality control for elimination of inferior materials. Although some of these issues can be alleviated by commercial solutions, most laboratories are not willing to purchase expensive equipment (that can cost tens of thousands of dollars) when germplasm banking is not a focus or obligation of their work. Through open hardware, individuals can fabricate standardized devices in-house with low cost, offering opportunities to begin or improve germplasm preservation, and facilitate repository development with community efforts through aggregated high throughput.

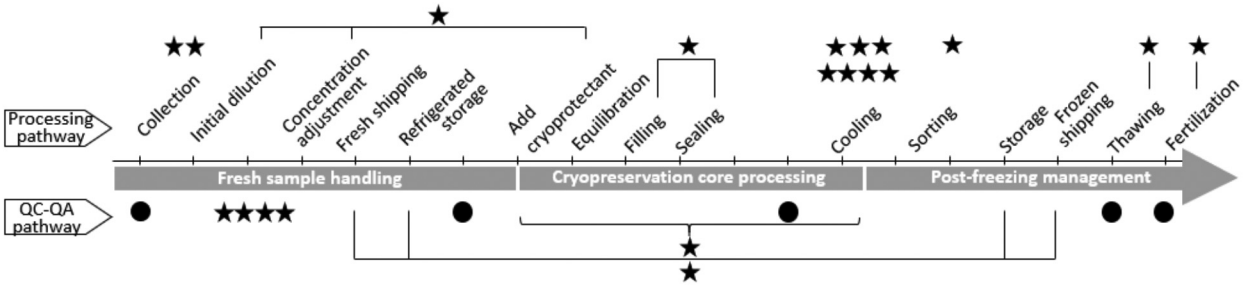

Figure 1. A generalized framework for technology development to support development of germplasm repositories for aquatic biomedical models. The processing pathway includes all activities of sample processing from gamete collection (left) through fertilization (right) with three major phases: fresh sample handing, cryopreservation core processing, and post-freezing management. The quality management (QC-QA) pathway includes repeated quality evaluation (QE) checkpoints that enable establishment of product quality control (QC), process quality assurance (QA), quality monitoring, and data transfer and management systems. Stars represent different hardware categories and black circles represent QE points.

MAXIMIZING THE GREEN POTENTIAL OF AQUAPONICS THROUGH NUTRIENT MANAGEMENT

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The rapid expansion of the aquaculture industry has brought about a heightened focus on the waste produced by high-intensity fish farming. In closed containment recirculating aquaculture systems (RAS), fish solids are mechanically separated and/or coagulated before being disposed as waste, with no subsequent revalorization. Recent developments to improve the reuse of RAS waste streams, for instance to fertilize downstream hydroponically grown plants (aquaponics), present an opportunity for innovation in waste treatment. In this research, we developed a novel, in-line, low-cost solid waste treatment system for freshwater RAS adapted from an enhanced biological phosphorus removal (EBPR) process. After an initial laboratory test using freshwater RAS solids, the system was operated in an aquaponics facility over a 3-month period, producing 450 kg of rainbow trout (*Oncorhynchus mykiss*) and 39 kg of lettuce (*Lactuca sativa*). Total suspended solids were reduced by 59.21% ±39.68 overall, stabilizing to 87.27% ± 9.95 over the duration of the greenhouse integration into the RAS loop. The removal of solids-bound nitrogen from the system averaged 39.91% ± 15.95 over the entire duration of the experiment (figure 1). Little residual biomass was produced (4.04 g ± 2.67 weekly) and the effluent pH was neutral (7-7.5). In addition to carbon and nitrogen removal, the system increased the solubility of trace nutrients creating a liquid fertilizer effluent highly suitable for aquaponics cultivation both in terms of concentration and bioavailability of nutrients. The present solids treatment system represents an improvement over existing solids removal methods which hitherto have been restricted to relatively costly strategies such as filtration followed by physical collection, or conversion to biomass that requires significant downstream processing. This study contributes to the expanding body of research on nutrient extraction from fish solids. By contextualizing this process as a means to deliver nutrients to a downstream greenhouse, we propose its use in aquaponics as a cost-effective remineralization strategy.

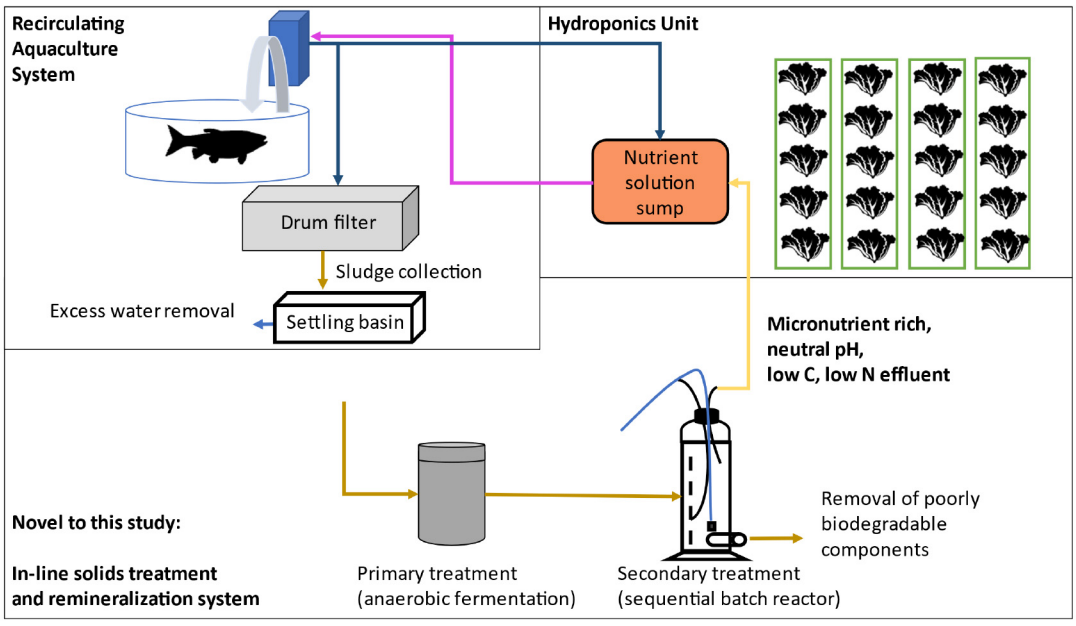

Figure 1. Overview of the solids treatment and remineralization system used in the current study.

EFFECTS OF SUPPLEMENTAL CARBOHYDRASE AND PROTEASE ENZYMES ON THE DIGESTIBILITY OF PLANT PROTEIN-BASED DIETS BY HYBRID STRIPED BASS *Morone chrysops* ♀ × *M. saxatilis* ♂

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Inclusion of plant-protein feedstuffs in aquaculture feeds has increased in recent decades, but the presence of indigestible carbohydrates and protease inhibitors in such feedstuffs can negatively affect their nutritional value to aquatic animals. Supplementation of exogenous enzymes in land animal feeds has been shown to influence feed intake, increase protein and energy digestibility, and improve growth rates and feed efficiency. However, information about the efficacy of enzyme additives in diets for farmed fish is still limited.

A 14-week digestibility trial was conducted to evaluate the effects of supplemental carbohydrase and protease enzymes in plant-based diets for Hybrid Striped Bass (HSB). Five test diets were formulated to contain 40% crude protein and 12% lipid with conventional soybean meal (SBM) or a combination of SBM and dried distillers grains with solubles as the main protein sources. To evaluate the effects of the enzymes additives, the following treatments were designed: no enzyme supplementation (negative controls), supplemental carbohydrase and protease (CP+), and supplemental carbohydrase and protease at 2X the recommended supplementation levels by the manufacturer (CP++). All experimental diets contained yttrium oxide (Y_2O_3) at 1% as the digestibility marker. Each diet was fed once daily to apparent satiation to quadruplicate groups of ~20 juvenile HSB (~100 g/fish) stocked in 180-L polyethylene tanks operating as a recirculating aquaculture system. Fecal samples were collected by stripping once a week until ~3 g dried fecal matter was obtained. Element and proximate composition analyses of the experimental diets and fecal matter are currently underway and apparent digestibility coefficients will be presented.

EVALUATION OF LOW-COST LED LIGHTS AS THE SOLE SOURCE LIGHT FOR PRODUCTION OF BIBB LETTUCE, *Lactuca sativa* var. *Capitate*, IN INDOOR AQUAPONIC SYSTEMS USING NILE TILAPIA, *Oreochromis niloticus*

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For aquaponics producers in northerly latitudes, or indoors in urban settings, artificial light is required for plant growth. Previous studies at Kentucky State University compared several technologies of grow lights and found that light-emitting diodes (LED) lights were significantly better than fluorescent, induction, or metal halide in terms of average plant weights. While the cost of LED technology is decreasing, specialized grow lights can be cost prohibitive for many producers, especially if a large number are required. To address this, a plant growth trial was conducted to compare the effectiveness of readily available and inexpensive LED lights to the more expensive horticultural LED lights on the production of Buttercrunch lettuce, *Lactuca sativa* var. *Capitate*, in aquaponic systems. The four different LED lights evaluated were the Neosol DS control (NEO; unit cost $1700) and Spider Farmer SF-2000 (SPI; unit cost $300), Fluence RAZRx (FLU; unit cost $364), and Designers Fountain 3500k (DES; unit cost $300) treatments. Four 1175-L systems were used with all four lights represented in each system in a complete block design. Adult Nile Tilapia, *Oreochromis niloticus* (586 g) were stocked in each system and fed a floating 32% protein diet at a rate of 60 g/m^2 of plant grow space/day. Plants were harvested after two weeks. At harvest, there were no significant differences (P>0.05) among light treatments (Table 1) in terms of average plant weight (g), total plant biomass (g), or plant growth per unit energy (g/m2/kWh). Since energy costs can be >25% of the variable costs in controlled environment aquaponics, the similar plant growth per unit energy (g/m2/kWh) of all three experimental lights compared to the NEO control make them all suitable alternatives. The similar plant growth results combined with a lower purchase price could make the DES a good candidate for a large-scale production trial.

Table 1. *Average individual plant weight (g), total biomass (g), total plant biomass (g) per square meter, total plant biomass (g) per kilowatt hour, total plant biomass (g) per square meter per kilowatt hour, and purchase cost of LED Lights for Buttercrunch Lettuce,* Lactuca sativa *var.* Capitate*, grown under four different LED grow lights.*[1]

Plant Variable	NEO	FLU	DES	SPI
Average Individual Weights (g)	62.6[a]	60.9[a]	54.1[a]	61.2[a]
Total Biomass (g)	938.8[a]	913.3[a]	811.32[a]	917.3[a]
Grams/m^2	1676.5[a]	1630.8[a]	1449.2[a]	1638.0[a]
Grams/kWh	36.5[a]	41.1[a]	43.7[a]	40.9[a]
Grams/m^2/kWh	977.2[a]	1100.2[a]	1169.7[a]	1096.5[a]
Unit Cost	$1700	$364	$100	$300

NEO=NeoSol DS; FLU=Fluence; DES = Designers; SPI=Spider Farmer

[1] Significant difference ($P \leq 0.05$) are indicated by different letters in rows.

RETAIL SEAFOOD SALES IN THE UNITED STATES

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While a large number of studies have investigated seafood consumption in various markets, surprisingly little is known about the types of seafood sold in retail outlets in the United States. or their product forms. This is particularly true for what is generally regarded as the most valuable product form of seafood, fresh. In this paper we analyzed a unique dataset on retail in-store seafood sales that includes information about three main product forms (fresh, frozen and shelf-stable products). Fresh seafood is important, as it makes up 43% of sales revenue (Table 1). Moreover, some species are almost exclusively sold fresh, with trout and lobster as prime examples. Fresh also includes the greatest diversity of species, and as such, is the most likely product form for new producers to succeed. However, overall sales are dominated by a few species, with salmon and shrimp dominating the fresh (27%) and frozen categories (43%), respectively, and tuna dominating the shelf-stable category (75%). There are also a large number of species with mostly small market shares. There are few differences in regional sales patterns for the main species, with notable exceptions such as whitefish in New England and crawfish in Louisiana and Texas. The degree of urbanization and income level appear as important drivers for seafood sales.

Table 1. Average U.S. retail seafood sales by product category, 2017-2019.

Product category	Annual revenue billion $ (st dev)	% of total	Annual volume 1,000 metric tons (st dev)	% of total
Fresh	5.30 (0.14)	43.3%	288.9 (8.39)	35.4%
Frozen	4.80 (0.08)	39.1%	341.6 (7.60)	42.0%
Shelf-stable	2.14 (0.01)	17.5%	184.2 (2.81)	22.6%
Total	12.2 (0.23)	100%	813.7 (13.4)	100%

IDENTIFICATION OF RECEPTORS OF PIRAB^{VP} BINARY TOXIN RELEASED BY *Vibrio parahaemolyticus* CAUSING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN *Penaeus vannamei* SHRIMP

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Acute hepatopancreatic necrosis disease (AHPND) in shrimp is caused by *Vibrio parahaemolyticus*, an enteric pathogen carrying a large plasmid which encodes a binary toxin, PirAB^{Vp}. The tertiary structure of PirAB^{Vp} toxin shows similarity to Cry^{Bt} toxin produced by *Bacillus thuringiensis* that causes lethal infection in insects. In insect, aminopeptidase N (APN) is one of the receptors of Cry^{Bt} toxin, but the receptors in shrimp foregut and hepatopancreas that bind to PirAB^{Vp} remain unknown. Upon mining NCBI database, five isoforms of insect homologs of APN in shrimp (*Penaeus vannamei*) were identified. Similar to insect APNs, shrimp APNs contain a transmembrane domain, a Cry Binding Region (CBR) and two signature amino acid sequence motifs. Phylogenetic analyses showed that crustacean APNs are similar to insect APNs but form a separate clade. *In silico* analysis of the tertiary structure of PirAB^{Vp} and predicted tertiary structure of shrimp APNs revealed all APN isoforms displayed the binding affinity to PirAB^{Vp}. Interestingly, following an experimental challenge of Specific Pathogen Free (SPF) *P. vannamei* shrimp with AHPND-causing *V. parahaemolyticus*, none of the five APN transcripts were detected in the foregut, and four out of five APN isoforms, APN-1, -2, -4 and -5 were detected by qRT-PCR in the hepatopancreas tissue.

The mRNA expression of APN-4 and -5 were significantly higher in an AHPND susceptible than in a tolerant shrimp line. Modelling of proposed biologically functional PirAB^{Vp} toxin and APN interactions coupled with the mRNA expression data suggest while APNs serve as receptors of binary toxin in hepatopancreas, and in the gut PirAB^{Vp} toxin likely binds to other receptors. In addition, lower mRNA expression of APN-4 and -5 in hepatopancreas of AHPND-tolerant line suggests a reduction in toxin uptake by epithelial cells in hepatopancreatic tubule may contribute to AHPND resistance. Collectively, these findings shed new insights on the molecular mechanisms of PirAB^{Vp} toxin involved in *V. parahaemolyticus* pathogenesis in shrimp.

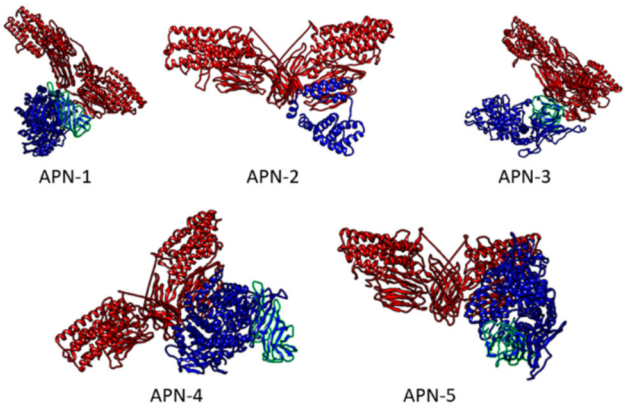

Figure 1. Interaction of PirAB^{Vp} toxin with APN-1-5 isoforms. PirAB^{Vp} was indicated by red color. APNs were indicated by blue color.

REPLACEMENT OF FISH OIL BY CAMELINA AND BLACK SOLDIER FLY LARVAE OILS IN DIETS FOR JUVENILE TOTOABA, *Totoaba macdonaldi*

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Fish oil (FO) has been used for decades as the major lipid source in aquafeeds; it is valued because of the richness in highly unsaturated fatty acids (HUFA), such as docosahexaenoic (DHA, 22:6n-3) and eicosapentaenoic (EPA, 20:5n-3) acids, that promote health, growth, and fillet quality of cultured marine organisms. Research on alternative sources of lipid to replace FO has increased to address sustainability, cost, and availability concerns. Among them, *Camelina sativa* and *Hermetia illucens* larvae oils have been tested in marine fish diets with promising results. Camelina oil contains up to 45% of α-linolenic acid (18:3n-3) and high n-3/n-6 ratio, highly desirable characteristics for an alternative lipid source. Black soldier fly larvae (BSFL) oil appears to be more palatable or attractable to fish compared to vegetable oils; it is rich in lauric (12:0) and linoleic (18:2n-6) acids, though this profile can be improved using substrates rich in omega-3 fatty acids. *Totoaba macdonaldi* is a newly cultured marine carnivorous fish, endemic to the Gulf of California, Mexico, whose industry is still developing. In the present study, the use of camelina and BSFL oils to replace FO in diets for *T. macdonaldi* was evaluated.

Four isoproteic (51%) and isolipidic (14%) diets were formulated based on a 2×2 factorial design with two lipid sources, camelina and BSFL oils, each tested at two levels of replacement, 30 and 60%. A control diet containing 100% FO as the lipid source was used as a reference. Fish with an overall initial mean weight of 3.0 ± 0.1 g were stocked at a density of 100 fish/m^3. Each experimental diet was randomly assigned to five replicate tanks. Growth performance and feed utilization were evaluated in terms of weight gain (WG), survival (arcsine square root transformed before analysis), and feed efficiency (FE), among other variables. Proximate composition and fatty acid profile of experimental diets, muscle, and liver were determined. Data were subjected to two-way ANOVA ($P \leq 0.05$), and Tukey's HSD test was used as the mean separation procedure. In addition, Dunnett's test was used to compare the treatment means to the control mean.

After 7 weeks, fish fed 30% replacement of FO by BSFL oil attained similar growth as fish fed the 100% FO control diet (Table 1). Muscle tissue reflected the fatty acid profile of the experimental diets; fish fed BSFL oil showed a high content of 12:0 (0.11 mg g^{-1} of wet tissue), while 18:3n-3 was higher in those fed camelina oil (0.27 mg g^{-1} of wet tissue). The fillet of fish fed 30% BSFL oil preserved their nutritional quality, with similar atherogenicity and thrombogenicity indices as the control fish.

Results of this study suggest that 30% of FO can be successfully replaced by BSFL oil in diets for *T. macdonaldi* without affecting growth and preserving its nutritional value.

Table 1. Growth performance, survival, and feed efficiency of *Totoaba macdonaldi* fed the experimental diets.

	WG (g)	Survival (%)	FE
Main effects			
Lípid source			
Camelina	52.5 ± 4.7	99.0 ± 2.1	1.1 ± 0.1
BSFO	55.7 ± 1.8	98.5 ± 3.4	1.1 ± 0.1
Replacement level (%)			
30	56.3[a] ± 5.1	98.5 ± 3.4	1.1 ± 0.1
60	51.9[b] ± 4.0	99.0 ± 2.1	1.1 ± 0.1
ANOVA *P* > *F*			
Lípid source (L)	0.1147	0.8514	0.3671
Replacement level (R, %)	0.0302	0.8514	0.5671
L × R	0.1224	0.8514	0.0153
Treatment means (Dunnett's test)			
Fish oil 100%	60.7 ± 2.5	100.0 ± 0.0	1.2 ± 0.1
Camelina / 30	53.2* ± 4.8	99.0 ± 2.2	1.1 ± 0.1
Camelina / 60	51.8* ± 5.0	99.0 ± 2.2	1.1 ± 0.1
BSFO / 30	59.4 ± 3.2	98.0 ± 4.5	1.2 ± 0.1
BSFO / 60	51.9* ± 3.5	99.0 ± 2.2	1.1 ± 0.1

Values for main effects are means ± S.E.M. of ten replicate tanks, or five for treatment means. Means with different superscripts within the same main effect column are significantly different ($P \leq 0.05$). *Marked treatment means are significantly different ($P \leq 0.05$) from the control diet (FO 100%) according to Dunnett's test.

ECONOMIC ANALYSIS OF A DECOUPLED TILAPIA *Oreochromis niloticus* - CUCUMBER *Cucumis sativus* AQUAPONIC SYSTEM

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Aquaponics, the integrated production of fish and plants, has gained attention and international interest. This system uses nutrient-rich effluent from the fish system as a fertilizer for plant production. While aquaponic set-ups vary greatly, the goal of commercial operations is profitability. Target markets for product are variable, depending on location, seasonality, and species or cultivars produced. Technological advances in aquaculture and horticulture continue to aid the development of the industry, but economic analysis remains scarce. This study investigated the current profitability and potential of a decoupled aquaponics operation, using one fish greenhouse stocked with tilapia, and one plant greenhouse producing cucumbers in a pilot-scale aquaponics system at the E.W. Shell Fisheries Center (Auburn, AL). Quantities of production inputs required for crops of tilapia (*Oreochromis niloticus*) and cucumbers (*Cucumis sativus* L. 'Delta Star') and quantities sold of each product were transformed into dollar amounts and entered into 12 monthly cash flows. Enterprise budgets were developed from the cash flow, augmented with fixed costs calculated from the initial investment schedule to obtain net returns to owner's labor and management. Research production of cucumbers was 5,544 kg of cucumbers after extrapolation to the full greenhouse area. Tilapia production was 3,002 kg and not expanded. A two-way sensitivity analysis indicated a tilapia price of $3.00/lb and a cucumber price of $2.00/lb was required to breakeven, covering variable plus fixed cost. Prior research had shown nutrient effluents coming from one tilapia greenhouse was sufficient to supply fertilizer for four cucumber greenhouses. Research results were extrapolated to four cucumber greenhouses fertilized by the one tilapia greenhouse. Total investment was $303,152. Tilapia returns were positive/negative in the short/long run. However, including cucumbers resulted in positive overall returns, Table 1. The cost of production covering all costs was $3.27/lb and $1.48/lb for tilapia and cucumbers respectively. Aquaponic tilapia–cucumber systems can be profitable, but rely heavily on market selling prices, quantity produced, and scale of operation.

Table 1. Summary enterprise budget values for one tilapia greenhouse providing fish effluent to fertilize four cucumber greenhouses. Values shown are based on selling prices of $3.00/lb and $2.00/lb for tilapia and cucumbers, respectively.

	Receipts $	Variable Costs $	Income Above Variable Costs $	Fixed Costs, $	Total Costs $	Net Returns $
Tilapia	19,856	15,175	4,681	6,466	21,641	(1,785)
Cucumber	97,779	44,891	52,888	27,376	72,267	25,512
Total	117,635	60,066	57,569	33,842	93,909	23,727

CLEAR-WATER FIXED FILM CAROUSEL PRODUCTION OF MARINE SHRIMP

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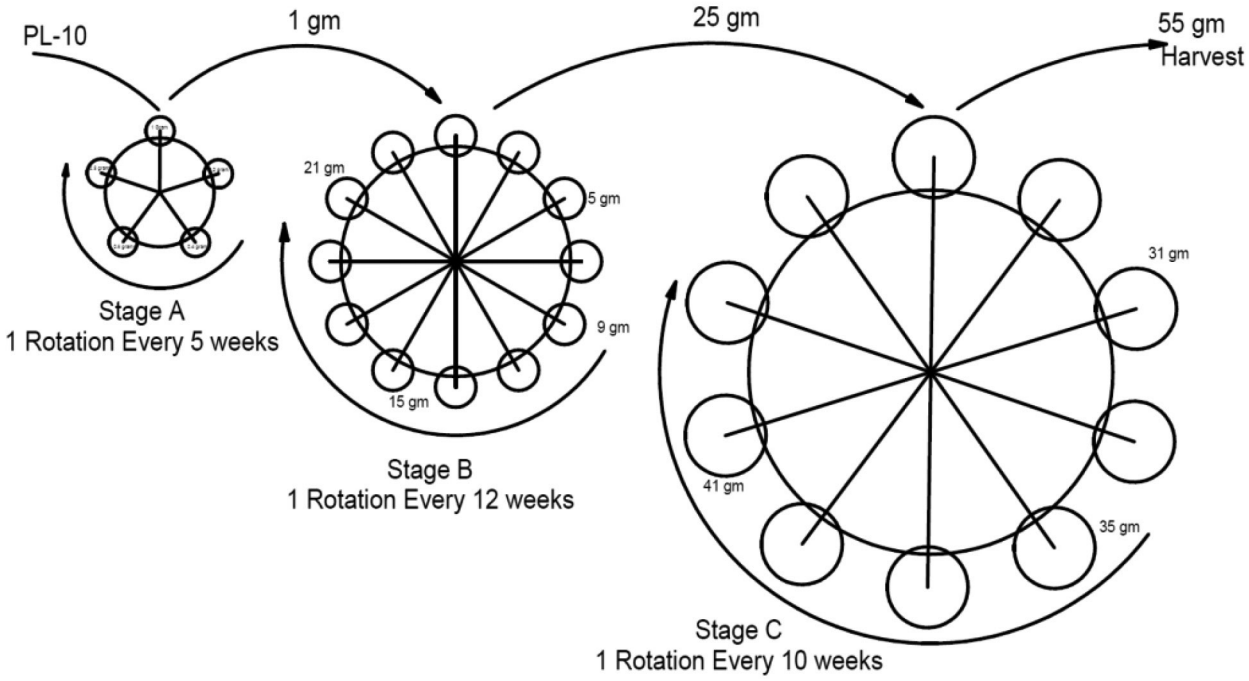
Conceptually a "carousel" consists of a cluster of tanks dedicated to shrimp growth through a designated size category. Tanks and filters are sized to support the highest feed rate in the size class. Tanks in a given carousel are "rotated" weekly until the shrimp complete the growth stage. Shrimp in the tank are then rotated forward to complete the next growth stage. Using fixed-film clear water technology, the carousel design is specifically targeted to produce a reliable weekly supply of shrimp for local sale. The carousel concept was generated as part of a USDA Small Business Innovative Research (SBIR) grant directed at improving indoor recirculating technologies to produce shrimp in tanks where temperature preclude pond production. The grant focuses on improvement of shrimp production densities in tanks supported by airlifted floating bead bioclarifiers. Weekly production targets in the range of 200-300 pounds are obtainable using polyethylene tanks and filters with production densities presumed at 10 kg/m^3. The number of RAS required range from 16-20, depending on the growth rate equations. Issues confronting potential producers include availability of post larvae for regular stocking of Stage A tanks, availability growth curves, and detection of food consumption deviations during growout.



SOYBEAN MEAL USE IN SHRIMP FORMULATION IS NOT A CAUSE OF WHITE FECES DISEASE IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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The objective of this study was to confirm the growth response, immune response, histopathology, and the expression of White Feces Disease (WFD, also termed White Feces Syndrome or WFS) in Pacific whiteleg shrimp (*L. vannamei*) fed diets containing different inclusions of soybean meal (SBM) at 0%, 15%, 30%, and 30% SBM supplemented with enzyme or health solutions.

A series of feed trials was designed to verify that SBM, even at high inclusions in *L. vannamei* formulation, has no linkage with WFD. Two trials were conducted, one in a closed clearwater tank system at Kasetsart University, Thailand and one in a pond-based hapa system at a commercial shrimp farm in Chachoengsao province, Thailand. SPF-certified shrimp postlarvae, PL12, were obtained from a local hatchery and transferred to the testing facilities.

In the first trial, SPF-PL12 were acclimated for 12 days in a common tank and shrimp (0.003g) were then stocked at a density of 120 PL/m2 or 80 shrimp/tank using four replicates under laboratory conditions. The shrimp were fed four times daily at a satiation rate. At the end of the 45-day feeding period, overall growth performance associated with different SBM inclusions was monitored. Harvest samples have an average weight of 3.28 g. Additionally, the immune responses and WFD related conditions such as *Vibrio* counts, EHP infection, and histology of the hepatopancreas, including Aggregated Transformed Microvilli (ATM) were determined. Furthermore, the effect of different SBM inclusion after a stress test with low dissolved oxygen (1.5-2mg/l) and a challenge test with *Vibrio parahaemolyticus* AHPND strain were examined. Finally, the nutrient digestibility of each dietary treatment was also analyzed.

In the second trial, SPF-PL12 were stocked in nursery tanks for 30 days at a stocking density of 1,250 PL/m^2. The shrimp (4.4g) were then stocked at a density of 1,320 shrimp/hapa or 110 shrimp/m^2 using four replicates under pond-based hapa conditions. The shrimp were fed four times daily at a satiation rate following the Cooperator's standard feeding guide. Sampling was carried out on a biweekly basis. At the end of the 66-day feeding period, overall growth performance, immune and health parameters associated with different dietary treatments were monitored. Harvest samples have an average weight of 20.1 g. Shrimp fed different SBM inclusions showed similar growth rates in both laboratory and field trials.

TEXAS CULTIVATED OYSTER MARICULTURE FARMER TRAINING

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COVID-19 has had severe economic impacts along the Texas coast specially the seafood industry and has raised new challenges in providing support and assistance to industry stakeholders. Texas recently passed HB 1300 that allows cultivate off-bottom oyster mariculture along the coast which allows the production of farmed oysters, but due to the pandemic, the industry has been slow to develop. In the wake of COVID-19 restrictions being lifted in along the coast, Texas Sea Grant has developed hands-on training and business workshops that is teaching farmers how to successfully develop their oyster business and navigate this new industry. With the new industry and type of farming that is being implemented, much technical assistance is needed in preparation, education, and training in beginning a oyster business, so Texas Sea Grant aims to assist with workshop trainings and business classes to provide technical support and troubleshooting to the farmers. Workshops have been conducted along the Texas coast to educate and train oyster farmers about off-bottom oyster culture industry. Texas Sea Grant also aims at educating the public and seafood industry on cultured oysters to potentially build partnerships with farmers and other stakeholders to further develop the industry.

Texas is the last coastal state to adopt oyster aquaculture, and with the state having one of the largest shorelines in the nation, there is much potential to for growth of the new industry. In 2017, oyster aquaculture produced $186 million dollars nationally, and the Gulf of Mexico aquaculture industry represented 22% of the national aquaculture production value (NOAA Fisheries, 2018). Texas has had a long history of being one of the largest producers of wild-caught oysters nationally with its many suitable areas for farms, but the new industry has considerably different techniques and methods that involve hands-on technical training and assistance that Texas Sea Grant will assist farmers in. Now that Texas has lifted restrictions on the COVID-19 pandemic, there is renewed potential for the industry to recuperate and get back to track.

VARIABILITY IN GROWTH AND IMMUNE RESPONSE OF *Oreochromis niloticus* UNDER THE DIETARY SUPPLEMENTATION OF BLACK SEED (*Nigella sativa*)

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Black seed (*Nigella sativa*) used as a natural immuno-stimulant in the feed of various farmed animals. In present experiment, effect of three dietary inclusion levels of *Nigella sativa* was assessed on growth and immune response of *Oreochromis niloticus* for 8 weeks. Experimental groups received three different levels of *Nigella sativa* i.e. 2, 3, and 4 % in feed while control group received feed without *Nigella sativa*. Growth parameters (weight (g), length (cm), feed conversion ratio and specific growth rate) and immunity response (white blood cells, lysozyme activity, and globulins protein) were investigated. All the recorded growth and immunity parameters of *Oreochromis niloticus* showed a significantly different outcome at the end of trial. Group fed by 4% black seed showed statistically highest increase in weight gain (2.05 gm±0.37), gain in length (0.32 cm ±0.16) specific growth rate (1.34±0.45) and best value of feed conversion ratio (2.27±0.56) followed by 3% and then 2% supplemented groups. Among the immunity parameters, group of fish received 4% black seed in diet showed statistically higher increase in white blood cell count (198 10^3/μL ±3.78), globulin protein (23.66 mg/dl ±1.21) and lysozyme activity (60.66 μl/ml ±1.20) followed by 3% and then 2% supplemented groups. Results of present study reveals that *Nigella sativa* improves the growth and immunity of *Oreochromis niloticus* significantly at 4 % inclusion level in feed.

OPTIMIZING EARLY WEANING OF BURBOT (*Lota lota maculosa*) LARVAE

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Burbot (*Lota lota maculosa*) are freshwater cod that have recently been identified as potential candidates to expand intensive freshwater aquaculture. However, burbot larvae require live food for several weeks before they can be weaned onto commercial feeds. To determine if dependance on live feed could be reduced, we evaluated two products, Gemma dry diet and a liquid artemia replacement (EZ *Artemia* spp.), for their ability to reduce the time of live feeding of burbot larvae. The effect of weaning on growth performance and survival was monitored for 70 days.

In trial 1, larvae were divided into six feeding regimes: a current standard rearing protocol of the Aquaculture Research Institute of University of Idaho fed live feeds (marine rotifers *Brachionus plicatilis* and *Artemia* nauplii.) for more than 40days before weaning onto the dry diet; a positive control group exclusively fed live feeds, negative control fed Gemma diet alone, treatment 1 fed *Artemia* nauplii and no rotifers, treatment 2 fed rotifers and no *Artemia* nauplii, treatment 3 fed rotifers, *Artemia* nauplii and EZ *Artemia* spp. Live feeds were administered from 11 days post hatch (dph) until introduction of Gemma diet at 21 or 40 dph and EZ *Artemia* spp. at 22dph. The stocking densities were 150 larvae/L and growth was assessed at 15, 25, 35, 42, 49 56, 63, and 70 days after stocking. Larvae in the standard rearing protocol had highest survival (>24%), followed by larvae fed exclusively live feeds (22.17%). Larvae fed rotifers, *Artemia* nauplii and EZ *Artemia* spp. resulted in significantly ($P < 0.05$) reduced survival. Use of EZ *Artemia* spp. in treatment 3 reduced the period of using live feeds by 17days when compared to the standard rearing protocol.

In Trial 2, additional feeding regimes were tested that aimed at further identifying feeding regimes that could reduce the use of live feeds while maintaining high growth and survival. Burbot larvae were divided into five feeding regimes: a standard rearing protocol fed live feeds for a prolonged period, treatment 1 fed *Artemia* nauplii and EZ *Artemia* spp., treatment 2 fed rotifers and EZ *Artemia* spp., treatment 3 exclusively fed EZ *Artemia* spp. and treatment 4 fed *Artemia* nauplii but introduced Gemma diet at 25dph. Live diets were administered at 11dph until when Gemma diet was introduced at 25 or 40dph and EZ *Artemia* spp. at 20 or 25dph. The stocking densities were 100/L and survival, and total length were assessed at 14, 28, 42, 56 and 70 days after stocking. Larvae fed live feeds for a prolonged period had significantly higher survival (>30%), followed by larvae fed *Artemia* nauplii and EZ *Artemia* spp. (20.7%), thirdly, larvae fed rotifers and EZ *Artemia* spp. had 17.5% survival. Larvae fed *Artemia* nauplii and Gemma diet had the least survival, but higher total growth.

Use of EZ *Artemia* in treatments 1 and 2 reduced the time and amount of using live feeds by 19 and 24 days, respectively when compared with the standard rearing protocol. Early use of Gemma diet in treatment 4 also reduced live feeding period by 19 days. The results demonstrate a potential to reduce the time of live feeding through incorporation of *EZ Artemia* and/or Gemma micro-diet as replacements for live feeds. This study provides a starting point for reducing live feeds in burbot larval culture.

FIRST SUCCESSFUL CAPTIVE SPAWNING AND LARVAL CULTURE OF THE COPPERBAND BUTTERFLYFISH *Chelmon rostratus*

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The copperband butterflyfish is a popular saltwater aquarium fish and is only available from wild collection. Wild specimens are commonly collected but are fragile and difficult to transition to artificial feeds. Thus development of captive culture methods is justified. This presentation will document the first successful captive spawning and culture of copperband butterflyfish larvae to the juvenile stage.

Copperband butterflyfish form monogamous pairs that spawn pelagic eggs. The eggs were collected using a surface skimmer made with 500 μm mesh. Eggs were harvested in the morning and quantified before being stocked into 29 L tanks.

The newly hatched prolarvae were 2 mm total length and lacked pigmented eyes and mouths. The larvae were robust and transitioned to exogenous feeding around 3 dph and were fed exclusively copepod nauplii *Parvocalanus crassirostris* for 50 days. At 10 dph, the larvae began to develop characteristics of the tholichthys stage; long spikes formed on the edge of the operculum and bony head plates began to form. Flexion occurred at 20 dph. Around day 38, incidental adult copepods that had escaped predation and matured in the tank began to disappear from the water column as the larvae continued to grow. The transition from tholichthys to settlement occurred rapidly around day 51 post hatch, when the first striped larva was identified. *Artemia* nauplii were introduced around 60 dph. The successful settlement of this species represents a significant step for ornamental aquaculture and the scientific community. Documenting this species' embryo and larval developmental and first successful culture protocols sets the foundation for future research and commercial aquaculture production efforts.

Figure 1. 1 dph copperband larva. Figure 2. 62 dph copperband juvenile.

DEVELOPING A CLINICAL DATABASE FOR FISH DISEASE PROFESSIONALS

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The objective of this project is to develop a clinical database for fish diseases that will allow fish professionals and veterinarians to track, record, and visualize fish disease outbreaks on their desktop or mobile device. The database and associated mobile responsive site will be designed by APAX Software located in Lexington, Kentucky. It will be programmed to track treatment methods, record if fish farmers applied the recommended treatment, and determine if the treatment was successful. Data will be collected from diagnostic laboratory cases in Kentucky and surrounding states and from surveys. The preliminary data have been collected from Kentucky State University's Fish Disease Diagnostic Laboratory, which include photographs and video footage compiled over the past 31 years of the laboratory's operation and will be linked to Oregon State University's educational website for fish diseases, www.fishpathogens.net . The presentation will introduce a general outline of the project and the planned capabilities of the clinical database. Supplemental material in the database will be accessible to the public and used as an educational tool designed to inform fish farmers of possible fish diseases. The long-term goal is to create a database that incorporates disease cases from other professionals, in the United States and other countries, to assist in diagnosing causes of diseases, and to visualize and interpret outbreak trends and treatment efficacy.

CHANGES IN FATTY ACIDS PROFILES OF FLORIDA POMPANO (*Trachinotus carolinus*) LARVAE REARED AT DIFFERENT SALINITIES

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As the United States production of warm water marine cultured fish increases, the demand for optimizing different culture aspects at different stages has been continuously arising. Florida pompano (*Trachinotus carolinus*) has been identified as a promising candidate for commercial scale aquaculture, but to date little information is available regarding its optimal salinity growth at the larval stage. Comparison of the fatty acid (FAs) profiles and patterns of use and conservation of the essential FAs of larvae at different stages (i.e., hatching, start of exogenous feeding, weaning etc.) and reared at various salinities will help identify the optimal salinity for growth, feed utilization and lipid metabolism and biosynthesis. The present study aims to describe and compare lipid and fatty acid use from Florida pompano larvae reared at various salinities. After 3 days' post hatch (dph) at 30 ppt, larvae were incubated at 10, 20 and 30 ppt in triplicate. Samples for FA analysis were collected every three days until weaning, which occurred 24 dph. Fatty acid composition was analyzed using a gas chromatography-mass spectrometry. We hypothesize, that larvae reared at low salinity (i.e. 10 ppt) would express more energetic needs (more use of saturated and monounsaturated FA) and a more selective conservation of essential FA in their membranes than larvae reared at optimal salinity (i.e. 30 ppt). By understanding the energetic requirements at specific salinities and different developmental stages, we will be able to optimize Florida pompano culture and fatty acids requirements for optimal growth and health.

METAANALYSIS OF DUCKWEED AS AN ALTERNATIVE FEED FOR FINFISH AQUACULTURE

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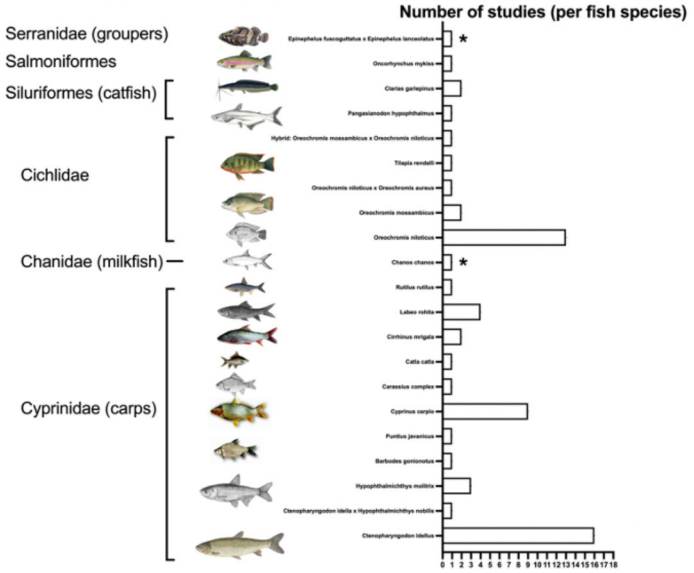
One of the primary sustainability challenges in aquaculture is replacing fish meal with plant based ingredients in aquafeeds. Plant based feeds often suffer with lower crude protein percentages and can cause dysbiosis in the fish gut microbiomes leading to suboptimal performance during growout. Duckweed (*Lemneaceae*) is a family of aquatic plants with high protein content and has been used successfully for various types of terrestrial livestock and fish feed. In this systematic review and meta analysis of 51 papers, we summarize the extent by which duckweed has been used in fish production including the species of fish tested, the growout stage of fish, method of application, and the countries where duckweed are endemic. Duckweed studies spanned a total of 17 species of fish (15 freshwater and 2 marine) which collectively are valued at 263 billion USD annually, and comprise 28% of total aquaculture production by mass. The average experiment length was 76 days (S.D. 44) with most fish at the fingerling life stage. Duckweed was fed to the fish either directly or allowed to graze on fresh growth, dried, as a fertilizer to the ponds, or fermented with newer studies opting for dried feed formulation. The most common formulation other than 100% was 20%. We performed an analysis of impacts of growth across 13 studies on tilapia. Of duckweed species used in experiments, the *Lemna spp* were the most common with *L. minor* and *L. gibba* most consistent. *Wolffia arrhiza* and *Spirodela polyrrhiza* were also more commonly used in experiments. The majority of duckweed species, especially from *Wollfiella* have not been tested as a fish feed. Future research should aim to describe methods of removing or mitigating heavy metal toxicity and anti-nutrients from duckweed. We hypothesize three opportunities to detoxify duckweed including 1) genetic engineering of the plant, 2) microbial treatments of feed stock prior to feed formulation, and 3) designing gut probiotics to enable fish to detoxify compounds in-vivo.



COMPARATIVE GENOMICS AND MICROBIOMES OF A HIGH VALUE FISH, PACIFIC CHUB MACKEREL (*Scomber japonicus*): A MODEL FOR MARINE FISH AQUACULTURE

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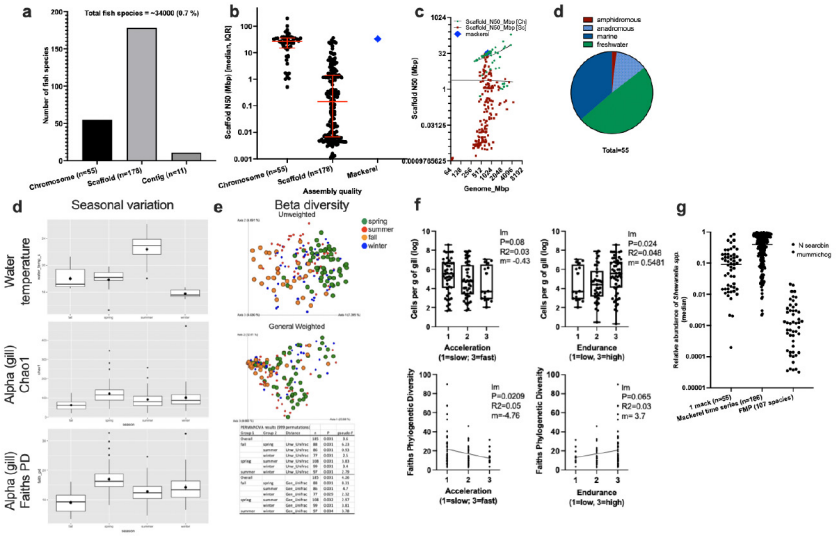
Scomber japonicus is an economically important fishery, consistently in the top 10 (by volume and price) globally, and is an emerging aquaculture species in Asia. Considering mackerel has a high omega-3 fatty acid content, is genetically related to tuna, has a similar life history to most marine aquaculture finfish, and does not grow more than ~40 cm in length, it has great potential as a model marine fish for aquaculture production. Despite an acceleration in high quality genome assemblies across the animal kingdom, there are currently only ~244 fish genomes available (out of ~34,000 species) with only 55 of these achieving high quality chromosomal-level assembly (Fig 1a). We sequenced and assembled a high quality chromosomal-level genome (788 Mbp, scaffold N50 33.19 Mbp, BUSCO 96.9%) using a combination of Oxford Nanopore long reads and Hi-C sequencing (Fig 1b-c). We compared the mackerel genome (fads and evol1 genes) to other published fish genomes (Fig 1d) to evaluate if there has been adaptation in lipid digestion efficiency or PUFA biosynthesis via Fads and Evol1 genes. Since mackerel can tolerate a wide temperature range (10-29 ºC), we also wanted to understand if local adaptation and subsequent divergence is occurring in mackerel populations in the Eastern Pacific. We compared full genomes of 22 mackerel across four seasons a year for three years for a total of 12 time points. We also evaluated the extent to which the microbiome varied seasonally by sampling at least four fish per month for three years from a fixed location (Scripps Institution of Oceanography Pier, La Jolla, CA). The microbiome (alpha diversity and beta) of the gill surprisingly varied most between spring (high diversity) and fall (low diversity) (Figure 1d-e). Lastly, we compared the gill microbial communities of mackerel to 101 local fish species, representing ~25% of the marine fish diversity in Southern California. Gill microbial biomass and diversity was negatively associated with swimming acceleration speed and positively associated with endurance in fish suggesting a potential trade off to fast swimming predatory fish (Figure 1f). Compared to the other 101 species, mackerel had much higher RA of the *Shewanella*.



PATHWAYS TO NET-ZERO: BRINGING CLIMATE ACTION INTO SHRIMP AQUACULTURE

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Current international climate goals cannot be met without mitigation in food-production systems. Agricultural greenhouse gas emissions are difficult to quantify and mitigate because they are primarily concentrated 'on farm'. With hundreds of millions of farms worldwide, the emissions are distributed among many actors producing food in many different ways. Recently, there has been an effort to quantify typical emissions intensities for particular food products and construct decarbonization trajectories for that food consistent with a global net-zero future.

Aquaculture has typically been excluded from global food system climate integrated assessment models, and studies of greenhouse gas emissions for the many aquaculture species are relatively few. This means that aquaculture has largely been left out of the narrative around climate impacts and mitigation opportunities.

To address this, both 'bottom-up' assessments of greenhouse gas emissions from different types of farms and 'top-down' assessments of aquaculture's emissions within the larger global macro-economic context are necessary.

We provide a framework for analyzing aquaculture's potential pathways towards decarbonization, using shrimp as a case-study.

This discussion focuses on initial characterization of emissions for shrimp aquaculture (focused on 4 types of shrimp production: extensive, semi-intensive, intensive, and hyper-intensive) and highlights the knowledge gaps critical to advancing climate sustainability in aquaculture.

STOCHASTIC MODELING AND FINANCIAL VIABILITY OF MOLLUSK AQUACULTURE

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Due to the limited control with the production process in mollusk farming, understanding the risks associated with production can be critical to a farm's success. Despite the industry's need for more knowledge, compared to freshwater finfish and crustaceans, limited attention has been given to economic aspects of mollusk production, such as productivity growth and production risk. Research that has been conducted on the economic viability of molluscan aquaculture often employed deterministic profitability models with sensitivity analysis used to measure risk. However, deterministic sensitivity analysis assumes variables are well known and can be represented by a single value, which can be inaccurate if inputs are highly correlated. Due to the characteristics of mollusk farming, it could be better fit by models that are a function of multiple random elements. Stochastic risk analysis can be performed utilizing Monte Carlo simulation in conjunction with sensitivity analysis or simulation scenarios can be compared. We applied both methods to compare three different environmental scenarios, three equipment systems, and four risk mitigation strategies for Gulf of Mexico oyster production.

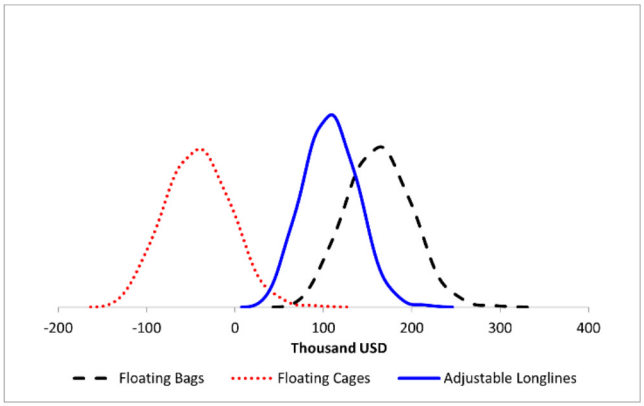

Figure 1. PDF of NPV across gear types with no environmental risk or insurance.

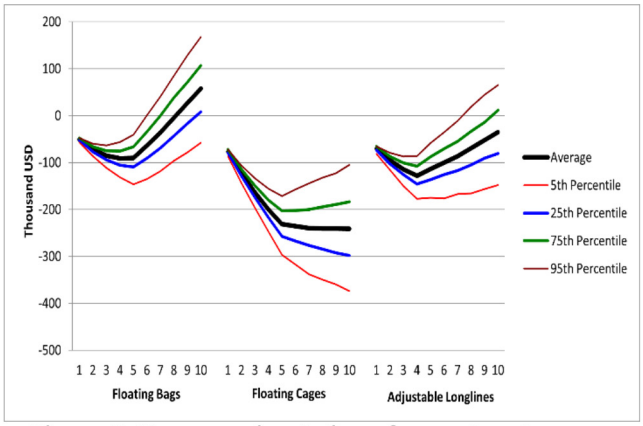

Figure 2. Ten year simulation of annual cash positions, across gear types, encountering high environmental risk level.

For our Gulf of Mexico case study, results showed that when no environmental risk was included, all three gear systems were profitable on an annual basis by the end of the 10-year time frame. The floating bag system had the highest average NPV (figure 1). When environmental events were introduced to the simulations, there was a predictable negative correlation between profit and the likelihood of environmental events. Floating cages were most adversely affected by these occurrences and had zero instances of a positive cash position by year 10 (figure 2). When risk mitigation strategies were introduced in the form of crop insurance, the economic difference between the four strategies were small. No insurance level had first order stochastic dominance.

As shown in our Gulf of Mexico example, differences in local environmental characteristics and production systems used can greatly influence profitability. For future applications of the model, we must be cognizant of the unique challenges to each region being analyzed and the species being farmed.

DETERMINING THE OPTIMAL BEHAVIOURAL BIOASSAY FOR THE COPEPODID STAGE OF PARASITIC SEA LICE

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The greatest barrier to growth in the salmon aquaculture industry is infestation by parasitic copepods known as sea lice. Sea lice can render fish unmarketable or vulnerable to secondary infections by feeding on the blood, skin, and muscle tissue of their host. Overuse of pesticides has resulted in drug-resistant strains, creating a need for new treatments. In the infective copepodid stage, sea lice must locate and attach to their host. Disruption of this behavior could be achieved by administering compounds to susceptible farmed salmon. Few behavioral bioassays are available to screen potential compounds. This project aims to reassess, develop, and compare behavioral bioassays to ultimately identify environmentally sustainable compounds for the abatement of these pests in salmon aquaculture.

In this project, bioassays were developed to measure the efficacy of anti-parasitic compounds in deterring sea lice from their salmon host. These bioassays were developed to target three measurable components of copepodid host search behavior; taxis, kinesis, and attachment. Taxis (general sea lice activity) was quantified using an arena bioassay, kinesis (directionality toward salmon derived cues) was assessed via a Y-tube maze, and attachment (physiological development required for infection) was analyzed using the development of a frontal filament in treatment bins. These bioassays were compared for reliability, variability, ease, and efficiency. Salmon "scent" (either salmon-conditioned-water or isophorone) was used in behavioral choice trials for positive control validation and comparison in all bioassays. Preliminary data suggest taxis to be a promising method of assessment of deterrent compounds through the response that sea lice exhibit to salmon derived chemical cues.

Reliable and validated behavioral bioassays are needed to assess compounds that could deter sea lice from finding and/or attaching to their host. Comparable bioassays and best practices would benefit researchers as well as industry. Identifying natural compounds that deter and reduce sea lice infestations will dramatically increase salmon aquaculture profitability and environmental sustainability.

SCALING SEAWEED AND SHELLFISH FARMING- IMPLICATIONS FOR RESOURCE USE

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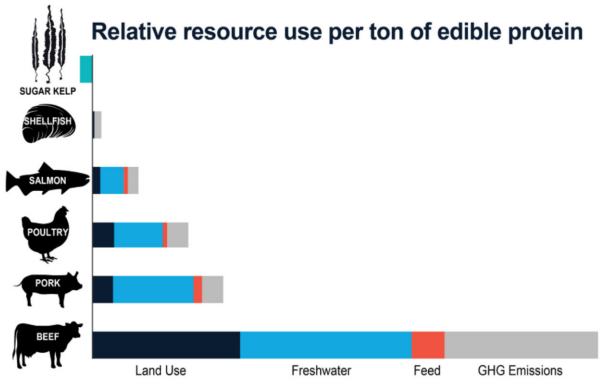
Seaweed and shellfish farming are distinct but complimentary tools used to mitigate climate change and disrupt resource-intensive feedstock production when brought to a meaningful scale. Both require no arable land, freshwater, or fertilizer and produce protein with the lowest resource use per ton of any food source while simultaneously playing a role in remediation of the surrounding water column. Both also face similar barriers to expansion.

Seaweed can be used as an alternative to animal feed and packaging material, displacing traditional crops and petroleum-based products. Accelerating growth of the seaweed industry could displace the use of commodities by 6.4 million tons and avoid over 1 million hectares per year of land use by 2030. Shellfish provide more protein than seaweed with significantly lower resource input than other animal sources and shell growth acts as a carbon sink. However, current domestic supply cannot meet demand and the US continues to import shellfish over long distances. This offsets the carbon footprint and resource use benefits of such a low-impact protein source.

This presentation will discuss the realistic potential for scaling seaweed and shellfish farming in order to provide alternatives that offset intensive resource use both during production and as an end-use product.



Graph 1. Credit Scott Lindell, Woods Hole Oceanographic Institute

The Seaweed Solution

Potential Climate Benefits from Scaling Seaweed Production

	Commodities replaced by seaweed (million tons)	Emissions avoided through commodity displacement ($mt CO_2 eq$)	Avoided land use through commodity displacement (hectares)	Potential sequestration benefit of avoided land use ($mt CO_2 eq$)
Human consumption (displacing vegetables)	2,800,000	1,900,000	160,000	520,000
Animal feed (displacing soy or corn)	2,000,000	5,100,000	540,000	1,800,000
Bioplastic (displacing petroleum)	1,600,000	4,100,000	440,000	1,400,000
Total	6.4 million tons commodities displaced	11 mt $CO_2 eq$ avoided	1.1 million hectares/yr avoided	3.8 mt $CO_2 eq$/yr potential sequestered

*Numbers are based on the methodology from Poore and Nemacek (2018) "Reducing food's environmental impacts through producers and consumers" and updated by the upcoming WWF's upcoming "Food Matters: Strategic Actions to Restore the Planet and Feed Humanity" publication.

2030 goal of 94 million tons

CASCADING EFFECTS OF A PANDEMIC ON SPORTFISH PRODUCTION IN KANSAS

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Long term effects of the aftermath from the COVID-19 pandemic are still not fully know. Due to mandatory leave and restrictive work environments there were shortages in production numbers of many sportfish species. Sorting the priorities considered the long term production needs as well as sportfish stocks projected as catchable in future years.

UTILIZING THE ONTOGENY OF DIGESTIVE ENZYME ACTIVITY AND DIGESTIVE TRACT MORPHOLOGY TO GUIDE LARVAL CULTURE PROTOCOLS FOR HOGFISH *Lachnolaimus maximus*

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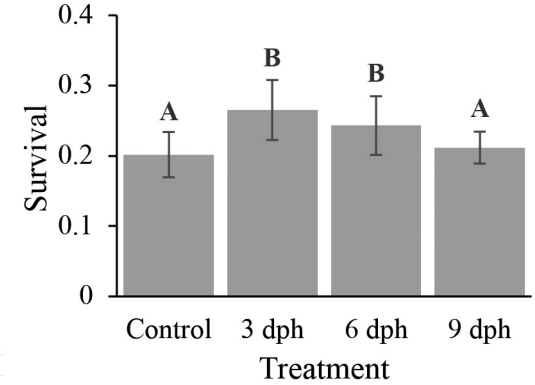
Hogfish (*Lachnolaimus maximus)* are large reef-dwelling wrasses endemic to Florida and the Caribbean. Florida *L. maximus* populations are popular targets for commercial and recreational fishers, which has led to increased management and concern regarding population status over the past several decades. Developing aquaculture protocols for this species would allow for the commercial production to meet market demands while simultaneously creating opportunities for stock enhancement. To date, no commercial culture protocols for *L. maximus* have been developed. First, larval development and nutritional requirements must be discerned for optimization of larval survival in the culture environment. Characterizing the ontogeny of digestive enzyme activity and digestive tract morphology will give insight into digestive capacity and potential nutritional protocols. Fertilized *L. maximus* embryos were collected, hatched, and raised on live feeds including copepods, rotifers, and *Artemia* nauplii for 45 days. Larvae were sampled throughout the trial to analyze growth, digestive enzyme activity, and digestive tract development. The activities of lipase, trypsin, and pepsin were analyzed using microplate spectrophotometric assays and digestive tract development was visualized using histology. The data from this trial was used to design a weaning experiment to transition *L. maximus* larvae from costly, labor-intensive copepod nauplii to more cost-efficient rotifers. Newly hatched *L. maximus* larvae were assigned one of four feeding regimes: copepods only (control) or rotifer transition at three, six, or nine dph with a three-day cofeeding period and six replicates per treatment. After 15 days, larvae were harvested and assessed for growth and survival. Larvae transitioned from copepods to rotifers at three and six dph had significantly greater survival compared to the other treatments (Figure 1). Total length was significantly lower in the control group compared to all other treatment groups. These results show that growing *L. maximus* larvae may be less laborious and costly to grow compared to other marine finfish that require prolonged periods of dietary copepods.



(+/- SE) alive at 15 dph (n=6, GLMM with Bonferroni distribution and estimated marginal means). Different letters above bars denote significance ($p<0.05$).

CHARACTERIZING THE DIGESTIVE ENZYME ONTOGENY AND GASTROINTESTINAL MORPHOLOGY TO INFORM LARVAL FEEDING PROTOCOLS FOR BLACKBANDED SUNFISH *Enneacanthus chaetodon*

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Blackbanded Sunfish (*Enneacanthus chaetodon*) are small ornamental centrarchids endemic to lakes and slow-moving water bodies from New Jersey to Central Florida. Their distribution has been increasingly fragmented due to habitat degradation, competition with non-native species, and in some cases, collection for the aquarium trade. Developing larval culture protocols is essential for the production of this species for the aquarium trade and for potential reintroduction to areas of extirpation. To gain insight into appropriate larval feeding and weaning protocols, the ontogeny of digestive enzyme activity and digestive tract morphology was assessed. *E. chaetodon* larvae were sampled 11 times from one-day post-hatch (dph) to 50 dph and analyzed for trypsin, lipase, and acid protease activity via spectrophotometric microplate assays. Histochemistry was used to visualize neutral and acidic mucopolysaccharides and gastric glands. The presence of gastric glands and acid protease activity indicated that *E. chaetodon* transitioned from agastric to gastric digestion at 40 dph. These data were used to design a trial to identify the appropriate timing for weaning larvae from live feeds to an inert microparticulate diet (MD). *E. chaetodon* larvae were assigned one of four feeding regimes: *Artemia* nauplii (control) or transition to MD at 36, 42, or 48 dph with a five-day cofeeding period (n=4). After feeding for 25 days, survival was greatest in the control group or when MD was introduced at 48 dph, and no differences in length were found (Figure 1). These data were used to design a dietetics trial to test the efficacy of commercially available MDs. 48 dph larvae were assigned one of four diets: *Artemia* nauplii (control), or one of three MDs (n=4). Certain MDs yielded higher survival than others and no differences in total length were found. Overall, larval *E. chaetodon* begin to transition to an adult mode of digestion around 40 dph and can be successfully transitioned to a MD after 48 dph without significantly affecting survival or growth.

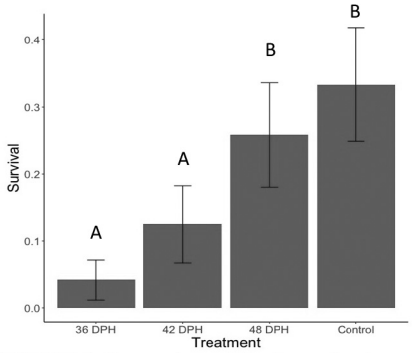

FIGURE 1. Proportion of *E. chaetodon* larvae alive (+/- SE) after 25 days of feeding different weaning regimes (n=4). GLMM with Bonferroni distribution and estimated marginal means). Letters above bars denote significance.

EFFECTS OF FEEDING A NOVEL FERMENTED SOYBEAN MEAL IN LOW INCLUSION FISHMEAL DIETS FOR PACIFIC WHITELEG SHRIMP *Litopenaeus vannamei*

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Pressures to reduce fishmeal consumption for sustainability reasons, combined with economic reasons, require intensive research efforts to find candidates for fish meal replacement. However, formulating low fish meal aquaculture feeds requires the use of combinations of several ingredients since most feedstuffs have been shown to have significant nutrient and functional limitations and cannot be used individually at very high levels in the diets of most aquaculture species. Fishmeal has always been the main source and the preferred choice of nutritionists for quality protein, above all in the formulation and especially in feeds for the youngest ages. Though, with the market volatility of fishmeal, the aquaculture feed industry is looking for cheaper sources of protein to substitute the fishmeal and this has become a priority. Additional renewable and sustainable protein alternatives are needed.

The use of a novel fermented soybean meal ME-PRO® along with low inclusion levels of marine proteins appeared to be a superior feed combination and analog to fishmeal and other marine proteins. The evaluation of several inclusion rates of ME-PRO® in broodstock, larvae and grow-out feeds have been evaluated in Pacific whiteleg shrimp, *Litopenaeus vannamei*. Data will be presented including examples of shrimp performance in recirculating aquaculture systems (RAS) with high stocking densities and final biomass of 4-5 Kg-shrimp/m^3.

USE OF HIGH PROTEIN DISTILLER'S DRIED GRAIN PRODUCT TO REPLACE POULTRY MEAL AND SOYABEAN MEAL IN PRACTICAL DIETS FOR THE CHANNEL CATFISH, *Ictalurus punctatus*

Sidra Nazeer*, Darci Carlos Fornari, Harsha S.C. Harsha S.C.Galkanda-Arachchige, Scott Tilton, D. Allen Davis

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As the ethanol industry matures and adopts new technologies, the co-products produced are also changing with their composition adjusted to meet feed industry needs. High protein distillers dried grain with yeast (HP42Y) is a relatively new product which is a variant of distillers dried grains that could be used as an improved protein source in catfish feed formulations. To evaluate the efficacy of HP42Y, a 10-weeks growth trial was conducted on the growth performance of juvenile catfish, *Ictalurus punctatus* (mean initial weight 1.80 ± 0.05g). In the growth trial, graded levels of HP42Y (0.00, 3.10, 6.20 and 9.30%) were used to replace poultry meal (PM: 6.00, 4.00, 2.00 and 0.00%) and other series of diets were used with HP42Y (5.00, 10.00, 15.00, 20.00, 30.00 and 40.00%) to replace soybean meal (SBM: 51.00, 46.49, 41.90, 37.40, 28.20, 19.20 %). Analysis of Covariance (ANCOVA) revealed a significant interaction between replaced protein (PM and SBM) and the inclusion level of HP42Y on biomass, mean final weight, weight gain, and FCR ($P<0.05$) of catfish. One-way Analysis of Variance (ANOVA) followed by Tukey multiple comparison test was used to test significant differences between treatment means of the tested variables for each diet type. In PM replacement series, complete replacement of PM resulted in poor performance, indicating a possible nutritional deficiency when the animal protein was removed. As a replacement for soybean meal, increasing levels of HP42Y only resulted in reduced growth of catfish when included in the diet at 30 and 40% respectively. The reduced performance at higher levels may be due to marginal levels of an essential amino acid, e.g. lysine, which warrants further review Results indicate that HP42Y is a good protein source when used at levels less than 30% of the diet.

GENETIC CHARACTERIZATION OF HETEROLOGOUS *Edwardsiella piscicida* ISOLATES FROM DIVERSE FISH HOSTS AND VIRULENCE ASSESSMENT IN A CHINOOK SALMON *Oncorhynchus tshawytscha* MODEL

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Edwardsiella piscicida is an emergent pathogen of finfish aquaculture. Although it is recognized as a pathogen with a wide host range, host associations driving genetic diversity remain unclear. This study investigated the genetic and virulence diversity of 37 *E. piscicida* isolates recovered from 10 fish species in North America. Multilocus sequence analysis (MLSA) was conducted using concatenated alignments of the *gyrB*, *pgi* and *phoU* sequences. MLSA clustered the tested isolates into six discrete genetic groups. In light of recent disease outbreaks in cultured salmonids, the virulence of each clade was evaluated in Chinook salmon *Oncorhynchus tshawytscha* fingerlings following intracoelomic challenge of ~10^6 CFU/fish. Challenged and control fish were monitored for 21d and microbiological and histological examination was performed on dead and survivor fish. Peak mortality occurred 3-5 days post-challenge (dpc) regardless of isolate or genetic group. *Edwardsiella piscicida* was recovered from all moribund and dead animals. At 21dpc, fish challenged with isolates from clades II, III and IV presented cumulative mortality ≥83. 3%, whereas isolates from clade I, V and VI resulted in cumulative mortality ≤71.4% (Figure 1). This study suggests an underlying genetic basis for strain virulence and potential host associations. Further investigations using other fish models and variable challenge conditions are warranted.

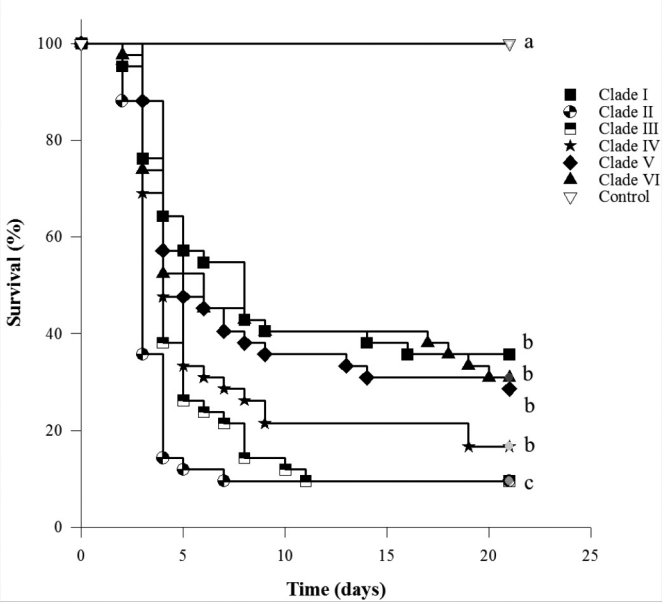

FIGURE 1. Average survival of Chinook salmon fingerlings challenged by intracoelomic injection with ~10^6 CFU/fish of 6 different clades of *E. piscicida* and PBS (control group) (n=14 per treatment isolate, 3 isolates/clade, n = 28 (2 tanks) for the control group). Different letters indicate significant differences in survival over the experimental period, based on the Kaplan Meyer survival analysis. (Survival analysis, SigmaPlot 11.0, $p < 0.05$).

VIRULENCE DIVERSITY OF *Flavobacterium columnare* GENETIC GROUP I and III TO DIFFERENT CULTURED RAINBOW TROUT *Oncorhynchus mykiss* STRAINS IN CALIFORNIA, USA

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Flavobacterium columnare is a filamentous Gram-negative bacterium responsible for significant economic losses in several freshwater fish and a re-emerging pathogen for wild and cultured salmonids in the US Pacific Northwest. *Flavobacterium columnare* strains have been assigned into four different genetic groups (GG) likely representing different bacterial species with some fish-host association. This study evaluated the susceptibility of four different rainbow trout (*Oncorhynchus mykiss*) strains cultured in California to three isolates typed in *F. columnare* GGs 1 and 3, the two more prevalent genetic groups affecting salmonids worldwide. The rainbow trout strains investigated are either native trout strains to California raised for conservation purposes, hatchery hybrid trout raised for sport fisheries or are imported strains selected for disease resistance or fast growth phenotypes. Virulence was determined through immersion challenges in two different strains of Steelhead trout, the Hoffer strain rainbow trout and the Shasta strain rainbow trout at 18°C. Central valley strain Steelhead trout, Eel river strain Steelhead trout, Hofer rainbow trout and Shasta strain rainbow trout challenged with GG1, presented a cumulative percent mortality (CPM) of 60-100%, 0-100%, 53.3-100%, and 100%, respectively. On the other hand, central valley strain Steelhead trout, Eel river strain Steelhead trout, Hofer rainbow trout and Shasta strain rainbow trout challenged with GG3, presented a CPM of 0-80%, 0-20%, 0-13.3%, and 0-26.7%, respectively (Table 1). *F. columnare* was recovered from gills and posterior kidney of moribund and recently dead fish in exposed treatments. Evaluation of up to 3 survivors in each treatment was performed to determine carrier status in gills and posterior kidney. The prevalence of *F. columnare* in survivors was 0% in both gills and posterior kidney of all challenged and control groups. The results suggest similar susceptibility to columnaris diseases in all strains of trout evaluated, and confirms GG1 greater virulence to trout when compared to GG3 isolates.

TABLE 1: Cumulative percent mortality (%) of different cultured rainbow trout challenged by *Flavobacterium columnare* genetic group 1 or 3 (n=15 fish per treatment isolate).

Fish species	GG1			GG3		
	Fc 80	Fc 81	Fc 117	Fc 101	Fc 206	Fc 208
Steelhead trout	100	93.3	60.0	0	0	80.0
Steelhead Eel trout	100	0	73.3	0	13.3	20.0
Hoffer rainbow trout	100	53.3	100	0	0	13.3
Shasta rainbow trout	100	100	100	0	0	26.7

BUILDING A MULTIDISCIPLINARY PATHWAY TO TILAPIA AQUACULTURE DEVELOPMENT IN KENTUCKY

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Most tilapia products (whole frozen fish, fresh and frozen fillets) eaten in the US are imported from China, Taiwan, Indonesia, Mexico, and Central and South America. Tilapia imports in 2018 were valued at $1.1 Billion, and US Tilapia sales were $39.4 Million. Tilapia imports more than quadrupled the last 30 years (56,000 to 230,000 Metric Tons [MT]) per year), and US Tilapia production remained well below 13,000 MT per year (6,554 MT in 2018). Though Tilapia is grown in Kentucky, production is minimal. In order to improve the livelihood and health of Kentuckians, and to expand local food production, the School of Aquaculture and Aquatic Sciences at Kentucky State University (KSU) started genetic improvement research to develop fast-growing Nile Tilapia (2015-19). The research demonstrated productivity advantages of genetic improvement methods used, and a superior cross that grew larger and faster than others tested was identified (Figure 1). These research findings led to a new and multidisciplinary Extension-based Tilapia Capacity Building Project (2021 to 2024) to bring the technology and products to the people that can use this – small-scale farmers, minority and limited-resource stakeholders, Extension personnel at KSU and University of Kentucky, and other public stakeholders and businesses. The project aims to stimulate local aquaculture growth, reduce economic and health disparities in minority and limited-resource families, and provide opportunities for development and delivery of training, education and Extension services in Tilapia Aquaculture, Marketing and Human Nutrition. This Extension project follows KSU's Mission as a historically black land-grant university to advance the Commonwealth of Kentucky, enhance society, and to impact individuals though teaching, research, and public service.

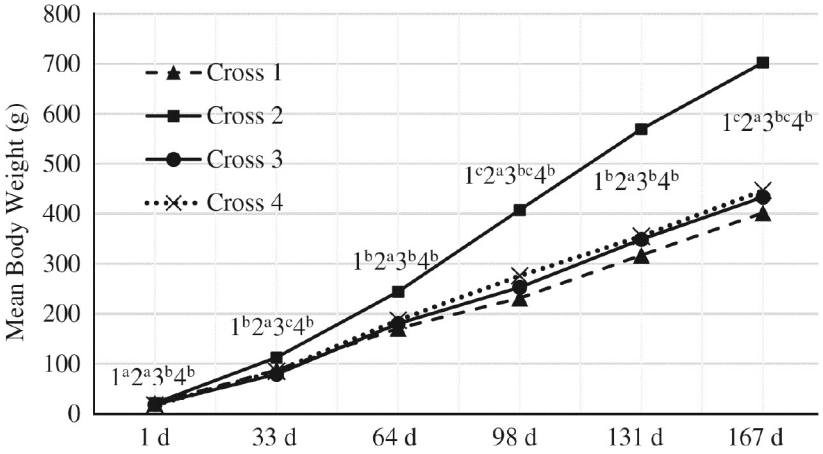

Figure 1. Growth of Nile Tilapia crosses. Different letter superscripts indicate significant body weight differences ($p < .05$). Modified from Novelo et al. (2021)

EFFECT OF DIETARY PROTEIN ON SPAWNING PERFORMANCE OF SINGIDIA TILAPIA *Oreochromis esculentus* (GRAHAM 1929) BROODFISH-KENYA

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Singidia tilapia (*Oreochromis esculentus*) endemic to Lake Victoria and once the most important commercial fish species in the Lake is now enlisted in the World Conservation Union Redbook (IUCN) of endangered species as critically endangered. To rescue this highly valued fish from total extinction, urgent conservational measures including captive propagation and reintroduction are required. This study was set out to investigate *O. esculentus* feed requirements for artificial breeding. Three feeds containing 20% CP, 25% CP and 30% CP were tested on 12 broodfish groups stocked in 12 nylon hapas (2x2x1 m^3) installed in earthen ponds. Females fed with 30% CP and 25% CP produced 40.1% and 36.3% more seed (eggs and york sac fry) respectively than females fed with 20% Crude protein (Table 1and 2). This study showed that the use of 25% crude protein diet is nutritionally adequate in *O. esculentus* seed production and that high protein diets were not beneficial in terms of seed output in Singidia tilapia. The study recommends the use of 25% crude protein level feed when producing seeds in *O. esculentus*.

Table 1*:* Absolute and relative fecundity of *O. esculentus* as affected by broodstock feed

Feed CP	Female Initial weight	Female Final weight	Female Mean Weight	Clutch size	Seeds/g Female	Seed/Female/ Day
20%	114.3± 3.47 ^{a}	216.8± 11.2 ^{c}	166.2± 9.33^{c}	503.0± 35.7^{b}	12.9± 0.33^{a}	16.8± 0.25^{b}
25%	114.5± 3.18^{a}	285.7 ±8.22^{b}	207.3± 15.2^{b}	685.8± 41.9^{a}	11.0± 0.19^{b}	22.9± 0.29^{a}
30%	113.8 ±5.65^{a}	314.5 ±6.06 ^{a}	251.1± 22.1^{a}	704.8±48.3 ^{a}	13.4± 0.16^{a}	23.5± 0.33^{a}

Means in the same column having different letters are significantly different ($P \leq 0.05$)

Table 2: Reproductive performance of *O. esculentus* broodfish subsequently fed with varying levels of crude protein during the spawning period of 120 days

Broodfish feed and reproductive parameters	Feed A	Feed B	Feed C
Total feed consumed (kg $hapa^{-1}$)	6.78 ^{c}	8.01 ^{b}	9.85 ^{a}
Clutch size ($no.clutch^{-1}$)	503.0^{b}	685.8^{a}	704.8^{a}
Seed/female day^{-1}	16.77 ^{b}	22.86 ^{a}	23.49 ^{a}
Seed g female	12.11 ^{a}	13.21 ^{a}	11.14 ^{a}
Seed yield ($no.m^{-2}$ day^{-1})	25.15 ^{b}	34.29 ^{a}	35.24 ^{a}
Seed output (no. g^{-1} feed)	1.78^{b}	4.14^{a}	3.46^{a}
Seed output(no. g^{-1} protein)	8.9 ^{b}	20.7 ^{a}	17.29^{a}
Protein Efficiency Ratio (PER)	12.25 ^{a}	8.11 ^{b}	12.43 ^{a}

Mean values in rows with same superscripts are not significantly different ($P>0.05$)

PRODUCTION QUOTAS AND REGULATORY RENTS IN NORWEGIAN SALMON AQUACULTURE

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Licensed production and production quotas are common in food production. When production has negative environmental impacts, these same measures are simple to manipulate to regulate aggregate production. However, command-and-control regulations are vulnerable to rent formation and production distortions. In aquaculture production, the regulatory rent mimics a resource rent. Command-and-control regulations remove property rights over fish stocks, a key feature that distinguishing aquaculture from the fisheries sector.

We develop a multiple-rotations optimal harvest and fish stocking model to investigate rent formation and production distortions across two regulatory regimes: unregulated and production quota regulated production. We parametrize the model and compare model predications in the unregulated and regulated regimes to recent developments in the Norwegian salmon aquaculture industry.

The private behavior predicted by the model under binding production quotas map closely to Norwegian industry developments since around 2012. Estimated regulatory the rents are substantial and suggest substantial wealth transfers to incumbent licenses owners, while simultaneously distorting production towards more intensive farming (greater stocking of fish, a shortening of the growth period, and smaller harvested fish). Stocking of fish is 11% greater, grow-out time 1.7 months shorter and fish weight 0.55 kilograms smaller than in a counterfactual regime when regulatory rents do not distort production decisions.

Our results point to important policy effects of command-and-control measures to limit production growth in aquaculture that might compromise policy objectives. Regulatory rents might be mistaken for resource rents, such as emerge in private fisheries, petroleum and other natural resource sectors. However, regulatory rents are temporary, and any tax regime need to be flexible enough to account for this as to not damage the future competitiveness of the industry. The rent is vulnerable to capture by competing markets, consumer substitution and cost escalation.

COMPARATIVE STUDY OF PHYSICO-CHEMICAL PARAMETERS AND TRACE METALS OF TWO DIFFERENT BOREHOLES IN TWO FARMS IN IKORODU, LAGOS STATE, SOUTHWEST NIGERIA

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The study examined the physicochemical parameters and trace metal contents of two boreholes in two different fish farms in Ikorodu. The samples were analyzed for pH, Turbidity, Electrical Conductivity, Total Dissolved Solids, Total Hardness, Total Suspended Solid, Total Alkalinity, Nitrates, Sulphates, Flourides, Phosphates, Residual Chlorine, Dissolve Oxygen, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Trace Metals like: Calcium, Magnesium, Sodium, Potassium, Zinc, Copper, Manganese, Iron, Nickel, Cobalt, Cadmium, Arsenic, Silver, Mercury, Lead, Chromium. were analyzed using Ultraviolent (UV) Spectrometer. The result obtained was compared with the World Health Organization (WHO) standard for drinking water. For sample A: pH value 5.5, Turbidity value 0.3 NTU and Sample B: pH value 4.2 and Turbidity value 0.2 NTU all fall below WHO permissible standard of 6.5 - 8.5 for pH and 5 NTU for turbidity. Trace metals such as Magnesium, Potassium, Zinc, Aluminum, cobalt, Arsenic and Mercury are not detected in the water samples while Calcium, Sodium, Copper, Manganese, Iron, Nickel, Cadmium, Silver, Lead and Chromium was detected in the analyzed samples using UV spectrophotometer. The result obtained was unsatisfactory when compared with World Health Organization (WHO) standard.

COMPARATIVE ANALYSIS OF WATER QUALITY PARAMETERS OF TWO COASTAL WATER BODIES (BAYEIKU AND OFFIN RIVERS) IN IKORODU DIVISION OF LAGOS STATE, SOUTHWEST NIGERIA

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Contamination of water bodies due to anthropogenic activities of man has been a major concern to mankind particularly in developing nations where regulations put in place by government are either not obeyed or no stringent regulations to monitor and punish the offenders. The biological wealth of a water body is mainly dependent on its physico-chemical parameters' quality. The Physico-chemical qualities of two waterbodies (Bayeiku and Offin Rivers) in Ikorodu division of Lagos State, Southwest Nigeria were sampled and analysed with the intension of determining the extent of pollution and the health of the water bodies. Five sampling stations each were established on both rivers and samples collected monthly for six months (March to August 2018). The physicochemical parameters were analysed using standard methods by APHA.

The parameters assessed are temperature, pH, turbidity, conductivity, hardness, salinity and dissolved oxygen (DO). The obtained result revealed that the average value of the parameters analyzed are; turbidity (29.71±23.03 NTU), conductivity (401.94±576.52 μS/cm), DO (33107.92±59091.09 mg/L), hardness (33107.92±59091.09) and that these values are above the WHO allowed limits except for temperature (29.09±1.65°C), pH (7.59±0.34) and salinity (3.05±3.45). One-way analysis of variance (ANOVA) and the 95% confidence level revealed that the value of each parameter was not statistically significantly different ($p > 0.05$) from one site to another. The variation of each parameter observed along the river was as a result of pollution from activities that take place along the river bank and the vicinity of the river.

OUT OF SEASON SPAWNING OF BURBOT *Lota lota* THROUGH TEMPERATURE AND PHOTOPERIOD MANIPULATION

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Intensive commercial production of burbot fingerlings will depend on consistent supply of eggs and larvae at multiple times during the year; however, no information on out of season spawning of burbot exists. The current study was designed to shift sexual maturation of burbot broodstock by six months from the natural spawning season through temperature and photoperiod manipulation. Results demonstrated that out of season spawning of burbot was successful. Broodstock, egg quality, and larval condition data were compared between out of season and in-season groups. For both production regimes, broodstock were sampled for condition factor and egg production, and embryos were monitored for development, viability, and survival. Larvae were reared to 35 days post hatch (dph) for growth and survival comparison. Pre-spawn condition factor for in-season fish was not statistically different from out of season fish, 0.81 (±0.03) and 0.89 (±0.03), respectively. Post-spawn condition factor for out of season females was not statistically different from in-season females, 0.83 (±0.06) and 0.66 (±0.07) respectively. However, post-spawn condition factor for out of season male broodstock was significantly higher, 0.82 (±0.04), than in-season fish, 0.64 (±0.02). Eggs produced relative to weight of pre-spawn female did not differ significantly between in-season and out of season production, 328.5 (±22.95) and 213 (±38.04) eggs/g, respectively. Fertilization did not differ significantly between treatments, averaging 90% for both in and out of season production. Egg survival was higher, 61.18% (±9.3), in the final week of incubation for out of season groups compare to eggs from in-season broodstock, 20.59% (±10.6). However, no difference in larval growth or survival was observed out to the 35dph termination point. Overall, egg quality and larval survival was not negatively impacted for broodstock spawned out of season. This is the first report demonstrating that burbot eggs can be produced outside the natural spawning season by altering photoperiod and temperature. As commercial production of this species develops, this will be an important tool to mitigate the risk of relying on a single annual spawning season and can ensure a consistent supply of burbot fingerlings throughout the year.

THE IMPACTS OF SUBLETHAL NITRITE EXPOSURE ON GOLDFISH MICROBIOMES

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Disease in aquaculture production threatens farms and undermines food supplies. Therefore, it is vital to understand immune health and disease transmission within farmed stock and into wild populations. Immune health is intertwined with numerous systems including the microbiome. Disruption of the microbiome, dysbiosis, can contribute to disease. A possible disruptor are agricultural pollutants which include excess nutrients, like nitrites. However, it is unclear how nitrites impact the microbiomes of various tissues and how these changes impact immune health. Additionally, a main portal for pathogen entry in fish is their nose; nasal infections can result in damage to the olfactory and nervous systems. This work aims to determine the impacts of sublethal levels of nitrite on the nasal microbiome and compare it to the gill and gut microbiomes.

Goldfish were held for 2 months under nitrite concentrations: 0.0 mM (control), 0.01 mM, 0.1 mM, and 1.0 mM. Tissue samples were collected from the nose, gills, and gut. The bacterial composition of the microbiome was characterized with high-throughput 16S rRNA gene sequencing. The gill microbiome showed no difference in community composition (Fig. 1). There was not enough evidence in the nose microbiome to determine a different composition, however, there appears to be a shift toward more diversity (Fig. 1). The gut microbiome showed a significant shift in composition as the level of nitrite increases. Sublethal levels of nitrite exposure significantly increases the diversity of microbial communities in gut microbiomes (Fig. 1). Therefore, environmental nitrites affect the composition of the microbiome in various tissues which could have detrimental consequences in normal physiological function in fish. Future experiments will increase sample size to better elucidate impacts to nose and gill microbiomes. We are working on developing probiotics to combat dysbiosis and prevent the negative impacts of nitrite exposure.

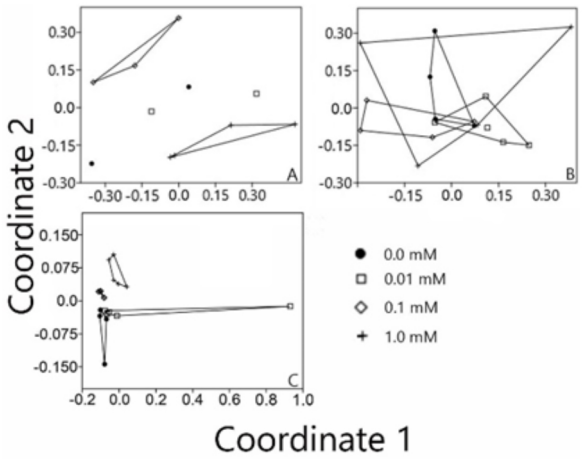

FIGURE 1. Non-metric multidimensional scaling (NMDS) of microbial similarity between tissues and treatments using a Bray-Curtis similarity matrix. (A) nose microbiome, (B) gill microbiome, and (C) gut microbiome. The gill and gut ordinations are fair; the nose ordinations are suspect.

POTENTIAL FOR USE OF AUTOMATED, HIGH-THROUGHPUT SPERM PROCESSING EQUIPMENT FOR CRYOPRESERVATION OF MICROALGAE

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Currently most microalgae are maintained and transported as live cultures. Some non-standardized, low-throughput methods exist for algal cryopreservation; high-throughput mechanisms and equipment are available for mammalian sperm that could be useful for microalgae. The sea hare, *Aplysia californica*, is a biomedical model that has a larval form requiring microalgae as food, and facilities need a method of raising and maintaining microalgal cultures without contamination. In collaboration with the National Resource for *Aplysia* (University of Miami), the AGGRC is researching methods and the effects of cryopreservation on two microalgal species, the diatom *Chaetoceros muelleri* and the haptophyte *Isochrysis galbana*. Microalgae were equilibrated in a penetrating cryoprotectant (CPA), 10% DMSO, + F/2 media for 20 min and packaged into 0.5-mL straws using a high-throughput Minitube MPP Quattro Filling and Sealing machine. Following equilibration, half of the samples were cooled at -40°C/min until reaching -80°C (CPA + Thaw) and subsequently stored in liquid nitrogen. The remaining samples (CPA + No freeze) were immediately diluted in F/2 media. Two days later, three straws of each species were thawed and diluted in F/2 media. All samples were cultured for 5 weeks and cell concentrations were tracked (Figure 1). CPA + Thaw cell concentrations was similar to the CPA + No freeze samples after a 2-week lag. Initially, the thawed *I. galbana* samples lost motility after freezing, which returned after 8 days. Both species appeared unaffected by the cryoprotectant but were hindered by the freezing process. The use of high-throughput equipment allows a single individual to fill and seal over 15,000 straws per hour. This allows for more efficient and standardized processing, storage, and transportation than with live cultures.

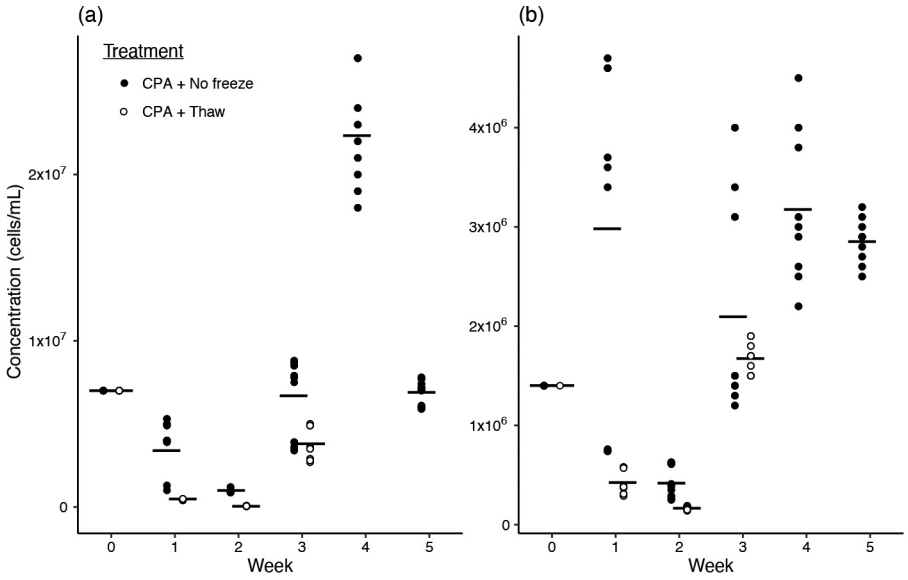

Figure 1. Cell growth of cryoprotectant-treated samples (CPA + No freeze) and thawed after freezing (CPA + Thaw) cultured at room temperature over the course of 5 weeks for the microalgal species (a) *I. galbana* (b) *C.* muelleri. The means of each treatment are denoted by a horizontal black line.

EVALUACIÓN DE DIFERENTES DIÁMETROS DE DIFUSORES EN SISTEMAS AIR-LIFT, UTILIZADOS PARA LA OXIGENACIÓN DE AGUAS EN CONDICIONES DE LABORATORIO

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ABSTRACT
The Air-Lift pumps have been implemented at present, due to the simplicity that they present at the time of their manufacture and also due to their low economic cost for the manufacture of them, so it was decided to conduct a study to analyze the efficiency of You are in oxygen transfer.

RESUMEN
Las bombas Air-Lift se han implementado actualmente, debido a la sencillez que estas presentan a la hora de su fabricación y también debido a su bajo costo económico para la fabricación de las mismas, por ello se decidió realizar un estudio para analizar la eficiencia de estas en la transferencia de oxígeno.

INTRODUCCIÓN

Las bombas "airlift" son un sistema utilizado hoy en día para elevar una mezcla de aire y agua a través de un tubo vertical, parcialmente sumergido en el líquido, en el cual es inyectado el aire comprimido en la tubería cerca del extremo inferior. Fawzy Sh y Jawdat A, 2017.

En el presente trabajo se diseñaran 4 modelos de bombas Air-lift con configuración en sus los diámetros de los orificios diferentes.

OBJETIVO

Diseñar, 4 modelos de bombas Air- Lift, evaluarlos y determinar la eficiencia de oxigenación.

METODOLOGÍA

El montaje y los ensayos, fueron realizados en el laboratorio de recirculación del programa de ingeniería en producción acuícola de la Universidad de Nariño, se diseñaron 4 modelos diferentes de Air-Lift con configuraciones distintas en cuanto al diámetro de sus orificios. (Tabla 1)

MODELO	Ø ORIFICIOS
A1	1/32"
A2	1/16
A3	1/16" Y 1/32"
A4	1/8"

Se diseña y se construye las bombas AirLift con tubería PVC de 1" y 2", luego se procede a realizar los ensayos correspondientes por cada modelo con ayuda de un compresor para el impulso de la mezcla aire-agua. Finalmente se analizan los datos obtenidos para de esta forma determinar cuál de las cuatro configuraciones diferentes da mejores resultados a la hora de la oxigenación del agua.

PERFORMANCE OF AQUACULTURE EFFLUENT FOR TOMATO PRODUCTION IN OUTDOOR RAISED BEDS

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Aquaculture is under great scrutiny as a source of nutrient pollution, specifically as a driver of eutrophication and harmful algal blooms in natural water bodies. The regulation of nutrient effluent is a major issue constricting the growth of the aquaculture industry in the US. Production facilities exceeding certain biomass load criteria are mandated to manage that effluent, which can be quite expensive and even cost prohibitive. Commercial field vegetable production techniques can be adapted to accept aquaculture effluent and grow crops for market.

In summer of 2019, effluent from a biofloc-style recirculating aquaculture system producing Nile Tilapia (*Oreochromis niloticus*) was used to perform two production trials in raised beds using tomato (*Solanum lycopersicum*) (figure 1). Each of the nine raised beds (6.1 m x 1.2 m x 0.3 m) and were filled with topsoil having a sandy loam to loamy sand texture. Irrigation lines were installed with one gallon per hour anti-clog drip emitters for each plant and set on a timer to water multiple times per day based on plant needs.

'Celebrity' tomatoes are a determinate, hybrid variety known for high yield, large size, excellent flavour and disease resistance. Differences in production were assessed for tomatoes grown using three different nutrient delivery techniques. Treatments included (1) a side-dressed granular fertilizer program, (2) fertigation with water-soluble nutrients, and (3) aquaculture effluent from a biofloc tilapia production system. Soil tests were conducted and raised beds were prepared with the appropriate amount of fertilizer for the production cycle, except for nitrogen, which was applied through the aquaculture effluent. Produce biomass, leaf greenness (SPAD), and foliar nutrient analysis data was collected as fruits reached marketable size. Comparative results will be presented.

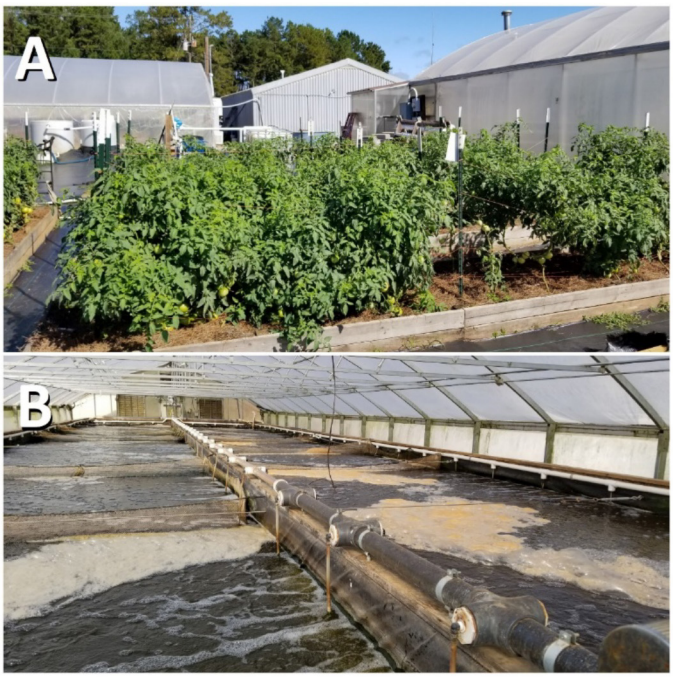

Figure 1. (A) Raised beds with tomatoes grown using aquaculture effluent. (B) Biofloc aquaculture production facility for growing tilapia.

ENHANCEMENT STOCKING OF JUVENILE TOTOABA (*Totoaba macdonaldi)* IN THE GULF OF CALIFORNIA

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Totoaba (*Totoaba macdonaldi)* is the largest member of the Sciaenidae family and are endemic to the Gulf of California (Sea of Cortez). Totoaba are very long-lived and may attain ages of up to 25 years or more, reaching sizes larger than 2 meters in length and 135 Kg. Pertaining to the croaker family, the Totoaba can create sounds with their well-developed swim bladder.

Totoaba swim bladder, also called Fish Maw or Buche, is highly prized by Chinese as an aphrodisiac and for fertility assistance, as well as a status symbol. The illegal fishing of Totoaba for the black market of Buches in the northern Gulf has driven the species into endangered population status. Commercial fishing was prohibited in 1975, but after many governmental impositions and regulations involving Totoaba and the by-catch of Vaquita, illegal fishing continues.

In attempts of protecting Totoaba and repopulating the species in the Gulf of California, one private company (Earth Ocean Farms - EOF) and two institutions from the public sector; Center for Reproduction of Marine Species of Sonora (CREMES) and the Autonomous University of Baja California (UABC) function as UMA´s (Ambiental Monitoring Units) and have closed the life cycle of Totoaba in marine hatcheries. These three entities have liberated more than 400,000 Totoaba in the Gulf of California. The UABC has released juveniles in Baja California between San Felipe and Puertecitos; CREMES near Bahia Kino in Sonora, and EOF in Mulege and Santispac in Baja California Sur. These efforts have aided in strengthening the population, in light of the continued illegal overfishing of Totoaba. The population of Totoaba is now observed 400 Kms to the south of its historical distribution. These three restocking sites act as recruitment areas, and the geographic distribution of recruitment increases the sustainability of the population.

It is imperative that illegal fishing of Totoaba cease immediately, and that the re-stocking programs continue in efforts of counteracting the effects of overfishing. It has also been recommended that mariculture of the species, as is the case with EOF, could produce a legal supply of swim bladder for the Asian market, and this legal trade should remove incentive to poach wild Totoaba for their swim bladders. This would be a win-win situation for the Totoaba and the Vaquita. With genetic traceability of juveniles produced by the UMA´s, the black-market trade of Totoaba swim bladders would decrease and eventually cease.

IMPLEMENTING DIDACTIC MATERIALS FOR TEACHING MEASURES OF CENTRAL TENDENCY IN THE CONTEXT OF AQUACULTURE

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Mathematics and Statistics have been traditionally stigmatized as abstract matters, difficult to apply and understand. However, the knowledge of both fields of study it is used, related, and interconnected with several aspects in our daily lives. It is critical to display how different subjects support each other to contribute to the analysis and solutions of problems. The aquaculture field exhibit a wide range of situations that interrelates mathematics and statistic knowledge and it could be used in education.

The department of Huila (Colombia) is recognized for the Magdalena River that run across its territory, and as one of the major tilapia aquaculture producers. Besides commercial aquaculture of tilapia, there are efforts for recovering and preserve artisanal fishing activity in the Magdalena River that have been developed by Enel-Emgesa and the Universidad Surcolombiana (USCO), at the Experimental Station of Hydrobiological Resources since 2017. Research on artificial reproduction is carried out at the station with native species of the Magdalena River, such as the bocachico (*Pimelodus reticulatus*), capaz (*Pimelodus grosskopfii*), dorada (*Salminus affinis*), pataló (*Icthyoelephas longirostris*) and peje (*Pseudopimelodus schutlzi*), to be used in restocking and potentially in fish farming.

We consider that the integration of knowing, doing and being is essential to develop the necessary skills in a world that increasingly requires citizens' commitment to social reality. The objective of the study is to evaluate the implementation of didactical materials that was developed using aquaculture data for the topic "Measures of central tendency" in statistics for students in grade nine. This work aims to strengthen the competencies related to recognizing the concepts and relationships between the mean, median and mode, as well as making simple inferences. A total of six lessons were developed to integrate statistical and aquaculture concepts (Table 1). The implementation of the didactic material includes an introductory video and the aquaculture contextualized lesson through a pedagogical guide. The implementation is being done virtually, and we will present the observations of this teaching-learning process.

Table 1 – Manual for teaching measures of central tendency in aquaculture.

Lesson	Aquaculture Topic	Statistical concept
1	Broodstock feed management	Random sampling, variable, types of variables, The Mean
2	Broodstock selection	The Median (Md)
3	Fish condition factor	The Mode (Mo)
4	Fish oocyte size distribution	Grouped data
5	Comparing two females' oocyte size distribution	The Mean, Median, and mode for grouped data
6	Stocking fingerlings	Review

MACROALGAL SULPHATED POLYSACCHARIDES MODULATE IMMUNE RESPONSE AND IMPROVE PATHOGEN RESISTANCE IN TILAPIA

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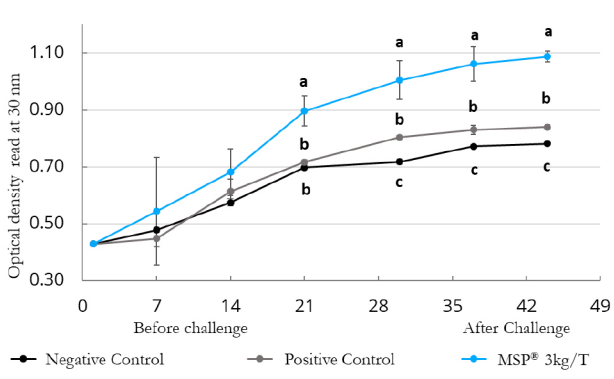
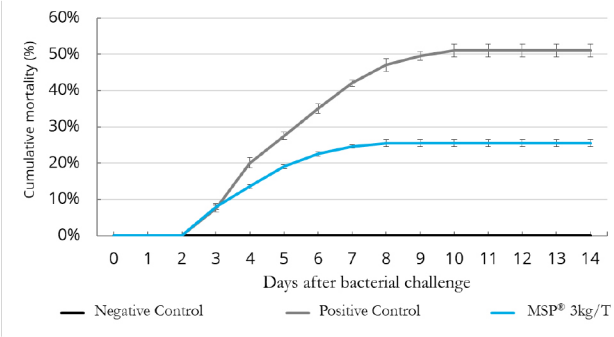
The cell wall of green and red marine algae is mainly composed of water-soluble sulfated polysaccharides with several biological activities, such as immunomodulation and strengthening of the intestinal barrier components respectively. Recent research has highlighted the potential of in-feed marine macroalgal polysaccharides to improve fish immune response, barrier integrity and resistance to pathogen 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 0.3% of green and red macroalgal polysaccharides at the MSP® group. Fish were fed four times a day at 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 reference. Blood samples were collected at days 1, 7, 14, 21, 30, 37 and 44 for respiratory burst analysis. Neutrophils oxidative radical production (ROS) were 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 were significantly higher in fish fed MSP® compared to the Positive Control group at days 21 and 30 before challenge (up to 25%) and days 37 and 44 after challenge (up to 29%). The mortality rate at day 14 was 49% lower in fed diets containing MSP® compared to Positive Control group (26% *vs* 51%). These results highlight the efficacy of MSP® to improve tilapia's resistance to pathogens.

Respiratory burst of Tilapia fed MSP® from green and red algae



Mortality of Tilapia under bacterial challange and fed MSP® from algae



MACROALGAL SULPHATED POLYSACCHARIDES MODULATE IMMUNE RESPONSE AND IMPROVE PATHOGEN RESISTANCE IN OLIVE FLOUNDER

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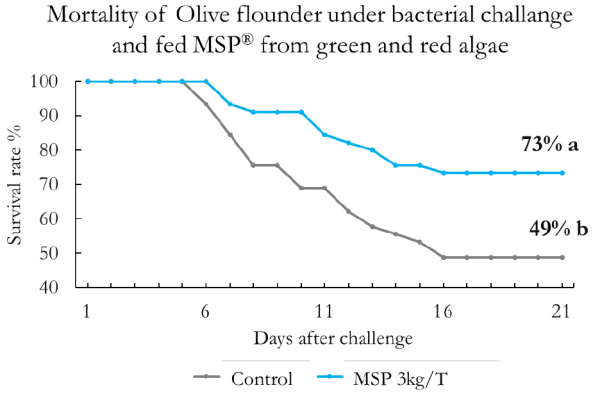
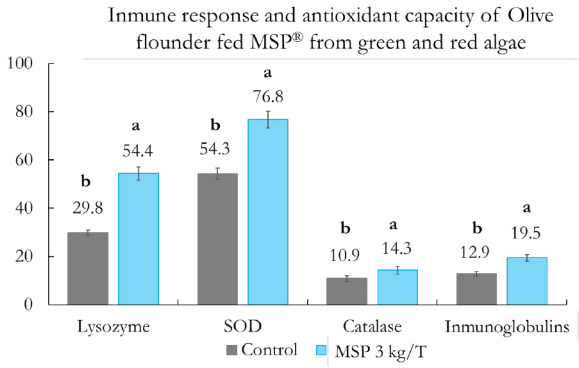
The cell wall of green and red marine algae is mainly composed of water-soluble sulfated polysaccharides with several biological activities, such as immunomodulation and strengthening of the intestinal barrier components respectively. Olmix has been able to isolate, identify and concentrate different macroalgal sulfated polysaccharides which are naturally available in the coast of Brittany (France) being it one of the most important sustainable macroalgae resources in the world. Macroalgal extracts are potentially useful in aquaculture to modulate immune response and to enhance epithelial cells integrity in the gills, skin and gut which are most important lymphoid tissue 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 the 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 0.3% of green and red macroalgal polysaccharides at 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, fish 44/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 week 12 before challenge for immune assay and antioxidant capacity. Lysozyme activity was quantified by turbidimetric method using *Micrococcus lysodeikticus*. Superoxide dismutase (SOD) and catalase activities were assessed by commercial kits, Sigma Aldrich and Biovision, respectively. Total immunoglobulin was quantified using micro-protein determination method. Lysozyme activity and total immunoglobulins were greater in MSP group by 45% and 34%, respectively. Likewise, SOD and catalase activities were increased by 29% and 24% by MSP®. Mortality rate was 33% lower in fed the MSP® (49% *vs* 73%). The use of MSP® improved the immunity, antioxidant capacity and pathogen resistance in olive flounder.

SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF LIMBAUGH'S DAMSELFISH (*Chromis limbaughi*), A POMACENTRID UNDER SPECIAL PROTECTION STATUS

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Limbaugh's damselfish, *Chromis limbaughi*, a member of the family Pomacentridae, is an ornamental fish endemic to the Gulf of California in Mexico. It is a beautifully colored fish, especially during the juvenile stages. The head and the anterior two thirds of the body are iridescent blue, and bright yellow in the posterior part of the body, including the dorsal, anal, and caudal fins. Limbaugh's damselfish is a relatively small-sized fish (10 cm maximum standard length), and unlike many damselfishes, it is relatively peaceful, which makes it compatible with many others fish species in the community tank, which are sought-after traits by saltwater aquarium hobbyists. For these reasons, *C. limbaughi* is one of the most exploited species for the aquarium trade in the Gulf of California. Regrettably, commercial trade of *C. limbaughi* is based solely on wild-caught specimens. Due to its endemism, conservation concerns have arisen, which prompted the Mexican government to place *C. limbaughi* in the list of ornamental fishes with special protection status for wildlife conservation NOM-059-SEMARNAT-2001. However, legal, small-scale fishing for *C. limbaughi* is still ongoing, not without suspicion that this species is being overexploited, because catch reporting is not appropriately enforced.

Because recovery from fisheries exploitation is a very slow process, captive breeding techniques developed for aquaculture are one viable option to mass produce fish seedstock, which would not only ensure a steady supply of captive-bred fish to meet the demand for marine ornamentals, but also it would alleviate fishing pressure and support stock enhancement of threatened species.

Thus, as a first step towards achieving the goal of reproducing *C. limbaughi* in captivity, this project investigated some aspects of its reproductive biology. After obtaining a fish collection permit for scientific purposes (Permit No. SGPA/DGVS/06436/17) from the Mexican Agency for the Environment and Natural Resources, we carried out seasonal fish samplings at San Esteban Island, Gulf of California, Mexico. Fish were caught by SCUBA diving using hand dip nets. After being euthanized with an overdose of MS-222, fish were individually weighed, measured, and dissected to determine the body indices, such as condition factor and gonadosomatic index. Each gonad was divided into two parts, one was used for the histological evaluation of the gonad developmental stage, and the other for the determination of the proximate composition and the fatty acid profile. With this information, reproductive biology parameters were determined, such as male to female ratio, length of the reproductive season, and size at first sexual maturity, among others. It is expected that information obtained from the fatty acid profiles of the gonads will provide insight into broodstock nutritional requirements, particularly for lipid and prominent fatty acids. Not less importantly, reliable information of actual field water quality conditions, under which maturation of this species takes place, was gathered in the present study, which will hopefully prove useful for the management of *C. limbaughi* broodstock in captivity.

ASSESSMENT OF SALINITY IMPACTS ON MICROBIAL COMMUNITIES ASSOCIATED WITH THE FLORIDA POMPANO (*Trachinotus carolinus*) IN LARVICULTURE

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Salinity presents economic and technical challenges in land-based recirculating systems in the U.S. warm water marine finfish aquaculture industry. In addition to influencing osmoregulation and osmotic stress of fish larvae, salinity can influence the larvae microbiome. Salinity is a primary abiotic determinant of diversity of free-living prokaryotic microorganisms within an aquaculture system since different microbial taxa thrive at different salinities. System microbiota affect colonization of fish tissues including skin, gills, and gut. Fish microbiota can also modulate host metabolic pathways and activate host-microbiota interactions in response to osmotic stress. Studies suggest that microbial populations vary between freshwater and marine water fish species, and those reared in lower salinities may comprise fewer beneficial bacteria and more opportunistic pathogens. Identifying favorable and unfavorable microbes in both the fish microbiome and aquaculture system at different salinities can have implications for health management and disease suppression during development in larviculture.

Our study aims to optimize hatchery production of Florida Pompano (*Trachinotus carolinus*) by determining changes in the microbiomes of the fish and tank water when subjected to different salinities in larviculture. Larvae were reared at different salinities of 10, 20, or 30 ppt in triplicate and larvae samples were collected every three days until time of weaning (24 days post hatch). Total genomic DNA (gDNA) was extracted from homogenized whole larvae samples using the Qiagen Blood and Tissue Kit. In tandem with larvae sampling events, environmental DNA (eDNA) was concentrated from 500 mL of tank system water using Smith-Root eDNA filter packs. Total eDNA was extracted from the filters using the Qiagen DNeasy PowerWater Kit. High-throughput DNA sequencing (16S metabarcoding on Illumina's HiSeq 2500 System) was used to identify the prokaryotic taxonomy within Florida Pompano gDNA and corresponding water eDNA from each salinity treatment.

We hypothesized that the microbial composition of Florida Pompano larvae and tank water would change between different salinities, with opportunistic pathogenic microbes such as *Vibrio* species being more abundant at 10 ppt. Additionally, bacterial members of the fish microbiome at important developmental milestones should be comparable to those found in the system environment. This study will help determine the lowest salinity required for successful hatchery production of Florida Pompano based on salinity tolerances specific to the fish larvae and a healthy microbiome. Our findings will also allow for the identification of targets for probiotics in near-future diet studies as well as potential early indicators of common aquaculture diseases.

DEVELOPMENT OF LARVICULTURE PROTOCOLS FOR REARING THE ECOLOGICALLY IMPORTANT LONG-SPINED SEA URCHIN *Diadema antillarum* WITHIN A NOVEL RECIRCULATING SYSTEM

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The long-spined sea urchin (*Diadema antillarum*) was once an abundant reef-grazing herbivore throughout the tropical western Atlantic. In 1983-1984, an unidentified disease affecting *D. antillarum* caused 93-100% mortality on Caribbean coral reef populations. This event resulted in a sudden lack of herbivory and contributed to ecological phase shifts from hard coral dominated to macroalgae dominated reef systems. Recovery has been slow or nonexistent and attempts to restore resilient coral reefs at scale would benefit from augmenting herbivory via establishment of sexually propagated *D. antillarum*. A critical first step in this direction is the ability to reliably produce this species from gametes. However, a difficult and lengthy larviculture (Fig. 1A) period of ~40 has resulted in inconsistent success over the past 30 years.

The purpose of this study was to develop protocols for rearing *D. antillarum* within a novel 1800 -L recirculating aquaculture system (RAS). An initial trial examining the effects of microalgae diet (*Tisochrysis lutea* and *Chaetoceros* spp.) densities on larvae stocked at 4 ml^{-1} in 40-L replicate tanks resulted in similar growth and survival between high density (40k cells ml^{-1}, n=5) and low density (10k cells ml^{-1}, n=5) treatments at 21 days post fertilization (DPF). The high-density treatment resulted in 6.6% growth per day and was used to establish a reference diet for subsequent trials. Further experimentation examined the effects of carbon-equivalent microalgae diet combinations on growth and survival. In two separate experiments, diets containing *Rhodomonas lens* outperformed the reference diet at 21-DPF and 42-DPF (Fig. 2). Data generated within the RAS were used to establish fundamental larviculture protocols that have since produced juvenile *D. antillarum (*Fig. 1B). Current efforts are focused on improving yields to achieve restoration relevant scale.

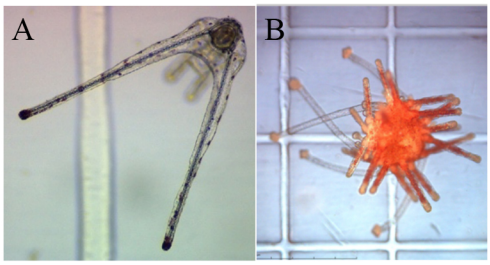

Figure 1. (A) 14-DPF *D. antillarum* larvae, (B) settled juvenile urchin

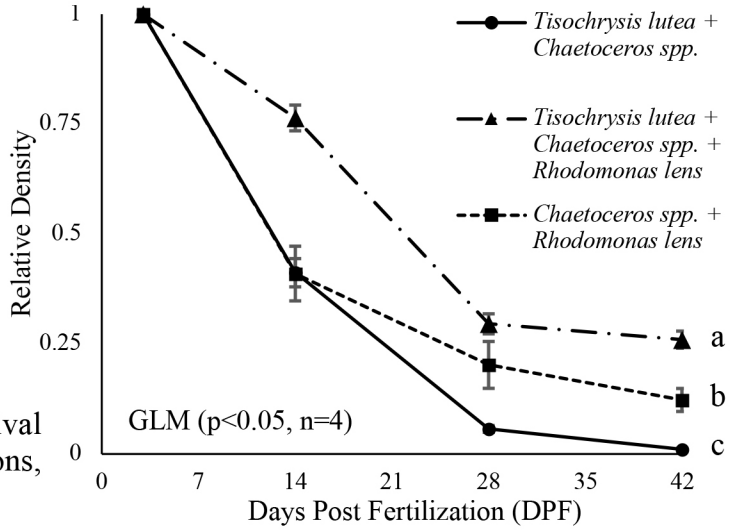

Figure 2. (right) Proportional survival over time across three diet combinations, error bars represent SEM.

FACTORS INFLUENCING PRODUCTION LOSS IN SALMONID FARMING

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Using a unique dataset, this paper investigates factors influencing production loss in Norwegian salmonid farming. The factors can be grouped into fish-specific factors (e.g. species, genetics, and generation), input factors (e.g. vaccines and smolt quality), environmental factors (e.g. geographical location), and managerial factors (e.g. ownership). The most important result is most likely that production losses to a large extent are explainable, as our best model has an R^2 as high as 0.826. This implies that it is possible to reduce production losses significantly. For the specific factors, vaccines reduce production loss, but their effect varies by production site. Production loss also varies with which smolt plant is providing juvenile fish, indicating that there is systematic quality variation among the providers of smolt. There is also significant variation in production loss between companies and production sites, and on average production losses are lower for larger companies and sea sites holding larger numbers of fish. An important point is that while some factors explaining production loss are controlled by the individual company, others are beyond their control. Some of these external factors are related to the regulatory system.

SEAFOOD MARKET IN BRAZIL

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Seafood consumption in Brazil has increased over time, but is still below the world average of 20.5 kg. per capita (FAO, 2021). Most of the increase comes from imports and aquaculture supply (Tab. 1). Imported farmed salmon and other whitefish (e. g. sharks and Alaska pollock), and farmed tilapia produced in Brazil are among the main groups driving the seafood consumption increase. Given a growing importance of fish as a protein and nutritional source, it is important to understand the seafood market boundaries and interactions between products in the market place. In this paper, the relationship between the price of salmon and other local fish groups (tilapia, spotted sorubim, weakfish flatfish, common snook, mullet, shark, sardine, and tuna) sold locally in the biggest wholesale market in Brazil is analysed, from February 2014 to January 2021. In addition, interviews with key stakeholders in the different levels of the value chain (from importers to restaurant owners) are carried out to identify potential opportunities and challenges for continued seafood consumption growth. The results from market integration tests point toward an imperfectly integrated fish market. Furthermore, from the interviews, flexibility in the restaurant's menu, and importers and distributors product bundles play an important role in the substitution possibilities among different fish products and species. The main challenges for future growth pointed out by the interviews are high fish prices (in particular compared to other sources of protein in Brazil), and a meat based food culture in Brazil.

Table 1. Brazilian seafood sources (fisheries, aquaculture, and imports) and exports over time. Source: FAO (2021)

Brazilian seafood sources:	1995	2000	2005	2010	2015	2017
fishery ('000 t)	607	667	750	785	840	850
aquaculture ('000 t)	46	172	258	479	590	650
import ('000 t)	264	182	140	264	306	384
export ('000 t)	25	52	86	28	31	38
Total seafood availability ('000 t)	892	970	1,062	1,500	1,705	1,846

References

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FACTORS TRIGGERING DISEASE OUTBREAKS IN AQUACULTURE PRODUCTION SYSTEMS

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In recent years, the adoption of intensive production practices has tremendously increased aquaculture production. However, with intensification comes added pressure on production systems, which often leads to infectious disease outbreaks. The pathogens causing diseases in aquaculture production systems are primarily categorized as bacteria, viruses, fungi, and parasites. The dynamic interactions of the elements constituting the epidemiologic triad namely host, pathogen, and the environment are key to disease outbreaks in any system (Figure 1). Citing reported cases, this retrospective review explored the subfactors of each key element and how the complex interplay among them might have contributed towards disease outbreaks in production systems. Factors discussed here include pathogen virulence, host-specificity, mutations, transmission routes, mechanisms of infection, methods of interaction, vectors, co-infections, stress, and sub-optimal environmental parameters off-balancing the epidemiologic triad. Proactive methods to enhance the health of the aquaculture species and biosafety measures to prevent the spread of infectious diseases are also mentioned. In nutshell, this review covers the epidemiological triad and their associated factors, the imbalance of which directly or indirectly culminate in disease outbreaks. It also discusses potential management aspects such as vaccination and biosecurity measures to minimize disease incidences.

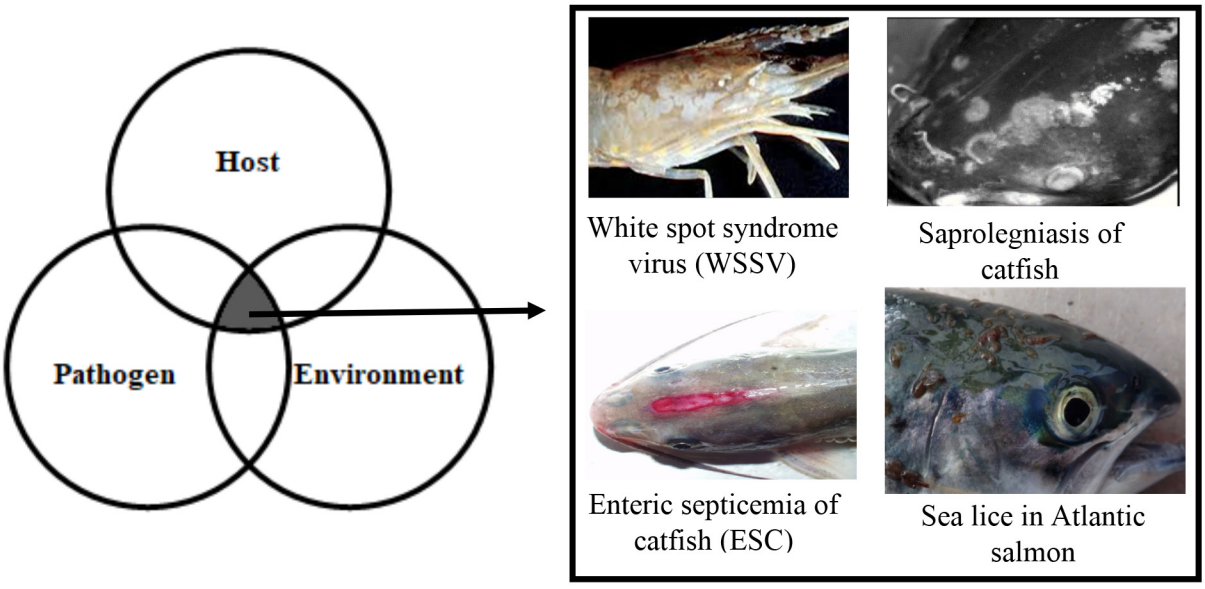

Figure 1. Imbalance of epidemiologic triad (host, pathogen, and environment) resulting in viral (WSSV), bacterial (ESC), fungal and parasitic diseases (Snieszko, 1974; Lightner, 1996; Hawke, 1979; Durborow et al., 2003; Erkinharju et al., 2020).

MEASURING CONSUMER WILLINGNESS-TO-PAY FOR LIVE SEAFOOD

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The live seafood market is an important market channel for fish producers in the Northcentral region of the US. This channel begins with the fish farmer, through a wholesaler, a broker or aggregator, retailer/restaurant, and ends with the consumer. Processing of the fish is done at the retailer/restaurant level and consumers who patronize live seafood markets are served fish products that have not undergone industrial processing or preservation, which is important for meeting their taste, freshness, quality, and food safety preferences. If these consumers are willing to pay premiums for different live seafood species, fish farmers can receive a high percentage of the dollar amount paid. This study focuses on 4 seafood species: Largemouth bass, hybrid striped bass, bluegill, and barramundi, which are major species produced in the Northcentral region.

A national online survey was conducted to assess consumer willingness-to-pay (WTP) for live fish. We collected information on consumer demographics and responses from a choice experiment. The experiment presented respondents with hypothetical purchasing choice scenarios where each choice scenario had four labeled alternatives and a no-buy option to simulate a real purchasing situation. Each species was presented with two attributes - price and source region (Northcentral vs non-Northcentral source). There was a total of 1,278 choices made by 215 respondents in the choice experiment.

Statistics of respondents show 62% are male, 80% are aged less than 45 years, 59% earn less than $100,000 annually, and 77% is white. The Asian racial/ethnic group was 6% of the respondents. In the choice experiment, hybrid striped bass was chosen the most (25.5%), followed by largemouth bass, 23.1%; bluegill, 22.2%; barramundi, 13.5%; and the none option, 15.6%. Two regression analyses, Random Parameters Logit (**RPL**) and Error Component Random Parameters logit (**EC-RPL**) were estimated with the choice experiment data.

RPL Model: Both the total and marginal WTP estimates show total WTP of $20.17 for hybrid striped bass, $19.37 for bluegill, $17.67 for largemouth bass, and $14.71 for barramundi. These estimates show consumers are willing to pay $15 - $20 for live fish while the reference prices used in the study range from $7 - $15, signifying a higher consumers' stated WTP for live fish species. The absolute values of marginal WTP, which give an idea of how fish prices would differ in the real market, range from $0.80 to $5.46 for the three species.

EC-RPL Model: The EC-RPL model incorporated demographics and results show that male, age less than 45 years, and $100,000+ income earners are more likely to purchase live fish. Asians are also found to prefer barramundi than the other species. Male consumers are willing to pay $20.50 for largemouth bass, $20.07 for hybrid striped bass, $23.64 for bluegill, and $23.14 for barramundi. High-income earners are willing to pay $5.69 for bluegill, $6.55 for hybrid striped bass, $6.73 for barramundi, and $8.90 for largemouth bass. The low WTP estimates for high income earners suggest that there is only a marginal difference between high- and low-income groups. Asians -consumers are willing to pay $12.39 for barramundi relative to non-Asian consumers.

PRELIMINARY RESULTS FROM A LARGEMOUTH BASS *Micropterus salmoides* BROODSTOCK NUTRITION TRIAL

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The global aquaculture value and quantity of largemouth bass (LMB) ranked 16 and 19, respectively in freshwater species in 2018, with a total production of 0.458 million tons in 2017, mostly produced in China (Hussein et al. 2020). However, unpredictable, and variable reproductive performance have been indicated as primary bottlenecks for the industry (Hussein et al. 2020). Nutritional status of the brooders is critical for spawning and egg quality since nutrients are transferred from the female to the egg (Migaud et al. 2013; Izquierdo et al. 2015). Aside from essential fatty acids and amino acids, there is potential for other dietary additives such as nucleotides to enhance broodstock nutrition. Nucleotides consist of a purine or a pyrimidine base, a ribose or 2´-deoxyribose sugar and one or more phosphate groups and have essential physiological and biochemical functions related to genetic information, energy metabolism and cell signaling (Li and Gatlin, 2006).

A pilot study to evaluate the impact of essential fatty acids and nucleotides in the LMB spawning performance was set up in a recirculating system composed of three tanks. Each tank was stocked with 100 fish, and fish were fed for 92 days, with three different diets (Diet 1 - commercial diet, Diet 2 - commercial diet supplemented with fish oil and DHA, and Diet 3 - commercial diet supplemented with a mix of fish oil, DHA and commercial nucleotides). Data collected during this pilot study is presented in the table below. Currently, we are conducting nutritional analysis on the feeds, fish eggs and fry collected for each treatment. While the results of this study were not shown to be significantly different, the increased number of fry obtained for each treatment may suggest an effect of the diets in the egg quality. These results are very encouraging for expanding the knowledge of the effect on the nutrition of reproductive performance of the largemouth bass.

Table 1. Preliminary results from pilot study.

Parameter	Diet 1	Diet 2	Diet 3	Total
Number of Spawns	45	39	50	134
Percentage of spawns (%)	33.6	29.1	37.3	100.0
Average Fry/mat				
First batch	882	3,484	3,021	
Second batch	2,412	5,914	3,137	
Number of Fry				
First batch	24,983	67,941	59,925	152,849
Second batch	32,146	82,383	69,497	184,026
Total number of Fry	57,129	150,324	129,422	336,875

TOXICITY EFFECTS OF SMOKED AND UNSMOKED FILTERED CIGARETTE BUTTS LEACHATE ON AFRICAN CATFISH (*Clarias gariepinus*)

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In this study, the toxicity of smoked and unsmoked filtered cigarette butts of various brands was conducted on *Clarias gariepinus.* The mean bodyweight of the fish was 4.72g. Leachate of cigarette butts was prepared by soaked 24 smoked (4.21g) and unsmoked (4.82g) cigarette butts in six litres of water for 24 hours. The leachate was serially diluted to obtain concentration of 8 cigarette butts per litre (8CB/L), (6CB/L), (4CB/L), (2CB/L), (1CB/L), (0.5CB/L), (0.25CB/L) and (0CB/L). Two hundred and forty (240) *C. gariepinus* of 21 days old were randomly exposed to the 8 different concentrations with ten fishes per treatment and replicated 3 times. T1 was exposed to 0CB/L, T2 (0.25CB/L), T3 (0.5CB/L), T4 (1CB/L), T5 (2CB/L), T6 (4CB/L), T7 (6CB/L) and T8 (8CB/L). The experiment was carried out for 96 hours. The result shows that the mortality of *C. gariepinus* increased with an increase in concentrations of the cigarette butts and time of exposure. Table 1 shows that there was 100% mortality in T8, T7 and 36.7, 33.3, 16.7, 13.3, and 6.7% mortality was observed in T6, T5, T4, T3, and T2 respectively after 96 hours of exposure while there was no mortality in the control treatment (T1). Table 2 shows the results of LC50 and $LC_{95.}$ LC_{50} value of 0.57 and LC_{95} value of 2.30 were obtained after 96 hours of exposure. The result of the physico-chemical water quality shows that there was no significant difference ($p>0.05$) in the mean temperature, dissolved oxygen and salinity of the leachate but there was a significant difference ($p<0.05$) in the mean pH among the treatments. This study shows that cigarette butts leachate is toxic to freshwater fish.

Table 1: Result of the percentage mortality of *C. gariepinus* response to different concentration of cigarette butts leachate

T	% Mlty at 24 hrs	% Mlty at 48 hrs	% Mlty at 72 hrs	% Mlty at 96 hrs
T1	0	0	0	0
T2	0	0	0	6.7
T3	0	0	10	13.3
T4	0	6.7	13.3	16.7
T5	6.7	16.7	23.3	33.3
T6	6.7	23.3	30.0	36.7
T7	100	100	100	100
T8	100	100	100	100

T (Treatment) Mlty (Mortality), hrs. (Hours)

Table 2: lethal concentrations of the cigarette butt leachate.

Time (Hrs)	LC_{50} (95% CL)	LC_{95} (95% CL)	Probit Equation	DF	TF
24	7.12 (3.47 - 0.90)	40.41 (2.32 - 1.40)	Y=1.81+5.36X	7	1.00
48	1.99 (2.1 - 0.46-)	6.76 (3.73 - 3.04)	Y=3.37+2.19X	7	3.58
72	0.96 (4.15 - 1.88)	3.41 (2.09 - 1.72)	Y=2.06+4.41X	7	7.41
96	0.57 (1.39 - 0.84)	2.81 (4.21 - 3.63)	Y=2.91+3.80X	7	12.49

LC (Lethal Concentration); CL (95% Confidence Limit); DF (Degree of freedom); T.F (Toxicity factor) = LC_{50} value of most toxic hrs/LC_{50} value of other periods

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

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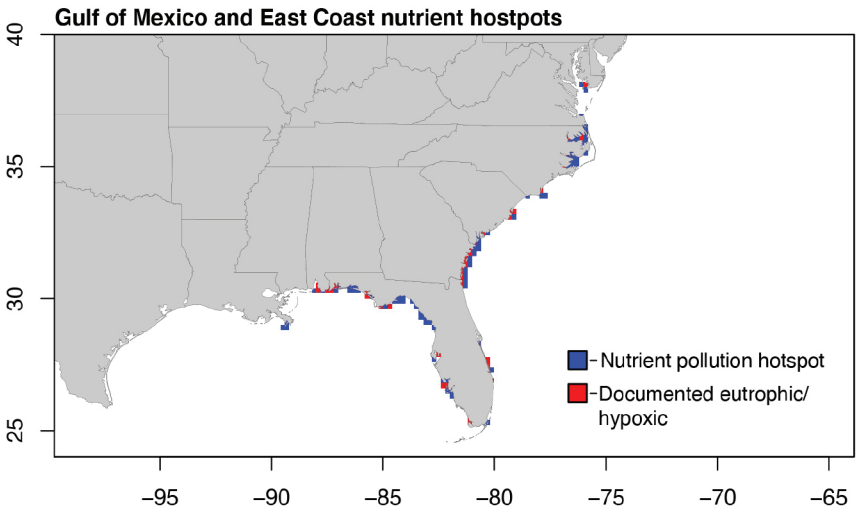
As the United States marine seaweed aquaculture industry develops, there is immense opportunity to site farms in locations that maximize the co-benefits of seaweed aquaculture, including nutrient assimilation. Seaweed aquaculture is capable of removing large quantities of nitrogen and phosphorus from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

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



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CATFISH CLCA5.2 mRNA SECRETED INTO MUCUS ALONG WITH p53

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The stringent regulations on the use of antibiotics call for new solutions to treat bacterial infections. Mucus is the first line of defense that fights pathogens in fish. Mucus consists of humoral factors that include metalloproteases that act as antibacterial peptides. CLCA genes are well conserved across species, including fish. We have identified catfish EST similar to zCLCA5.2 isoform, a putative soluble protein secreted in the fish mucus. CLCAs are known to be stress-inducible genes in other species and are shown to induce cytokines in the Staphylococcus aureus disease model. The CLCA protein family is intimately tied to mucus secretion and goblet cells. P53 is known to bind the CLCA promoter and induce its expression in response to stress. This study investigates the channel catfish CLCA regulation after *Aeromonas hydrophila* infection in a time-dependent manner. Interestingly, we find that cCLCA 5.2 and p53 and p21 mRNA are packaged in mucus secretions. The CLCA expression is significantly higher in gills and mucus at 8 and 12 hrs, directly correlated with P53 and P21. Also, we found that the expression of all three genes increased in the head kidney compared to the tail kidney suggesting a role in an immune response. Overall, we speculate that cCLCA5.2 in the mucus is a potential antibacterial peptide that can inhibit bacterial pathogenesis.

ANALYSIS OF WILD UNDERSIZED OYSTER HARVEST IN TEXAS FOR INFORMING FARM SIZE REGULATIONS

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For many years, Texas has relied on the harvest of oysters in natural habitats approved by the Texas Department of Health Services. Due to increasing value of oysters, The Texas Parks and Wildlife Department (TPWD) was directed in 2019 to develop appropriate regulations for an off-bottom oyster mariculture program to become effective in September 2020. Regulatory issues of concern include size-based harvest, where the legal market-size cultivated oyster is 2.5 inches and legal market-size harvested (i.e., wild) oyster is 3.0 inches. TPWD considers this size difference as a critical challenge because there is a potential that undersized oysters acquired from public reefs could be sold through mariculture operations. An analysis of oyster violations was conducted to determine if undersize oyster harvesting is problematic in Texas and if this information could be used to deter undersized oysters from the wild harvest being illegally mixed into cultivated oyster production. An open-record request was made to TPWD for oyster violations data. The oyster violations data set had 6,483 recorded citations issued between November 1, 1985 and April 15, 2020. Of these citations, 4,029 (i.e., 62%, Figure) were for undersized or take/possession violations. The difference in harvest size (wild vs cultivated) may lead to a further increase in the undersized harvest, which is the number one category for violations. The use of current harvest documentation paired with enforcement should minimize chances of mixing wild with cultivated oysters, thus protecting the species and the natural resource.

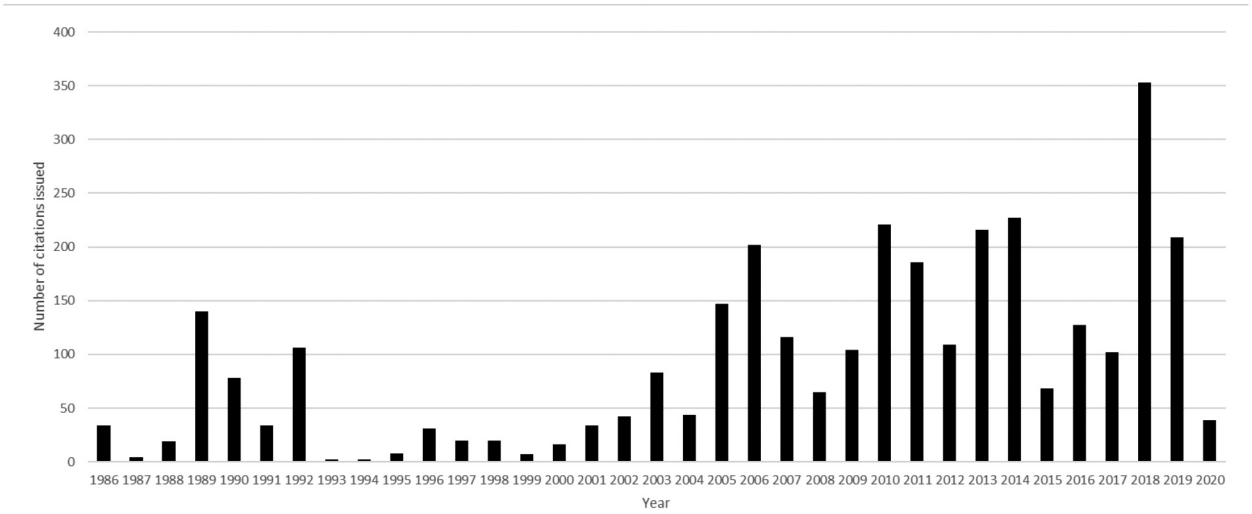

Figure. Total citations issued for undersized or take/possession of small oysters during the public season (Nov.1st - April 30th); data from TPWD oyster violations (Nov. 1985-April 2020).

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 projects at the facility will be reviewed.

ENGINEERING OF A ZERO-DISCHARGE COUPLED AND DECOUPLED AQUAPONICS SYSTEM PROTOTYPE

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There has been much debate regarding whether aquaponics systems should be coupled or decoupled, particularly for commercial food production. Decoupling allows the fish and plant components to be managed separately, thus enabling optimal growing conditions for each. It also allows for zero waste to come from operating the RAS, which in its entirety becomes used to drive the hydroponics system. However, decoupling does little to improve upon the fish growing conditions from a traditional recirculating aquaculture system (RAS). On the downside, it actually removes the added filtration that plants provide in a coupled aquaponics system. By incorporating hydroponics directly into the RAS of a decoupled aquaponics system, we can further control nitrate levels as well as use the plants to take up various contaminants or other pollutants that might otherwise persist in the aquaculture system water.

This prototype examined the feasibility of growing tomatoes in Bato buckets ("Dutch buckets") using aquaponics. With this system we were able to monitor the nutrient content available for plants in both the coupled and decoupled aquaponic system as well as optimize the aerobic mineralization of fish waste in order to maximize nutrient availability. Successful operation of this prototype system has shown the sustainability and potential cost savings for investment in this model for aquaponics food production.

SURVEY CAPTURES SOUTH CAROLINA OYSTER CONSUMER PREFERENCES

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In August 2020, over twelve hundred (1210) consumers in South Carolina were asked about their oyster consumption. Of the consumers surveyed, 905 (75%) were oyster consumers and 305 were non-oyster consumers. The high percentage of oyster consumers was somewhat surprising, but this finding was reinforced by a separate Clemson study which found that South Carolinians consume almost twice the amount of oysters per capita versus the U.S. average (Cheplick et al., 2020).

Survey results showed that oyster consumer demographics were consistent with other studies with respect to education and household income, but not age. South Carolina oyster consumers tended to be under 45 years of age, while other surveys suggest that younger adults consume less seafood (Jahns et al., 2014).

The survey found that 76% of oyster consumption occurred away from home, at restaurants and oyster roasts. Previous studies had also pointed out that most seafood consumption occurs away from home—between 62% (Zhang et al., 2004) and 90% (Richards, 2020), but the number of responses including “roasts” was surprising, at 25% of all responses.

Another finding was that most oyster consumers preferred their oysters cooked (75%) versus raw. This finding is especially important for South Carolina oyster producers, as most of their oysters are marketed to the raw, on the half-shell market.

Finally, availability and price were the main factors limiting oyster purchases. Restaurant consumers mentioned price (48%) and availability (48%) limit their purchases while those eating oysters at home responded that lower prices (51%) and increased availability (63%) would encourage more oyster purchases.

Table 1. Age of oyster consumers (n=905)

Age	Percent
Under 25	15%
25 to 44 years of age	53%
45 to 64 years of age	23%
65 years or older	8%

Table 2. Where and how oysters are eaten (n=905)

Where oysters are eaten	Percent
Home	24%
Away from home	76%
Responses including "roasts"	25%
How oysters are eaten	
Percent consumed raw	25%
Percent not consumed raw	75%

Table 3. Factors limiting and encouraging oyster purchases

Factors limiting restaurant purchases	Frequency
Fresh oysters not available	48%
Price	48%
Food safety concerns	29%
Not prepared the way I like	20%
Factors encouraging home purchases	**Frequency**
More availability of fresh, local oysters	63%
Lower prices for oysters	51%
The oysters are preshucked	38%
Information how to shuck and prepare	27%
The oysters are precooked	20%

UTILIZATION OF OPEN-FORMULA DIETS FED TO FLORIDA POMPANO *Trachinotus carolinus* TO EVALUATE MICROALGAE OIL SUBSTITUTION FOR FISH OIL

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There is interest in reducing the use of rendered fish oil (FO) in fish feeds. There are an increasingly diverse array of alternatives; however, suitability requires accurate information on the nutritive value of a prospective alternative. There is increasing interest in utilizing microalgal oils as a partial replacement/supplement to FO, particularly the heterotrophic microalgae *Schizcochytrium*

The specific objectives of the study were: 1) to evaluate performance of four experimental fishmeal (FM) free diets, each with different supplementary oil sources relative to an open-formula FM based diet; and 2) evaluate performance of the four different oil supplements relative to each other.

Five experimental diets were formulated as iso-nitrogenous and iso-lipidic. The control diet (FM) contained FM and FO and other ingredients common in commercial feeds. The remaining four diets were FM free and varied only in lipid containing either fish oil (FO), algal oil (AO), soy oil (SO), or an algal/soy oil (50/50) blend (A/S). The AO (Veramaris®) is a liquid product extracted from whole cell, heterotrophic microalgae *Schizochytrium* sp. The SO diet served as a negative control. The A/S diet was evaluated to determine if the very high levels of DHA found in AO are necessary. Oil supplements were incorporated at 10.8% of the dry diet.

The experiment was a completely randomized design with four replicate tanks. Tanks were stocked with 20 pompano (= 4.1g) and fed four times/day *ad libitum*. The experiment lasted 85 days. Data were analyzed as a one-way ANOVA. The FM control was tested against the other four experimental diets using Dunnett's t-test. The diets differing only in oil sources were tested against each other using orthogonal contrasts. Significant differences were declared at $P<0.05$.

Of the AO, SO, and A/S diets, only the SO diet was significantly different than the FM control in almost all parameters evaluated. The protein productive value (PPV) of AO was not different than the FM control, whereas FO, SO, and A/S were.

The results of the contrasts indicate performance of the SO fed fish were lower than the other oil sources (Table 1). The data suggest within the similar diets varying only between supplemental oil sources that Veramaris® algal oil is a suitable alternative to FO, and that diluting it to a 50/50 blend with SO resulted in no decreases in performance other than in PPV.

Table 1. Orthogonal contrasts. Table values represent the probability (P-value).

Diet	Weight Gain	FCR	Feed Intake	PPV
FO vs AO	0.255	0.072	0.941	0.303
FO* vs SO	0.001	0.004	0.015	0.004
FO vs A/S	0.995	0.514	0.672	0.062
AO* vs SO	<0.001	<0.001	0.018	0.001
AO* vs A/S	0.255	0.225	0.727	0.008
SO vs A/S*	<0.001	<0.001	0.036	0.202

(*) treatment resulting in better performance.

DEVELOPING A WEST AFRICAN REGIONAL AQUACULTURE LEARNING CENTER IN GRAND BASSA COUNTY, LIBERIA: AN UPDATE

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In Liberia women comprise more than 50% of the agricultural labor force and nearly 67% of the labor force in trade and commerce. The ongoing inability to access needed resources such as land, labor, and capital, as well as technology and market opportunities presents serious challenges with adverse implications for household food security, nutrition, and income. Although rich with fertile soil and abundant water resources Liberia is currently dependent on importing approximately 90% of it's national food needs. Additionally, the unemployment rate, particularly among the youth exceeds 70% and represents a national security risk. The Government acknowledges these are unsustainable conditions. In 2014, the Bureau of National Fisheries (BNF) released their Fisheries and Aquaculture Policy & Strategy plan which promotes the development of sustainable aquaculture for subsistence and commercial purposes to provide greater food security, and greater employment and export opportunities.

AWF is actively engaged in a working coalition of partners to bring an Aquaculture Learning Center to Liberia, West Africa to be housed at Grand Bassa Community College (GBCC). The coalition brings together leaders from the Liberian BNF, the Association of African Agricultural Economists, the Grand Bassa County Association in the America's, Solar Afric, Inc., GBCC, and AwF.

GBCC was established in 2008. Programs offered include those in science and technology, engineering, mathematics, nursing, TVET, ICT, education, agriculture, and entrepreneurship. GBCC is currently transitioning from a 2-year Community College to a 4-year degree seeking University and wishes to add aquaculture and sustainable fisheries to their existing curricula, develop a demonstration facility, and to seek recognition as a Center of Excellence (COE) in Aquaculture. As a COE it is envisioned GBCC will serve as a self-sustaining teaching, research, and demonstration facility.

A multi-year multi-phase plan is in development that would utilize the resources at the COE to support a regional ALC for access by all West African nations utilizing both hands-on and web based platforms. Training would include activities up and down the value chain including fish production, pond construction, pond management, feed production, seed production, business plan development, enterprise management, group action, market access, processing, storage, and food safety and hygiene.

REVITALIZING LOUISIANA'S SOFT SHELL CRAB INDUSTRY WITH EXTENSION AND RESEARCH ON THE TRANSMISSION OF *Callinectes sapidus* REOVIRUS 1 (CsRV1) AMONG BLUE CRABS

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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 (floats or tables) until they shed their hard shell in order to grow. The production of soft shell crab in Louisiana, and across the United States, has declined over the past decade. In Louisiana, the number of soft shell crab producers has decreased from 300 individuals in the 1990's to less than 40 individuals today. The decline in producers has been attributed to hurricanes and storm damage, as well as an aging workforce in the industry. Our efforts have combined extension and outreach with lab-based bioassays to increase resiliency of the industry.

To help new soft shell producers and increase success for those still in the industry, we have worked to capture the institutional knowledge of existing shedders and update best management practices. We have developed outreach materials (i.e, factsheets and videos) summarizing key information on how to produce soft shell crabs. Information provided includes the basics such as the costs to get started, the basic system design, and the overview of crab shedding; and more advanced topics covering water quality and diseases, the major attributors to large mortality events in shedding systems.

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 located in Maryland, Virginia, and Louisiana. We have conducted experiments focusing on the transmission of CsRV1 among crabs in shedding systems using scaled recirculating aquaculture systems. We have examined virus threshold transmission, waterborne transmission, and the impact of salinity on transmission. All transmission studies have relied upon Real time qPCR (RT-qpcr) for detection of CsRV. Preliminary data have shown that virus transmission is spread through water and is lethal to a crab within 2-3 weeks.

PRELIMINARY RESULTS FROM A LARGEMOUTH BASS *Micropterus salmoides* JUVENILE PRODUCTION

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The Largemouth Bass (LMB), *Micropterus salmoides* is a centrarchid freshwater species native to North America and the most sought-after sport fish in the U.S. In addition to being a prized sport fish, the demand for LMB in the food fish market, particularly fresh Asian markets, has increased dramatically in recent years. (Quintero et al 2017). However, unpredictable, and variable reproductive performance have been indicated as primary bottlenecks for the industry development in many cultured fish species and particularly in largemouth bass (Izquierdo et al. 2001, Hussein et al. 2020). Some of the aspects that could improve the seed quality and seed production are broodstock nutrition and feeding. Nutritional status of the brooders is critical for spawning and egg quality since nutrients are transferred from the female to the egg (Migaud et al. 2013; Izquierdo et al. 2015).

A pilot study to evaluate the impact of essential fatty acids and nucleotides in the largemouth bass spawning and juvenile production was performed. LMB brooders (300 fishes) were stocked in RAS (64.0 ±1.0 °F), and fish were fed for 92 days, with three different diets (Diet 1 - commercial diet, Diet 2 - commercial diet supplemented with fish oil and DHA, and Diet 3 - commercial diet supplemented with a mix of fish oil, DHA and commercial nucleotides).

This work presents the evaluation of the effect of the diets on the larval and early juvenile development of largemouth bass. Data collected during pilot juvenile production is presented in the table below. Currently, we are conducting growth curve evaluations and statistical analysis of data. Results of this study regarding the number of juveniles obtained for each treatment may suggest an effect of the diets in brooders, but it is not significant due to the lack of replicate tanks treated with the experimental diets.

Table 1. Preliminary results from pilot study.

Parameter	Diet 1	Diet 2	Diet 3
Initial biomass, 15 dph (Kg)	1.5	1.8	1.6
Final biomass, 82 dph (Kg)	205.7	348.9	305.4
Initial individual weight (g)	0.05	0.09	0.06
Final individual weight (g)	3.8	3.6	3.7
Survival (%)	82.5	72.9	62.0
Final juvenile	54,184	94,718	84,620

COMPARING BLACK SOLIDER FLY (*Hermetia illucens*) LARVAE VS PREPUPAE AS SUPPLEMENTS IN THE DIETS OF LARGEMOUTH BASS JUVENILES

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Black solider fly larvae (BSFL) and prepupae (BSFP) are increasingly being used as sustainable ingredients in terrestrial and aquatic animal diets, particularly as fishmeal becomes less readily available/more expensive. There are some indications that BSFL can impart health benefits to fish, including mitigating intestinal enteritis in some salmonids fed plant protein-based diets. Most research focuses on BSFL as opposed to BSFP. BSFP are a more advanced, non-feeding larval stage that proceeds their emergence as adult flies. It is likely that BSFP have more chitin, based on having a harder/darker cuticle, but this has not been definitively confirmed.

BSFL and BSFP were produced on site from the same batch, using the same food, and under the same conditions; the only difference was their developmental stage. Full fat BSFL and BSFP were analyzed for their proximate composition. Using this information, four isonitrogenous and isolipidic diets were formulated to include a fishmeal-based control diet (D1), soybean meal-based diet (D2), and soybean meal-based diets with BSFL (D3) or BSFP (D4) included at 11.90% and 9.83%, respectively.

Trial was 8 weeks and the survival, growth, feeding efficiency/feed intake, intestinal/liver histopathology, muscle/liver biochemical/mineral composition, and expression of genes responsible for lipid metabolism were measured. Survival was high (95.5-100%) but growth was D1 > D2 > D3 > D4, with each treatment significantly different than the others. Lauric acid (C12), which is high in BSFL/BSFP, accumulated in the muscle and liver in fish fed D3 and D4, while mercury was detected in the liver of fish fed D4. Intestinal histology in D3 had less densely arranged villi compared to those fed D1 and D2 (Fig. 1), but no liver abnormalities. Fish fed significantly less on D4 while liver histology revealed inflammation and necrosis in a third of the fish sampled. D4 fish had significantly shorter/less densely packed villi (Fig. 1). These adverse effects might be related to excessive chitin and thus is not recommended as a supplement in the diets of largemouth bass. Relatively high amounts of lauric acid in both BSFL and BSFP may have also suppressed their appetites, as observed in other terrestrial and aquatic animals. Further research is warranted to ascertain the influence of lauric acid in fish, because this may require BSFL to be defatted to ensure sufficient growth.

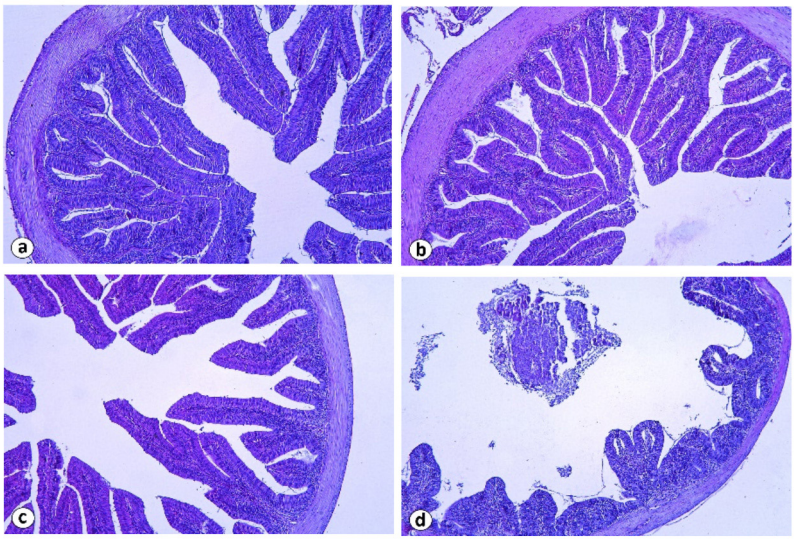

Figure 1: Intestinal histology of largemouth bass fed D1 (a), D2 (b), D3 (c) or D4 (d) after 8 weeks.

NUTRITIONAL CONTRIBUTION OF BIOFLOC TO BLUEGILL, *Lepomis macrochirus*, AND IMPORTANCE OF UNINTERUPPTED AERATION IN BIOFLOC SYSTEMS

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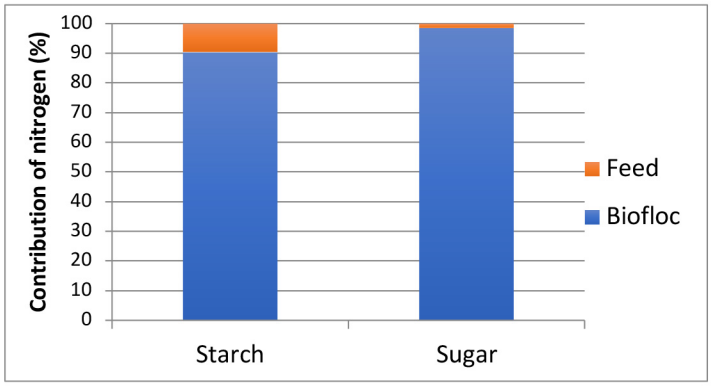
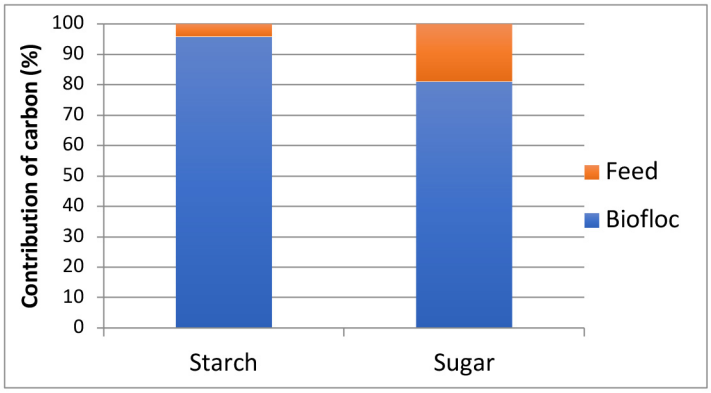
Bluegill is a popular sportfish in Arkansas and food fish throughout the world. They are mostly cultured in earthen ponds where they consume various zooplankton and insects. There is interest in year round production, but in more temperate areas, this must be done indoors where complete diets must be provided. While this may increase feeding cost, it may be possible that bioflocs can provide supplemental nutrition to bluegills, but this has not yet been established.

In this study, three treatments were compared; a clearwater flow through control; biofloc technology with corn starch (BFT-Sta) or biofloc technology with sugar (BFT-Sug). The carbon sources were added at carbon to nitrogen ratio of 15. The bluegill juveniles (5.3 g) were cultured in 100L circular tanks and were fed to apparent satiation twice daily for 32 days. Over 32 days, water quality was measured, and the bacterial composition was identified on the final day. The survival, growth, hepatosomatic index, liver histology and isotope analysis were performed.

Ammonia-N was significantly lower in the control (0.23 mg/l), but the mean ammonia levels in the BFT treatments (0.57-0.69 mg/l) were within safe levels for bluegill. However, on day 22 there was unusually high rainfall and subsequent flooding that tripped the electrical fuse and caused a temporary stoppage to the aeration. This led to dissolved oxygen reaching 0.2 mg/l in the BFT treatments compared to 5.5 mg/l in the control. The aeration stoppage also caused an ammonia spike of around 1.5 mg/l in the BFT treatments compared to 0.3 mg/l ammonia in the control. This water quality deterioration caused survival dropping to 63 and 65% in the BFT-Sta and BFT-Sug, respectively, whereas survival in the control was 98%. It was also noticed that feed intake was lower in the BFT treatments, before and after the aeration stoppage.





These findings indicate that biofloc conditions may not be optimal for bluegill; however, there was evidence bluegill obtained some nutrition from the bioflocs. Moreover, the carbon source influenced the contribution of carbon and nitrogen. Significantly more nitrogen and carbon were obtained from bioflocs produced from corn starch and sugar, respectively. This may indicate differences in the nutritional composition of the bioflocs.

FORTY YEARS OF FLUCTUATION IN THE OCCURRENCE OF TWO VARIOUS METAZOAN PARASITES: *Contracaecum multipapillatum* (NEMATODA) AND DIGENEAN METACERCARIA N1 IN WARM WATER FISH CULTURE IN THE ISRAELI FARMS

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We present field observation results from October 2019 to February 2021 in Israeli fish farms. Surprisingly, two metazoan parasites, the larvae of Contracaecum multipapillatum and unidentified metacercaria N1, which disappeared from Israeli fish farming 30 years ago, were commonly found in the current study. The reappearance of these two helminths is likely related to changes in the management policy related to new regulations regarding bird-of-prey species. It means that farmers could have prevented parasitic infections by keeping away piscivorous birds from their fish ponds and by treating these ponds with appropriate compounds against the affected invertebrates.

FISH WELFARE: THE USE OF CO2 TO AVOID FISH SUFFERING DURING PREPARATION TO PROCESSING

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Fish welfare has become a significant concern during the last decade. The issue of slaughter is getting the interest of many consumer organizations and fish growers, and suppliers. We need to take careful consideration to food fish slaughtering, and not only for an ethical reason. Numerous quality traits are in place already at the end of fish life. Nonetheless, other traits can be developed while the post mortem muscles are turning into consumer produce. Therefore, it is necessary to investigate ways to avoid the suffering of the fish before their processing. To improve the efficiency of the immobilizing and stunning process of the fish at the entrance of the fish plant, we investigated the use of CO_2 in combination with low temperature. When combining CO_2 to 2°C water temperature, fish were stunned and permanently immobilized within 20 min or less, while their core temperature was 3.5°C. Our results suggest that combining low temperature and bubbling CO_2 in the fish receiving tank of the processing plant might be more appropriate for animal welfare. Therefore, we suggest continuing our experiments in the fish processing plant.

A PACIFIC INTERNATIONAL OCEAN STATION

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World fisheries agencies have long warned of the depletion of wild fish stocks in our oceans due to overfishing to meet rapidly growing human demands for seafood. On the other hand, we recognize the potential in aquaculture for providing safe, sustainable seafood supplies to the world. Seafood aquaculture already supplies total yields about equal to caught fish. Still, conflicts with near-shore and on-shore usages, navigation lanes, commercial fisheries, and feed and waste discharges in local waters are imposing limits to its expansion.

The obvious solution is to develop ocean aquaculture in deep, voluminous ocean waters farther from shore. But the proof of economically sustainable and environmentally protective best practices is needed before commercial operators undertake the risks of deep ocean aquaculture operations.

Blue Revolution Hawaii is advocating the Pacific International Ocean Station (PIOS) deployment in Hawaiian Exclusive Economic Zone (EEZ) waters 35-75 miles offshore on the lee side southwest from the Hawaiian Islands. PIOS would be a pilot test station for the eventual deployment of commercial ocean resources production platforms in ocean waters.

The 35-75-mile-band of EEZ ocean waters provides a 20,000 square-mile region along the southwest side of the Hawaiian Islands that may ultimately be designated for commercial-scale ocean resources production operations. This area lies in the vacant zone between existing Hawaii-based day-boat and longline fishing operations. It is situated far from near-shore usages, navigation lanes, and marine mammal and reef preserve areas.

The PIOS' mission is to support a test fleet of submerged multi-trophic aquaculture systems for growing pelagic species of fish from fingerlings to harvesting. An elevated semi-buoyant core platform (similar to oil/gas drilling platforms) will hover above storm-surge wave heights. It would provide housing and operations space for up to 200 scientific researchers, ocean monitoring personnel, and fish cage operations workers. In addition, an outer line of tethered ocean wind turbines can provide electricity for on-platform activities.

The PIOS Station research would include ongoing monitoring and data-gathering of climate, marine life, and ocean environment. In addition, it would test, design, and validate best practices for sustainable and environmentally protective operations for deep ocean aquaculture and other ocean resources production.

COST ANALYSIS OF MARINE COPEPOD NAUPLII PRODUCTION AS A FIRST FOOD SOURCE FOR MARINE FINFISH LARVAE

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Most marine finfish require live feed during the first feeding phase of the larval cycle – a period of extreme importance to the continued growth and development of marine finfish aquaculture. Live feeds must provide adequate nutrition, stimulate a feeding response, and be small enough to be consumed by larval fish. Currently, most cultured marine fish are raised with rotifers as their first feed due to the ease and lower costs associated with rotifer production. However, rotifers lack the proper nutritional profile required by marine fish and must be enriched prior to feeding, are not easily digested by all marine fish, and, generally, do not move in a manner that elicits a feeding response in marine fish larvae. On the other hand, copepod nauplii are the preferred prey of many wild marine fish larvae, provide marine fish larvae with proper nutrition without enrichment, and move in a manner that marine fish larvae associate with prey leading to a feeding response.

Research trials have shown that multiple high value species, including several snappers, groupers, and marine ornamental species, can be cultured with greater success using copepod nauplii as a first live feed as opposed to rotifers. To date, the use of copepod nauplii in commercial marine finfish aquaculture has been limited by inconsistent and costly production. This research evaluates system design and production costs associated with intensive production of *Parvocalanus crassirostris* and *Oithona colcarva* copepod nauplii. Costs are based on production systems, techniques, and research trials performed at the University of Florida Tropical Aquaculture Laboratory. Both production systems analyzed employed overlapping batch production processes designed to produce a continuous supply of nauplii for larval marine finfish producers. Both system construction and production costs are analyzed for all three system components: water-mixing for production of saltwater, algal production, and nauplii production. The analysis evaluates potential system and per unit production cost reductions associated with access to natural saltwater sources, scaling of production, and the potential use of microalgae concentrates as opposed to live algae for feeding.

RESEARCH VERIFICATION IS EFFECTIVE AS A TOOL FOR EXTENSION PROGRAMS IN AQUACULTURE

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Research verification programs have been used by Extension professionals to evaluate the effectiveness of research-based management recommendations in a number of different aquaculture species. The University of Arkansas at Pine Bluff pioneered research verification in aquaculture in 1993 with channel catfish and later developed a baitfish research verification program. Extension professionals in Alabama (Alabama Cooperative Extension System) have carried out a number of research verification programs using traditional single batch and multiple batch production systems for channel catfish as well as a program that targeted farmers using new alternative production technology (intensively aerated ponds). In addition to providing a platform on which to examine current best management practices and Extension recommendations from Land Grant Universities, research verification is valuable in identifying gaps for which further research is needed. Perhaps the best benefit of research verification programs are the comradery and relationships developed between cooperating commercial producers and Extension personnel that can lay the ground work to facilitate future collaborative efforts related to technology transfer in the aquaculture industry. Several aquaculture research verification program case studies in Alabama and Arkansas will be summarized and discussed in the context of the value to commercial producers and Extension programs at state Land Grant universities.

A PRELIMINARY EVALUATION OF THE EFFECTS OF OYSTER CAGE AQUACULTURE ON SEAGRASS (*Halodule wrightii*) COLONY HEALTH

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Harte Research Institute/NOAA CCME
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Seagrass is essential habitat for many different marine species and is found in shallow coastal waters having high light penetration and low wave action. These conditions are also conducive to oyster aquaculture. As Texas develops commercial oyster farming, it is important to understand the effects of oyster aquaculture on seagrass health and to determine whether this activity negatively impacts this form of essential marine habitat. The goal of this study was to characterize the effects of a research-scale oyster aquaculture farm with adjustable longline system cages on the health of adjacent seagrass colonies in Copano Bay, TX. This was accomplished by using typical methods for the monitoring of seagrass health as well as relevant water quality monitoring and characterization of sediment transport in the farm area. Four seagrass beds (*Halodule wrightii*) were identified at various distances (100 m "upstream", directly adjacent (zero m), 30 m, and 60 m "downstream") from the research farm were monitored for 1) change in seagrass density, canopy height, max leaf length, 2) turbidity, water temperature, light intensity, salinity, DO, conductivity, dissolve nutrient concentrations (e.g., NH_4, NO_2, NO_3, PO_4), and 3) current direction, sediment deposition rate, and sediment texture analysis. In general, results showed only minor differences in dependent variables over the 18-week sampling period. No significant difference ($P \geq 0.05$) was shown in maximum leaf length of seagrass, water quality variables, current direction, and sediment texture analysis. Seagrass leaf length and sediment deposition rate at the end of the study period was significantly ($P < 0.05$) higher for the 60-m downstream colony. Both the 30-m and 60-m downstream colonies had significantly ($P < 0.05$) denser seagrass coverage. The 100-m upstream seagrass colony had the numerically lowest density. These results suggest that differences in indicators of seagrass health were either unaffected by proximity to the research-scale oyster farm or beneficial and that higher sediment deposition rate (e.g., the 60-m downstream site) did not decrease seagrass colony density. Overall, it can be concluded that an oyster aquaculture farm of the type, size, and at the location described in the present study had no major negative impact on seagrass colony health.

DIVERSITY AND INCLUSION IN US AQUACULTURE

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This session is organized by members of the Diversity and Inclusion (DI) committee of the United States Aquaculture Society (USAS) and welcomes all USAS members and conference participants. It seeks to provide a safe and inclusive environment and forum where questions related to diversity, equity, and inclusion in the society can be discussed freely. The first part of the session will include a presentation of the results of a recent diversity survey conducted by the committee and an open discussion of practical issues related to DI experienced by USAS members throughout their careers. This part of the session will also consist of a round table discussion on possible actions that could be taken at the level of USAS. The second part of the session will include presentations of ongoing initiatives to increase DI in US Aquaculture and a round table discussion of coordination and opportunities for collaborations.

PEDIGREE RECONSTRUCTION AND ESTIMATES OF GENETIC PARAMETERS FOR EARLY GROWTH TRAITS IN GULF OF MEXICO EASTERN OYSTER FAMILIES REARED COMMUNALLY

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The eastern oyster, *Crassostrea virginica*, supplies major markets throughout the United States. In recent decades, the species has been declining throughout most of its range including in the Gulf of Mexico, the largest producing region in the US. The SALT consortium is developing a breeding program to support commercial off-bottom harvests in the Gulf of Mexico with eastern oyster bred for improved performance in different salinity environments. The program is evaluating the effectiveness of a family selection approach where families are mixed at fertilization and reared communally until phenotyping.

In this work, 102 males and 102 females collected from 17 natural reefs from across the northern Gulf of Mexico between San Antonio Bay (Texas), and Cedar Key (Florida) were bred according to 51 non-overlapping 2 males x 2 females mini-factorial crosses to produce 204 families. Crosses were produced by strip spawning and in vitro fertilization between August 31 and September 3, 2020. Families produced on the same day were pooled after fertilization for larval culture. The protocol for setting larvae involved harvesting larval tanks daily during 4 days to capture pediveliger larvae as soon as an external foot was observed. Samples of the daily harvests of each setting group (16 spawning date x setting day combinations, 1,600 samples totals) were taken on October 10 (38-41 days post fertilization, dpf) for genetic analysis. All 16 fractions were then pooled for further nursery culture and another 1,600 spats were randomly sampled on December 7 (96-99 dpf) and their height, length, and width were recorded. Parents and all offspring were genotyped at 192 Single Nucleotide Polymorphism markers and pedigrees were reconstructed using a likelihood ratio approach.

Parentage analysis revealed that 96 sires and 80 dams contributed to the sampled offspring. Contributions of individual sires ranged from 0.07% to 5.93% while contributions of individual dams were from 0.07% to 7.00%. The estimate of heritability of (log transformed) height using a simple animal additive model was 0.26 ± 0.04. Length and width were strongly correlated to height (phenotypic and genetic correlations between height and length were $r_p = 0.89$ and $r_g = 0.92 \pm 0.02$ respectively; correlations between height and width were $r_p = 0.77$ and $r_g = 0.89 \pm 0.04$). Estimates of heritability of length and width analyzed with height as a covariate were 0.36 ± 0.07 and 0.21 ± 0.04, suggesting occurrence of genetic variation for both traits.

LUNAR PERIODICITY IN REPRODUCTION OF RED DRUM IN AQUACULTURE

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Lunar periodicity in spawning is documented in several marine species. Red Drum (*Sciaenops ocellatus*) are in Sciaenidae, or the drum family. Red Drum use acoustics in their courtship and spawning behavior, and their acoustical spawning activities have been recorded in wild populations and linked to the lunar phase. Texas Parks and Wildlife has fish hatcheries for stock enhancement along the Texas coast of the Gulf of Mexico. Broodstock are housed in recirculating aquaculture systems indoors with controlled photoperiods and thermal cycles to induce volitional spawning. These broodstock tanks have no lunar phase illumination, yet lunar phase has a significant effect on the number of spawning events recorded by this species at the Sea Center Texas facility in Lake Jackson. An ANOVA test was run, producing a p-value of 0.0317.

Using the Fisher's Least Significant Difference test (alpha of 0.05), it was found that Red Drum spawning events are significantly more frequent during the full lunar phase when compared to the new and first phases.

This information can be used to maximize production. By working with the lunar cycle, thermal fluctuations can be used in conjunction with the lunar phase to increase spawning events on a schedule. Strategically preparing fertilized ponds to have zooplankton blooms when larvae are at first feeding stage can ensure that resources are successfully utilized.

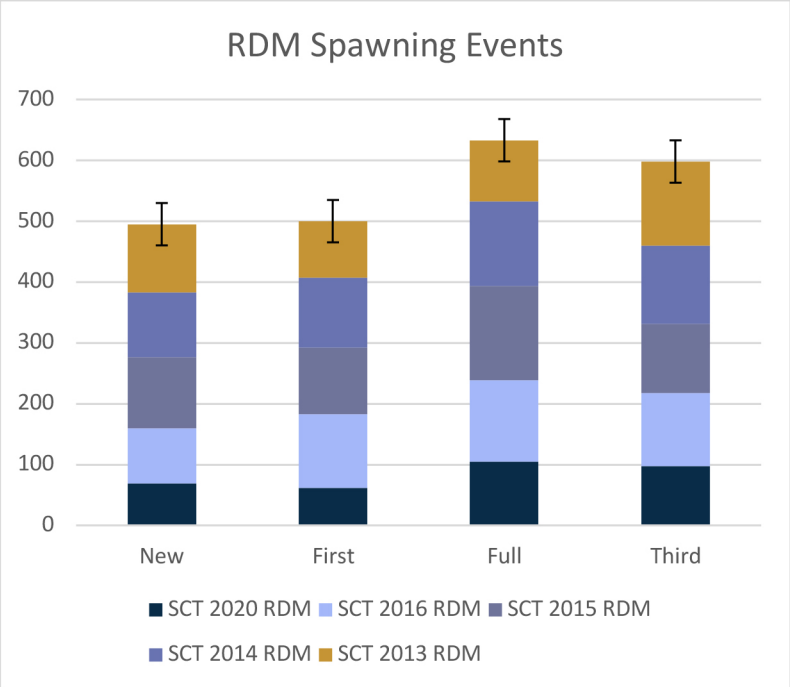

Figure 1. The total number of spawning events by Red Drum for each of five years grouped by lunar phase with standard error bars for each phase.

BASAL SOURCES OF ENERGY AND THEIR TRANSFER PATHWAY FOR HILSA FISHES (*Tenualosa ilisha*) IN THE UPPER MEGHNA RIVER ESTUARY IN BANGLADESH

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The stable isotope ratios (‰) of the hilsa fish species significantly varied according to their standard length in both δ13C and δ15N. It reveals that their food source and trophic level change as of their standard length. The map of δ13C and δ15N very interestingly expresses that benthic microalgal cells of 0-63µm is a primary basal source of energy for most of the Hilsa species in the study area.

The hilsa shad (*Tenualosa ilisha*) is the national fish of Bangladesh, being diverse in the south-east Asian and Persian Gulf region along with the Vietnam Sea and China Sea due to its diadromous migratory features. This species contribute more than 1% of GDP as a single fishery in Bangladesh. Hilsa is primarily planktivore species. However, no research could not conclude the type of source materials fuel primarily to Hilsa Fish. We applied stable isotopic signatures as a pioneer study to determine primary energy source materials and trophic relation to Hilsa fish.

Significant variation of δ13C and δ15N in Hilsa fish tissue and their standard length suggest that their food source and trophic level changes in line with their standard length (Fig 2). The map of δ13C and δ15N (Fig. 3) revealed that benthic microalgal cells of 0-63µm is a clear basal source of energy for Hilsa in the study area.

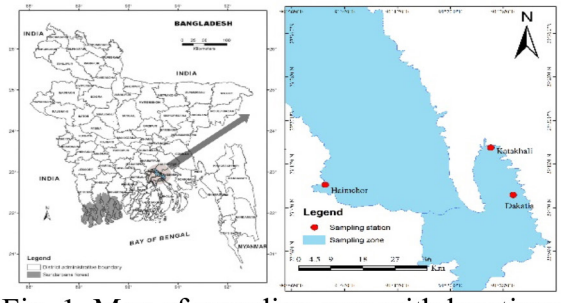

Fig. 1. Map of sampling area with locations.

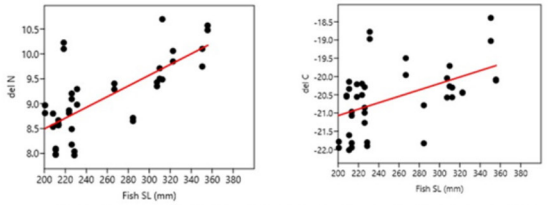

Fig. 2. Changes of δ15N (‰) and δ13C (‰) against standard length (mm) of Hilsa fish observed during the study.

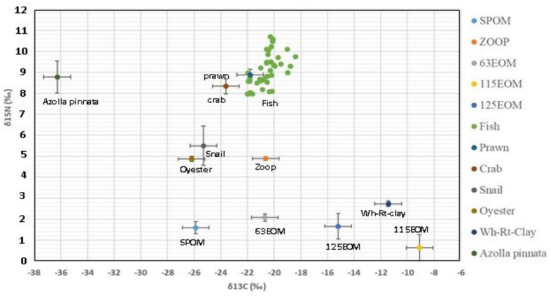

Fig. 3. δ13C (‰) and δ15N (‰) transfer pathway in the Meghna River. Bars indicate standard deviation of each variables.

MMEERSET PHASE ONE: DEVELOPING PLATFORM-BASED OFFSHORE AQUACULTURE USING A MULTI-USE APPROACH AT STATION PADRE

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Using a multi-use business approach, offshore oil and gas platform sites were investigated to develop the first platform-based offshore aquaculture pilot project in the Gulf of Mexico (GOM). Our investigators performed a comprehensive feasibility assessment of two platform sites (North Padre Island 975 and 969, located at 26°50'31N, 96°58'47W and 26°49'57N, 96°56'23W, respectively) to support site-specific development and provide economic and spatial analyses of broad interest to potential investors/stakeholders. Primary deliverables produced during this project include a user-friendly economic model and regional maps identifying suitable areas for platform- and non-platform associated offshore development specific to the Station Padre study site in the northwest GOM.

Our work concluded that: 1) the economics of an offshore platform-based aquaculture system producing Cobia (see Figure 1) could be cost competitive under a range of assumptions, but the economics of Red Drum are significantly more challenging; 2) site characterization revealed favorable conditions for an offshore aquaculture farm and no conflicts that would preclude farm development at Station Padre; and 3) ocean parameters measured are characteristic of highly exposed, open ocean conditions, and aquaculture infrastructure installed on site will need to withstand this high energy environment.

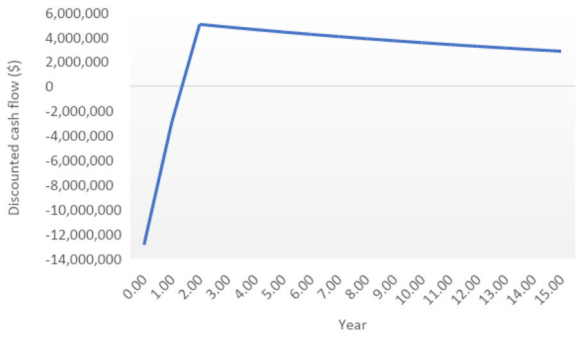

Figure 1

CHANGING ATTITUDES FOR OFF-BOTTOM OYSTER AQUACULTURE IN TEXAS

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Eight public meetings were held in coastal counties of Texas to identify through written surveys potential user conflicts and potential solutions to allow for off-bottom culture of oysters. A majority of respondents (67.6%) were generally familiar with aquaculture. Before the presentation (Pre), a similar majority had a positive opinion of general aquaculture (GA, 65.1%) and bivalve aquaculture (BA, 63.0%) as compared to a negative opinion of aquaculture (3.8%) or bivalve aquaculture (2.1%); the remainder indicated no opinion or mixed opinion (Fig.). After the presentation (Post), there was a large increase (~25% absolute value) in positive perception of both general aquaculture and bivalve aquaculture (Fig.). Much of the increase came from those that had no opinion initially, although there was also a reduction in those that initially had a negative opinion. A primary reason noted for a change to a positive opinion was the alleviation of a concern, many of which were environmental in nature. This change of opinion indicates the importance of reaching target audiences, especially those with mixed or no opinions, with information that assists in forming evidence-based decisions. It is key for ensuring that actions, such as oyster culture in coastal Texas, will be accepted by stakeholders and the community (i.e., social license to operate). This research project was supported by a Gulf States Marine Fisheries Commission award (ACQ 210-039-2017-TAMU) and was reviewed by the TAMU-CC Institutional Review Board.

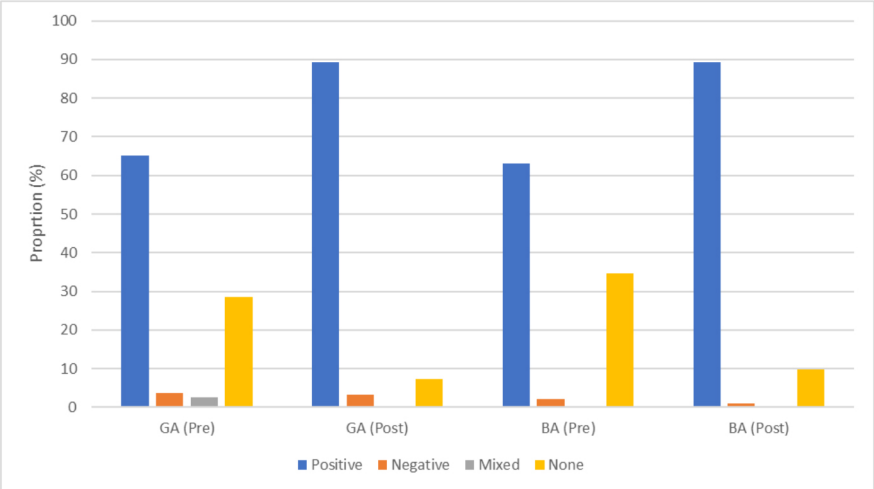

Figure. Proportion (%) of responses indicating an opinion (positive, negative, mixed, or none) pre-presentation (Pre) and post-presentation (Post) of general aquaculture (GA) and bivalve aquaculture (BA). Number of responses was 238 (Pre GA), 216 (Post GA), 243 (Pre BA), and 215 (Post BA). Note the increase of positive responses from pre-presentation to post-presentation for both GA and BA, as well as the reduction in negative and none responses.

SUBSTRATE PREFERENCE FOR SETTLEMENT OF PONDEROUS ARK *Noetia ponderosa* LARVAE

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Previous research on ponderous ark *Noetia ponderosa* culture indicated a bottleneck at metamorphosis using traditional hard clam *Mercenaria* culture techniques. Earlier research found no survival using downweller systems, but settlement and surivavl did occur on the macroalgae *Ulva* and on tank bottoms with or without added natural silt or a benthic diatom assemblage including *Amphora*. Ponderous ark larvae produce a byssus sheet for attachment similar to byssal threads of blue mussels. Therefore, this project evaluated in a commercial setting the settlement preference for ponderous ark larvae on natural rope, synthetic rope, sand substrate, or no substrate.

Ponderous ark, *Noetia ponderosa*, broodstock (Fig.) were collected from the wild and conditioned. Spawning occurred volitionally as well as induced by thermal shock. Larvae were cultured using standard hard clam culture techniques. Determination of larval competency is difficult, therefore when larvae reached ~140-200 µm shell length they were transferred to experimental setting systems. Microalgae was added daily along with partial water exchanges during the following 3-4 weeks. After this period, settled larvae were enumerated.

The initial trial found that substrate (nylon rope and sand) containing tanks (n=3) had an average of 9219 (±4515, s.d.) settled larvae, whereas the control tank (n=1) with no substrate had 5280 settled larvae. A second trial will be presented in which replicate (n=3) tanks containing all substrates, as well as an area with no added substrate, were utilized. This project will contribute to developing a setting protocol for ponderous ark, which will assist shellfish hatchery operators who may consider producing ponderous ark seed. This project was supported by a Gulf States Marine Fisheries Commission award (ACQ-210-039-2020-TAMU).

Figure. Ponderous ark *Noetia ponderosa* broodstock collected from the wild.

SHOULD THERE BE A CONCERN FOR TILAPIA CULTURISTS REGARDING CONTAMINANTS OF EMERGING CONCERN

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Concerns about water quantity and quality for aquaculture are increasing due to climate change and population growth. Surface water sources are increasingly dominated or dependent on treated wastewater effluent, potentially influencing downstream uses. Wastewater effluent generally contains trace levels of anthropogenic compounds, typically referred to as contaminants of emerging concern (CEC), for which our knowledge of their impacts is still evolving. Therefore, introduction of CEC from surface waters influenced by wastewater effluent is a potential concern for cultured fish health as well as humans who consume farmed fish.

Bioconcentration and depuration of the pharmaceutical diltiazem (DTZ) and the perfluorinated compound GenX (ammonium 2,3,3,3-tetrafluoro-2-heptafluoropropoxy propanoic acid) were examined in fingerling tilapia (*Oreochromis mossambicus*) using a controlled time sequence exposure (1 μg L^{-1}, 1, 3, 6, 12, 24, 48, 78 and 96 hr) and non-exposure (1, 12, 48 and 96 hrs after max exp time) in freshwater (DTZ & GenX) and brackish water (~16 ppt, GenX only). A semi-static flow-through tank system was utilized that consisted of triplicate 10-gal tanks connected to a single 30-gal sump tank for each exposure and depuration experiment. Fish carcass, blood plasma, liver, and muscle were analyzed in both exposure and depuration phases.

DTZ bioconcentration was greatest in liver >plasma >carcass >muscle. The biological half-life ($t_{1/2}$) of DTZ in tilapia muscle was 18.8 hrs, indicating the compound is processed relatively quickly. Based on the 96 hr DTZ uptake by fingerlings, human exposure to the highest DTZ muscle concentration would be ~6 orders of magnitude below the lowest daily human therapeutic (120 ppb) dose. Plasma values were on the same order of magnitude as the human therapeutic dose, implying potential effect on the fish and productivity (verification needed).

GenX bioconcentration was greatest in plasma >liver >carcass >muscle. Muscle was found to have the highest $t_{1/2}$ followed by carcass, plasma, and liver. The rate of uptake and depuration was affected by salinity, raising concern for euryhaline organisms. Fish muscle (fillet) GenX concentration at 96 hrs in freshwater was 0.14 ppb and at 16 ppt it was 0.312 ppb. Based on the 96 hr GenX uptake by fingerlings, human exposure to the highest GenX muscle concentration would be ~2 orders of magnitude below the chronic daily exposure limit (0.08 ug kg-day^{-1}).

The projections and improved bioaccumulation models for farmed fish from this research will provide aquaculturists with knowledge to make pro-active management decisions regarding water quality in the future, while improving our general understanding of human exposure to CEC from nontraditional water use. This project was supported by a USDA-NIFA.

INSTALLATION AND MANAGEMENT OF A SEMI-COMMERCIAL SCALE PILOT OYSTER FARM IN TRES PALACIOS BAY, PALACIOS, TEXAS

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In 2019, commercial off-bottom oyster farming was legalized in Texas by House Bill 1300. Texas Parks and Wildlife Department (TPWD) approved regulations to create the framework for the industry in May 2020. To aid in facilitating a successful launch of this new industry, research was needed to evaluate the feasibility of oyster farming in Texas, determine best management practices for Texas bays, and to identify potential issues and their possible solutions. A semi-commercial scale pilot oyster farm was established in Tres Palacios Bay in Palacios, Texas to evaluate two types of oyster aquaculture methods, floating cages and an adjustable long-line system, for growing oysters in natural waters in Texas.

Before installing the farm, an appropriate site was selected based on water depth, bottom characteristics, salinity regime, and TPWD criteria to protect critical habitats such as seagrass, natural oyster reefs, and rookeries. After all permits for the site selected had been approved, installation of the farm began. The adjustable long-line system and floating cages used at the farm had different installation methods and each method had unique challenges. Some of these challenges included sourcing materials and finding contractors that could perform the installation and were willing to travel to remote sites. When installing the adjustable long-line system, different methods (water-jetting or pile-driving) and their pros and cons were considered.

Management of the farm included regular and seasonal gear maintenance, developing a seasonal desiccation regime to reduce biofouling, regular tumbling and grading of oysters, and dividing oysters to reduce densities as they grow. Hurricane protocols were also implemented several times throughout the season.

FIGURE 1. Aerial view of the pilot oyster farm in Tres Palacios Bay, Palacios, Texas.

BEHAVIORAL CONDITIONING THROUGH HABITUATION TO INCREASE POST-RELEASE SURVIVAL OF HATCHERY-REARED RED DRUM *Sciaenops ocellatus*

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Three sets of experiments were conducted from July to December 2016 to investigate whether habituation and habitat complexity in rearing ponds (0.2 ha) affected post-stocking predation of hatchery-reared red drum, *Sciaenops ocellatus*. Three structurally different habitat treatments: 1) non-vegetated (normal rearing environment), 2) artificial seagrass, and 3) artificial seagrass with predator exclusion cages were used to assess if habitat complexity influenced growth and condition. Survival from predation was assessed by exposing red drum to free-roaming predators (pinfish, *Lagodon rhomboides*) in experimental 'wild' ponds (0.2 ha) for 24 hours. Fish growth (TL) was found to be affected by trial (i.e., temporal) but not by any treatment ($P < 0.0001$ and $P = 0.178$, respectively). Prior to the introduction to red drum into experimental 'wild' ponds with predator exposure, condition factor (K) of fish was found to be affected by trial and treatment with an interaction ($P < 6.95e\text{-}16$, $P = 0.0013$, $P = 0.006$, respectively). Condition factor was significantly lower in the artificial seagrass treatment with predator exclusion cages ($K = 0.82$), as compared to the non-vegetated and artificial seagrass treatments ($K = 1.06$ and $K = 1.09$, respectively), prior to predator exposure. Although condition factor may have been affected by the addition of predator exclusion cages, data suggests that survival during culture increased with habitat complexity. In conclusion, habitat complexity improved behavioral mechanisms (i.e., foraging, predator-avoidance), thus increasing post-release survival of hatchery-reared red drum.

TIMELY ESTIMATION OF POST-RELEASE SURVIVAL FOR HATCHERY-REARED JUVENILE FISHES: APPLICATION TO COMMON SNOOK *Centropomus undecimalis*

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Effective adaptive management of aquaculture-based fisheries enhancement programs requires timely estimation of the impact of stocking activities. For estuarine finfishes, post-release survival estimation may be particularly challenging due to low recapture rates. In southwest Florida, aquaculture-based enhancement has been explored to aid population recovery of common snook (*Centropomus undecimalis*) after mass mortality events due to cold stuns and red tide because snook support a valuable recreational fishery. To guide effective snook enhancement, we apply a novel approach for release activities of hatchery-reared juveniles to guide timely estimation of post-release survival.

Following replicate weekly release designs, juvenile snook marked with passive integrated transponder (PIT) tags have been used in hypothesis-driven release experiments to identify the influence of release locations, times, and procedures on post-release survival and behavior. Stocked snook are monitored with marine-adapted PIT tag antenna arrays that generate recapture histories that inform multistate mark-recapture models after 1 year of post-release monitoring.

Short-term differences in survival among the first few weeks after release primarily influence the overall impact of stocking activities (e.g., Figure 1), but fish size, tag size, pre-release acclimation to habitat, environmental conditions at release sites, the use of predator-exclusion cages, and residency behaviors of hatchery-released fish have influenced survival and detectability. The highest survival rates have been observed for individuals released in lower reaches of tidal creek systems in the spring, indicating these areas may provide ideal release sites for juvenile snook at that time.

Further identifying optimal release locations, times, and procedures will promote adaptive management of enhancement programs and maximize the impact of informed, strategic stocking on receiving populations.

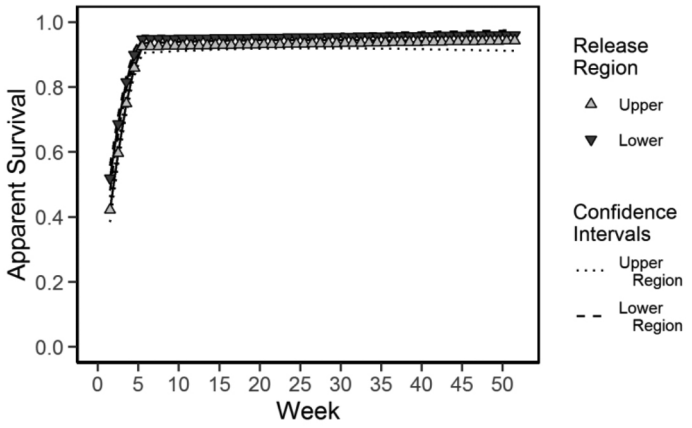

Figure 1. Weekly apparent survival estimates from a multistate open robust design model for a cohort of common snook exhibiting non-resident post-release behaviors.

RAPID AMPLIFICATION OF *Enterocytozoon hepatopenaei* (EHP) INOCULUM IN SHRIMP (*Penaeus vannamei)*

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Enterocytozoon hepatopenaei (EHP), the etiologic agent of Hepatopancreatic Microsporidiosis (HPM) has emerged from an obscurity to the forefront of infectious diseases in shrimp aquaculture in just over ten years. As the disease spreads across Asia, and more recently in the western hemisphere, there is an urgent need to prevent its further spread and develop genetically resistant line of shrimp.

EHP is an obligate parasite and currently there is no *in vitro* culture method to propagate the parasite in a robust and reproducible manner. The inability to produce a large quantity of inoculum to carry out an experimental challenge is an impediment in screening for EHP resistance in shrimp and in conducting other laboratory experiments.

We describe here a simple yet robust method to generate a large quantity of EHP inoculum in approximately 30 days using live shrimp. This method involves directly injecting EHP inoculum into the hepatopancreas of Specific Pathogen Free shrimp (*Penaeus vannamei*). At fifteen, thirty, and forty-five days post-injection, EHP-infected hepatopancreata were dissected and an aliquot of hepatopancreas tissue was taken to assess infection level by histopathology and quantitative PCR. The remaining tissues at each of the three time points were used to challenge SPF *P. vannamei* shrimp via feeding. Following feeding EHP-infected tissue, animals were sampled at 15, 30, and 45 days post-challenge with infection status determined by H&E histology and quantitative PCR. The data shows that feeding SPF *P. vannamei* with hepatopancreas tissue originally derived from EHP-injected *P. vannamei* will provide a large pool of infected tissue within 30 days. This tissue can then be prepared into inoculum and made available for experimental bioassay work. The method is robust enough and overcomes the bottleneck of EHP inoculum availability for screening resistance in shrimp. Until an *in vitro* culture method for EHP is developed, the method described can be used to generate a large quantity of EHP inoculum for an experimental bioassay.

COMPARATIVE ANALYSIS OF STALKED AND ACORN BARNACLE ADHESIVE PROTEOMES

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Barnacles are stationary crustaceans that aggressively colonize marine surfaces. The Pedunculata (stalked) and Sessilia (acorn) orders contain the majority of barnacle species. Both orders rely on a proteinaceous adhesive for surface attachment, but the composition of the adhesive and how it varies across the evolutionary tree is incompletely understood due to a lack of genetic data. With the recent genomic sequencing efforts for one Pedunculata (*Pollicipes pollicipes*) and Sessilia (*Amphibalanus amphitrite*) species, we begin to address this knowledge gap. *P. pollicipes* is a species of commercial interest in Europe where it is a delicacy, whereas *A. amphitrite* contributes to biofouling which costs marine industries millions in extra fuel expenditure and maintenance activity.

We reanalyzed mass spectrometry proteomic samples of P. pollicipes and A. amphitrite with the recent genomic information and compared the resulting adhesive proteomes, revealing 87 and 161 proteins identified for A. amphitrite and P. pollicipes, respectively. 82% of proteins had homologous proteins identified in the adhesive of the opposite species, indicating a high degree of conservation (Fig 1A). The proteins thought to drive adhesion were examined for similarity in amino acid composition (Fig 1B). Glycine rich proteins (GrCP1 and GrCP2 groups) cluster together, while the two groups of leucine rich proteins (LrCP1 and LrCP2) mostly separate from one another. Proteins do not separate by species, indicating that the amino acid composition of these adhesive proteins is conserved.

This presentation will compare and contrast the adhesive proteomes of these two divergent barnacles in greater detail.

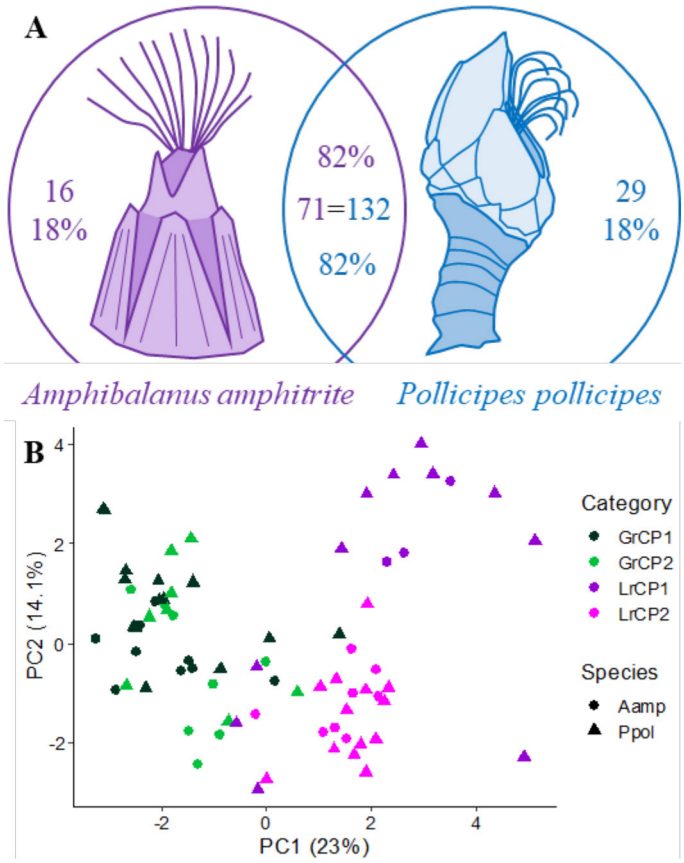

Figure 1. Overview of the adhesive proteome of *A. amphitrite* and *P. pollicipes*. **A)** Overlap of the homologues of proteins identified in each species. **B)** PCA analysis of the amino acid composition of barnacle adhesive proteins.

FEASIBILITY ASSESSMENT FOR INTENSIFICATION OF THE SMALL-HOLDER EXTENSIVE SHRIMP FARM AREA

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About half of the 2 million hectares of the global shrimp farming area is in the tidal zone and often in what was tropical mangrove forest developed into shrimp ponds. Most of this area is comprised of small extensive farms that average yields of less than 500 kg/ha/year. The area surrounding Cau Mau and other provinces in the Mekong Delta is characterized by more than two hundred thousand hectares of such small farms that occupy from about 1 to 3 hectares each employing the farm owners and providing their families with subsistence income. These yields are relatively small compared to more intensive, often mechanically aerated, shrimp ponds elsewhere in Asia. In Thailand typical average yields of 15 mt/ha/year of shrimp suggests that the clearing of mangrove in the Mekong Delta is both excessive and environmentally destructive. This study examined the potential transition of extensive farms to more productive farming methods that would improve farm income, require less mangrove conversion, and provide an opportunity to increase shrimp production by allocating human and land resource to more efficient and productive farming methods. The results of engineering and financial planning studies showed that existing farms less than 2.8 ha were unable to justify investment in more intensive farm configurations, but that farms over 6 ha with yields of 7 to 15 mt/ha/yr had the potential to amortize investment in construction of more intensive farms, employ farms and workers with more compensation, stabilize sustainable pond maintenance, protect remaining mangrove forest, restore mangrove areas, and reduce the contraction of the Vietnamese coastline caused substantially by pond sedimentation in the extensive pond area.

DEVELOPMENT OF A RECIRCULATING AQUACULTURE SYSTEM FOR BIVALVE LARVAL CULTURE

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Typical bivalve larval hatchery practices rely on pumping near-shore coastal waters to support flow through larval production. In such applications, suboptimal ambient water quality can cause impaired hatchery/larviculture performance. The application of recirculating aquaculture systems (RAS), have long been investigated to allow reuse of larval production water, thereby minimizing impacts from fluctuating ambient coastal waters. This presentation will follow the progression in development of such a system in Virginia, integrating programming from the Virginia Institute of Marine Science Eastern Shore Lab, the Virginia Tech – Virginia Seafood Agriculture Research and Extension Center, Virginia Sea Grant, and Oyster Seed Holdings, LLC.

FRESHWATER MUSSEL CONSERVATION AT THE SAN MARCOS AQUATIC RESOURCES CENTER IN TEXAS

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Freshwater mussels are a highly imperiled group of organisms with over 70% of the nearly 300 species in the US and Canada in need of conservation. Texas is home to around 50 species of freshwater mussel, with roughly one third of those species considered either threatened or endangered. Freshwater mussels provide a breadth of ecological services to aquatic ecosystems, and filter water continuously. Elevated conservation statuses of these vital species have triggered a rise in conservation efforts nationwide by federal, state, municipal, and academic entities, with research, monitoring, and propagation at the forefront of these important conservation efforts. Efforts by the Mussel Program at the USFWS San Marcos Aquatic Resources Center (SMARC) center around research and propagation. Research onsite strives to inform conservation strategies by providing essential information regarding life histories, environmental tolerances, and toxicity testing of target and surrogate species. Propagating research animals on site reduces impacts to wild populations from specimen collection and allows biologists to streamline propagation systems and approaches. Each mussel propagation facility is unique in water supply, climate, and building type, so each season and new propagation system built provides a lesson for future improvements.

Figure 1. Mussel food ponds are vital to the survival of freshwater mussels at SMARC and provide algal particles to mussels constantly (left). Pond equipment provided a lesson in weatherproofing after freezing over during record Central Texas winter storms in February 2021 (right).

POTENTIAL MITIGATION OF WATERBORNE IRON TOXICITY IN CATFISH AQUACULTURE THROUGH APPLICATION OF BENTONITE CLAY

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Among different water quality parameters (typically hypoxia and high ammonia) responsible for limiting the fish productivity and performance, water-borne iron is the one of the prime concerns. When a certain threshold for iron is exceeded in the aquatic environment, it can incite a number of toxic effects in fish. Consequently, mitigating the toxic effects of iron overload is a major challenge to fish farmers, toxicologists and water quality managers. As such, we investigated the potential application of bentonite (a low-cost natural clay with superior adsorption properties) to bind and reduce iron load from channel catfish (*Ictalurus punctatus*) culture units, and thereby reduce iron induced toxicity.

Based on a bentonite dose testing pilot experiment- two doses 0.2 g/L and 0.4 g/L bentonite were investigated in this study. Fish were exposed to 9.5 mg/L high environmental iron (as Fe^{3+} representing 25% of determined 96 h LC_{50}). In brief, there were four experimental groups (i) Control (Con), (ii) Fish exposed to high environmental iron (HEI), (iii) Fish exposed to HEI and bentonite application at 0.2 g/L (HEI-$B_{0.2}$), and (iv) Fish exposed to HEI and bentonite application at 0.4 g/L (HEI-$B_{0.4}$). Each of these groups was performed in four replicated tanks (250 L), with 30 channel catfish (*Ictalurus punctatus*) (av. wt. 15-18 g) in each tank. Fish were sampled after 3, 7, 14 and 21 days. Results show that bentonite applications at both doses were effective in reducing iron load from water in a time-dependent manner. However, the time course for the response was shortened for the 0.4 g/L bentonite. Relative to HEI wherein ammonia homeostasis was disrupted, HEI-$B_{0.4}$ groups were able to maintain ammonia excretion rate efficiently. HEI also resulted in electrolytes imbalance in the plasma and inhibition of ion-transporter enzyme Na^+/K^+-ATPase, which was re-stored to control level in HEI-$B_{0.2}$ and HEI-$B_{0.4}$ groups. A remarkable increment in iron accumulation in plasma, gills, liver and muscles was noted in HEI, which was maintained at basal level in HEI-$B_{0.4}$. Protective effects of 0.4 g/L bentonite against HEI were also evident by histological analysis of gills. These could potentially explain an efficient iron mediated toxicity countervailing response in HEI-$B_{0.4}$ group. Overall, these data suggest that iron induced toxicity can disturb several physio-biochemical, ion-regulatory processes and iron homeostasis; the application of bentonite at 0.4 g/L can alleviate elevated waterborne iron toxicity in the catfish culture system.

TRIPLETAIL, BERMUDA CHUB, SPADEFISH, AND ATLANTIC CROAKER: NEW SPECIES UNDER INVESTIGATION FOR CULTURE AT TEXAS A&M UNIVERSITY

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Tripletail, Bermuda chub, spadefish, and Atlantic croaker are all species that have unique characteristics that may make them suitable for commercial aquaculture production. Tripletail are a highly desirable sport and foodfish that is popular among recreational anglers due to the texture and quality of their flesh, yet a unique live style of floating among debris at the surface severely hinders any substantial commercial wild harvest. Bermuda chub are somewhat unique among marine fish in that they are primarily herbivorous, feeding on brown algae, and this means they have potential to be grown commercially using sustainable, non-animal protein sources. Spadefish are an abundant but underutilized marine species that has regionally devoted followings as a foodfish, so much so that wild stocks are considered overfished in several countries, yet most of the U.S. population has never heard of them let alone had the chance to eat one. Atlantic croaker is unique in that it is a popular foodfish, but are also extremely popular as a marine baitfish and have great market potential for both purposes.

Join us for a brief overview of these fish species, their potential in culture, and insights gained during their investigation as potential commercial production species for Texas and elsewhere.

EVALUATION OF A NOVEL SLOW-RELEASE SPAWNING AID, OVAPLANT-L®, IN MARINE FISFISH REPRODUCTION

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Ovaplant-L® is the brand name for a new "liquid implant" that can be substituted in place of larger cellulose-based implants to induce spawning in fish. Ovaplant-L® contains a synthetic peptide analogue of salmon gonadotropin-releasing hormone (sGnRHa) in a novel, sustained-release excipient available from Syndel USA. Many fish species that have great economic significance in aquaculture do not reproduce spontaneously in captivity, or naturally reproduce under conditions that are not easily recreated in a hatchery setting. The use of hormones to induce spawning in fish is critical to the successful culture of many captive fish species that require hormone administration to complete final gamete maturation.

In this presentation, we will discuss some of the disadvantages of using traditional cellulose based implants compared to a liquid implant. As with any new product, safety to the culture animal must be evaluated prior to use. We will present the results of a study that evaluated the safety and gross pathology of the novel, sucrose-based excipient in Ovaplant-L® intended to deliver time-released spawning peptides in warmwater marine fish, using the commonly cultured red drum as a model species.

Finally, the results of studies using Ovaplant-L® to induce and synchronize ovulation in captive female cobia and improve spawning rate and fecundity in captive Atlantic croaker will be presented.

APPARENT NUTRIENT AND ENERGY DIGESTIBILITY OF COMMERCIAL FEED INGREDIENTS WITH OR WITHOUT PROTEASE (JEFO) IN RAINBOW TROUT

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One of the most important aspects in evaluating the effectiveness of alternative feed ingredients is the determination of digestibility. Several alternative feed ingredients have been tested in aquaculture feeds to replace fishmeal for sustainable aquaculture. Nevertheless, imbalanced amino acid profiles, poor digestibility and palatability, and presence of anti-nutritional factors (ANFs) limit their use in aquafeeds. Therefore, one of the strategies is to supplement enzymes for improving nutrient digestibility. Among enzymes, proteases have potential use in reducing ANFs, such as protease inhibitors, and breaking down macromolecular proteins. The efficacy of supplemental protease across a wide range of protein ingredients has not been previously investigated. Therefore, this study was conducted to evaluate the effects on apparent digestibility coefficients (ADCs) of dry matter, crude protein, amino acids, and gross energy when dietary protease was added to 17 different protein ingredients using rainbow trout as a model species.

In vivo digestibility was determined for 17 ingredients with and without protease supplementation (175 g kg^{-1}, Jefo Nutrition Inc., Quebec, Canada) fed to rainbow trout. The ingredients consisted of two feather meals, two poultry by-product meals, two meat and bone meals, sardine meal, menhaden meal, black soldier fly larvae meal, *Methanococcus maripaludis* single cell protein, soybean meal, canola meal, distiller's dried grains with solubles (DDGS), cottonseed meal, peanut meal, sunflower meal, and algae (*Spirulina* sp.) meal. A batch of test diet containing 30% test ingredient and 70% reference diet mash (combined on a dry-matter basis) was prepared and analyzed. Trout (average weight, 250 g) was used in the digestibility trial. Each of the experimental diets (reference and 34 test diets) was fed to two replicate tanks of fish in a completely randomized design to apparent satiation. Feces were expelled from each fish using gentle pressure on the lower abdomen of fish. ADC of diets and ingredients, for dry matter, protein, amino acids and energy were calculated. Apparent digestibility was calculated using fecal material pooled from 30 fish/tank, and all data are expressed as the mean ± standard error of the mean (SE). Data were subjected to a Student's t-test to test for protease effect using SPSS Version 20.0 (SPSS Inc., Chicago, IL, USA).

ADC of dry matter for rainbow trout ranged from 51.0–86.6% for animal products and single cell protein and 33.1–70.1% for plant products without protease supplementation. ADC (without protease supplementation) of protein and energy ranged from 55.4–84.5% and 58.1–90.2%, respectively, for animal products and 70.0–83.8% and 32.9–76.0%, respectively, for plant products. Supplementation with the commercial protease (175 mg protease complex/kg of diet) resulted in ingredient-specific ADC increases for dry matter, energy, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, alanine, aspartic acid and glutamic acid, with most ingredients having improved digestibility of at least one amino acid. Protease supplementation had the most profound improvement on ADCs for soybean meal, including dry matter and most individual amino acids.

In conclusion, supplementation with the protease complex resulted in ingredient-specific ADC increases for dry matter, energy, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, alanine, aspartic acid, and glutamic acid, with most ingredients having improved digestibility of at least one amino acid. Protease supplementation had the most profound improvement on ADCs for soybean meal, including dry matter and most individual amino acids. Overall, this research demonstrates the benefit of the evaluated protease supplementation on the digestibility of feed ingredients commonly used in rainbow trout and other commercially cultured fish feeds, although the degree of improvement in digestibility varied among ingredients.

NAVIGATING PERMITTING REQUIREMENTS FOR OFFSHORE SHELLFISH AQUACULTURE: CHALLENGES AND OPPORTUNITIES FACED BY THE VENTURA SHELLFISH ENTERPRISE PROJECT

Robert M. Smith,* Everard Ashworth, Brian Pendleton, Doug Bush, Linda Santschi, Ph.D, Ralph Imondi, Ph.D, Brooke Ashworth, Diane Windham, Scott Lindell, and Blake Stok

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Over the past six years, the Ventura Port District and its partners sought to establish the Ventura Shellfish Enterprise (VSE) project, a new offshore mussel shellfish farm in federal waters that would eliminate or reduce permitting hurdles for private shellfish companies and designed to accommodate participation by members of the Ventura working waterfront community in turn-key, pre-permitted parcels for mussel culture. As part of this project, the team sought to navigate the complicated state and federal permitting process associated with offshore federal aquaculture. This included extensive engagement with the U.S. Army Corps of Engineers, California Coastal Commission, U.S. Food & Drug Administration, National Oceanic and Atmospheric Administration (NOAA), California Fish & Game Commission, California Department of Fish & Wildlife, and numerous stakeholders and non-governmental organizations.

This presentation will discuss the permitting path for offshore aquaculture and some of the key issues that applicants will need to consider as they seek regulatory approvals. While the VSE project was focused on shellfish aquaculture in Southern California, many of the issues associated with resolving use conflicts are germane to other forms of commercial offshore aquaculture as well. This presentation will discuss the lessons learned during that process as the Ventura Port District engaged in outreach with key stakeholders, like commercial fishing interests and environmental NGOs, and addressed concerns raised by regulatory agencies. It will also detail the Ventura Port District's decision to pivot towards supporting NOAA's Aquaculture Opportunity Area process and role to support private aquaculture in the Santa Barbara Channel going forward to land product at Ventura Harbor in support of its working waterfront.

DEVELOPMENT OF IMPROVED COMMERCIAL FEEDS FOR RAINBOW TROUT *Oncorhynchus mykiss*. A CASE STUDY ON BALANCING COST, PERFORMANCE, AND SUSTAINABILITY

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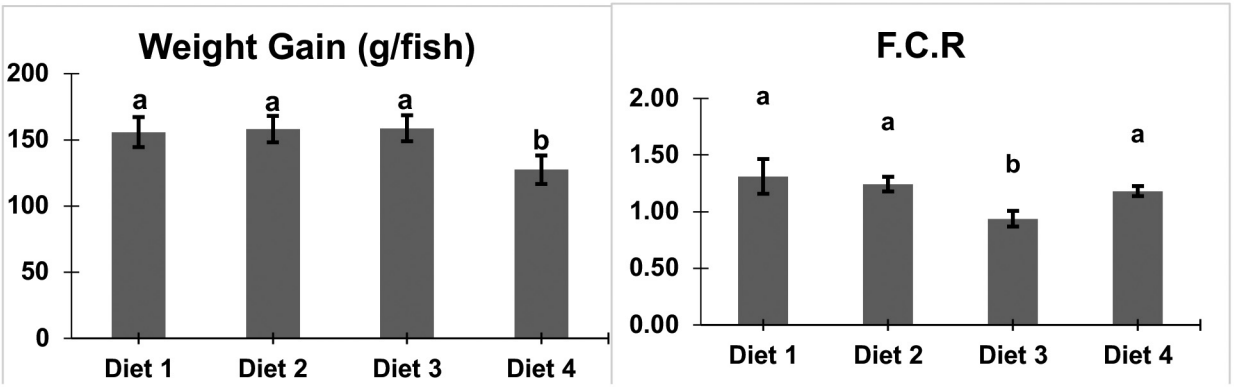
The state of static production volume and highly volatile market conditions of rainbow trout (*Oncorhynchus mykiss*) production in the US leaves farmers with few options for increasing the profitability of their businesses. It is well established that feed costs are the largest single variable cost, lowering trout feed costs as profits shrink is an industry priority. Four experiments were conducted at the Hagerman Fish Culture Experiment Station evaluating 16 commercially-produced trout feed formulas with the objective of optimizing the feed cost – fish performance balance. The primary objectives of experiments 1-4 were to measure the effects of feeding alternative formulas to rainbow trout on intestinal morphology and innate immunity, fecal turgidity, followed by performance during the juvenile lifestage, and growout lifestage, respectively.

Experiment 1 resulted in no statistical differences in all measurements except for higher oxidative radical production in fish fed Diet 4. Experiment 2 resulted in statistical differences in weight gain and FCR; and numerical differences in fecal durability. Experiment three resulted in significantly improved nutrient digestibility in Diet 4 while maintain statistically similar fish performance and fecal particle size distribution as Diet 1. Although the growth performance, feed utilization, fillet yield and whole-body composition results were not statistically different (P>0.05), practical numerical differences were observed in Experiment 4. Taken together, these results suggest that based on growth and feed utilization, fish fed diet groups 1 and 3 tended to perform better than fish fed the diet group 4.



UNDERSTANDING THE CEMENTING PROCESS OF THE ACORN BARNACLE *Amphibalanus amphitrite*: RECENT ADVANCES TOWARD A HIGH QUALITY GENOME

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The acorn barnacle *Amphibalanus (=Balanus) amphitrite* is an established fouler with a cosmopolitan distribution in tropical and sub-tropical marine environments. As a well-studied marine invertebrate in the laboratory, *A. amphitrite* is an ideal model for understanding its settlement, growth and robust adhesion to a wide variety of substrates. Of particular interest is the adult barnacle cement, which comprises a complex mixture of lipids, carbohydrates and proteins. In recent years, the number of proteins identified at the adhesive interface of acorn barnacles has been greatly expanded to cover several classes of proteins including enzymes, pheromones, and a number of previously identified "cement proteins" whose exact functions are unknown. A persistent bottle-neck to understanding how the proteinaceous interface is formed and functions as an effective marine adhesive is the current lack of proteome depth, i.e. many peptide hits during proteomic mass spectrometry analysis cannot be traced to identifiable proteins in limited transcript databases. Therefore, we seek to increase the coverage of proteome depth based on a high-quality *A. amphitrite* genome.

Previously, our research group presented early results of a draft genome of *A. amphitrite* based on Pac-Bio Single Molecule, Real-Time (SMRT) Sequencing. We now expand on those results by incorporation of Hi-C sequencing (a chromatin confirmation capture protocol utilizing proximity ligation of genomic information) as well as high-quality RNA sequencing using PacBio Iso-Seq. Combined, these sequencing efforts provide a high-quality genome of this marine arthropod (genome size: ~ 1Gb, 32 chromosome pairs) and, consequently, much greater protein depth for identification to understand the complex nature of the adhesive interface and the process of settlement and growth. Beyond the understanding of the acorn barnacle genome, these results provide a roadmap for development of high-quality genomes of other non-model organisms to account for contaminants and troublesome DNA repeat sequences.

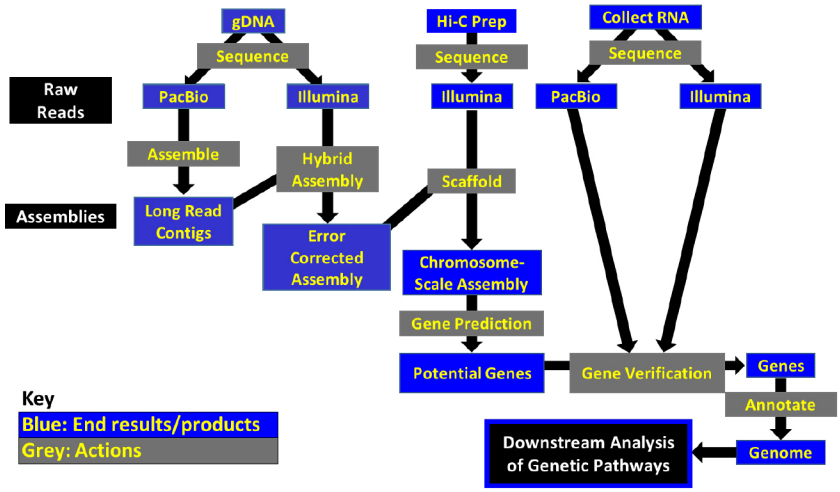

Figure 1. Overview of genomic sequencing pipeline combining long-read, (PacBio), short-read (Illumina), and chromosomal-level genomic data.

BIOENERGETICS OF YELLOWTAIL JACKS *Seriola spp.* TO IMPROVE PRODUCTION EFFICIENCIES IN AQUACULTURE

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Species of yellowtail jacks, collectively referred to by their common genus as *Seriola*, are produced throughout the world in tropical and temperate marine waters in both land-based and offshore aquaculture systems. As *Seriola* have grown in popularity in the aquaculture industry, fish farmers are increasingly looking to improve the overall production efficiencies of these high-value marine fish. To optimize the aquaculture performance of a fish species, it is critical to understand the energetic demands, especially under specific environmental conditions. Optimal rearing conditions for fish species vary depending on multiple factors, including species-specific life history. While some bioenergetics research has been conducted in the past on several temperate species of *Seriola* (i.e., *S. lalandi, S. quinqueradiata, and S. dorsalis*), there is virtually no information on the bioenergetics of the *S. rivoliana*, commonly known as the Almaco jack. Almaco jack has been identified as one of the prime candidates for warm water marine finfish aquaculture development in the U.S., and it is currently being produced commercially in Hawaii, USA. Results of ongoing bioenergetics research on *Seriola* will be presented, including assessment of the aerobic performance of *S. rivoliana* under a range of water temperature conditions; comparable to temperatures encountered by both ocean-based and land-based producers. Comparative bioenergetics analysis with other *Seriola* species will also be discussed. The collaborative research results will provide novel insights on optimal rearing temperatures, water velocities (i.e. optimal swimming speeds), and overall bioenergetics to improve the production efficiency of *Seriola* species under commercial settings. This research presentation was supported by the U.S. Department of Agriculture, Agricultural Research Service by cooperative agreement number 59-6034-9-007 with Florida Atlantic University's Harbor Branch Oceanographic Institute.

DEVELOPMENT OF PILOT-SCALE YELLOWTAIL SNAPPER *Ocyurus chrysurus* AQUACULTURE GROWOUT TECHNOLOGY TO INCREASE RESILIENCE FOR FISHING COMMUNITIES OF THE SOUTHEAST U.S.

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The yellowtail snapper, *Ocyurus chrysurus*, is an iconic and highly-valued marine fish species harvested primarily in the South Atlantic, Gulf of Mexico, and the Caribbean. It is a well-known and popular species amongst commercial and recreational fishing communities throughout these regions. In particular, yellowtail snapper is one of the most sought after and valuable fish species for the Florida Keys commercial fishing fleet, the largest commercial fleet from Texas to North Carolina. Historically the yellowtail snapper fishery of the South Atlantic has been considered relatively healthy and not undergoing overfishing. Seasons for the fishery remained open year-round allowing for the commercial fleet, seafood processors, distributors, and end users to rely upon this species on a steady basis. However, in recent years the commercial season for yellowtail snapper in the South Atlantic was forced to close early to prevent the commercial annual catch limit from being exceeded. Such closures had direct negative impacts on all segments of the fisheries supply chain, from the commercial fleet to processors, distributors, and the entire working waterfront community of these regions as a whole. In an effort to provide increased resiliency for working waterfront communities, aquaculture production of marine fish species has been identified as a potential alternative to supplement income for communities that have historically relied solely on wild-catch fisheries. As part of a project funded by the NOAA Saltonstall-Kennedy grant program, results will be presented outlining efforts aimed at establishing reliable and cost-effective aquaculture production of yellowtail snapper in an effort to build upon the existing high-value market for this species and offer opportunities for market expansion, stabilization, and product diversification. Progress on yellowtail snapper broodstock capture, spawning, larval rearing, fingerling production, and nursery stages will be discussed.

DIETARY LYSINE REQUIREMENT FOR JUVENILE FLORIDA POMPANO *Trachinotus carolinus* FED FISHMEAL FREE DIETS

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Alternative protein sources are implemented as a substitute or complement to fishmeal in order to improve feed sustainability. However, critical nutritional requirements such as indispensable amino acids are limited when alternative protein sources are added in aquaculture feeds. Lysine is often one of the first limiting amino acids in feeds formulated with high levels of plant proteins. Therefore, the objective of this study was to determine the dietary lysine requirement for Florida pompano, *Trachinotus carolinus*. In the trial, eight diets were prepared with increasing lysine inclusion levels from 1.35 to 2.34% of the diet. The feeding trial was conducted in a semi-closed recirculating system comprising of 36 circular tanks (750L) at Claude Peteet Mariculture Center. Twenty Florida pompano juveniles (mean weight = 13.07 ± 0.46 g) were stocked into each tank and assigned to quadruplicate tanks in a randomized design. Fish were fed 4 times per day and daily ration was adjusted to apparent satiation. Sampling of fish occurred every 2 weeks to adjust feed inputs for growth and mortalities. The growth trial lasted for 56 days. At the conclusion of the trial, fish were weighed and 4 fish per tank were euthanized in ice for measurement of whole-body composition analysis. Statistical analyses were conducted using SAS (V9.3 SAS Institute, Cary, NC). Initial weight, final weight, percent weight gain (PWG), thermal growth coefficient (TGC), feed conversion rate (FCR), feed intake and survival were analyzed using a one-way analysis of variance (ANOVA). Significance level was set at $P<0.05$. Results indicated that significant differences were observed amongst treatments for final weight, PWG, TGC, FCR and survival (Table 1). Means in a column with different superscript letters are significantly different according to Tukey's multiple range test (Table 1).

Table 1. Growth of Florida pompano fed graded levels of lysine for 56 days.

Dietary Lysine (%)	Initial Weight (g)	Final Weight (g)	Weight gain (%)	TGC	FCR	Feed offered (g)	Survival (%)
1.35	13.00	33.43^{B}	156.53^{B}	0.0588^{B}	2.79^{A}	55.31	82.50^{AB}
1.47	13.04	41.66^{AB}	219.77^{AB}	0.0758^{A}	2.23^{B}	63.62	95.00^{A}
1.59	13.07	41.91^{AB}	221.21^{AB}	0.0762^{A}	2.19^{BC}	62.97	91.25^{AB}
1.71	13.02	43.76^{A}	236.38^{A}	0.0798^{A}	1.98^{BC}	60.91	82.50^{AB}
1.86	12.96	43.55^{A}	234.68^{A}	0.0792^{A}	2.06^{BC}	62.10	86.25^{AB}
2.02	13.22	48.78^{A}	269.76^{A}	0.0878^{A}	1.67^{C}	59.14	75.00^{B}
2.18	13.05	44.61^{A}	241.57^{A}	0.0811^{A}	1.85^{BC}	57.96	78.75^{AB}
2.34	13.24	44.30^{A}	234.00^{A}	0.0797^{A}	2.01^{BC}	61.84	86.25^{AB}
P-value	0.9923	0.0045	0.0009	0.0007	<0.0001	0.3398	0.0360
PSE[1]	0.2626	2.1588	16.5645	0.0042	0.1449	2.5728	3.9857

[1]PSE: Pooled Standard Error

THE IMPORTANCE OF INTANGIBLE ASSETS ON SALMON EXPORT: A FIRM LEVEL PERSPECTIVE

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Seafood is the world's most traded food product group, with Norway being the world's second largest producer of seafood, as well as the leading producer of farmed salmon. As the Norwegian aquaculture industry better tackles capacity problems, controls lice- and disease problems in the production, the industry is anticipated to have a high potential for future growth. A significant share of future production is expected to be exported to foreign markets demanding high quality nutritious products. In the latter years, the reputation of the product, an important part of the exporting firms' intangible assets, has become more important for the industry as consumers pay more attention to sustainable production.

This paper studies the relationship between intangible capital and export performance at the firm level for export of fresh salmon from Norway in the period 2013-2017. We decompose the exporting firms' intangible capital into different parts such as the value of production licenses, research and development and goodwill (reputation) and relates these to important trade margins such as export value, unit value and the size of the exporters' network of buyers.

Our results show that there exist a strong positive relationship between the size of the firms' intangible capital and important margins of trade at the firm level, indicating that investments in research and development, as well as the reputation of the product, are important factors for expanding trade growth at the firm level. Our results are of importance to managers in the industry that seek to ensure continued trade growth, as we document that higher profit margins can be created through investments in intangible assets, such as goodwill.

ZEBRAFISH CLCAs STRESS RESPONSE UPREGULATES INTERLEUKINS

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Bacterial diseases cause significant economic loss to the aquaculture industry. There is a need to find new solutions that can replace antibiotics. Epithelial tissues play a vital role in host defense mechanisms. One of them is a barrier function that protects inhibits pathogenesis. Epithelial cells also secrete humoral factors into the fish mucus that fight bacteria and other pathogens. In this study, we investigate the role of zebrafish CLCA genes in bacterial infections. CLCA genes are well conserved across species, including fish. CLCAs are predominately expressed in epithelial cells and goblet cells. CLCA1 has been shown to increase pro-inflammatory cytokines upon its activation in a Staphylococcus aureus disease model. We have evaluated the expression of three zCLCA family members in various zebrafish tissues infected with *Aeromonas hydrophila*. We found that the zCLCA1's highest expression is in the intestine compared to gills or skin, unlike zCLCA5.1 or zCLCA 5.2. CLCAs have metal-binding motif HEXXH, which is conserved across species, including zebrafish. Interestingly, we find that zCLCA 5.1 and zCLCA5.2 expression is significantly lower in tissues but upregulated in mucus and its stress response directly correlated with upregulation of IL-1β, IL-6, IL-8, and IL-13 in various tissues. Our immunofluorescence and gene expression analysis data indicate that zCLCAs, like other CLCAs, are at cell-cell junctions zCLCA1 and zCLCA5.1. They also express transcript variants that act as antibacterial peptides (zCLCA 5.1 and 5.2) that induce immune responses in fish mucus to inhibit bacterial pathogenesis.

RECIRCULATING AQUACULTURE SALMON NETWORK (RAS-N): EMPOWERING US LAND-BASED SALMON PRODUCTION

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Currently, at least a half dozen land-based, Atlantic salmon RAS production facilities throughout the US are in operation or in advanced planning and permitting phases. Collectively, this represents over $2.5 billion in investment in this emerging US industry, which is expected to contribute significantly to domestic seafood production, national food security, reduction of US seafood trade deficits and local economic development. However, for Atlantic salmon RAS in the US to be successful and economically feasible, the industry must overcome biological and technological hurdles, gaps in knowledge, and be continuously optimized, which requires transdisciplinary R&D, education, workforce development and extension. To help build capacity for this rapidly expanding industry, NOAA/Sea Grant has funded a national public-private consortium, consisting of academia, industry and federal labs across the US - the Recirculating Aquaculture Salmon Network (RAS-N). Since its inception in September 2019, RAS-N's mission has been to facilitate the growth of environmentally sustainable and economically feasible Atlantic salmon production in this country, in order to provide better food security and reduce the current trade deficit associated with salmon imports. A plethora of stakeholder-focused activities have been conducted to solicit industry input, including two network workshops (one in-person and one virtual), sessions at national conferences, and a dedicated salmon RAS website (ras-n.org) that provides updated technical information, links to relevant research and outreach to lay audiences. RAS-N continues to work closely with US stakeholders to maintain, promote and expand its holistic hub of knowledge that will integrate past, current and future research as well as extension, outreach & education, and workforce training to promote the successful growth and stability of the Atlantic salmon RAS sector and, more broadly, US aquaculture. To this end, working groups of industry, academic and federal experts have been established to assess specific barriers and knowledge gaps. These respective groups have established a plan to identify barriers and suggest resolutions. They have also contributed their collective assessments and solutions/strategic approaches. To date, the network has expended considerable effort compiling stakeholder input and research-driven data on priorities, barriers and areas of concern and generated a Concept Paper, with contributions from the various industry-academic-federal working groups that summarize this input. Education, extension and workforce development issues have also been thoroughly examined. The final deliverable of this collaborative program will be the development of a detailed Road Map/Strategic Plan that will help, federal/state agencies, policymakers and industry identify and responsibly allocate resources to promote an economically and environmentally sustainable land-based US salmon industry. This presentation will review the activities of RAS-N and solicit stakeholder engagement and input.

DYNAMICS OF PRICE VOLATILITY SPILLOVER IN THE U.S. CATFISH MARKET

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Aquaculture/farming of catfish (specifically the Channel catfish, *Ictalurus punctatus*) is an important agribusi- ness, especially in the Southeastern U.S. Farmed catfish is the largest segment of the U.S. aquaculture in- dustry. The catfish industry was considered to be an exemplary success story of U.S. aquaculture.

Figure 1 shows the trend in water acreage (acres) of cat- fish farms and production (in thousand pounds) from 1999 to 2019. Though there is a slight but noticeable increase in farm production in the last four years, the industry has shrunk substantially from the peak acreage achieved during 2003.

Feed grain prices started to rise dramatically around 2008 that led to increased cost of production of catfish (Engle and Stone 2013). Volatility of corn and soybean prices also increased during this period. Since selling to catfish processing plants is the most important marketing outlet for farmers, volatility in feed grain prices can be expected to impact processor price through farm prices. Therefore, volatility of feed grain prices is an important factor in the U.S. catfish market. Moreover, since the catfish industry is small compared to feed grain markets, direction of spillover/transmission of volatility can be assumed to be unidirectional, from input markets to catfish markets. Volatility of prices can act as an indicator of price variability in the future. Higher volatility implies increased uncertainty about the direction and magnitude of price change for farmers and processors, thus impairing their ability to form realistic expectations about the market for their product. In this study, we analyze the volatility spillover from feed markets (corn, soybean and fishmeal) to catfish farm price and processor price. A unique feature of our study is the use of volatility impulse response functions (VIRF) to characterize volatility spillovers. Hafner and Herwartz (2006) developed the VIRFs to trace the time path of the effects of independent shocks on covariances. We use the VIRFs to understand spillovers during periods high and low volatility in feed prices. We compiled monthly time-series data on catfish farm price, processor price, corn price, and soybean price from USDA sources. We used the data on price of imported fishmeal available from the National Marine Fisheries Service (NMFS) Foreign Trade database. The results will show differences in price volatility spillover during times of high and low feed price volatility. The results can serve as indicators to the vulnerability of catfish markets to volatility shocks.

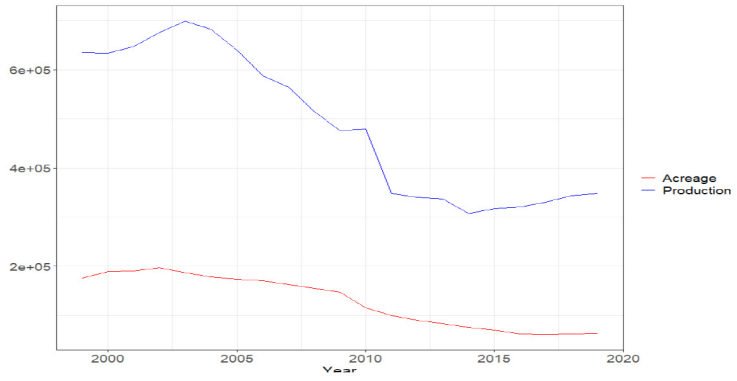

Figure 1: U.S. Catfish Acreage and Production: 1999-2019

DETERMINING INFLUENCIAL FACTORS IN CONSUMERS SEAFOOD PURCHASING DECISIONS

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Food safety has become one of critical issues being discussed all around the world. Currently 1 in 10 people in the world will fall ill after eating contaminated food resulting in the deaths of 420,000 people every year (WHO, 2014). Consumers are exposed to an influx number of positive and negative information regarding food safety, this makes it critical to understand the perceptions and preferences of these consumers. These influences come from media sources, recall information, and development of policies to safeguard the food supply. Issues of declining consumer trust in the food system stems from concern about new policies, technologies, and perception of current agricultural practices and how it impacts the quality and safety of the food supply (Smith et al., 2010; Rivera et al., 2018). The introduction of legislation as well as the implementation of the Hazard Analysis Critical Control Point (HACCP) is a government response to changing the way producers conduct themselves within the food safety system and their response. The focus of HACCP although geared towards producers, consumers should be aware of the policy because of the potential impacts that unsafe foods can have on consumer health and the economy. The consumer knowledge of food safety policies is critical for consumers to understand how policy makers are working to protect them and working to move from reaction to prevention of food safety issues (FDA). Although food safety has been presented as a global problem, in order to understand food safety and the impact that it has on consumers, this study will use Texas as area of the study. There are multitude of reason for selecting Texas as the study area. Geographically, Texas is the biggest state in United States and recently we saw rapidly increasing trend growth in population of due to migration to metropolitan areas in Texas. These areas have had population changes ranging from 2% to about 3% from 2016-2017. In 2018, it was expected that 43.70% of the population growth that occurred within Texas was due to migration to major cities (Valencia, 2019). The goal of the proposed project is to understand consumer preferences in terms of food safety issues and food safety regulations in seafood. To achieve this goal, the study is understanding consumer perception of existing food safety issues and control measures in the seafood market and determining the change in consumers' willingness to pay for products as information about food safety changes. This study takes a qualitative research approach to understand the consumer and their perceptions of current existing food safety issues and control measures in the seafood market while also determining if there is a change in consumers' willingness to pay for products as information about food safety changes. This was done in two parts, using focus groups to gather baseline data and the development of four different consumer surveys with a focus on different products such as seafood, cheese, meat, and produce. The data will be analyzed using descriptive statistics, each individual consumer survey will also be broken down into four individual sections, demographics, consumer purchases and preferences, consumer food safety knowledge and attitudes, and consumers' willingness to pay. The detailed results will be presented in the conference.

NUTRITIONAL VALUE OF BLACK SOLDIER FLY (*Hermetia illucens*) REARED IN TWO DIFFERENT SUBSTRATES, AND THEIR EFFECT ON GROWTH PERFORMANCE, IMMUNE RESPONSES, AND INTESTINAL MICROBIOTA WHEN PARTIALLY REPLACING FISHMEAL IN DIETS FOR JUVENILE RED DRUM (*Sciaenops ocellatus*)

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Black soldier fly larvae (BSFL) has been identified as a prominent ingredient for aquafeeds due to its high productivity, relatively low feed conversion, and for growing on a wide range of substrates. These features allow the BSFL to convert waste biomass to an ingredient with an adequate nutritional profile: In the current study, BSFL were reared in Brewer's spent grains (BSG) and a commercial substrate (CFD) as a control. Protein content and the amino acid profile of the BSFL meal were similar regardless of what substrate was used; however, a higher lipid concentration was observed when feeding the BSFL with BSG (33% crude lipid) compared to the commercial substrate (26%).

For the comparative feeding trial, a reference diet was formulated to be similar to a commercial diet, having its protein provided by menhaden fishmeal, poultry by-product meal, soybean meal, and soy protein concentrate. The two BSFL meals replaced 65% of fishmeal in the experimental diets. Groups of 12 fish (initial weight ~5.6 g) were stocked in 15, 38-L aquaria, and the three experimental diets were distributed in a completely randomized design (n=5). After 8 weeks, weight gain (% of initial weight) and feed efficiency (FE) were significantly impaired by the inclusion for both BSFL treatments (BSG: 522%; 0.70) (CFD: 597%; 0.77) when compared to the control (682%; 0.82); while fish fed BSFL-BSG had the lowest production performance (Figure 1). No significant differences were detected for the immunological assays in the plasma, production performance indices, or whole-body composition among treatments; however, redfish fed the reference diet had higher mineral content compared to fish fed both BSFL meals. Digesta was aseptically sampled representative fish in each aquarium on the 9th week and bacterial DNA was extracted to assess possible differences in microbial communities by the denaturing gradient gel electrophoresis (DGGE) method and next-generation sequencing (NGS). DGGE results showed that fish fed BSFL-BSG and BSFL-CFD had similar intestinal bacterial communities (89%), but both treatments were noticeably different than fish fed the reference diet (69%). Data from NGS are currently being processed, and more results will be evaluated. In the current study, the partial replacement of BSFL reared in BSG and CFD had reduced growth performance and FE compared to fish fed the reference diet.

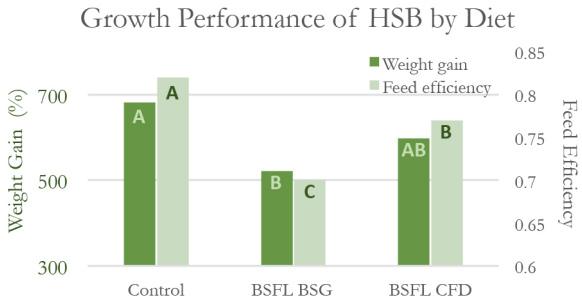

Figure 1. Weight gain and feed efficiency of red drum fed experimental diets with BSFL meal reared in two substrates.

EFFECTS OF DIETARY GLUTAMATE ON THE GROWTH PERFORMANCE AND IMMUNE RESPONSE OF HYBRID STRIPED BASS, (*Morone chrysops* × *M. saxatilis)*

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Certain dispensable or nonessential amino acids synthesized by the body have been demonstrated to provide some enhanced physiological roles in animals when supplemented in the diet, and thus have been termed functional amino acids. Glutamate has been identified as a possible functional amino acid for fish. Therefore, an 8-week feeding trial was conducted to evaluate the effects of varying levels of dietary glutamate on growth performance, immune responses, and whole-body composition of juvenile hybrid striped bass. Three diets were formulated from practical ingredients to contain 43% crude protein and 12% lipid, to which L-glutamate was supplemented at either 0, 1.0 or 2.0% of dry weight. All diets were randomly assigned to quadruplicate groups of 15 juvenile hybrid striped bass (HSB) initially averaging 5.65 g/fish in 110-L aquaria connected as a recirculating aquaculture system and fed twice daily at a percent of body weight which approached apparent satiation. A preliminary *in vitro* study also was conducted to evaluate if immunological responses of head-kidney isolated leukocytes from HSB would be affected by incremental levels of glutamate, glutamine and their combination in the culture media. Leukocytes were incubated overnight in a complete cell culture medium containing physiological levels of plasma amino acids as a control, while experimental groups were incubated with the reference media coupled with separate glutamate (1.0, 2.0, and 5.0 mM) and glutamine (1.0, 2.0, and 5.0 mM) supplementation, as well as a combined glutamate and glutamine (1.0 mM) treatment.

HSB fed diets containing incremental levels of glutamate did not present differences in terms of percent weight gain (average of 649% of initial weight), feed efficiency (FE) (average of 0.75), whole-body composition, hepatosomatic index (HSI), or plasma amino acid composition. However, intraperitoneal fat (IPF) ratio was significantly ($P < 0.05$) higher in fish fed the basal diet (5.75%) when compared to those fed the diet supplemented with glutamate at 2.0% (5.01%). Fillet yield was also significantly higher in fish fed the basal diet (31.7%) when compared to those fed the diet supplemented with glutamate at 1.0% (29.9%).

In vitro intra- and extracellular superoxide anion (O_2^-) production were linearly correlated with the addition of increasing glutamate levels while the coupled treatment of glutamate and glutamine (1.0 mM) produced the greatest respiratory burst response of head-kidney- derived leukocytes. The current experiment suggested a negligible *in vivo* response of HSB fed diets with elevated levels of glutamate while *in vitro* immunological responses to glutamate addition were more apparent.

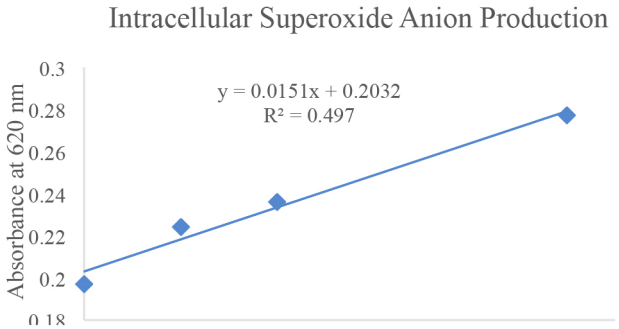

Figure 1. Intracellular superoxide anion production (O_2^-) of head kidney leukocytes incubated in a complete cell culture media with increasing levels of glutamate (mM).

EVALUATION OF GROWTH PERFORMANCE, CONDITION INDICES AND BODY COMPOSITION OF JUVENILE RED DRUM (*Sciaenops ocellatus*) FED FISHMEAL- AND FISH OIL-FREE DIETS

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Global increases in aquaculture production has led to heightened demand and increasing costs of fishmeal and fish oil products for use in the diets of highly carnivorous fish species, such as the red drum. Therefore, viable alternative protein and lipid sources are needed to substantially replace fishmeal and fish oil in commercial diets. In the current study, a commercial reference diet containing 51% crude protein (CP) on a dry-matter basis and six isonitrogenous (50% CP) experimental diets that completely replaced menhaden fishmeal and/or menhaden fish oil were evaluated with juvenile red drum. The experimental control diet (Diet 2) contained menhaden fishmeal at 28.3% of dry weight and 6.5% menhaden fish oil. Diet 3 had all of the menhaden fishmeal replaced with a combination of peptide product, poultry by-product meal and soy protein concentrate but contained menhaden fish oil at 7.7% of dry weight. All other experimental diets were isolipidic (13% crude lipid) and contained no fishmeal or fish oil which was replaced by various combinations of canola, flax, and algal oils.

In the comparative feeding trial, groups of 15 fish (~4.4 g/fish initial weight) were stocked in 28, 38-L aquaria fashioned as a recirculating aquaculture system with quadruplicate aquaria randomly assigned to each diet and fed for an 8-week duration. At the end of the trial, juvenile red drum fed the commercial reference diet exhibited significantly ($P<0.05$) reduced percentage weight gain, fillet yield, feed efficiency (FE), protein conversion efficiency (PCE), and survival compared to fish fed all experimental diets as analyzed by one-way ANOVA (Figure 1). Red drum fed the six experimental diets did not show any significant differences in any growth parameters. Fish fed the commercial reference diet also showed significantly ($P<0.05$) higher viscerosomatic indices, including hepatosomatic index (HSI) and intraperitoneal fat (IPF) ratio than fish fed most experimental diets that replaced fishmeal and/or fish oil. Whole-body proximate analysis revealed no significant differences in crude protein content; however, red drum fed the commercial diet had a significantly higher ash and lower whole-body lipid composition than fish fed all other diets. The current study provides evidence that juvenile red drum can be successfully raised using practical diets devoid of fishmeal and fish oil.

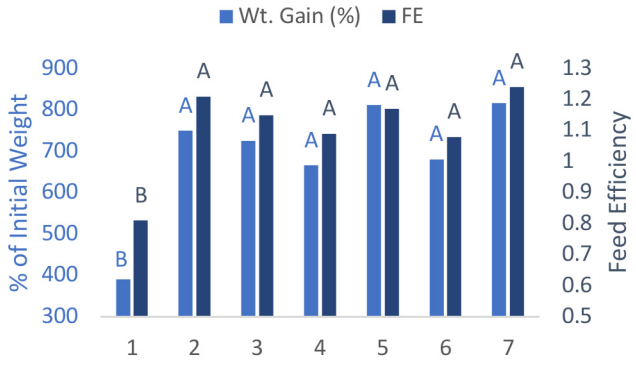

Figure 1. Percentage weight gain and feed efficiency of juvenile red drum fed experimental diets replacing fishmeal and fish oil for 8 weeks. Diet 1 = commercial reference; Diet 2 = experimental control diet containing both fishmeal and fish oil; Diet 3 = experimental diet containing fish oil but no fishmeal; Diets 4-7 = No fishmeal and no fish oil with various combinations of canola, flax, and algal oils.

ASSESSMENT OF UNIQUE LOUISIANA OYSTER *Crassostrea virginica* POPULATIONS FOR ADAPTATION TO LOW SALINITY

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Eastern oysters (*Crassostrea virginica)* support a critical commercial industry and perform critical ecosystem services in coastal estuaries, but changing estuarine conditions call for increased farm management strategies. In particular, climate models and local management strategies predict increasing freshwater in productive oyster areas, creating a need for strategies to maintain production despite water quality changes. Although highly tolerant to a wide range of salinities and salinity variation, more frequent exposure of oysters to extreme low salinity (<5) may impact overall sustainability within some estuaries. This study assesses unexamined populations of oysters from assumed low-salinity areas for population-specific tolerance to low salinities. Spat (<25mm) from four populations were grown in an off-bottom longline system in an intermediate salinity (10-20) and a low salinity (<6) environment for twelve months. Monthly sampling of growth and mortality showed no significant difference in growth rate between populations at either site; however, there were differences in cumulative mortality between populations at the low salinity site. Oysters from the site of origin with the closest matching salinity regime to the low salinity grow out site had significantly lower mortality than the other three populations tested. This finding suggests potential for adaptation to lower salinity by oysters frequently exposed to those conditions at their site of origin. Further differences in mortality between populations may be explained by unidentified local salt wedges at the oysters' sites of origin or an exacerbated effect of increased temperatures that oysters in deeper waters may not be accustomed to. The identification of further oyster populations able to tolerate low salinity conditions would facilitate selective breeding using diverse stocks specifically suited to the areas where they will be grown to promote efficient restoration, development of aquaculture, and industry interests.

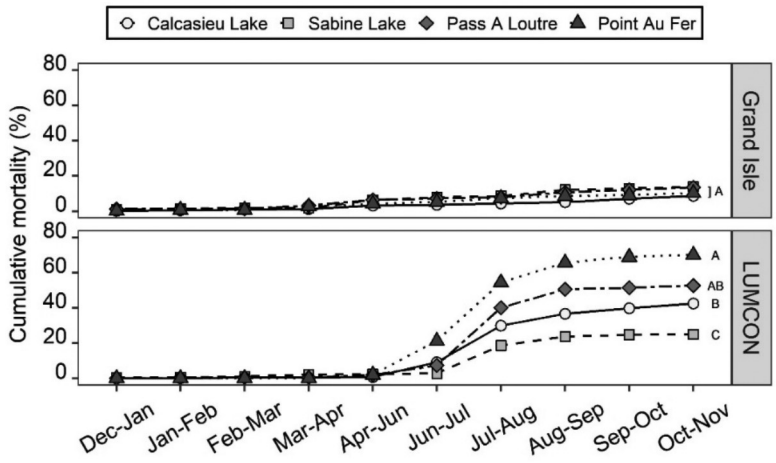

Figure 1. Cumulative mortality (%) of oysters from Calcasieu Lake, Sabine Lake, Pass A Loutre, and Point Au Fer stock populations. Different letters denote statistical differences (sites analyzed separately).

INDUSTRY RESEARCH, ENGAGEMENT AND EDUCATION NEEDS

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Sea Grant invests around $20 million in federal funds annually to support aquaculture, research, education and engagement. To better respond to industry needs in these functional areas, Sea Grant is developing a research, education and engagement plan. The plan is being developed using information collected from multiple sources including an internal needs assessment of the Sea Grant Network and an external needs assessment of the industry, non-governmental organizations and federal agencies, and the use of existing planning documents which identify research, education and engagement needs. The three sources of information will be used to develop the final plan. Once complete, the plan will be shared with the National Sea Grant College Program to use in their deliberations when developing their competitive funding initiatives. The National Aquaculture Association has been a critical partner during the process of developing the plan. The presentation will present a high-level summary of the results of the final plan.

RISK ASSESSMENT OF VIRAL ENCEPHALOPATHY AND RETINOPATHY INTRODUCTION AND SPREAD IN MEDITERRANEAN SEABASS FARMS

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The objective of this study was to use risk assessment to determine the biosecurity risk associated with Viral encephalopathy and retinopathy (VNN) introduction and spread in Mediterranean seabass production, and to identify the control measures to manage the risks.

Biosecurity plans for prevention of introduction and further spread of disease pathogens involve knowledge on risk assessment of disease hazards in the region, likelihood of introducing the infectious agent into the production system, epidemiology and transmission routes, critical points for introduction and control. VNN was identified as the most important disease of seabass in the western, central, eastern and southern Mediterranean areas in terms of production and economic impact due to factors such as the high mortality and morbidity by literature, survey, and expert opinion.

A group of 10 experts consisting of fish health specialists, veterinarians, biologists, and epidemiologists were asked to provide their opinion regarding disease hazards, likelihoods of disease introduction and spread in the Mediterranean seabass. Expert knowledge elicitation (EKA) approach was used to elicit expert opinion and perform the risk assessment. Risk matrix was used to present the overall risk estimates by integrating the numerical scores for the likelihood of disease introduction and the economic consequences. The experts to identify the points at which VNN introduction and spread could occur and be prevented. At each action point, experts gave a weight for potential biosecurity measures concerning its feasibility and effectiveness for controlling VNN

The main risk pathways differ by type of production for which the likelihoods of introduction are similar for hatchery and pre-growing, but different from the likelihoods for on-growing. Intake of water, live fish and eggs, vehicle transporting live fish, human, equipment, and high-risk purchasing were identified by the experts as the risk pathway for all types of production. This illustrates a clear recommendation to encourage focusing on introductory risk.

The economic consequences of VNN depend on the type of production of the facility. The consequences of VNN introduction were regarded most devastating in economic terms for hatcheries and pre-growing units and the risk estimate was in general high or very high for these productions. For on-growing the risk estimates were regarded medium to high. Measures to comply with introduction entail requirements of reliable health certificates and quarantining newly acquired fish upon arrival. The measures for disease management entail removing dead fish daily, preventing direct contact between quarantined fish and the other fish on the facility, separate water flow between quarantined fish and the other fish on the facility, and follow-up investigation of disease outbreaks.

BUILDING A DIGITAL TRACEABILITY SOLUTION

Wyllys Chip Terry, Ph.D.

The Centers for Disease Control estimates *Vibrio (parahaemolyticus & vulnificus)* causes 80,000 illnesses and 100 deaths in the United States every year, most commonly from eating raw or undercooked seafood such as oysters. However, according to the co-chair of the Interstate Shellfish Sanitation Conference: *"Our current [tracking] system…is inadequate…. More than half of all traceback investigations fail because the information in the value chain is lost."*

With an SBIR Grant from NOAA, Shellfish Solutions piloted a digital traceability and marketing system that is lower cost than the existing largely handwritten system and more powerful: Saving users significant amounts of time while improving compliance.

The value extends beyond regulatory compliance. QR codes embedded on the regulatory tags gives farmers/harvesters the opportunity to tell their unique story directly to chefs and consumers--connecting the consumer with the producers of their food.

The increasing toll on consumer health from unsafe food is leading companies and regulators to look for a high quality, easy to use, and cost-effective digital traceability system. Without such a system many companies will not be able to meet the increasing regulatory requirements such as those from the new Food Safety Modernization Act.

The session will be a presentation on your experiences building this solution, what we learned about the traceability regulations, and the challenges companies face as they move from pen and paper to digital solutions.

ASSESSING THE EFFECTS OF AN AUTHENTIC PROJECT-BASED INTERVENTION ON SECONDARY STUDENTS' UNDERSTANDING OF ECOSYSTEMS AND THEIR ATTITUDES TOWARD AND INTERESTS IN STEM

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There is a need for secondary schools to provide more authentic, hands-on experiences in science, technology, engineering, and mathematics (STEM), and specifically, project-based investigation (PBI) environments in the classroom that focus on real-world problems relevant to students' experiences, interest, and lives that manifest the Next Generation Science Standards (NGSS) following practices they prescribe. This study investigated how, to what extent, a contextualized aquaponics PBI (APBI) 10-week model unit affected high school students' attitudes toward STEM in general, and aquaculture and aquaponics in particular, and interests in future STEM-related disciplines and/or STEM career pathways. This study also measured changes in students' understanding of standard-based ecological relationships and concepts concerning interactions in ecosystems and specifically the phenomena carrying capacity and bacterial nitrification process.

Using a quantitative methods, quasi-experimental research design, three different student groups who participated in the authentic, hands-on APBI intervention (i.e., treatment groups) were given a pre- and post-attitude/interest survey (N=55). Further, the treatment groups and control group were given a pre- and post-content-aligned test (N=88) which measured changes in students' ecological knowledge. The results revealed that the intervention contributed to the treatment group students' positive attitudes toward STEM in general, and aquaculture and aquaponics in particular, and developing an interest in STEM disciplines and/or STEM career pursuits. Results also demonstrated that the project-based intervention was an effective method to provide meaningful learning and content understanding of standard-based ecological concepts and relationships.

The intervention design and findings in this study may provide educators new insights and ideas on how to incorporate and use contextualized, aquaponics project-based instruction (APBI) as a teaching and learning tool. In addition, APBI can offer engaging curricula that articulates NGSS.

GERMPLASM REPOSITORIES AND COMMERCIAL MARKETS FOR GENETIC RESOURCES OF AQUATIC SPECIES: A ROLE FOR OPEN TECHNOLOGIES?

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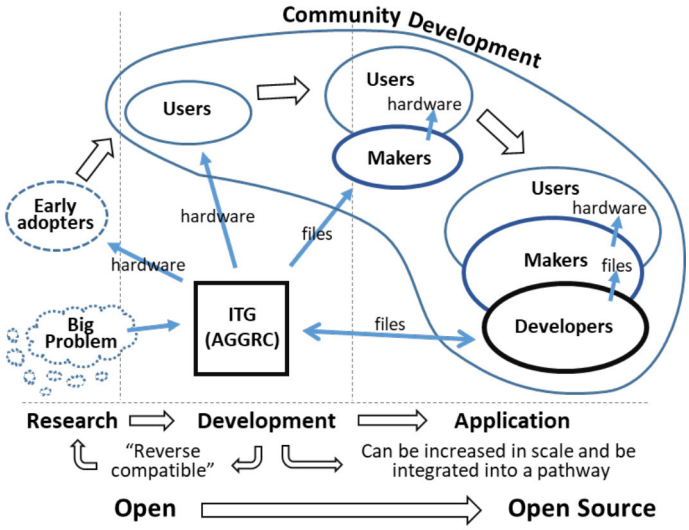
Some challenges are immense, and defy resolution despite offering tremendous opportunity. The development of germplasm repositories to protect genetic resources of aquatic species is such a challenge. Despite 70 years of cryopreservation research, fish and shellfish have only minimal frozen collections, although there are thousands of publications, primarily addressing creation of freezing protocols. This is in stark contrast to livestock such as dairy for which massive collections exist that drive multi-billion dollar global markets for improved genetics. The lack of repositories suppresses advances across aquaculture, conservation programs, natural fisheries, biomedical models, and efforts to address food security and poverty alleviation. Recognition of this as an immense challenge (not likely addressed by current approaches) is a step towards resolving it. Because large problems such as this are beyond the resources of single entities, other models are required to address them. An emerging model involves use of distributed networks to combine the efforts of large, interconnected communities that share common motivation. This approach was used to develop the Linux operating system in the 1990s through open-source software development driven by thousands of volunteer computer programmers. This sharing and community-based approach was in direct response to the limitations of proprietary development. The tremendous success of Linux provided impetus for other open-source projects, and the experience gained opened doors to expand distributed development. This spirit has emerged in renewed form with the advent of new consumer-level design and fabrication technologies that can enable study, distribution, production, modification, improvement, and commercialization (all based on licensing agreements) of computer-aided design (CAD) files shared over the internet. As such, these open technologies provide a powerful alternative to traditional research and proprietary development to combine efforts across multiple communities to establish repositories (Figure 1).



FAILURE TESTING THE MODIFIED DECOUPLED RECIRCULATING AQUACULTURE SYSTEM

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The Modified Decoupled (MDC) recirculating aquaculture system (RAS) is a closed system configuration with a sludge digestion capability. In aquaponics, decoupling the fish system from the plant system allows for independent control over water quality in both loops. This decoupled configuration works well for schools offering aquaponics classes in a STEM program, all levels of home operations, and marine applications, like shrimp, where a high degree of water reuse is desirable. The MDC 2000 in this study was made up of a 260 gallon tank, an Endurance® 2000 floating bead filter, an airlift designed for 30% lift, and a sludge digestion basin (operated in an aerobic configuration) fed by a pneumatic exchange mechanism. The MDC 2000 was tested in house over a 7-month period and a maximum feed rate of 1 pound per day (1.33lb/ft^3 media/day) was reached which was just shy of the goal of 1.5 lb/ft^3/day. It was concluded that the system was limited by recirculation flow at feed loadings beyond 1 pound/day. This was seen in suddenly rising nitrite levels (0.061 to 3.02 ppm) as recirculation flow rate decreased (6.4 to 5.55 gpm) at a feed loading of 1 pound per day. A high submergence to lift ratio decreases the efficiency of an airlift. This will be addressed in trial 2 by raising the tanks in order to achieve 20% lift for the airlift.

MDC 2000 Aquaponic Fish System

DISTRIBUTION OF MARINE MUSSELS SPECIES OF THE GENUS MYTILUS ALONG THE CHILEAN COAST

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The chilean native blue mussel *Mytilus chilensis*, is an economically important marine resource in Chile. The *M. chilensis* aquaculture began in 1943 in Chiloé Island, southern Chile, and the aquaculture production increased rapidly from 3,864 t in 1993 to 398,274 t in 2018, equivalent of 29,3% of the total Chilean aquaculture production. Therefore, and because the relative recent description of two species of mussels *Mytilus galloprovincialis* and *Mytilus edulis* in chilean waters, there is a need to carry out a surveys to establish the actual distribution of these two exotic mussels along the Chilean coast and evaluate their actual and potential effects on the biodiversity along the coast and on the fasting growing mussel (*M. chilensis*) aquaculture industry. Once the evidence that these mussel species arrived to a coastal location it is important to study their distribution and urgently to investigate their origin and its potential for bioinvasion along the chilean coast.

Samples of 30 adult mussels from 36 locations along almost 2,500 km of Chilean coast were analyzed using two nuclear markers (*Me 15-16 and Myti*) and two mitochondrial markers (*16S and COIXba*). The results indicate that from the 1030 mussels analyzed 76% belongs to the Chilean native *M. chilensis*; 17% to the mediterranean mussel *M galloprovincialis*, 4% to the northern hemisphere *M. edulis* and only 2% to hybrids between these species of mussels. The highest number of hybrids were detected between *M. edulis* and the native mussel *M. chilensis*. Also samples of cultured mussels from 8 aquaculture production centers were analyzed, within the X Region (southern Chile), where over 95% of the Chilean mussel aquaculture production is obtained. Among these mussel aquaculture centers, the results indicated that 96% of the mussels were the native Chilean mussel *M. chilensis*, 3% *M. galloprovincialis* and 1% of hybrids. Interesting is that among these hybrids, there were *Mytilus trossulus* alleles detected (a mussel species that inhabits the cold waters in the northern hemisphere), as described earlier by other authors. Also a high plastic variability among mussels shell (different morphology and coloration) within pure species was observed which precludes the use of these phenotypic traits in taxonomy. Two of the mussels detected can be detrimental for the chilean mussel aquaculture. *M. galloprovincialis* has been declared one of the world´s 100 worst invasive species and *M. trossulus* have lower rates of somatic tissue and shell weight.

Research founded by FONDECYT 1170194.

COMPARATIVE ANALYSIS OF SOYBEAN MEAL EFFECTS IN COMMERCIAL STRAINS OF RAINBOW TROUT *Oncorhynchus mykiss*

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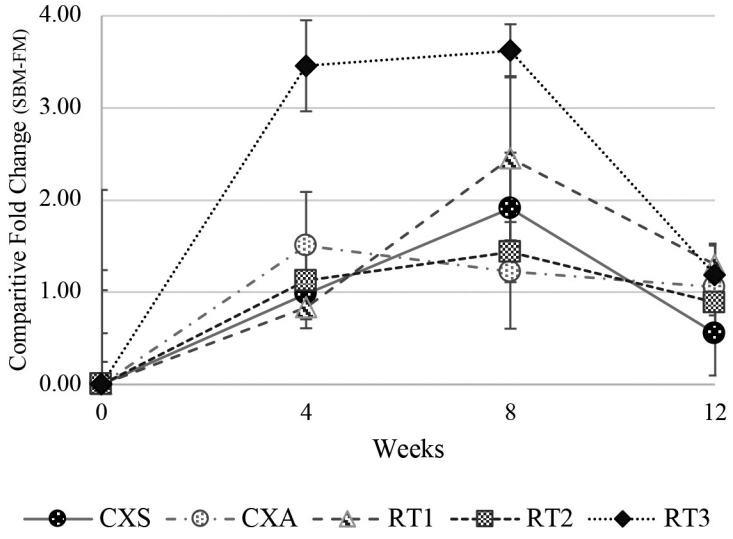
A strain of rainbow trout (CX strain) at the Hagerman Fish Culture Experiment Station has been selected for growth on plant-based feeds for ten generations. We compared fish from the CX strain that were age (CXA) and size (CXS) matched to commonly available commercial strains selected for growth (RT1, RT2, RT3). We compared differences in oral tolerance, intestinal inflammation and overall growth between fish fed a fishmeal (FM) or soybean-meal (SBM) diet for 12 weeks. Fish (5.7 ± 0.2 g) of each strain were randomly assigned to FM or SBM diet groups and fed daily to satiation. Tissues from each experimental group were sampled every four weeks to assess gene expression, growth, histology and enzyme activity. The CX strain had higher growth rates compared with similar feed consumption among all groups. Transcription of intestinal and hepatic biomarkers for inflammation and stress peaked at 8 weeks among most strains fed the SBM diet and declined by 12 weeks as shown in Figure 1 with intestinal calcium-binding S100I2 expression. Enzymatic activity followed gene expression but remained elevated at 12 weeks. Semiquantitative histological examination indicated initial signs of enteritis in gut epithelial tissues.

Figure 1. Intestine S100I2



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

Rose Upton*, Jack C. Koch, M. Teresa Gutierrez-Wing, and Terrence R. Tiersch

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

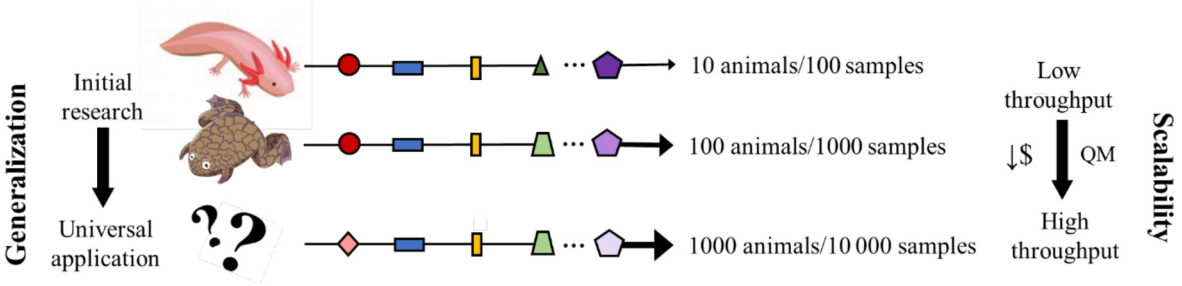

FIGURE 1. Strategy for developing scalable and generalizable germplasm repositories. Different shapes represent refinement of the cryopreservation process, however, each pathway need not be redeveloped from scratch. A generalized and scalable pathway for repositories can be developed for other species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

HOW CAN WE INTEGRATE GERMPLASM REPOSITORIES INTO THE CONSERVATION LANDSCAPE?

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The climate crisis currently at play worldwide has led to a long list of stressors on biodiversity in a wide array of ecosystems. Aquatic environments are often sensitive to seemingly small changes, with many organisms being affected by disruptions to habitat. Germplasm repositories have the potential to advance and complement current conservation efforts, at a relatively low cost, by preserving the genetic diversity of species and allowing correction of genetic effects caused by decreasing population sizes. For example, by reintroducing sperm collected before a disturbance event, captive breeding programs can improve genetic diversity of captive and reintroduced wild populations (Figure 1). While this approach has been used successfully in some species, this approach is often overlooked and has experienced a slow uptake in aquaculture and conservation. National stock centers, such as the *Ambystoma* Genetic Stock Center (University of Kentucky), can provide models for such repositories. *Ambystoma mexicanum* is a valuable research tool for improving treatments for spinal cord and limb injuries, however, is also a relevant example for conservation efforts as this species is considered critically endangered in its natural setting. Our goal is to develop generalizable cryopreservation pathways that can be applied to biomedical model organisms and can be extended to imperiled aquatic species such as amphibians, fishes, gastropods and corals.

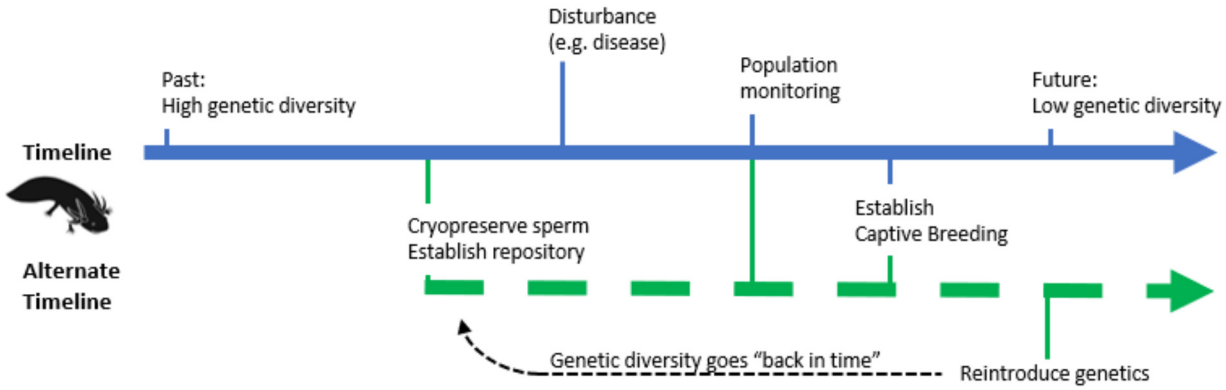

FIGURE 1. Possible timelines with and without sperm cryopreservation to capture genetics in response to potential or actual population disturbance. With decreasing population size, wild and captive populations lose genetic diversity over time, however with addition of cryopreserved sperm from before a disturbance event, the genetic diversity is improved.

A COMPREHENSIVE BIOCHEMICAL AND HISTOLOGICAL CHARACTERIZATION OF WILD SETTLEMENT STAGE LEPTOCEPHALUS LARVAE OF BONEFISH (*Albula vulpes*)

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Currently, there is little known about the early development of the near-threatened bonefish (*Albula vulpes*). Bonefish belong to the order Albuliformes, joined by tarpon, ladyfish, and eels. Members of Albuliformes share a unique leptocephalus larval stage, which is not well understood. This study will support conservation efforts to replenish wild bonefish populations by contributing to a comprehensive research collaboration with the Bonefish and Tarpon Trust that explores all aspects of the bonefish life cycle. Health at the larval stage is crucial to adult bonefish health and identifying major lipids used for energetic processes can help to develop feeds for future captive populations. In order to gain a better understanding of the nutritional requirements for leptocephalus larvae, samples of wild *A. vulpes* larvae were collected at Long Caye Island in Belize. Settlement stage leptocephalus larvae were preserved in formalin and frozen. A total of 36 larvae samples were used to determine various biochemical characteristics including lipid class breakdown, fatty acid profiles, and glycosaminoglycans (GAGs) abundance; each of which play a role in early developmental processes such as cellular membrane formation. Total lipids and lipid class breakdown were determined using thin layer chromatography and flame ionization detection (FID) techniques and fatty acid analysis was conducted using gas chromatography/mass spectrometry (GC/MS) techniques. To determine GAG content in each sample, GAGs were isolated from the samples, separated by class, and measured using SAX-HPLC coupled with fluorescence detection techniques. The remaining larvae were preserved in formalin and underwent histological analysis to determine age and key morphological characteristics such as olfactory pit length and ocular diameter using a scanning electron microscope (SEM). Both biochemical and histological characteristics of wild *A. vulpes* larvae are presented in this study for the first time, helping to better understand the nutritional requirements of this species. In the future, these types of analyses can be applied to other members of Albuliformes like eels to identify nutritional requirements at this stage of development. The information obtained in this study will aid in future studies regarding larval bonefish diets. Our findings will also facilitate the initial step in the development of adequate aquaculture systems for captive bonefish, ultimately leading to improved management strategies for wild bonefish habitats.

IMPACTS OF COVID-19 ON THE U.S. CATFISH AQUACULTURE INDUSTRY

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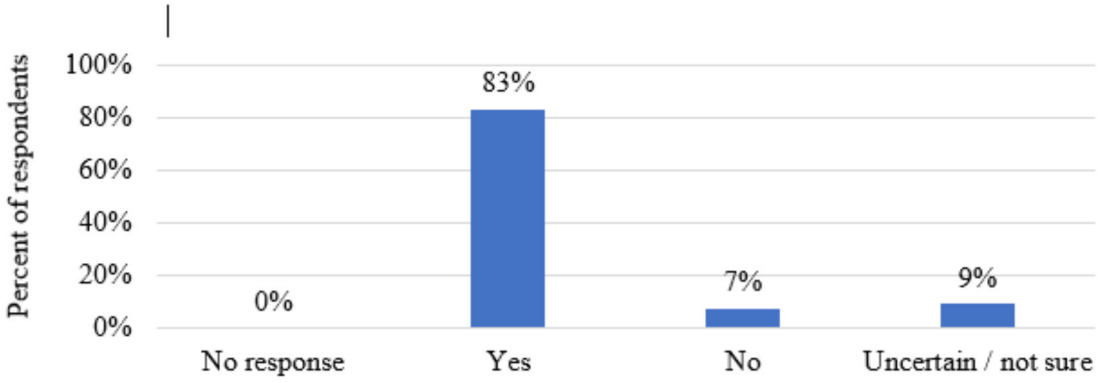
In response to the COVID-19 disease pandemic an effort was launched to assess the impacts on U.S. aquaculture producers. As the largest sector of U.S. freshwater aquaculture, the catfish industry was a critical sector to include in this assessment. A majority (83%) of catfish respondents did report being impacted by the pandemic. The disruption of traditional marketing channels for the catfish industry resulted in loss of revenues reported by 77% of catfish producers. Lost sales reported ranged from $1,001 to more than $1 million. The disruptions and loss of revenue resulted in ripple effects that had subsequent impacts on farm labor and production activities (as well as goods and services). Eighty-six percent of respondents reported that prices would be reduced as market-sized catfish held in ponds would grow beyond the size that brings a premium price. Catfish respondents to the surveys indicated challenges with labor; ranging from terminations to disruptions in labor due to illness or fear of illness. Producers also reported anticipated disruptions to production activities that would follow farms for the 2-year product cycle. Comments submitted by producers noted increased costs for production inputs, particularly feed.

This presentation will cover the findings regarding the impacts of COVID-19 on U.S. catfish aquaculture businesses.

Q1. Has your farm or business been impacted by the coronavirus disease (COVID-19)? (n = 54)

- No response : 0%
- Yes : 83%
- No : 7%
- Uncertain / Not Sure : 9%



TELOCYTES: A NOVEL PLAYER IN PRESERVING THE HOMEOSTASIS OF RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL MUCOSA

Nicole Verdile[1]*, Rolando Pasquariello[1], Gloriana Cardinaletti[3], n Tiziana AL Brevini[2], Emilio Tibaldi[3], Fulvio Gandolfi[1]

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Intestinal epithelium undergoes a rapid, constant, and complete renewal, driven by a specialized population of intestinal stem cells (ISCs) that are housed in well-defined niches made of epithelial and stromal cells. We previously described the spatial organization of the rainbow trout (RT) ISCs, identifying the epithelial and stromal niche components in this species. Here, we expand that knowledge focusing on a newly identified stromal population known as telocytes that has recently been identified as an active player of the intestinal mucosa.

Segments of proximal and distal intestine were collected from 5 female rainbow trouts (Oncorhynchus mykiss) weighing around 250g. Sections were stained with hematoxylin-eosin (HE), Periodic Acid Schiff-Alcian Blue at pH 2.5 (PAS-AB 2.5), Mallory's triple stain, Crossman's trichrome, toluidine blue and silver nitrate. Samples were also subjected to in situ hybridization for pdgfra, foxl1 and lgr5, that are known to selectively identify intestinal telocytes and telocytes' subsets. Finally, small fragments of proximal and distal intestine were examined with transmission electron microscopy (TEM), looking for telocytes peculiar ultrastructural characteristics.

Slender, elongated cells distributed in the peri-epithelium region around the folds base and along their whole length, were detected in both gut regions. These cells showed affinity for all the histochemical staining and expressed pdgfra (fig. 1). TEM further confirmed their identity as telocytes. They created a network extending from the folds base to their apex. Some of them expressed also foxl1, while others expressed lgr5, indicating the presence of different subsets. Telocytes positive to both pdgfra and foxl1 were located around the fold base close to sox9^{+} cells, that we previously identified as the real rainbow trout ICS. This suggests that some telocytes may support the epithelium renewal and homeostasis. Telocytes positive to foxl1, lgr5 and pdgfra were selectively located in the folds apex, implying a different role aimed at the maintenance of the tip epithelium.

Our data showed that two different telocyte subpopulations are present in the RT intestinal mucosa: one forms a peri-cryptal signaling source, possibly promoting and modulating ISC proliferation. The other, located at the fold apex, may be involved in modulating cell differentiation and anoikis. Taken together our results suggest that telocytes could represent relevant targets for the morphological evaluation of diet effects on RT gut health.

Supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 828835 and by the AGER-2 Sushin project, cod.2016-0112.

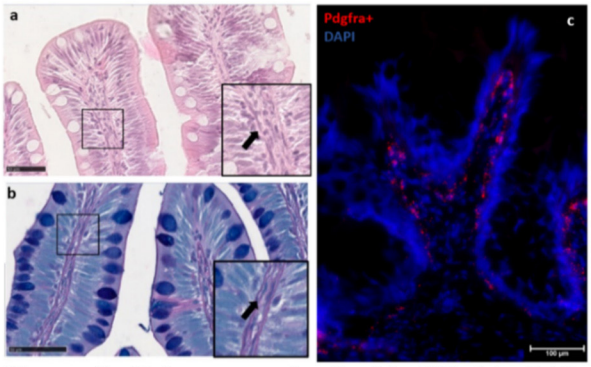

Figure 1: Telocytes stained with HE (a), PAS-AB 2.5 (b) and Pdgfra in-situ hybridization (c)

PERFORMANCE OF JUVENILE TOTOABA, *Totoaba macdonaldi*, FED HYDROLYZED SOY PROTEIN AND BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEALS, IN PLACE OF DIETARY FISHMEAL

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The search for feedstuffs alternative to fishmeal continues to be a priority for the aquaculture industry. Among them, soy protein has been extensively investigated, but the presence of anti-nutritional factors and amino acid imbalances have limited its inclusion. However, processing techniques, such as enzymatic hydrolysis and heat treatment, may help remove or inactivate anti-nutrients and break down protein into small peptides. Hydrolyzed soy protein (HSP) has been shown to improve growth and survival of fish. Another interesting alternative feedstuff is black soldier fly (BSF) (*Hermetia illucens*) larvae meal, which has recently been identified as an excellent protein source for farmed fish and crustaceans. Totoaba (*Totoaba macdonaldi*), a carnivorous marine fish endemic to the Gulf of California in Mexico, has been targeted for aquaculture mainly due to its fast growth rate and meat quality. The present study evaluated the use HSP and BSF meals as substitutes for fishmeal in diets for totoaba.

A control diet was formulated to contain 50% crude protein and 14% crude fat, using fishmeal as the main protein source. Then, 15 and 25% of fishmeal was replaced by HSP meal. Also, 25 and 40% of fishmeal was replaced by BSF meal. Juvenile *T. macdonaldi* (2.5 ± 0.03 g, overall initial mean weight) were reared in a recirculating aquaculture system consisting of 250-L tanks, with four replicate tanks per treatment, and a stocking density of 100 fish/m^3. Fish were fed the experimental diets to apparent satiation for 7 weeks. Results indicate that HSP meal is an adequate source of nutrients for totoaba when used to replace 15% of fishmeal, resulting in good growth, survival, and feed conversion ratio (FCR) (Table 1). In contrast, growth and feed utilization of fish fed 25% HSP meal and 25 or 40% BSF meal were significantly inferior (P <0.05) to those of fish fed the control diet. This is, to our knowledge, the first report on the use of BSF meal in diets for totoaba. Despite the fact that these results were not encouraging, further research should be conducted testing BSF meal levels below 25%, and perhaps incorporating a prior hydrolysis treatment for BSF meal.

In conclusion, the results of the present study indicate that fishmeal can be partially replaced by HSP meal without negatively affecting growth of juvenile totoaba.

TABLE 1. Biological performance of *T. macdonaldi*.

Diet	Weight gain (g)	Survival (%)	FCR
Control	57.3 ± 2.6	98.8 ± 1.3	0.9 ± 0.04
HSP 15%	56.9 ± 1.4	97.0 ± 1.2	1.0 ± 0.01
HSP 25%	44.1* ± 0.7	97.0 ± 2.0	1.2* ± 0.03
BSF 25%	45.7* ± 1.0	95.0 ± 1.6	1.1* ± 0.03
BSF 40%	40.3* ± 1.1	97.0 ± 1.2	1.3* ± 0.02

*Indicates significant differences (P < 0.05), when compared to the control diet (Duncan test).

UTILIZATION OF AQUAPONIC PROJECT BASED INVESTIGATIONS IN SECONDARY SCHOOLS TO PROMOTE STUDENT INTEREST AND UNDERSTANDING OF STEM CONCEPTS

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Hands-on educational experiences help students relate to scientific concepts in more tangible and effective ways. When teaching about the complex relationships that exist in aquatic ecosystems; educators can implement the culture of fish and plants in an aquaponics system to reinforce classroom curriculums and more effectively reach learning targets. Utilizing aquaponics as an applied, project based investigation (PBI) experience promotes student comprehension of broad scientific concepts, including those defined in the Next Generation Science Standards (NGSS). Aquaponics project-based investigations (APBI) teach students about the importance of cycling nitrifying bacteria, the carrying capacity of an ecosystem, and the complexities of aquatic animal husbandry in relation to science, technology engineering and math (STEM) concepts. This intervention shows how APBI promotes student development of interdisciplinary connections and contextualized problem solving.

Recirculating aquaponics system components, fish, and plants were delivered by Kentucky State University's Aquaculture STEM Extension staff to four rural high schools located in multiple districts in central Kentucky, USA. The participating classes were biology or agriculture-based and contained an average of 30 students each. Students engineered the system design and collected data including water quality parameters, feeding rates, and biomass of the fish and plants throughout the 12-week unit. Two separate APBI trials were investigated: 1) Eight week large system, whole-class investigation; 2) Four week small system, student group investigation. The APBI central driving research question throughout this project was: How does nutrient input affect the carrying capacity of our aquaponics ecosystem? This question was used as a guide for the intervention design and it facilitated the learning objectives within the benchmark lessons. Students observed how feeding rate (nutrient input) can affect the water quality and productivity of their living systems. Students were also able to discover that aquatic systems have limits in terms of nutrient input and when the carrying capacity of a system is met, harm can come from excessive waste accumulation. These factors were emphasized when the scale of the system was reduced for the small tank investigation and the students had to accommodate for reduced water volume and the increased rate of waste toxicity.

The inconstant nature of an aquaponics system allows students to better understand the multifaceted interactions that naturally occur between the water, plants, fish and bacteria. This APBI encouraged students to use analytical reasoning skills in conjunction with STEM based problem-solving to ensure the health and success of their aquatic ecosystem.

INTEGRATED SEQUENCES UNCOVER GENOMIC REGULATION AND EPIGENOMIC MODIFICATION OF SEXUAL SIZE DIMORPHISM IN YELLOW PERCH

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Sexual size dimorphism (SSD) has been the most common phenotypic dimorphism across taxa and theory has long suggested that the SSD is facilitated by chromosomes. However, our recent studies showed that hormonal-induced neo-males with female genotype (XX♂) and normal males (XY♂) exhibited no SSD in yellow perch, where females naturally grow significantly fast and larger than males. Why are phenotypic traits correlated with phenotypic sex instead of genotype (chromosomes) sex of an organism? Does steroid exposure in early life epigenetically modulate subsequent gene expression which in turn regulates lifetime SSD? What is the mechanism of sex-bias gene regulation of this nature and fish SSD evolution in general? The aim of this project is to unravel the nature and mechanisms of SSD by integrating phenotypic experiments, genomic, epigenomic, and physiological approaches.

The whole genome of a XX genotype female yellow perch was sequenced using Illumina and PacBio platforms. We adopted hybrid assembly strategy to improve the assembly quality with the Illumina short and PacBio long reads. A total of 1326 high quality contigs were obtained, with 1.33 Gb in length and 2.8 Mb of N50. Of these, a total of 531.68 Mb (39.79 %) repeat sequences were identified and 45,608 consensus protein-coding genes were predicted in yellow perch genome. As a result, 43,296 predicted genes (94.93%) were successfully annotated. Using the genome as a reference, RNA-seq and related analysis of 96 samples of large females (LF), small females (SF) and regular males (RM) were performed. PCA plotting of three groups in liver showed that the SF was much closer to RM instead of LF, indicating that the gene expression patterns of liver in SF and RM were similar. Furthermore, it could be inferred that the growth rates of these two groups may be related to the gene regulation. Hormonal analysis of the three groups showed similar patterns. We also conducted RNA-Seq and hormonal analysis of males, females and neo-males and results showed that steroid exposure during the critical period of sexual differentiation could epigenetically modulate subsequent hormonal responses and gene expression. In addition, we performed BS-seq of the males, females and neo-males to exam how epigenetically-modulated hormonal responses and gene expression regulates SSD throughout the lifespan. The female samples showed different methylation patterns with the other two groups, and the numbers of C methylated in CpG context in female are much lower than neomales and males. These results support our hypotheses: 1) Sex-biased or sex-specific gene expression is partially responsible for SSD; 2) The differences in sex-biased or sex-specific gene expression are associated with or the results from estrogen-mediated regulation; 3) Steroid exposure during the critical period of sexual differentiation can epigenetically modulate subsequent hormonal responses and gene expression; and 4) Epigenetically-modulated hormonal responses and gene expression in turn regulates SSD throughout the lifespan.

INITIAL ASSESSMENT OF NUCLEAR MAGNETIC RESONANCE-BASED METABOLOMICS AS A TOOL FOR THE IDENTIFICATION OF SKIP-SPAWNING BIOMARKERS

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Estimating reproductive potential is an integral but challenging task in fish stock assessments. Evidence suggests that several species may skip spawning in response to changes in nutritional, environmental, or anthropogenic conditions, which may have serious effects on the assessment. Accurate estimates of skipped spawning within a population is, therefore, a critical component to fishery recruitment assessments. Previous methods for the estimation of skipped spawning include macroscopic assessments of different reproductive phases and monitoring migration to spawning grounds. The degrees of skipped spawning typically associated with these methodologies are extremely variable, resulting in somewhat inaccurate estimates of the relative proportions of skipped spawners and therefore recruitment. We are currently working on the development of a novel analytical approach to quantify the reproductive potential of fish populations.

Nuclear Magnetic Resonance (NMR)-based metabolomics is a high-resolution analytical technology that can detect changes in metabolic profiles induced by a variety of factors including disease, xenobiotic toxicity, and nutritional status. The physiological and hormonal changes occurring during spawning within the gonad can significantly alter the tissue metabolic profiles. NMR-based metabolomics has the capability of identifying specific metabolic "fingerprints" as well as identifying and quantifying potential biomarkers which will clearly differentiate skip spawning females from spawning individuals. We intend to apply this technique to address additional questions in broodstock management and selection.

In a preliminary study, we have analyzed 24 ovarian tissue extracts at 4 different maturation stages from a species of interest in the U.S. South Atlantic: Vermilion Snapper (*Rhomboplites aurorubens*). Polar extracts were analyzed using NMR-based metabolomics. Principal Component Analysis (PCA) was performed on the processed NMR spectra and the resulting PCA scores plot is shown (Fig. 1). Our results show that the metabolite profiles of all 4 ovarian maturation stages evaluated in this study were significantly different from each other, thus demonstrating that NMR metabolomics has the potential to clearly discriminate between ovaries at different maturation stages.

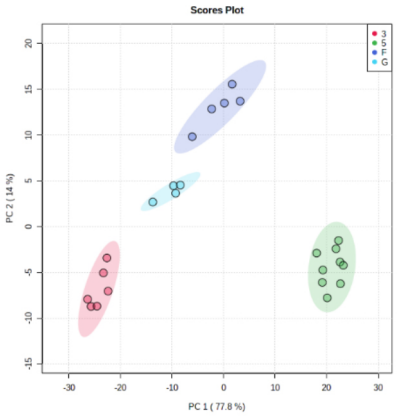

Figure 1. PCA score plot of vermilion snapper ovary extracts (polar extracts) showing the separation among the 4 different ovary maturation stages evaluated: hydration (red, n=6), regeneration (green, n=9), developing (blue, n=5), germinal vesicle migration (light blue, n=4).

EVALUATING PREFERENCES FOR FISH DISEASE TREATMENT OPTIONS AND SUCCESS RATES AT KENTUCKY STATE UNIVERSITY FISH DISEASE DIAGNOSTIC LABORATORY

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Local aquaculture allows farmers to provide a fresh fish product for their community, but as with any cultured animal, disease issues arise; therefore, it is essential that farmers understand fish health management. To assist fish farmers in raising healthy fish, Kentucky State University (KSU) offers a fish disease diagnostic laboratory (FDDL) service to farmers in Kentucky and other states. Fish health professionals at the KSU FDDL obtain fish and/or water samples from farmers via an Extension visit to the farm, or farmers send samples to the KSU Aquaculture Research Center. Samples are analyzed in the FDDL and a final diagnosis is given to the farmer along with a treatment recommendation. Once a treatment option has been recommended to the farmer, a short survey will be sent inquiring what type of treatment was preferred and if the treatment chosen was successful. The objectives of this study are to 1) better understand the needs of small-scale fish farmers in Kentucky and other states, including which disease treatments are most effective, 2) help educate farmers on pathogen clinical signs and treatment options, and 3) evaluate which treatments are preferred among fish farmers. This information, obtained by survey, will allow researchers to better understand fish disease treatment efficacies and treatment preferences of aquaculturists.

FEED MANAGEMENT OF *Litopeneaus vannamei* IN A HIGH DENSITY BIOFLOC SYSTEM

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In commercial shrimp production, one area with the highest potential for improvement is through proper feed management. Avoiding feed wastage is important for saving money as well as properly managing water quality. The purpose of this study was to observe the growth response of *Litopenaeus vannamei* to different feed inputs in an intensive production system. Shrimp were fed varying percentages of a standard feeding ration, starting at 30% and incrementally increasing up to 150%. The standard feeding ration was determined using assumptions that shrimp would double in weight weekly until reaching 1g, then gain 1.3g per week with a feed conversion ratio of 1.2.

The growth trial was conducted in an outdoor recirculating aquaculture system consisting of 24, 750L tanks stocked at 160 shrimp/m^2, and grown for 63 days. Treatments consisted of seven varying feeding rates (30% to 150%) that were split into four feedings per day, and an eighth treatment that was fed 150% of the standard feeding ration using a belt feeder that fed six times per day. Treatments were randomly assigned using an online number randomizer.

Significant differences in final weight and feed conversion ratio were observed. Regression analysis performed on the first three feeding rates determined that natural productivity was responsible for approximately 2.8g of total individual growth. Results also indicated that increasing feed amounts over 100% of the standard ration led to an increase in final weight, while still achieving a desirable feed conversion ratio (Figure 1). The results from this trial indicate that shrimp can be grown at high densities in biofloc systems while still achieving acceptable growth, final weight, and feed conversion ratios. This trial also indicates that growth response continues to increase as feed inputs are increased, meaning that feeding protocols could be optimized by increasing the standard feeding ration.

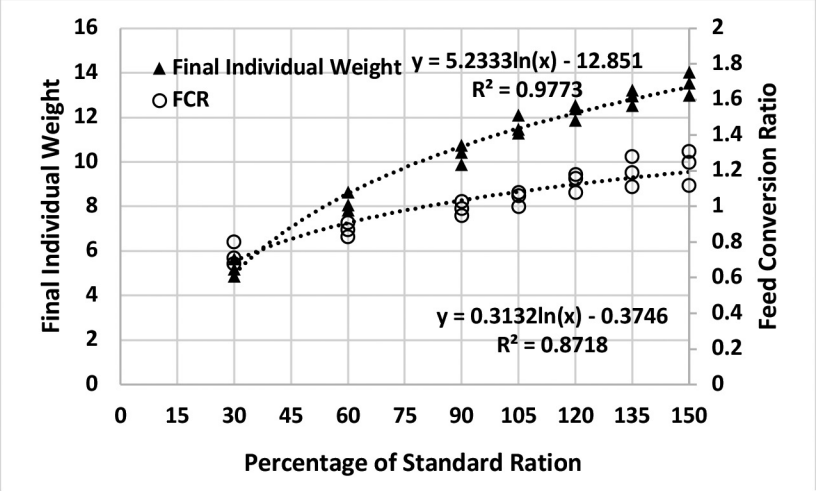

Figure 1. Relationship between percentage of standard ration fed and final individual weight, and feed conversion ratio.

USE OF METABOLICALLY MODIFIED CANOLA OIL AS A REPLACEMENT FOR FISH OIL IN PRACTICAL DIETS OF PACIFIC WHITE SHRIMP *Litopeneaus vannamei*

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As aquaculture expands and intensifies, fish oil production cannot increase to meet demand, which means that aquaculture must find an acceptable alternative oil source that meets the dietary requirements of the animals being fed. The efficacy of metabolically modified canola oil (MCO) has not been extensively tested in shrimp. Hence, the objective of this research was to quantify the effect of MCO on shrimp growth performance at various menhaden fish oil (FO) and menhaden fishmeal (FM) replacement levels. Ten diets were formulated and tested in both clear and green water systems. In the first experiment, all ten diets were evaluated under clear water conditions. At conclusion results indicated shrimp reared on treatments with above 90% FO replacement had significantly lower growth and feed utilization likely due to a nutritional deficiency of long chain highly unsaturated fatty acid (LC-HUFA) docosahexaenoic acid (DHA). In the second experiment, five experimental diets were utilized in a green water growth trial, with FO replacement levels from 75% to 95%. At conclusion, no significant differences were observed between treatments that indicate an effect of treatment on shrimp growth and survival, suggesting that all replacement levels were successful.

In each of these trials, tissues and feeds were analyzed for fatty acids (FA).Lipid results indicated that the diets containing 90% FO replacement or higher in the clear water trial were very close to the nutritional LC-HUFA requirement but were deficient in DHA. Significant differences in shrimp growth were observed in the clear water growth trial, but not in the green water growth trial. This difference could possibly be attributed to the consumption of algae from the system, supplementing the contribution from the diets and meeting nutritional requirement for limiting fatty acids like DHA. Whole shrimp were kept from the green water growth trial to be used for human sensory analysis. Cooked shrimp samples were evaluated on appearance, juciness, texture, flavor, and overall acceptability. There were no significant differences between sensory parameters. Four diets were selected for palatability testing in order to observe consumption response to different ingredients in experimental feeds. No significant differences were
observed.

Results of this research confirm that albeit MCO has sufficient DHA as a pure oil source, the dilution effect of native oils results in the 100% replacement being deficient in DHA. MCO is not yet suitable to fully replace fish oil in shrimp diets but can be replaced at levels up to 90% without large sacrifices in growth and survival. Because results from these experiments are conflicting, more research with this product is warranted. Further experiments in green water conditions should evaluate the lipid profile of shrimp that are not fed any feed in order to determine how much of the EFA nutritional requirement is fulfilled by the natural foods present in green water systems.

NEW LIVE FISH TRADE REGULATIONS FOR THE STATE OF ARKANSAS

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The live fish trade industry is a global market where fish and other aquatic organisms move via various pathways for a variety of uses. As markets expand and new pathways for moving undesirable species and pathogens emerge, regulators struggle to find a balance in protecting the natural resources without impeding commerce and overburdening the aquaculture industry. In 2019, Fisheries staff from the Arkansas Game and Fish Commission began working with a core group of aquaculture industry representatives to overhaul the aquaculture regulations for the state of Arkansas. The last major overhaul of the existing regulations occurred in the 1970's.

A joint approach of combining regulators and multiple aquaculture industry representatives together allowed for a deeper understanding of the challenges faced by all and the development of more comprehensive and practical regulations. The new regulations cover more pathways than simply aquaculture farms. They incorporate live haulers, wholesalers, distributors, pond consultants, and other live fish trade avenues as these entities also serve as pathways for moving undesirable species. In addition to new permits, clear instructions regarding paperwork during transit and record keeping, new fish health requirements, and updated species lists were part of the regulations package.

The new regulations went into effect January 1st, 2021. The development and implementation of the new regulations would not have been as comprehensive, successful, and effective without the industry representatives involved from the beginning and throughout the entire process. Hopefully in the future more regulatory entities will follow a joint approach and process when developing/updating live fish trade regulations.

EVALUATING KENTUCKY EXTENSION AQUACULTURE AND FISHERIES CLIENT INFORMATION REQUESTS FROM 2017 – 2020

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Kentucky County Extension offices receive numerous requests for information regarding the management of ponds and lakes. Client interest in private pond and lake management issues is greatest during the months of March to October. Information requests recorded from University of Kentucky Agriculture and Natural Resources Agents and from the public were tallied into the following subject areas: aquaculture, pond and lake management, aquatic plant and algae control, and aquatic natural resources management. Due to the large number of pond plant and algae control requests received, these were tabulated separately from those of pond and lake management. Data were collected to estimate the percentage of inquiries pertaining to each subject area. Table 1. Provides these results.

During 2017 - 2020 aquaculture information requests averaged 17.5% of total inquiries recorded and ranged from 22% in 2017 to 16% in 2020. Four-year averages of pond management and aquatic vegetation control information requests were similar at 39.5% and 38.8%, respectively. Numbers of these requests between years were relatively consistent and differed ≤ 5%. Pond and lake management and aquatic vegetation control information requests when combined totaled 78.3% of the client inquiries recorded. Aquatic natural resource management-based inquiries averaged < 10% for the three years data were collected. These data suggest the need for significant Extension programming effort to address private pond/lake management and aquatic plant control issues during the spring and summer months.

Table 1. Extension client and agent information inquiries percentage by subject 2017 – 2020.

Year	Aquaculture	Pond management	A. vegetation control	A. natural resources	Total
2017	22%	41%	38%	X	307
2018	18%	36%	41%	5%	264
2019	14%	40%	39%	8%	279
2020	16%	41%	37%	5%	380
Average	17.5%	39.5%	38.8%	6%	1,230

THE DIETARY SUPPLEMENTATION OF VITAMIN C AND BENTONITE IMPROVE THE FISH GROWTH AND ALLEVIATE IRON TOXICITY IN RAINBOW TROUT

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Aquaculture is the world's fastest-growing food-producing industry, accounting for half of all fish supplies in both the United States (US) and the rest of the world. Rainbow trout production in the US was about 21,600 tons in 2012, with a total value of $79.7 million dollars. A reoccurring problem in many areas includes high iron in the water. Iron (Fe) is an essential element for fish; however, excessive amounts can exert toxic effects in fish, such as causing lipid peroxidation in vital tissues and cells. Vitamin C is a powerful antioxidant while bentonite clay acts as a chelating agent. The overall objective of the study was to mitigate iron toxicity via dietary intervention (vitamin C and Bentonite).

An eight-week feeding trial was conducted to evaluate the response of trout to iron after supplementation of dietary bentonite and vitamin C at different inclusion levels. A total of 560 rainbow trout juveniles (average weight: 5.06 ±0.42g) were distributed into 7 groups with four replicates (20 fish per tank). Fish fed seven isonitrogenous (45% crude protein) and isolipidic (20% lipid) diets at three different levels (500 ppm, 1500 ppm, and 3000 ppm) of vit. C with or without 2% bentonite and/or 0.25% iron. (i) Control (Con; fed with commercial diet), (ii) diet supplemented with low vit. C and iron (ConFe; 500 ppm; LVC; 0.25% Fe), (iii) with bentonite and iron (BenFe; 2%; Ben; 0.25%; Fe), (iv) with medium vit. C and Fe (MVCFe; 1500 ppm; MVC), (v) MVC with bentonite and iron (MVCBenFe), (vi) HVC with iron (HVCFe; 3000 ppm vit. C), and (vii) HVC with bentonite and iron (HVCBenFe).

At the end of the feeding trial, production performance of Trout fed Con, BenFe, MVCBenFe, HVCBenFe, was significant to ConFe (Fig. 1). The highest growth was seen in HVCBenFe group. Also, the iron load in the liver was significantly higher in ConFe group. Histopathology of liver in ConFe (Fig.

2) group showed necrosis (nec), congestion (con), hemorrhage (he), and vacuoulization (V) compared to the other groups. Overall, dietary supplementation of vit. C and bentonite can be an effective approach to mitigate iron toxicity in the aquaculture trout industry.

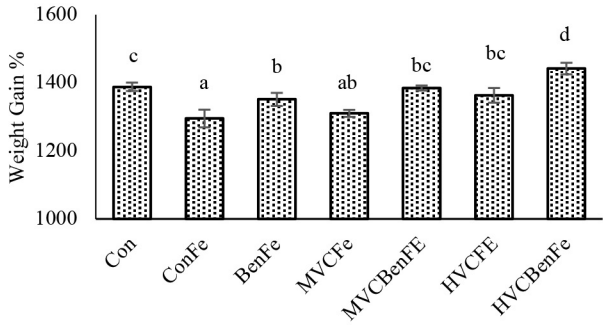

Fig. 1. Weight gain % of trout

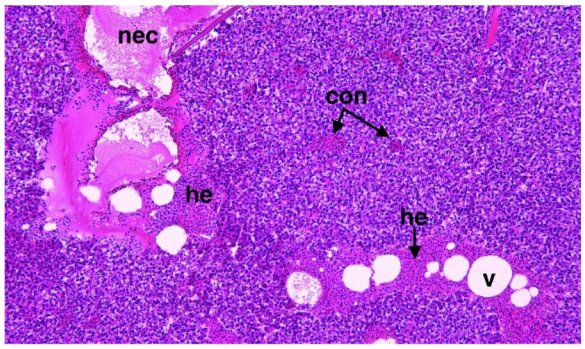

Fig. 2. Histopathology of liver of trout fed with Iron based diet.

SUPPLEMENTATION OF A COMMERCIAL PREBIOTIC AND PROBIOTIC AS WELL AS THEIR COMBINATION ON GROWTH PERFORMANCE, IMMUNE RESPONSES, AND INTESTINAL MICROBIOTA OF RED DRUM (*Sciaenops ocellatus*)

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Prebiotics and probiotics have been long investigated with aquatic species to improve health and diminish the usage of antibiotics. In the present study, the potential synergism between the beneficial bacteria contained in probiotics with the fermentable complex carbohydrates (or prebiotics) and their metabolites were explored in red drum using the commercial products Bactocell™ and Grobiotic®-A. Four experimental diets were formulated and supplemented with either 1 g kg^{-1} of Bactocell, 20 g kg-1 of Grobiotic®-A, or their combination. A non-supplemented basal diet served as the negative control. Groups of 12 juvenile red drum with an average weight of 5.5 g/fish were distributed in 16, 38-L aquaria. Each of the four experimental diets were assigned to four replicate tanks for 8 weeks with daily rations divided into two feedings and adjusted weekly according to total biomass. At the end of the feeding trial, production performance, whole-body proximate composition, plasma immunological responses, and intestinal microbial community were evaluated. Data were analyzed as a mixed model, having a 2 × 2 factorial design (absence or presence of probiotic or prebiotic, as the main factors) and the disposition of the aquaria was used as a statistical block.

Red drum fed diets supplemented with probiotics had better growth performance than those fed the non-supplemented diets, and a higher protein content in their whole-body composition. No differences were observed for feed efficiency, survival, whole-body lipid and ash, or protein conversion efficiency. Fish fed diets supplemented with the prebiotic had a lower concentration of circulating protein in plasma. The intestinal microbiome was assessed with the denaturing gradient gel electrophoresis (DGGE) method and next-generation sequencing (NGS) targeting the V4 region of the 16S rRNA gene. The bacterial community of fish fed diets containing the probiotic, and the combination of probiotic and prebiotic, were 91.5% similar to the other two experimental diets, according to the disposition of the DNA amplicons in the DGGE gel. NGS data indicated alpha and beta diversity were significantly affected by dietary treatments. A higher relative abundance of the lactic acid genus *Pediococcus* sp. was observed for fish fed diets supplemented with the prebiotic. Differences were also detected in the predicted functions of the microbiota, where the relative abundance of 52 pathways were significantly higher for fish treated with the prebiotic and 18 pathways for fish fed the probiotic. Many of these pathways involved the biosynthesis of essential amino acids like lysine and methionine and the biosynthesis of nucleotides. Even though no potential synergistic effect was observed for the supplementation of both commercial products, the individual inclusion of the prebiotic and probiotic positively affected growth performance and intestinal health of red drum.

LARGE-SCALE PRODUCTION AND COMERCILIZATION OF MONOSEX YELLOW PERCH

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Yellow perch (YP) *Perca flavescens* are members of the Percidae or perch family, native to North America, and very common in the northwest to Great Slave Lake and west into Alberta. This species is a particularly important aquacultural and ecological species in the Great Lakes Region (GLR) and the Midwest USA. The demand for yellow perch has remained very high in the region. One reason in particular hindering expansion has been relatively slow growth of currently cultured populations of this species. Using current yellow perch strains, only 60% of the fish cultured in aquaculture operations reach market size in a normal growth cycle (16 months), with the remainder below market size. This is an inefficient use of resources, feed, and operational costs, and leads to marginal profits at best.

Yellow perch female monosex culture has considerable potential for increasing the efficiency and profitability of yellow perch aquaculture, since females grow ~40% to ~50% faster than males in aquaculture systems in year 1 and the advantage is even more pronounced in year 2. Funded by USDA and NOAA-Sea Grant, Ohio Center for Aquaculture Research and Development (OCARD) at OSU South Centers has developed technology that can generate large numbers of fast-growing, all-female yellow perch populations. A growth performance test of the all-females vs. mixed-sex group showed that all-females grew 26.3% faster than the mixed group, and 66.0% faster than males. In the past few years, OCARD created a large number of neomale broodstock of yellow perch with a female genotype and is using the large numbers of superior neomale broodstock to produce a commercial-scale of all-female monosex yellow perch and transferring them to aquaculture industry for demonstration. A few aquaculture partners have been identified for the farm demonstration and commercialization. All-female monosex populations will significantly benefit the aquaculture industry.