Aquaculture America 2020

Hawai'i Aquaculture:
A Tradition of Navigating with Innovation, Technology and Culture

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THE NATIONAL CONFERENCE & EXPOSITION OF

U.S. Aquaculture Society — A Chapter of the World Aquaculture Society

National Aquaculture Association

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WELCOME

Welcome to Hawaii for Aquaculture America 2020! On behalf of the United States Aquaculture Society, the National Aquaculture Association, the Aquaculture Suppliers Association and Hawaii Sea Grant it is with great pleasure that we welcome you to the city of Honolulu. It has been 15 years since last we held Aquaculture America in Hawaii, a place many believe to be the birthplace of aquaculture. We are excited to return to the Hawaii Convention Center in this idyllic location just off Waikiki beach in the shadow of the volcano Lēʻahi.

The theme of our conference is Hawai'i Aquaculture: A Tradition of Navigating with Innovation, Technology and Culture. We come together here on O'ahu to celebrate the people, the traditions, the culture of Hawaii and to learn about the history of aquaculture on the islands.

Our program chairs, Dr. Aaron Watson, Dr. Matt Hawkyard, Dr. Maria Hawes, and Mr. Paul Zajiceck have worked very hard to bring you a diverse program that provides all of our traditional sessions but also adds several special sessions on Native Peoples Aquaculture and Hawaiian Fish Pond Production. In addition, we are providing a Fish Health Management and Aquaponics Principles and Practices Workshop. This year we have more than 725 abstracts with 70 technical and producer sessions in 15 rooms running contiguously while a tradeshow with over 175 exhibitors highlights the continued technological advancement of the aquaculture industry. A new feature of this year's conference is a video competition for Hawaiian high school students. The student winner's videos will be displayed on video monitors throughout the convention center.

There will be two plenary sessions at the beginning of day 1 and 2 of the conference. We are very fortunate to have Professor Lilikala Kame'eleihiwa as our first plenary speaker. She will help us navigate Hawaii's aquaculture origin story and highlight how culturally important fishponds or Loko i`a, and the fish they produce, are to the history of Hawaii. Hawaii is also known as a source of innovation and technological advancement in aquaculture. The development of specific pathogen free (SPF) shrimp, which is now a 30 million dollar a year export, began here. We are honored to have Dr. Jim Wyban as our second plenary speaker known widely as "the father of SPF shrimp". His presentation will tell the SPF story of the past and present and plot a course through the future of SPF shrimp production in Hawaii and the world.

On day two our first Plenary speaker is Keliʻi Kotubetey, the assistant Executive Director of the Heʻeia fishpond who will explain the function and production of food in this 800-year-old fish pond and continue our theme of Hawaii aquaculture. Our second speaker, Dr. Mark Lyons, President and CEO of Alltech will talk about, "A Planet of Plenty" and again connect history with technological advancement in shaping the aquaculture industry's role in global protein production.

Our sincere thanks go to the many companies and organizations that have provided support for Aquaculture America 2020 and especially our conference Premier Sponsors: Blue Aqua, Zeigler, Darling and Kemin. Please visit them in the tradeshow and thank them for their ongoing support of Aquaculture America.

Michael Denson, Aquaculture America 2020 Steering Committee Chair
On behalf of the Steering and Program Committees

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ABSTRACTS

EFFECT OF STRAIN ON GROWTH RATE OF CHANNEL CATFISH *Ictalurus punctatus* FEMALE × BLUE CATFISH *I. furctatus* HYBRIDS

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Growth is an economically important trait in aquaculture. The channel catfish, *Ictalurus punctatus*, ♀ × blue catfish, *I. furctatus*, ♂ hybrid catfish have become common in the U.S. aquaculture industry accounting for 50-75% of the U.S. catfish production. Performance of hybrid catfish can be further improved through strain selection and likely through recurrent reciprocal selection. This study was conducted to compare the growth rate of the channel catfish female × blue catfish male hybrid and determine the effect of family, genetic type of dam and genetic type of sire on the body weight of hybrid progeny. In general, strain of sire effects were more influential than strain of dam effects, which also should be considered in practical catfish genetics and breeding programs.

MOLECULAR EFFECTS OF DIRECT UTILIZATION OF SUGARCANE BAGASSE PULP AS ECONOMIC FEED ON GROWTH, IMMUNITY AND ANTIOXIDANT CAPACITY IN (*Litopenaeus vannamei*) UNDER INTENSIVE CULTURE

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Biofloc technology (BFT) has been developed recently as a promising technology to enhance animal production especially, shrimp species. BFT rely on using an economic carbon source to promote the development of heterotrophic bacteria which can enhance water quality, animal growth, reduce stress and prevent diseases. Studies on the molecular effects of biofloc (BF) addition on shrimp physiology, growth, immunity and reduce stress are limited; a field known as nutrigenomics. In our study, we attempted to study the differential changes of expression of different immune and antioxidant genes upon direct utilization of sugarcane bagasse pulp by *L. vannamei* larvae reared for 45 days under two different densities (400 and 700). Our data have shown that after 45 days of rearing, antioxidant gene *sod* and *gpx* were highly expressed (50 and 6 fold change respectively) in the high-density control group (700) as compared to the low-density control group (400) which indicate that high level of reactive oxygen species was generated in the high-density control group. Besides, the high-density control group showed a lower expression level of immune gene *bgp*, *proph* and *lys* (0.4, 0.5 and 0.8 respectively) in comparison to the low-density control group. On the other hand, BF treated high-density group showed lower levels of antioxidant genes *sod* and *gpx* (0.9 and 1.1 respectively) and higher levels of immune genes *bgp*, *proph* and *lys* (3.5, 5 and 1.2 respectively) in comparison to the BF treated low-density group. Furthermore, normalizing gene expression values of BF treated groups in relation to control non-treated groups have shown that BF treatment had higher immune gene expression of *lys*, *proph* and *bgp* under low density treatment (2, 2 and 2-fold change respectively) and high-density treatment (2, 20 and 4 fold change respectively). Not only improving immunity and reducing stress in the high-density group but also, growth of shrimp was also enhanced after BF treatment in terms of growth performance, survivability and feed utilization.

To sum up, this study has shown that direct addition of sugarcane bagasse pulp as a direct feed to *L. vannamei* for 45 days was associated with enhancing shrimp growth performance, survivability and reducing stress in accordance to underline molecular changes in expression of antioxidant and immune genes and we, therefore, strongly recommend the use of sugarcane bagasse pulp as an economic and feasible source of carbon in *L. vannamei* intensive rearing.

Acknowledgements: The research was performed within the “Development and Research Application of bioFloc Technology for increasing shrimp production in Egypt (EGY-DRAFT)” project, which is financially supported, by the Science & Technology Development Fund (STDF), Ministry of Scientific Research, Egypt. (Agreement No. 25305, Reintegration Grants). The authors are grateful for all the support.

DEVELOPMENT AND EVALUATION OF WHITE BASS *Morone chrysops* GENETIC RESOURCES TOWARD THE SELECTIVE IMPROVEMENT OF HYBRID STRIPED BASS

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White bass (*Morone chrysops*) is a parental species of hybrid striped bass, a fish of increasing commercial importance throughout the US. A chief constraint to the expansion of hybrid striped bass production arises from the use of wild-catch parents in breeding programs. This is costly, unsustainable and leads to uncontrolled variation in the offspring. Our goal is to advance progress in the genetic improvement of hybrids by building additional white bass resources to facilitate selective breeding for agriculturally-important traits. Toward our genomics goal, we created a first-generation white bass genome assembly as well as multiple transcriptome datasets to complement our genome assembly. Improvement of genetic maps and development of molecular markers to discriminate sex, parentage and other critical traits are ongoing.

Toward our fish genetics resource goal, wild white bass gathered from Arkansas, Texas and Alabama along with available domesticated strains are being used to establish a base breeding population for familywise evaluations of growth and nutrient utilization on alternative, sustainable diets. To evaluate existing fish stocks and create an even year-class, approximately 100 white bass full-sib families were spawned. Fish were reared in a completely indoor, controlled, bio-secure environment, with larviculture (rotifers and artemia) transitioning to manufactured starter diet. At 247 days post-spawn, white bass were transferred to 250 L tanks (culture depth 100L initial & 200L final based upon fish density) at 1 family/tank and switched to a custom fishmeal free (FMF) diet. Mixed family controls (n=4 tanks each) were evaluated simultaneously and fed either the custom FMF diet or a commercial control diet (45/16% protein/lipid) diet. Fish were fed to apparent satiation once daily. Gains were evaluated over a seven-month period. Distinct differences in growth performance was observed among wild and domestic populations. Wild (male) x domestic (female) cross families performed significantly better when fed a fishmeal free diet then did their reciprocal crosses, and to a similar degree as that of pure domesticated white bass and those fed a control diet. Pure wild cross families had the worst growth performance during this evaluation period. Top performers from each family were selected for propagation.

The combination of these resources substantially expands the genetic toolbox for white bass, while the application toward white bass breeding will aid in its improvement through selective breeding. Progress on white bass resources will be presented and discussed.

THE MICROALGAE AND THEIR BIOLOGICAL, ECOLOGICAL AND ECONOMIC POTENTIAL

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Microalgae are photoautotrophic organisms with high potential biological, ecological and economic. Due to its great applicability microalgae has been the focus of many studies in recent years. The most common application of microalgae is verified in aquaculture for direct or indirect feeding of aquatic organisms of economic interest. Environmental applications of microalgae include CO_2 biofixing and production of biofuels. Studies are being carried out for the use of microalgae in wastewater treatment of industrial process numbers, for biological detoxification, heavy metal removal and as bioindicators. In agriculture the biomass can be used as a soil biofertilizer. Species commonly used in aquaculture such as *Nannochloropsis oculata* and *Thalassiosira weissflogii* present a high absorption capacity of atmospheric carbon dioxide.

The biotechnological potential of microalgae is mainly due to the identification of various substances synthesized by these organisms. The immense biodiversity and the consequent variability in the biochemical composition obtained in the biomass microalgal crops, allied to the genetic improvement in large scale, have allowed certain species are used commercially. The main commercially cultivated microalgae are *Chlorella* and *Arthrospira* genera for addition to health foods and *Haematococcus pluvialis* to obtain astaxanthin. In this sense, crops of microalgae have been carried out to produce biomass both for use in food preparation and for obtaining high value natural compounds in the market worldwide. Among the many compounds can be cited: polyunsaturated fatty acids (PUFA), carotenoids, phycobilins, polysaccharides, vitamins, sterols and various natural bioactive compounds, such as antioxidants, cholesterol lowering, among others which can be used in functional foods due to their nutritional and pharmaceuticals properties. PUFA have a very promising market in biotechnology, especially in the food industry functional.

Because they are capable of synthesizing toxins, microalgae can produce a range of bioactive molecules with antibiotic, anticancer, anti-inflammatory, antiviral, cholesterol-lowering, enzymatic and other pharmacological properties. Beta carotene can accumulate up to approximately 10% in halotolerant species such as *Dunaliella* genus and has application as a dye and may act as a pro-vitamin A, antioxidant product and against degenerative diseases such as cancer. Too many compounds of commercial interest can be obtained by cultivating microalgae and the interest in sustainable technologies brings the need to continuous search for strains capable of synthesize large quantities of specific compounds e the knowledge to enhance the synthesis of these products. In this way, they are also research is needed to develop and commercial scale production systems to enable some of the known systems. It is therefore important to identification of products that can be extracted from microalgae, as well as possible biological activities through clinical, metabolic and toxicological studies.

EFFECTS OF CRYOPROTECTANTS AND FREEZING RATES FOR PRESERVING SPRERMATOGONIAL STEM CELLS OF BLUE CATFISH

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In the United States, catfish farming has surpassed 65% of total freshwater aquaculture production, where the channel catfish ♀ × blue catfish ♂ hybrid constitutes nearly 70% of the harvest. Unfortunately, sacrificing males is the only way to extract testes to obtain sperm to make hybrids. Thus, our long-term goal is to develop xenogenesis resulting in xenogens to increase reproductive efficiency for catfish hybridization. Having spermatogonial stem cells (SSCs) frozen in germplasm repositories will help facilitate this initiative. In this study, we first examined the effects of permeating cryoprotectants (DMSO, ethylene glycol, glycerol, methanol) and cryoprotectant concentrations (1 M, 1.3 M, 1.6 M) on post-thaw cell viability. Secondly, the non-permeating agents, trehalose and lactose, were tested at different concentrations (0.1 M, 0.2 M) in conjunction with egg yolk (10%) and bovine serum albium (1.5%). Finally, different cell freezing rates (-0.5, -1, -5, -10°C/min) were tested. Our results showed that DMSO 1 M generated the most cells after cryopreservation (Fig. 1A) and had the highest viability recovery index (Fig. 1B). Post-thaw cell viability increased (70 ± 3.2%) when lactose 0.2 M and egg yolk where incorporated into the cryo-media. The best freezing rate was -1°C/min. Together, these finding show that catfish SSCs can be frozen for future cell transplantation trials. Frozen cells will also become a valuable resource to conserve genes for catfish genetic selection programs and conservation efforts.

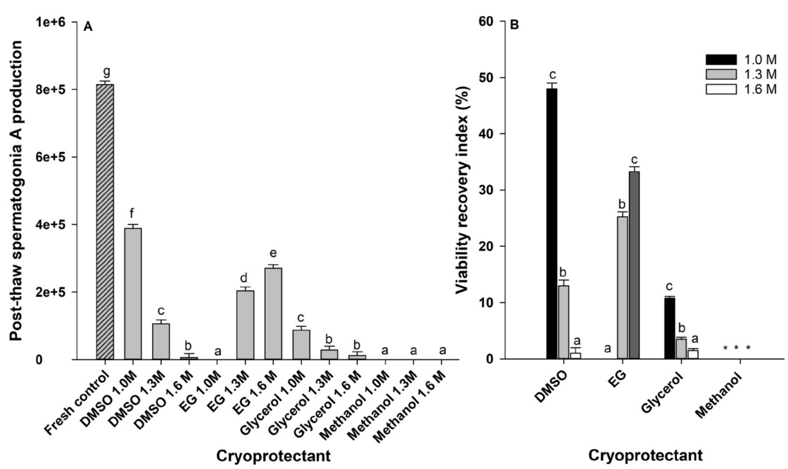

Fig. 1. The effects of permeating cryoprotectants and cryoprotectant concentrations on post-thaw blue catfish spermatogonia production (A) and post-thaw viability recovery index (B). Bars represent means ± standard error. Bars with different superscripts are significantly different (P < 0.05).

EVALUATING *Spartina alterniflora* IN DECOUPLED AQUAPONICS TO REMEDIATE REUSED SHRIMP (*Litopenaeus vannamei*) WATER

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Marine shrimp recirculating aquaculture systems are densely stocked to ensure they are economically feasible. With intensive feed inputs and repeated water use, contaminants such as nitrogen, phosphorus, and heavy metals, including copper, chromium, cadmium, iron, manganese, nickel, zinc, and lead, can accumulate. Such issues have been shown to negatively affect shrimp production. The purpose of this two-month-long decoupled aquaponics trial was to evaluate *Spartina alterniflora* production and water remediation effectiveness in reused, brackish shrimp water.

Four plants were floated on rafts in each of 24, aerated buckets (18.9-L) that were randomly assigned to one of six treatments (four replicates per treatment). The 2 x 3 factorial design consisted of two sources of water (clean, artificial saltwater [SW] or reused water [RW] from a 19-m^3 biofloc raceway) and three levels of fertilizer application (no fertilizer [NF], fertilizer [F], and fertilizer plus nitrate [FN]. A standard hydroponics fertilizer was applied in the F and FN treatments, and sodium nitrate was used in the FN treatments to examine what effect excess nitrate may have on plants. Temperature, pH, salinity, and DO data were collected 3 times per week; dissolved NO_3, NO_2, NH_3 PO_3, and K concentrations were measured once per week and plant performance (number of leaves and stems, dry and wet weight of stems and roots, number of dead leaves and stems, leaf chlorophyll concentration index and plant height) were determined at the beginning and conclusion of the trial. Changes in plant performance were analyzed using a two-way (two by three factor) analysis of variance (ANOVA) while the other parameters were analyzed using a two-way repeated-measures ANOVA.

There was a significant (*P>0.05*) differences in pH, salinity, and DO across treatments. The SW Source water had significantly greater mean ± SD shoot dry weight (SW: 10.068 ± .415; RW: 8.514± .415; P=0.01) and number of leaves (SW: 51.3 ± 1.940; RW: 41.9 ± 1.9; P=0.001). Fertilizer type had a significant effect on the number of leaves (FN: 50.6 ± 2.39; F: 42.0 ± 2.35; P=.012), dry root weight (NF: 3.731 ± .225; F: 3.026 ± .225; P=.029), and number of dead leaves (FN: 22.30 ± 1.38; F: 17.34 ± 1.36; P=.038). These results suggest that *S. alterniflora* performs best in clean saltwater compared to reused shrimp culture water. Plants have a variety of effects on water quality, and the plants do respond to excess dissolved nutrients, which is a common characteristic of reused water. Further research is needed to examine what components of reused water could be changed to facilitate better plant production and remediation potential.

QUANTIFIED SWOT ANALYSIS OF MAJOR AQUACULTURE SPECIES IN SOUTH AFRICA

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South Africa produces both marine and freshwater aquaculture species, with a combined production output of 5 418 tons of food fish in 2015. Key aquaculture species reportedly cultured include; 4 marine Species - Abalone, Dusky Kob, Mussels and Oysters and 7 freshwater species - Tilapia, Trout, Catfish, Marron crayfish, Carp, Koi-carp and Ornamental species. Key aquaculture food fish species – Abalone, Dusky Kob, Mussel, Oyster, Salmon, Trout, Tilapia, Catfish and Marron Crayfish were identified, and subjected to quantitative and qualitative analyses of SWOT factors by adapting the quantified SWOT analytical method coupled with the Multi-Attribute Decision Making Method MADM (SWOT-MADM). Internal and external assessment factors were determined; followed by data collection; performance normalization; determination of benchmarking and SWOT coordinates values. Trout and Tilapia are positioned in quadrant I, showing a combination of competitive strengths and market opportunities for aquaculture development in South Africa. Abalone, Oyster and Marron Crayfish are situated in quadrant II; thus, they possess market opportunities but weak in competitive position as aquaculture species. Salmon and Dusky Kob are positioned in quadrant III; therefore, both species are weak in competitive strengths and faced with more threats factors rather than market opportunities. Finally, Mussel and Catfish are in quadrant IV; therefore, both species have competitive strengths but are facing more threats than opportunities. Catfish recorded the highest competitive strengths due to internal assessment factors such as; high FCR, ability to withstand very high stocking density, rapid growth rate, high survival rate, hardiness and versatility of production systems and locations. Abalone and Marron Crayfish on the other hand, both exhibited more market opportunities as compared to other key aquaculture species due to external assessment factors like high pricing and good financial indicators. The study is expected to add to existing body of knowledge and guide aquaculture industry stakeholders to make informed decisions with regards to feasibility of aquaculture species in South Africa.

FEEDING HABITS AND DIGESTIVE ENZYMES IN THE GUT OF *Chrysichthys nigrodigitatus* IN OYAN DAM, ABEOKUTA, OGUN STATE, NIGERIA

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The feeding habits of 100 silver catfish, *Chrysichthys nigrodigitatus* (Lacépède, 1803) from Oyan dam were investigated. Stomach contents were identified and analyzed by occurrence methods and the prominence of Individual food items was determined by the ranking index. Only 15% of the specimens had empty stomachs while 21% had full stomach, others had stomachs in varying degrees of fullness. Sand was 11% of total volume in the stomach of *chrysichthys nigrodigitatus* species, which suggests a benthic feeding habit. The high percentage occurrence of Insects (arthropod) established *Chrysichthys nigrodigitatus* as an omnivore.

Assay was conducted to determine the distribution and specific activities of digestive enzymes in different gut regions of *chrysichthys nigrodigitatus*. Amylase, lipase and protenase were present in different gut and regions at varying quantities and with specific activities. Amylase is high in the stomach, while lipase and protenase in the posterior region. There were significant differences (P<0.05) in specific activities of digestive enzymes. The wide distribution of enzymes in *Chrysichthys nigrodigitatus* specimen reflects its ability to digest the carbohydrate, protein and lipid portion of its food. The viscerosomatic and hepatosomatic indices were carried out for *Chrysichthys nigrodigitatus* analyzed based on the total body weight and organ weight of the fish with condition factor (K) ranging between 1.87 and 1.99 respectively.

The coefficient factor of the length weight relationship, linear equation shows a correlation between weight and length, with correlation (r=0.78) indicating a strong association between the two variables and the regression co-efficient of determination R^2=0.611 indicating that 61.1% of the variations recorded in weight can be explained by length.

PHENOTYPIC CHARACTERIZATION AND NATURAL DIET OF *Chrysichthys nigrodigitatus* IN EPE LAGOON, LAGOS STATE, SOUTHWEST, NIGERIA

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Identification of species plays a key role for the behavioural study. Different methods are used for identification but metric counts and morphometry are considered as earliest and authentic methods for the identification of species (Naymann, 1966). Meristic counts mean anything that can be counted while morphometry is the external measurement of an organism (Talwar and Jhingran, 1992). Morphological characters have been commonly used in fisheries biology to measure discreteness and relationships among various taxonomic categories.

The Phenotypic characterisation and natural diet of *Chrysichthys nigrodigitatus* in Epe lagoon were investigated between March and September 2017. The major fishing methods employed for collecting the 124 specimens were cast netting and set netting. The total lengths ranged from 15.00 to 36.3 cm (mean size 26.186 ± 0.3744 cm) while the standard lengths ranged from 10.9 to 29.4 cm (mean size 19.841 ± 0.316+1 cm). The length-weight relationship was described by the equation: Log W = a + b Log TL. The value of the coefficient of regression 'b' for this species was nearly 3 thus, indicating isometric growth. The mean condition factors ranged from 0.68 to 0.79. The food organisms consisted of phytoplankton, crustaceans, molluscs, plants material and fish parts.

The population of males was significantly ($p < 0.05$) higher than females in the lagoon.

ENTREPRENEURIAL OPPORTUNITIES IN SEAWEED AQUACULTURE; THE CASE STUDY OF HAWAIIAN GROWN ASPARAGOPSIS TAXIFORMIS AT NELHA

Alexia Akbay, Simona Augyte, Jonathan Simonds and Kirk Muller

Emerging research has shown that *Asparagopsis taxiformis*, a tropical red macroalgae, has the potential to reduce livestock GHG emissions by ~80% with just a small inclusion rate of 0.5% in feed. However, wild harvesting of large quantities of *A. taxiformis* is economically unsustainable and can damage coastal marine ecosystems. While *A. taxiformis* has been shown to significantly decrease methane production in cattle, critical gaps in cultivation knowledge, and its complex life-cycle, have kept this seaweed from becoming a commercially viable product.

Symbrosia has spent the past year stabilizing biomass cultures and obtaining baseline parameters and specific physiological tolerance limits for temperature, light, seawater flow rate, photoperiod, and nutrient additions. Both lab and out-door tank trials are currently underway at NELHA (Natural Energy Laboratory of Hawaii Authority) for optimizing conditions as part of its efforts to develop and optimize large-scale, land-based tank production of local Hawaiian strains of *A. taxiformis*. Our unique site at NELHA allows access to the nutrient-dense deep sea water needed to cultivate seaweed and plenty of year-round sunshine for *A. taxiformis* that thrives in this sub-tropical coastal climate.

Biomass produced will be utilized for both animal health trials with the USDA (e.g., productivity increases and disease prevention) and as the initial source of Symbrosia's revenue. Our market research has elucidated interest from consumer-facing beef and dairy brands, farmers in regions where regulation is on the horizon (California, the EU and New Zealand), as well as small-scale farmers in the North East and Hawaii that are interested in reducing their livestock emissions.

Overall, our proprietary solutions will help demonstrate the technical feasibility of scaling up the aquaculture system to a commercial level with the fewest energy, resource, and labor inputs. Worldwide, as seaweed aquaculture continues to grow (estimated at $11.7 billion annually), Symbrosia is committed to contributing its expertise to this increasingly important domestic and global industry. With the support of the world's first sustainable aquaculture accelerator (HATCH), VC investors, early adopters, and a number of champions throughout the supply chain, Symbrosia aims to provide a commercial source of *A. taxiformis* as an increasingly important climate mitigation strategy.

EVALUATION OF SALT-INCORPORATED DIETS ON GROWTH, BODY COMPOSITION AND PLASMA ELECTROLYTES OF BLACK SEA BASS *Centropristis striata* REARED IN A SEMI-PILOT SCALE LOW-SALINITY RECIRCULATING AQUACULTURE SYSTEM

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A feeding trial was conducted to investigate the effects of dietary salt supplementation on growth, survival, body composition, and plasma electrolytes of black sea bass *Centropristis striata* reared under sub-optimal salinity conditions of 10-12.5 g L^{-1} in a semi-pilot scale RAS. Four test diets were formulated and prepared with different levels of supplemental sea salt (99.86% NaCl, Cargill Salt, Minneapolis, MN): 0% (control), 2.5%, 5%, and 7.5% dry wt. In addition, two control diets were tested for fish raised in full-strength seawater (34 g L^{-1}) in an independent RAS; one was a 0% salt formulated diet and the second was a premium commercial diet (Skretting, Canada). All test diets were isonitrogenous (46% crude protein) and iso-lipidic (12% crude lipid). A 25-m^3 automated reservoir system was used to supply brackish water to the low-salinity RAS. Twelve tanks (vol = 2-m^3) of the low-salinity RAS were each stocked with juvenile black sea bass (mean wt. = 19.6 g) at a density of 100 fish per tank and at a starting salinity of 34 g L^{-1}. The RAS salinity was gradually decreased at a nominal rate of 1 g L^{-1} per day until the target salinity of 10 g L^{-1} was reached in 24 days. Six tanks (2-m^3) of the RAS supplied with full-strength seawater were each stocked with 100 fish from the same cohort to serve as a control. After the 8-month feeding trial, fish grown at 10-12 g L^{-1}, were fed their respective diets for an additional 5 weeks under adverse low-salinity conditions in which salinity was further decreased gradually from 10 g L^{-1} to 4 g L^{-1} in 30 days.

Percent survival over the initial 8 months remained high (93-100%) among treatments, with no significant differences. The highest weight gain (825%) among treatments was observed for fish fed 2.5% salt in low salinity, whereas the lowest weight gain (765%) was found for fish fed 5% salt. In comparison, fish raised in full seawater showed weight gains of 788-813%. However, no significant differences ($P > 0.05$) in weight gain were observed among fish under any diet or salinity treatment. Plasma osmolality (mOsm kg^{-1}) for fish raised at salinities of 10-12.5 g L^{-1} or 35 g L^{-1} ranged from 336-357 and was not significantly different. No significant differences were observed in plasma electrolytes concentrations among fish fed the various diets, except bicarbonate, which was significantly ($P < 0.05$) higher for fish fed the commercial diet in full seawater. When challenged with a further reduction in salinity from 10 to 4 g L^{-1} over a period of 5 weeks, fish a 0% salt diet showed poor survival (29%), not significantly different from fish fed diets with 2.5% (42% survival) and 5% salt (60% survival). However, fish fed diets with 7.5% salt showed significantly higher survival (67%) than fish fed 0% salt (29%). The results suggest that black seabass can be acclimated to and raised at a low salinity of 10 g L^{-1} with no effects on long-term growth performance, or on fish plasma electrolyte concentrations compared to fish raised in full strength 34 g L^{-1} seawater. Salt-incorporated diets, however, improved survival under extreme low salinity (~ 4 g L^{-1}) challenge conditions. These findings have potentially important implications for rearing black sea bass in low salinity RAS and for the siting of black sea bass RAS growout operations.

INFLUENCES ON THE TIMING OF FERTILITY OF AN INTERTIDAL KELP *Alaria marginata*

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The cultivation, production, and marketing of seaweed and seaweed products will likely play an important role in the future of mariculture in Alaska. The common intertidal seaweed *Alaria marginata*, shows promise for commercial production. However, the environmental and physical conditions that stimulate the release of spores of *A. marginata* is a significant knowledge gap.

The purpose of this research is to determine the environmental conditions that influence fertility and the release of zoospores for *A. marginata*. Thalli from two small bays were gathered weekly for two months. Within each bay, two subsites were visited, one on the more exposed side of the bay and the other on the protected side of the bay. Physical measurements, including stipe length, sporophyll number, fertile sporophyll number, and sorus area were taken weekly. Spore release was conducted on the most fertile sporophyll from each thallus. It is hypothesized that water temperature and light intensity are strong influencers of spore release, however, nutrient concentrations and salinity are being analyzed for their possible influence of *Alaria's* fertility. Preliminary results show that spore release peaked at the beginning of the field monitoring season. Minimal and often zero spore release was observed after June. Furthermore, there were large differences in characteristics related to reproductive potential, such as the number of sporophylls and the area of fertile material on sporophylls, between sites.

Knowing when kelp species are fertile is very critical to mariculture. It is key for industry members to know when wild stock should be harvested to most effectively produce seed in the hatchery.

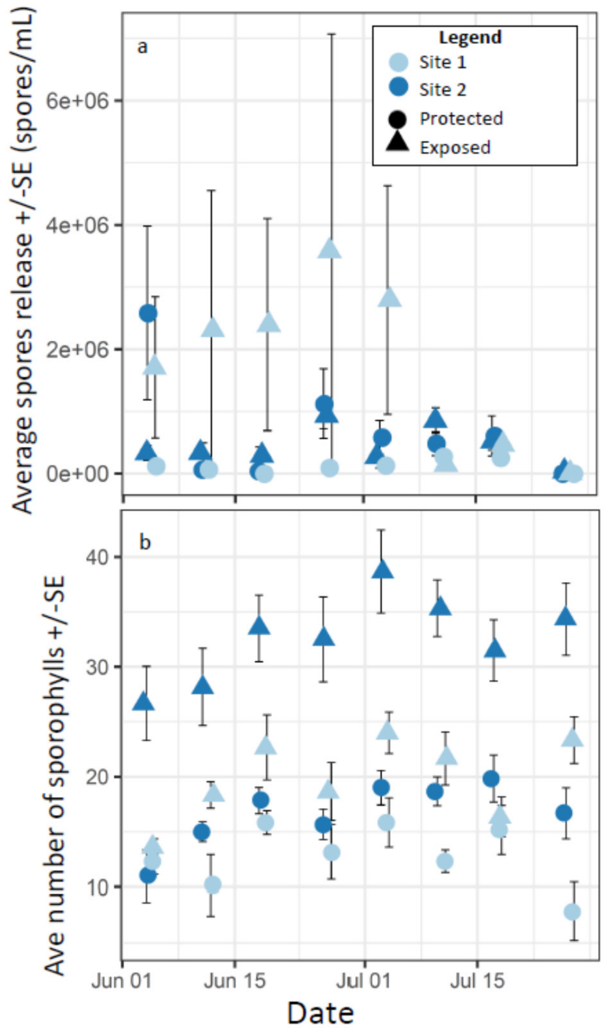

Figure 1. a) Non-zero spore b) Ave. number of sporophylls through June and July.

LARGE-SCALE CLIMATIC EFFECTS ON TRADITIONAL HAWAIIAN FISHPOND AQUACULTURE

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Aquaculture accounts for almost one-half of global fish consumption. Understanding the effects of climate fluctuations and climate change on aquaculture is therefore critical for the sustainability of this important food resource. Our objective was to understand the role of the 2009 El Niño in tropical coastal estuarine environments in the context of aquaculture practices in Heʻeia Fishpond, Oʻahu Island, Hawaiʻi. This was the first study examining climate effects on traditional aquaculture systems in the Hawaiian Islands. Data from two adjacent weather stations were analyzed with *in situ* water quality instrument deployments spanning a 12-year period (November 2004 – November 2015). We found correlations between two periods (May and October 2009) with extremely high fish mortality (i.e., 'fish kills') at Heʻeia Fishpond and slackening trade winds in the week preceding each fish kill event, as well as elevated sea surface temperatures (SST), 2-3 °C higher than background periods (March-December 2009). We posit that lack of trade wind-driven surface water mixing promoted enhanced surface heating and stratification of the water column, leading to hypoxic conditions and stress on fish populations. Impact on fish was amplified because they were contained within net pen enclosures. Elevated SST and interruption of trade winds have been previously linked to the onset of El Niño in Hawaiʻi. Our results provide empirical evidence regarding El Niño effects on the coastal ocean as well as on aquaculture. These findings can inform resource management efforts about potential impact of climate variation on aquaculture production.

MANAGEMENT OF SPONTANEOUS AUTOPOLYPLOIDY IN WHITE STURGEON *Acipenser transmontanus* CONSERVATION HATCHERIES

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Spontaneous autopolyploidy is a condition of genome duplication (increased chromosome number) that occurs between parents and progeny within a species in the absence of hybridization or induction using traditional methods of thermal, pressure, or chemical shocks to produce triploid fish. This condition is known to occur in nine sturgeon species worldwide, including white sturgeon (*Acipenser transmontanus*) in the Columbia River Basin in the United States and Canada. Although this species is normally octoploid, having 8 copies of each chromosome (8N), spontaneous autopolyploid white sturgeon (12N) have been recently confirmed in conservation and commercial white sturgeon production facilities in California and the Pacific Northwestern US and British Columbia, Canada. Some of these 12N individuals are fertile and when spawned with 8N fish can produce 10N progeny, which may have reduced fitness, performance, and reproductive viability. This condition in white sturgeon results from retention of the second polar body during meiosis, the same mechanism intentionally used in hatchery programs to produce sterile triploid (3N) fish. Although spontaneous autopolyploidy in white sturgeon was initially documented in hatchery programs that artificially spawn adult broodstock, a small number of wild (naturally-produced) 12N fish have recently been confirmed.

We briefly discuss the detection and causes of spontaneous autopolyploidy for white sturgeon in the conservation aquaculture setting and describe six general and 13 specific Hatchery Best Practices to reduce the incidence and risks of spontaneous autopolyploidy in such programs. Recommended Best Practices involve a range of activities from rigorous screening of broodstock and progeny groups to collectively holding pre-spawning male and female broodstock and maintaining strict ranges of environmental conditions and handling protocols in the hatchery before, during, and after ovulation and spawning. While spontaneous autopolyploidy in conventional conservation hatchery programs can pose risks to recipient populations, implementing Best Hatchery Practices can significantly reduce the incidence and risks of 12N fish. Additional focused studies to better understand the relationships between specific hatchery practices and the incidence of spontaneous autopolyploidy will help sturgeon culturists and hatchery managers balance short-term production and population rebuilding goals with the need to meet long-term population restoration and viability targets.

CONSERVATION AQUACULTURE OF WHITE STURGEON *Acipenser transmontanus* IN THE COLUMBIA RIVER BASIN, USA AND CANADA: A 30-YEAR RETROSPECTIVE

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Conservation aquaculture programs for white sturgeon (*Acipenser transmontanus*) in American and Canadian waters of the Columbia River Basin (Basin) have evolved considerably since their inception during the late 1980s. Early programs focused on assessing the feasibility of aquaculture to protect and restore imperiled or endangered populations. As the reliability of sturgeon culture techniques improved, programs diversified to address more specific demographic and genetic goals, increase year class representation and population viability, and support the reestablishment of multiple use fisheries. Despite multiple challenges and successes of these programs over the past 30 years, no inclusive review or synthesis of program design, operations, and outcomes has been produced.

To address this information gap, we summarized key aspects of 10 white sturgeon conservation aquaculture programs in American and Canadian waters of the Basin to facilitate communication and coordination and to guide ongoing and future programs. We report information gathered from a survey questionnaire regarding: 1) program history, 2) goals and objectives, 3) monitoring and evaluation programs, 4) significant findings, 5) unexpected results, 6) lessons learned, and 7) future needs and concerns. The survey was sent to over 30 entities directly involved in white sturgeon hatchery programs including state, federal, and provincial fisheries agencies, Native American tribes and tribal commissions, federal, private, public and provincial hydropower companies and utility districts, and private sector and academic entities. As of 2019, the 10 reported programs have been operating from 6 to 30 years, with 8 of 10 the programs operating continuously since their inception. Significant accomplishments include the prevention of extinction, increased abundance and year class representation, incorporation of native genetic diversity into recipient populations, development and refinement of aquaculture, monitoring, and evaluation techniques for various white sturgeon life stages, successful implementation of repatriation programs and streamside rearing techniques, and the reestablishment of fishery opportunities.

PATHOGENICITY OF *Oligoflexus tunisiensis* BACTERIUM ON FRESHWATER FISHES

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Oligoflexus tunisiensis is the first culture isolate and the only bacteria strain in the newly described class *Oligoflexia,* as of the time of the experiment. This bacteria was isolated from a 0.2μm filtrate of a sand gravel suspension collected from the Eastern margin of the Sahara desert. However genome sequence of the bacteria shows that it encodes the gene for the ABC transporter of the amino acid−RND- type efflux pump system – which is widely known among gram-negative bacteria to promote multidrug resistance in pathogenic bacteria. Also the genome sequence of the bacteria showed a higher similarity (67% identity and 99% coverage) to the fish pathogenic bacteria *Pseudomonas aeruginosa* –a well-known antibiotic resistant bacteria in some freshwater aquaculture fishes, and the causative agent of some important freshwater fish diseases. The aim of this present study was to ascertain the pathogenicity of the bacteria, *Oligoflexus tunisiensis* on freshwater fishes.

Using the infection challenge trial, three freshwater fishes (*Heterotilapia buttikoferi, Nimbochromis venustus* and *Epalzeorhynchos bicolor*) of average weight 3±0.5g were challenged intraperitoneally with 10^8 cfu/fish bacterial cells of *O. tunisiensis*, and observed for their response to the infection.

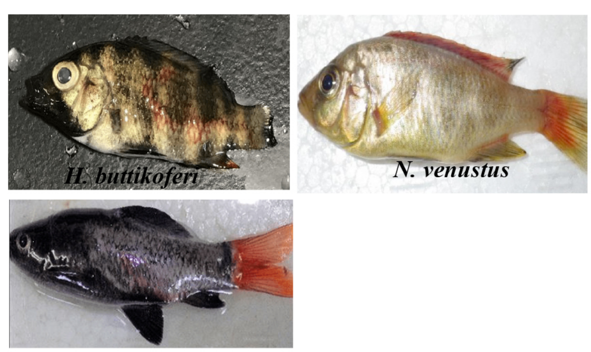
48−72 hours after post inoculation, *Oligoflexus tunisiensis* infected samples showed some strong pathological and clinical symptoms such as haemorrhagic septicemia, tail rot, degradation of some internal organs and mortality. In order to justify that these clinical signs observed were elicited by *O. tunisiensis*, we used the traditional culture-independent technique to retrieve the bacteria from the liver, spleen and kidney of the affected samples.

Our results indicated that *O. tunisiensis* significantly influenced the observed symptoms post-infection.

TABLE 1: Percent mortality after post-inoculation with *O. tunisiensis*

Treatment	(cfu/ml)	H. buttikoferi	N. venustus	E. bicolor
O. tunisiensis				
Live cell	1.5×10^8	87.01	93.05	90
Supernatant	-	73.2	53.13	70
Heat-inactivated cell	1.5×10^8	0	0	0
Heat-inactived supernatant	-	0	0	0
A. hydrophila				
Live cell	1.5×10^8	100	100	100
PBS	-	0	0	0

FIGURE 1: Observed clinical signs hours after post-inoculation



“EVALUATION OF THE BIOLOGICAL PERFORMANCE OF SHRIMP *L. vannamei* IN SYSTEMS CULTURE SANITIZED WITH DIFFERENT RESIDUAL CHLORINE CONCENTRATIONS

Juan Pablo Apún-Molina*, Manuel Jimenez Leyva, Juan Carlos Sainz Hernandez, Alejandro Urias Camacho and Jorge Herrera Olmos

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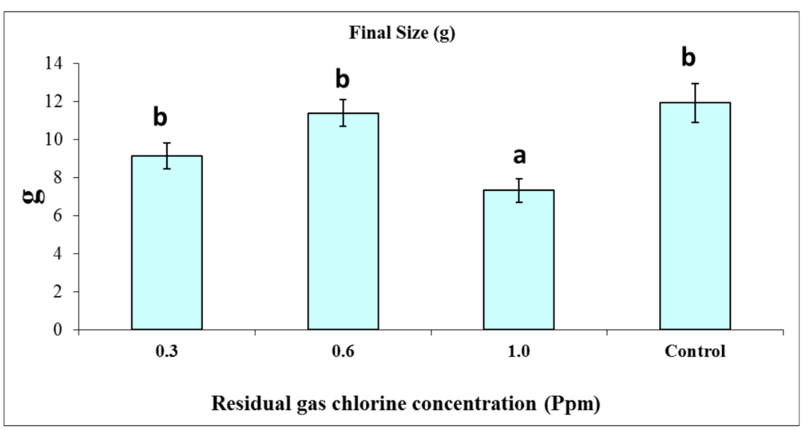
In the aquaculture industry the biggest obstacle to overcome for a future increase in productivity are the diseases. Many of them, caused by viruses, bacteria, fungi, parasites and even diseases undiagnosed or just emerging that have affected the aquaculture industry and will continue to impact to the industry as it grows and develops trying to increase production to meet the food needs of the population. In 2013, there were millions of losses in the shrimp industry in Mexico due to mortality caused by two diseases: EMS and WSSV (Early Mortality Syndrome and Syndrome White Spot Virus, respectively). It is estimated that in that year, 70 to 80% of shrimp production. The affected states were Nayarit, Sinaloa and Sonora with more than 100 thousand hectares of shrimp aquaculture production. These states produce 80% of the shrimp at the level national and 2/3 of this production is obtained through aquaculture.

Moreover, a supposed resistance has no chance if in hatcheries and farms they do not correctly manage the microbiological community of their crops, letting *Vibrio* continue being the dominant genus in the cultivation water and therefore in the digestive system of the shrimp and this it extends to the producer’s premises, where the probability that this is dedicated or can control the microbiological community of its ponds. Many producers they are investing correctly in maternity and pre-fattening systems, however, the vast majority still does not handle these phases in his favor and ends up sending organizations with a higher probability of to get sick.

The disinfection of the cultre systems observes great relevance in these times, since there is a direct relationship between pathogenic bacteria and culture shrimp diseases.



ASSESSMENT OF TRANSMISSION RISK IN WSSV-INFECTED SHRIMP *Penaeus vannamei* UPON COOKING

Luis Fernando Aranguren Caro[1]*, Hung N. Mai[1], Joshua Lin[1], Linda Nunan[1], and Arun K. Dhar[1]

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White spot syndrome virus (WSSV) has been a threat to global shrimp industry since it was discovered in Taiwan in 1991. The economic impacts of WSSV has now surpassed over $15 billion globally. Thus, major shrimp importing countries around the world have enacted regulations to prevent further spread of WSSV in those countries through imported and potentially virus containing commodity shrimp. Recently, cooked shrimp originating in WSSV-endemic areas has turned out to be positive for WSSV by PCR. However, as of now, there is no published report describing the risk of WSSV transmission via cooked shrimp. We, therefore, conducted a study to evaluate the infectivity of cooked WSSV-infected shrimp. Specific Pathogen Free (SPF) Pacific white shrimp (Penaeus vannamei) known to be infected with WSSV was cooked at a boiling temperature for 1, 3, 5, 10 and 30 minutes, respectively. Uncooked shrimp (i.e. 0 min exposure to boiling temperature) served as a positive control. After cooking, the cooked shrimp was used to experimentally infect SPF P. vannamei shrimp through an oral feeding at 5% of the biomass of the tank. The data from experimental challenge showed that while animals from 0-minute treatment (Positive control) tested positive for WSSV by qPCR and H&E histology, animals from 1, 3, 5, 10 and 30-minute treatments were negative for WSSV. Mortality data confirmed that only 0-minute treatment displayed acute mortalities, in contrast, 100% survival was recorded in SPF shrimp challenged using cooked shrimp exposed to boiling temperatures at 1, 3, 5, 10 and 30 min. These finding suggested that cooking shrimp at boiling temperature for at least 1 minute can prevent the risk of WSSV transmission to healthy shrimp.

ASSESSING INFECTIVITY OF FECAL STRING OBTAINED FROM EHP INFECTED *Penaeus vannamei* SHRIMP AT DIFFERENT SALINITIES

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Enterocytozoon hepatopenaei (EHP) is an emerging pathogen that affects *P. vannamei* shrimp in many SE Asian countries including Indonesia, Vietnam, China, Thailand, India and Malaysia. In the western hemisphere, EHP was reported for the first time in 2016 in farmed *P. vannamei*. Infected shrimp exhibit reduced feeding and severely retarded growth. EHP is an enteric pathogen that replicates in the cytoplasm of tubule epithelial cells in the hepatopancreas. Anecdotal evidence suggest that EHP seems to be more prevalent in grow-out ponds where the salinity is high (>15 ppt) compared to grow-out ponds with low salinities (<5 ppt). . Considering that *Penaeus vannamei* shrimp is an euryhaline species which is now farmed worldwide in wide range of salinities, we were interested to determine if EHP is able to replicate in low salinities.

In this study, we described an experimental infection using fecal strings from known EHP-infected *P. vannamei* as a source inoculum. Specific Pathogen Free (SPF) *P. vannamei s*hrimp were maintained at different salinities including 2 ppt, 15 ppt and 30 ppt and continuously challenged using fecal string over a period of three weeks. Infectivity of EHP containing fecal strings to animals reared at different salinities were confirmed by conventional PCR and histology. PCR data showed positive results in shrimp maintained at 2ppt, 15ppt and 30 ppt with higher prevalence at 2 ppt. H&E histology where animals reared at 2 ppt salinity displayed higher levels of infection compared to animals reared at 15 and 30 ppt, respectively. The results showed that EHP can replicate well in shrimp maintained at low salinities.

GROWING TOGETHER AQUAPONICS: A JOB TRAINING AND EMPLOYMENT PROGRAM FOR STUDENTS WITH INTELLECTUAL AND OTHER DEVELOPMENTAL DISABILITIES

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The purpose of this poster session is to describe a unique and creative way of utilizing the multiple conceptual skill sets that aquaponics can provide to young learners with disabilities. These skills can then lead ultimately to employment, independence, and improved quality of life. The Growing Together Aquaponics Program provides job training and employment opportunities to high school and college students with intellectual and other developmental disabilities (ID/D) (i.e., Autism Spectrum, Deaf, Blind, Cerebral Palsy, etc.). Growing Together has developed a unique community-wide partnership between Slippery Rock University of Pennsylvania and the North Country Brewing Company, both located within the Slippery Rock Borough, Pennsylvania. The brewery provides valuable land space for the aquaponic system, and employment opportunities within its three restaurants. Their canning facility houses two unique aquaponic systems. One located within the cannery, the other located on a deck above the Tap Room restaurant and bar. This fully inclusive, community-based program demonstrates to community members and patrons of the potential for successful employment skills and abilities of individuals with disabilities independently managing an aquaponic facility.

The university's role is to provide mentor who support, when necessary, high school students with disabilities as they learn how to operate an aquaponic system start to finish, fish to crops, business to distribution. Mentors are typically special education, recreation therapy, or related fields of study earning service-learning credits for specific college credits. Ten local school districts also send students with ID/D to the program five days a week further developing partnerships in an aquaponic demonstration model. The program also provides training opportunities to adults with ID/D who are residents in group home settings as well as classes for middle school students and senior citizens.

Students enrolled in the program are certified in the Serv Safe food handling certification program and progress through a series of developmental steps with the ultimate level demonstrating stewardship and leadership skills by then teaching classes to middle school students and community seniors during weekend classes. All crops grown within the two systems are provided to the North Country Brewing Company restaurants throughout western Pennsylvania.

Future and immediate plans call for the development of a medium sized greenhouse and aquaponic system in the middle of downtown Slippery Rock and adjacent to the North Country Brewing Company Pub. This will provide the opportunity to sell crops to regional restaurants and businesses, subsequently hiring our program graduates.

THE MARKET FOR SALMONIDS: SALMON TROUT AND IT'S RELATINSHIP TO ATLANTIC SALMON

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Atlantic salmon is the main species in Norwegian as well as Chilean aquaculture. However, in both countries large trout or salmon trout is the second most important species, although an increasingly distant second. In many ways this is surprising as the salmon trout appears as the poor cousin which do not seem to do too well but that still not go away. In this paper we investigate the market relationship between Atlantic salmon and salmon trout investigating market integration as well as volatility transmission.

The results indicate that the markets for Atlantic salmon and salon trout is highly integrated, with the price for salmon trout being determined by the price of Atlantic salmon. On average, salmon trout has a price discount, has higher price volatility and higher seasonality, in production and accordingly appears as a poorer performing species economically. However, there are periods where it is doing significantly better, indicating that it may be a useful tool in spreading risk. This is further emphasized by different export patterns in terms of main markets as well as product forms.

VENTURA SHELLFISH ENTERPRISE: AN OVERVIEW OF THE CHALLENGES TO SITING LONG LINE MUSSEL FARMS IN FEDERAL WATERS OFF CALIFORNIA

Everard Ashworth,* Brian Pendleton, Doug Bush, Linda Santschi, Ph.D., Ralph Imondi, Ph.D., Richard W. Parsons, Brooke Ashworth, Paul Olin, Diane Windham, Scott Lindell, Laurie Monarres, and Robert Smith, Esq.*

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Increasing the supply of safe, sustainably produced domestic seafood is a priority for the National Oceanic and Atmospheric Administration (NOAA) and the Department of Commerce. The Ventura Shellfish Enterprise (VSE) project is an initiative that seeks to permit commercial mussel shellfish aquaculture operations consistent with this objective. The farms are to be located in federal waters outside Ventura Harbor, California. The permits will be held by the Ventura Port District and leased to qualified companies, who must land all product within Ventura Harbor and follow Best Management Practices developed in concert with the US Army Corps of Engineers, California Coastal Commission, NOAA, and National Marine Fisheries. The current complexities and costs associated with the aquaculture permitting process represent a significant barrier to expansion of the industry. The VSE project seeks to address several regulatory and planning challenges that effectively limit the development of domestic marine shellfish culture.

The key elements of this innovative project include:

1. Developing a technically sound strategy to successfully obtain government entitlements necessary to establish twenty 100-acre aquaculture permits in federal waters of the Santa Barbara Channel, proximate to Ventura Harbor;
2. Implementing this strategy and obtain the necessary permits and entitlements, and complete associated environmental review documents;
3. Developing an effective monitoring and reporting program to monitor environmental impacts and evaluate project progress;
4. Collaborating with the Food and Drug Administration (FDA) and NOAA's Seafood Inspection Program to ensure future landed product has a pathway for compliance with the National Shellfish Sanitation Program (NSSP) guidelines for shellfish grown in federal waters; and
5. Preparing grower/producers for successful farming of the growing areas through business planning, training, and technology transfer.

When fully operational, the project is anticipated to provide up to 20 million pounds per year of Mediterranean mussels (*Mytilus galloprovincialis*) that will be landed in Ventura Harbor. This presentation will provide a focused review of the significant permitting and operational design challenges facing the VSE project, and describe the tools used to date to manage each project element.

ARTICULATING NEEDS TO EMPOWER PRACTITIONER-LED MANAGEMENT OF LOKO I‘A (HAWAIIAN FISHPOND) RESOURCES AND PRACTICES

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In response to practitioner desires to compile research needs for fishponds, we aimed to facilitate a year-long process to support practitioners to voice their needs, articulate their priorities, and create pathways for resilience in their places and practice. This process resulted in the first comprehensive compilation of the research ideas and needs within the community of fishpond managers, landowners, and stewardship organizations to inform adaptation of fishpond practices toward their resilience and sustainability in the face of a changing climate. The final assessment report is highly collaborative and co-produced, with initial inquiries directly from expert kia‘i loko (fishpond stewards and practitioners) who are actively restoring and managing loko i‘a (fishponds) in Hawai‘i, and refined in partnership with researchers who are experts in their own fields of inquiry and scientific methods.

This effort seeks to inform the development of future research, monitoring, and planning for loko i‘a throughout Hawai‘i to endure dynamic and unpredictable environmental conditions. Our process and key outcomes will be presented to offer lessons learned about network-building, cultural frameworks that uphold an evolving traditional practice, and deep partnership between community and academic institutions. We hope these findings can inform decision-making and galvanize support for place-based aquaculture both locally and globally.

LOKO I‘A: INDIGENOUS AQUACULTURE AND MARICULTURE IN HAWAI‘I

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Loko i‘a are an advanced, extensive form of aquaculture found nowhere else in the world.[1] They enhance nearshore areas and were essential components of traditional food systems in Hawai‘i, providing food security and community resilience. In the wake of the social and political upheaval following the overthrow of the Hawaiian monarchy in 1893, loko i‘a production dropped. By 1970, loko i‘a only produced 20,000 pounds of fish--less than 1 percent of what they produced at their peak.[2] Today, the majority of sites are highly degraded, some completely covered and unrecognizable as fishponds. Barriers to restoration include: altered watersheds and diversion of water, invasive species, permitting processes that are not well-designed to accommodate restoration, and loss of generational knowledge for management and care of loko i‘a.

Over past decades, communities worked to restore loko i‘a around the islands and reclaim the knowledge and practice of loko i‘a culture. In 2004, recognizing the current challenges and in an effort to increase collaboration, restoration and food production, kia‘i loko (guardians/caretakers) formed the Hui Mālama Loko I‘a, a network of loko i‘a from six Hawaiian islands. As an ever growing network of committed and skilled site-based caretakers the Hui Mālama Loko I‘a has improved and accelerated the loko i‘a stewardship movement.

Loko i‘a practice is the result of over a thousand years of generational knowledge, experimentation and adaptation and reflects a deep indigenous understanding of the environmental, ecological and social processes specific to our islands[3]. Their revitalization today goes hand-in-hand with the revitalization of Hawaiian language, arts, architecture and diet. Loko i‘a are thus celebrated for their past and future potential to provide opportunities for Native Hawaiians and the larger community to renew ‘āina momona, an abundant, productive ecological system that supports community well-being. A panel of kia‘i loko will discuss various aspects of progress and the next steps for Hui Mālama Loko I‘a and the reactivatoin of loko i‘a throughout Hawai‘i.

1 Patrick V. Kirch, Feathered Gods And Fishhooks (Honolulu: University of Hawai‘i Press, 1985).
2 Fishponds," Ahupua‘a, Fishponds and Lo‘i, directed by Nā Maka o ka ‘Āina, produced by Nalani Minton (Honolulu: 1992).
3 Reflection from practitioners of local non-profit Paepae o He‘eia, formed in 2001 to restore and mālama He‘eia fishpond on O‘ahu.

TOXICITY OF WASTE DRY CELL BATTERY ON THE PHYSIOLOGY OF *Clarias gariepinus* FINGERLINGS

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Dry cell battery is the most common source of household energy to power electronic devices and torchlights in both urban and rural communities in Nigeria and the Waste Dry Cell Battery (WDCB) indiscriminately discharged into water bodies which could have attendant consequences to aquatic inhabitants. The acute toxicity (96hrs LC_{50}) of the Water Soluble Fractions (WSFs) of WDCB was investigated on *Clarias gariepinus* fingerlings. WDCB powder was macerated in distilled water at room temperature and supernatant (WSFs) obtained.

A range finding test of the WSFs was conducted on *C. gariepinus* fingerlings from which five definitive concentrations (5.00, 2.50, 1.25, 0.63 and 0.31g/L) were obtained. 120no *C. gariepinus* fingerlings (mean weight, 9.77±0.42g; length, 6±0,05cm) in twelve (12) circular plastic tanks were exposed to the graded concentrations of the WSFs with 0.00mg/L which served as control.

Exactly 1ml of blood was obtained via cardiac puncture and used to assay blood parameters (RBC, PCV and Hb) and serum enzymes (ALP, AST and ALT) activities of the exposed fish. Behavioral signs observed include restlessness, air gulping, and erratic swimming. The 96hrs LC_{50} of WSFs to *C. gariepinus* fingerlings was calculated as 0.83g/L with upper and lower confidence limit of 1.43 and 0.48g/L respectively (Figure 1). There was significant ($P<0.05$) increase in the activities in both blood cells and enzymes (Figure 2) of *C. gariepinus* exposed to WSFs compared with the control which predicts a possible physiological alteration that can be used as biomarkers of the potent toxic properties of WDCB to fish.

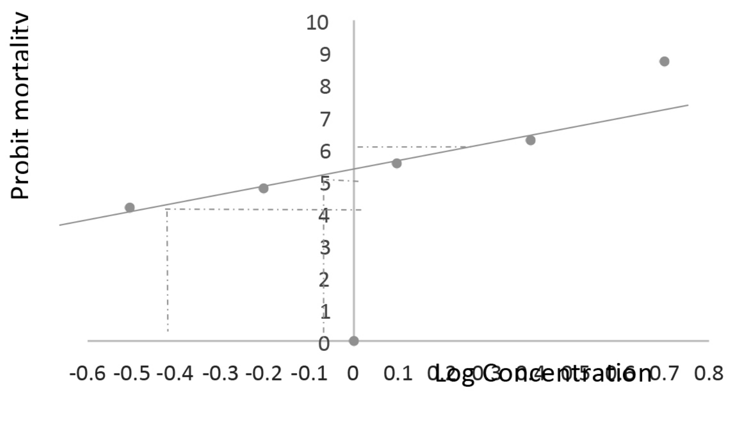

Figure 1: A Graph of Linear Relation of Probit Mortality against Log Concentration of *Clarias gariepinus* Fingerlings Exposed to various Acute Concentrations of WSFs of Waste Dry Cell Battery for 96h LC_{50}.

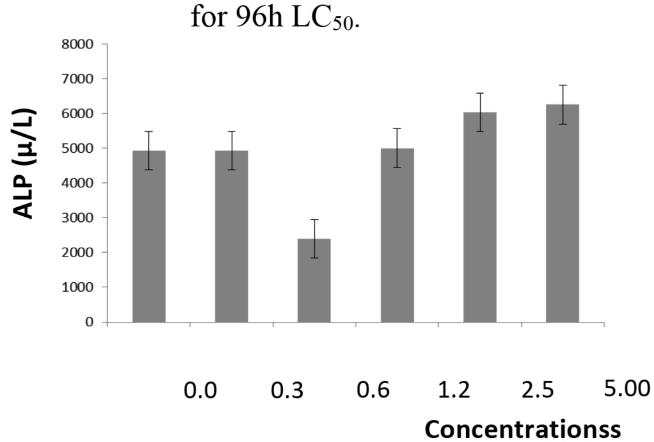

Figure 3: Alkaline Phosphatase Activities in the Gills of *Clarias gariepinus* Exposed to Acute Concentrations of WSFs of WDCB

POPULATION CONNECTIVITY OF SUGAR KELP, *Saccharina* SPP. IN THE NORTHWEST ATLANTIC FOR CULTIVATION AND BREEDING

Simona Augyte*, J.-L. Jannink, X. Mao, K. Robbins, M. Hare, S. Umanzor, M. Marty-Rivera, S. Lindell, D. Bailey, C. Goudey, C. Yarish

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An understanding of genetic diversity of natural kelp populations is critical for making recommendations for breeding and cultivation efforts of the growing seaweed aquaculture sector in the US. An important component of the ARPA-E's MARINER project is selectively breeding *Saccharina spp*. in the Northwest Atlantic in order to improve overall productivity for biofuels, feeds and food. Historical records indicate the presence of regional kelp ecotypes based on physiological tolerance, specifically temperature. We made collections of 13 wild *Saccharina* spp. populations via SCUBA along the New England coast. These parental populations were also used to make over 300 hybrid crosses that were planted at several farm locations. We then used genome-wide single nucleotide polymorphism data to identify the finer population structure of the kelp in the Gulf of Maine and Long Island Sound.

An assessment of the sequence diversity revealed distinct genetic variation between the Gulf of Maine and Southern New England, confirming that Cape Cod acts as a barrier to kelp gene flow. Furthermore, based on the analysis of molecular variance (AMOVA), we found the largest variance (58%) within sites. We also observed admixture among five sub-populations (Figure 1) and isolation by distance in the Gulf of Maine.

Future steps for this project include skim sequencing the haploid phase of the kelp life cycle to identify trait heritability and phenotypic diversity observed for both morphological traits and tissue composition. Furthermore, we plan to place our sequence data into a larger context to include samples from sites in Europe and Asia.

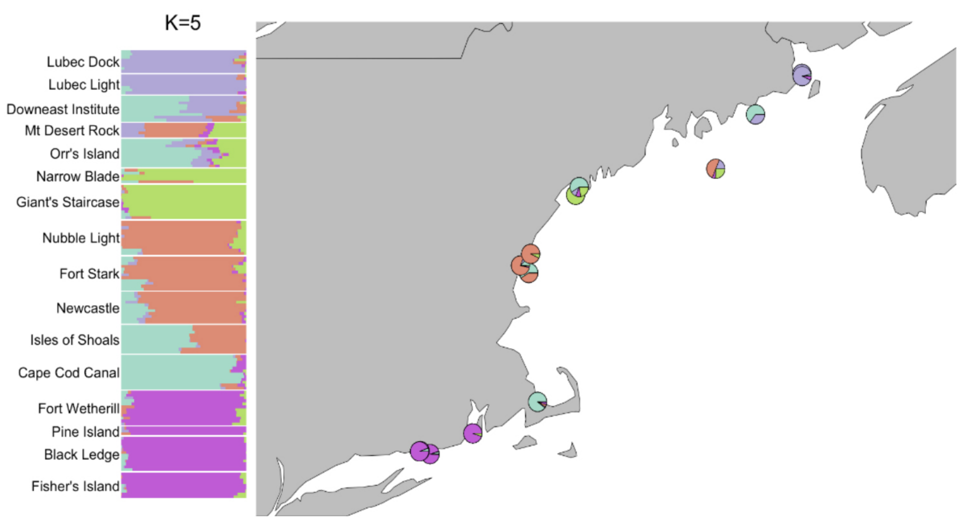

Figure 1. Admixture proportions estimated for kelp samples for K=5 with pie plot at their approximate geographic locations.

EFFECT OF AERATION ON WATER QUALITY, AND ROMAIN LETTUCE PERFORMANCE IN AQUAPONICS AND HYDROPONIC SOLUTION

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Oxygen within the root zone is an important requirement to keep roots alive for enhanced nutrient uptake and plant growth. In water-based systems, oxygen of the water is maintained by blowing air through the water with the aid of diffusers or air stones. Where aeration is not maintained, dissolved oxygen (DO) concentration (mg/l) reduces. At very low DO, water quality parameters such as nitrates, pH, and electrical conductivity (EC) are greatly affected. These changes affect plant growth and productivity. The objectives of this study were to 1) compare water quality of two nutrient sources in response to aeration and no aeration, 2) to assess the growth, stomata conductance, SPAD and yield of Romain lettuce in the systems. The experiment was a 2x2 factorial arrangement in a randomized completed block design with four replicates. The results indicate that no aeration led to a reduction in DO level in both aquaponics and hydroponics systems (1.12 mg/l and 3.46 mg/l respectively). No aeration caused no statistically significant change in EC for either system but led to 70 mg/l reduction in nitrate than aeration in aquaponics. Additionally, no aeration led to a 1 ºC increase in temperature than the aerated for both systems but decrease in pH by 1 unit in only aquaponics system. Generally, plant height decreased in no aeration across systems whereas average SPAD value was higher by 3 units and 1 unit in no aeration than aeration aquaponics and hydroponics respectively. Leaf temperature and stomata conductance showed no statistically significant differences among the treatments. Although root length was statistically different between aeration and no aeration, root dry mass was not. Also, shoot weight per plant was about 50% and 29% more in aeration than no aeration for fresh and dry mass respectively. In conclusion, the study revealed that aquaponics-based system was more affected by a lack of aeration than hydroponics system. Also, no aeration had more effect on productivity related traits than physiological related traits except SPAD of the lettuce plants. It was observed that no aeration reduced tipburn which is a major challenge in hydroponic Romain lettuce production. Therefore, no aeration should be explored further. Future research should introduce an oxygen zone around the root area. This might counter the negative effects of no aeration in deep water culture system.

COMPARISONS BETWEEN AQUAPONICS AND CONVENTIONAL HYDROPONIC CROP YIELD: A META-ANALYSIS

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Aquaponics is a relatively new system of farming which has received ardent research attention due to its potential for sustainability. However, there is no consensus on comparability between crop yields obtained from aquaponics (AP) and conventional hydroponics (HP). Meta-analysis was used to synthesize the literature for comparability between AP and HP crop yield. Factors responsible for differences were also examined. A literature search was done in five databases with no time restriction in order to capture any publication on AP and HP crop yield comparisons. The search was however, limited to publications in English, Journal, and Conference articles. Study characteristics and outcome measures of food crops were extracted. A natural log response ratio effect size measure was used to transform study outcomes. An unweighted meta-analysis through bootstrapping was used to calculate overall effect size and its confidence interval. Between-Study heterogeneity (I^2) was estimated using a random effects model. Sub-group and meta-regression were used assess moderators. The results showed that although crop yield in AP was lower than conventional HP, the difference was not statistically significant. There were differences in effect of aquatic/fish species, HP system type, type of grow media, and crop species on AP crop yield. Nutrient supplementation led to similar or even higher aquaponics crop yield than conventional hydroponics. These findings are vital information source for choosing factors to include in an AP study. These findings also synthesize the current trends in AP crop yields in comparison with conventional HP. However, drawing conclusions on the overall effect size must be done with caution due to the use of unweighted meta-analysis.

EVALUATION AND PROPOSE THE OPTIMUM LEVEL OF DIETARY FUNCTIONAL FEED ADDITIVES IN JUVENILE OLIVE FLOUNDER, *Paralichthys olivaceus*

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The present two experiments were conducted to evaluate and propose the optimum level of the different dietary functional feed additives in olive flounder. First experiment was conducted to evaluate 7 different functional feed additives in olive flounder fed with the low fishmeal diet formulated according to the other previous experiment. Including a control group, 8 groups of fish with three replicates were randomly distributed in 24 rectangular 35 L tanks (15 fish/tank). Fish averaging 5.26±0.17 g (mean ± SD) were fed 8 experimental diets; a basal diet without feed additive was used as a control (CON), CON + encapsulated sodium butyrate (ESB), CON + gamma-aminobutyric acid (GABA), CON + selenium yeast (SeY), CON + yeast extract nucleotide (YEN), CON + yucca meal (YUM), Song-gang bio stone (SGS) and protease (PRT). After 8 weeks feeding trial, growth performance of fish fed GABA, YEN and SGS diet were significantly higher than those of fish fed CON diet. Non-specific immune responses of fish fed GABA, YEN, and SGS diets were significantly higher than those of fish fed CON diet. Growth and immune related gene expression of fish fed GABA, YEN and SGS diets were significantly higher than those of fish fed CON diet. Cumulative survival rates of 7 days bacterial challenge test of fish fed GABA, YEN, YUM, SGS and PRT diets was significantly higher than those of fish fed CON diet. In the second experiment, fish averaging 2.1±0.07 g (mean ± SD) were randomly assigned into 8 groups and fed eight experimental diets; CON diet, and seven other diets supplementing YEN at 2.5 g ($YEN_{2.5}$) and 3.5 g ($YEN_{3.5}$), SGS at 2.5 g ($SGS_{2.5}$) and 3.5 g ($SGS_{3.5}$), GABA at 200 mg ($GABA_{200}$) and 250 mg ($GABA_{250}$), and oxytetracycline at 4 g (OTC) per kg diet. Growth performance of fish fed $YEN_{3.5}$, $SGS_{2.5}$, $SGS_{3.5}$ and OTC diets were significantly higher than those of fish fed CON diet (*P*□0.05). Non-specific immune responses of fish fed $YEN_{3.5}$, $SGS_{2.5}$, $SGS_{3.5}$ and OTC diets were significantly higher than those of fish fed CON diet. Digestive tract trypsin activity of fish fed $YEN_{2.5}$, $YEN_{3.5}$ and $SGS_{3.5}$ diets were significantly higher than those of fish fed CON diet ($P<0.05$). Growth and immune related gene expression of fish fed $YEN_{3.5}$ and $SGS_{3.5}$ diets were significantly higher than those of fish fed CON diet. Intestinal villi length of fish fed $YEN_{2.5}$, $YEN_{3.5}$, $SGS_{2.5}$, $SGS_{3.5}$ and OTC diets were significantly higher than those of fish fed CON diet (*P*□0.05). Cumulative survival rates of 8 days bacterial challenge test of fish fed $YEN_{3.5}$, $SGS_{3.5}$ and OTC diets were significantly higher than those of fish fed CON diet. These results indicated that dietary 3.5 g yeast extract nucleotides ($YEN_{3.5}$) and 2.5 ($SGS_{2.5}$) or 3.5 ($SGS_{3.5}$) g Song-gang stone per kg diet could replace antibiotics, and have beneficial effects on growth, immune responses, histology, disease resistance and gene expression in the low fishmeal diet of olive flounder.

A REVIEW ON DIETARY PROBIOTICS AS ANTIBIOTIC REPLACERS IN AQUACULTURE

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Four feeding trials were conducted to evaluate the effects of dietary probiotics as antibiotic replacers in Japanese eel *Anguilla japonica*, Nile tilapia *Oreochromis niloticus*, and whiteleg shrimp *Litopenaeus vannamei*. In the first experiment, growth performance, non-specific immune responses, immune-related gene expression and cumulative survival rate of Japanese eel fed *Bacillus subtilis* WB60 (0.5$\times 10^7$ CFU/g) with mannanoligosacharide (5 g/kg) was significantly higher than those of fish fed control (CON) diet. However, there were no significant differences with the oxytetracycline (OTC) diet. In the second experiment, growth performances, non-specific immune responses, cumulative survival rate of Nile tilapia fed *B. subtilis* at 1×10^8 (CFU/g) and *Lactococcus lactis* at 1×10^8 (CFU/g) diets were significantly higher than those of fish fed CON diet. Immune-related gene expression and enzyme activity of these diets were significantly higher than those of CON and OTC diets. In the third experiment, growth performance, non-specific immune responses and cumulative survival rate of whiteleg shrimp fed *B. subtilis* at 1×10^8 (CFU/g) and *L. lactis* at 1×10^8 (CFU/g) diets were significantly higher than those of shrimp fed CON diet. Immune-related gene expression and histology of shrimp fed these diets were significantly improved compared to those of shrimp fed CON and OTC diets. In the fourth experiment, growth performances, non-specific immune responses, immune-related gene expression and cumulative survival rate of Nile tilapia fed *B. subtilis* at 1×10^8 (CFU/g), *Enterococcus faecium* 1×10^7 (CFU/g), and *E. faecium* 1×10^8 (CFU/g) were significantly higher than those of fish fed CON diet, however, there were no significant differences among fish fed the probiotic and OTC diets. Based on these results, *B. subtilis* at 10^8 CFU/g, *E. faecium* at $10^{7\sim8}$ CFU/g, and *L. lactis* at 1×10^8 CFU/g could be ideal probiotics in terms of the growth performance, immune responses, enzyme activity, disease resistance and gene expression, to replace antibiotics in Japanese eel, Nile tilapia, and whiteleg shrimp.

INTEGRATING THE CULTIVATION OF MULTIPLE CROPS ON LONGLINE STRUCTURES: TRIALS WITH MUSSELS *Mytilus edulis* AND SUGAR KELP *Saccharina latissima*

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Mussel (*Mytilus edulis*) farming and sugar kelp (*Saccharina latissima)* farming have been two rapidly expanding sectors of marine farming in the Northeastern U.S. over the past ten years. The number of mussel farms has climbed from two or three raft-based farms in Maine to more than a dozen in that state, and an additional four offshore farms in NH, MA and RI. There were no seaweed or kelp farms a decade ago, and now there are more than 40 across New England. Markets are robust for both crops and the alternatives are a dwindling and erratic wild supply or imports. Given that both these crops are individually being grown on similar longline structures, integrating the cultivation of the crops can be advantageous for several reasons; (1) better space utilization of limited permitted sites – "3D farming", (2) shared use of the capital costs of expensive anchors, lines, and buoys, (3) better business risk management via crop diversification, and (4) lower risk to protected species by using fewer vertical lines per unit of production. The additional benefits of using multiple complementary nutrient bio-extractive crops are improved ecosystem services such as improved water quality, and provision of structures resulting in nursery and foraging habitat for other species.

Our research focused on developing and demonstrating innovative gear that efficiently integrated two different crops into an offshore lease area by using multiple growline systems. Our engineering and *in-situ* trials developed methodologies for efficient management and harvest of a dual-crop culture system.

POSSIBLE APPLICATION OF INVASIVE DREISSENIDS AS PROTEIN SOURCE ALTERNATIVE IN INTENSIVE FRESHWATER FISH FARMING PROJECTS: GROWING DYNAMICS AND NUTRIENT VALUE OF MUSSELS *IN SITU* AND FEEDING WITH ALGAE

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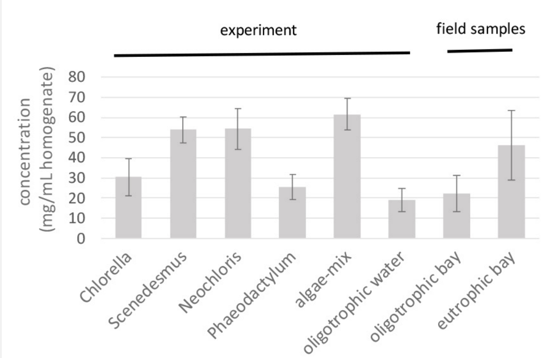
Invasive Dreissena species (*D. polymorpha*, *D. r. bugensis*) are dominant freshwater filter feeders playing intermedier role in the food chain. Their biomass (dry mass without shell) reaches around 88 t/year in Lake Balaton the largest shallow lake in Central Europe, where dreissenids are main food sources for carp, bream and roach. We found that the protein content of the soft tissue was high (55-60% dw) enough to count this animal as a potential protein source alternative provided by freshwater ecosystem service for feeding fry in intensive fish farming.

In the present study, we investigated the type and quantity of algae that yields intensive growing, higher weight and therefore more energy source biomacromolecules of dreissenids, which is beneficial in fish bait composition. Monocultures of those algae were used, which can be found with high number in the natural environment of dreissenids and can be easily cultured in laboratory conditions.

First, using flow cytometry, we decided the optimal algae concentration that dreissenids consume daily, when we measured the growth of the animals in a mid-term (3 months) microcosmos study. Results were evaluated in the view of data obtained from animals living in the western eutrophic and eastern oligotrophic bays of Lake Balaton.

The optimal algae concentration for mussel growth was 1-3x10^7 algae cell/L/day. Results showed that length increment, wet weight, protein and carbohydrate content of the mussels were highly correlated. Protein levels were similar in mussels getting lake water in laboratory and *in situ* (Fig. 1). Absolute values of all energy source materials measured were the highest in case of feeding with *Scenedesmus rubescens* and *Neochloris conjuncta*, and the algae-mix containing equal concentration of the algae species used. These results were comparable with those originated from the eutrophic lake water. Carbohydrate data related to wet weight showed that the most effective algae was *Scenedesmus*, whereas *Chlorella minutissima* proved the best evoking the relative fatty acid level. In conclusion, considering natural conditions, amount of biomacromolecules could be deliberately enriched in dreissenids fed on selective algae monocultures.

Figure 1. Dreissenids protein content



METABOLISM OF BREVETOXINS IN *Mercenaria campechiensis*

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Brevetoxins, the causative agent of neurotoxic shellfish poisoning (NSP) produced by the marine dinoflagellate *Karenia brevis*, are a significant concern in seafood safety due to the increasing number and severity of red tide events in the coastal southeastern United States. The commercial shellfish industry is particularly vulnerable to these events as a result of the sessile, filter-feeding life cycle of clams and oysters. To prevent NSP, shellfish harvest areas are closed when *K. brevis* density exceeds 5,000 cells/L and re-opened when the shellfish toxicity assessed by mouse bioassay (MBA) is < 20 MU/100g. Until recently, the NSP MBA has been the only National Shellfish Sanitation Program (NSSP) approved method for regulatory NSP testing. An ELISA that determines the composite B-type brevetoxins has been approved recently as a limited use method for NSP testing in Eastern oyster (*Crassostrea virginica*), sunray venus clams (*Macrocallista nimbosa*), and hard clams (*Mercenaria mercenaria*). Brevetoxins are extensively metabolized in hard clams and Eastern oysters. The most persistent and abundant metabolites contributing to overall toxicity are identified as cysteine and taurine conjugates of B-type brevetoxin. These metabolites, BTX-B1, BTX-B2, and S-desoxy BTX-B2, have been identified as biomarkers of brevetoxin exposure in oyster and hard clam and correlate well with the composite toxin measurements by ELISA. In this study, metabolism of brevetoxin in *K. brevis* bloom-exposed southern hard clams *(Mercenaria campechiensis)*, a species of growing commercial interest, was examined by N2a cytotoxicity assay, ELISA, and LC-MS. Brevetoxins are metabolized in *M. campechiensis* and the major metabolites are cysteine and taurine conjugates of brevetoxin. Results suggest that *M. campechiensis* and *M. mercenaria* metabolize brevetoxins similarly and BTX-B1, BTX-B2, and S-desoxy BTX-B2 could serve as biomarkers of brevetoxin exposure in *M. campechiensis* for confirmation by LC-MS.

ALABAMA MARINE RESOURCES CLAUDE PETEET MARICULTURE CENTER: INSTITUTION FOR RESEARCH, STOCK ENHANCEMENT AND PARTNERSHIPS

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The Alabama Department of Conservation and Natural Resources/Marine Resources Division (ADCNR/MRD) maintains the Claude Peteet Mariculture Center (CPMC) located in the northern Gulf of Mexico in Gulf Shores, Alabama. The primary goal of CPMC is to produce larvae and juveniles of recreationally important finfish species from native broodstock for wild stock enhancement. Additionally, some fish production may be used for age and growth, movement, and habitat use studies.

Facilities include a 2135m2 building containing rooms for broodstock maturation and spawning, algae production, live food production, egg incubation, larval rearing, and juvenile holding. Multiple recirculating pump and filtration units can support small-scale replicated studies. The hatchery compliments additional infrastructure including: 35, 80m2 lined ponds, a greenhouse complex containing recirculating systems, and two water supply pipelines; 2080 L/min of brackish water and 2275 L/min of full-strength seawater from the Gulf of Mexico.

ADCNR/MRD has provided pond and greenhouse space to researchers from in-state academic institutions to study water quality and feeding schedules and nutrition studies. ADCNR/MRD is available to investigate additional partnerships with industry, federal and state agencies, and other academic institutions to advance sustainable marine aquaculture.

ONLY TIME WILL TELL: SUCCESSES AND FAILURES OF RESTORING NATIVE OYSTER POPULATIONS IN HABITAT LIMITED SYSTEMS

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Historically, Olympia oysters (*Ostrea lurida*) played an important economic, ecological, and cultural role as Washington's only native oyster. Yet, due to overexploitation, loss of habitat, and other human-related factors, only ~5 % of the once-known beds remain in Puget Sound. In 2012, the Swinomish Indian Tribal Community began a small-scale Olympia oyster restoration effort with the intent of eventually establishing self-sustaining populations that could act as larval sources for additional sites. During the onset of the project, we initiated a monitoring plan to track survival and growth of outplanted seed by site. Initially encouraging results lead to the expansion of our restoration project and research objectives. Preceding work addressed the following goals: (1) determine when our population of oysters were brooding, (2) investigate local larval supply and distribution to better target habitat enhancement efforts, (3) continue to monitor oyster growth and survival while examining surrounding habitat for signs of recruitment, and (4) quantify parameters of ecological change before and after restoration. These oyster populations successfully brood at low temperatures and late-stage larvae were documented in areas surrounding restoration sites. Despite the presence of larvae, we have not found definitive evidence of successful recruitment in the area. Specifically, we last seeded our sites in 2017, yet we did not record signs of new cohorts in 2018 or 2019 (Fig. 1). Quantifying ecological change due to restoration efforts is more difficult when the population does not expand naturally. Our combined results highlight the importance of consistent monitoring and analysis, as we were able to identify the lack of recruitment and respond by developing innovative approaches to enhance methods for successful future restoration.

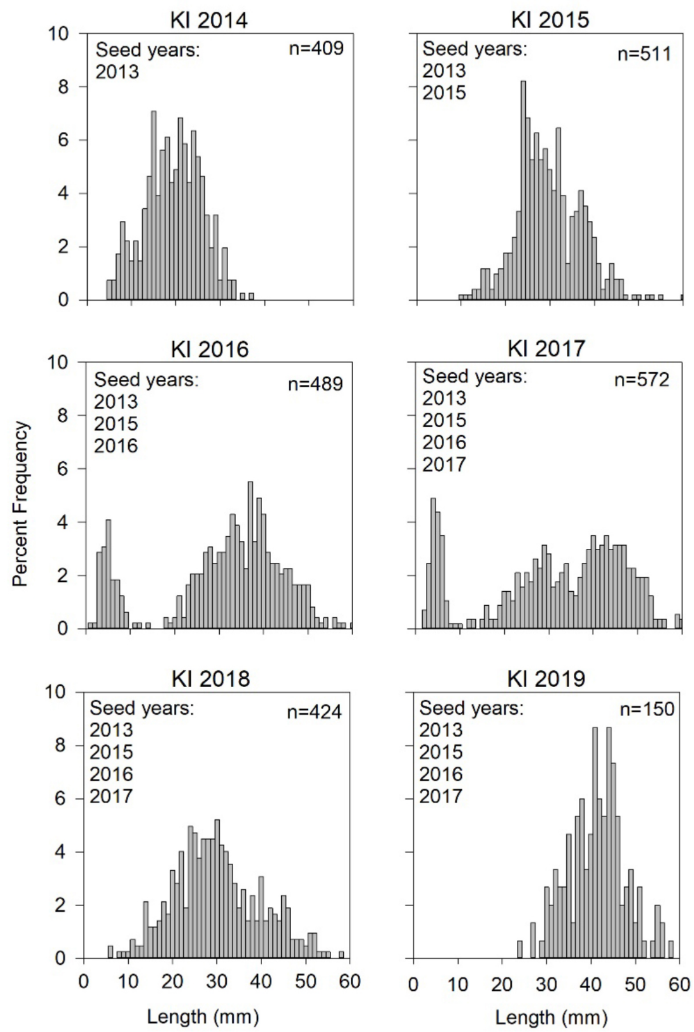

Figure 1. Temporal change in length frequency of Olympia oysters on Kiket Island (KI). Note evidence of new cohorts, due to seed outplanting, by year.

CLINICAL CHEMISTRY: A HOLISTIC DIAGNOSTIC APPROACH TO FISH HEALTH IN AQUACULTURE

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Diagnostics play an important role in aquatic animal health management and disease control. Despite advances in identifying pathogens, the aquaculture sector currently has very limited tools for assessing fish health and is heavily reliant on histopathology; a lethal, labour intensive technique that that does not facilitate rapid fish health assessment. Therefore, the objective of the work undertaken was to study the use of serum clinical chemistry diagnostic techniques as biomarkers for fish health and welfare. The fish gill is a multi-purpose organ that plays a dominant role in osmotic and ionic control, acid-base regulation and nitrogen waste excretion in addition to promoting gas exchange. Gill disease often has a multifactorial aetiology and a complex histopathological manifestation.

In this particular case, blood biochemistry was used to investigate the clinical significance of complex gill disease (CGD) in aquaculture reared Atlantic salmon to determine relevant blood biomarkers for early diagnostics. Blood was taken from fish with no clinical signs of any pathology (n=30) and from fish with confirmatory clinical appearance of CGD (n=30).

Serum biochemical analysis from CGD fish showed significant increases ($p<0.05$) in typical markers of excretory dysfunction such as concentrations of ammonia, urea and creatinine. Subsequently, activities of ALP (alkaline phosphatase), ALT (alanine aminotransferase) and LDH (lactate dehydrogenase) as markers for liver damage and overall health status were increased. Moreover, increased concentrations of minerals such as magnesium and calcium and electrolyte such as chloride indicated impaired osmoregulatory function of gills. Levels of serum iron were significantly decreased suggesting increased red blood cells production caused by low oxygen intake.

In this study the clinical significance of blood biochemistry was demonstrated as a promising approach to fish heath diagnostics and was correlated with organ/tissue damage. We propose applying the clinical chemistry approach and expanding it to haematological analysis to facilitate monitoring and early detection of health challenges in salmon aquaculture.

IS AQUAPONICS A SUSTAINABLE OPTION WHEN COMPARED TO CONVENTIONAL MONOCULTURES?

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Six years of research performed in a Small Unit Production Aquaponics (SUPA) in both high altitude desert (Negev, Israel 2012 - 2015) and tropical rainforest conditions (Hilo, Hawaii 2016 -present) demonstrates the challenges and opportunities of integrated fish and vegetable production in comparison to conventional monocultures.

Long term and sustained production trials indicate potential economic viability and a reduction in environmental impact. Inputs (infrastructure, energy, water, feed) and outputs (fish, vegetable, waste) in the two SUPA systems were monitored. Two commonly cultured fish, 1. hybrid striped bass (*Morone saxatilis* x *M.chrysops*) and 2. tilapia (*Oreochromis niloticus*, *O. aureus* and their hybrids) were grown with 55 species and varieties of vegetable. Standard operational procedures including integrated pest management are under development.

Preliminary comparisons of the economics and environmental impacts of aquaponics vis-a-vis monoculture are presented and discussed. Production output, water, land, nutrient, energy and capital utilization were comparable or superior to monocultures indicating that SUPA can be a mainstream food security resource.

OPTIMIZATION OF FEEDING FREQUENCY FOR THE INTENSIVE CULTURE OF LARVAL PACIFIC LAMPREY *Entosphenus tridentatus*

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The Pacific lamprey is an ancient fish of great importance to the ecosystems and indigenous cultures of the U.S. Pacific Northwest. Pacific lamprey have declined in abundance and range from historic levels, thus leading to increased conservation efforts for the species. Efforts to conserve this native fish include development of culture techniques, which the present research aims to address. The larval stage of this species occurs in freshwater and can take up to seven years to reach metamorphosis in the wild. During the larval stage lamprey are filter feeders and a slurry of active dry yeast paired with a fine larval fish diet (4:1) is commonly employed as a standard lamprey diet in the hatchery. This project tested two levels of feeding frequency through two experimental trials with 65 days post hatch (DPH) larvae (Trial 1) and 803 DPH larvae (Trial 2). The trials compared the control feeding frequency of two feeding events per week to a high frequency of five feeding events per week. Trials were 8 weeks long. Survival, growth, condition factor, whole body lipid and fatty acid profile (Trial 2 only) were measured at the end of the trials.

For the younger fish in Trial 1 the high frequency feeding regime lead to heavier larvae relative to the control frequency larvae, but there was no difference in length, condition factor or survival. Higher feeding frequency increased both length and weight of the older larvae of Trial 2, but did not affect survival or condition factor. More feedings also elevated whole body lipid content and altered the fatty acid profile of the older larvae. Results from both trials will be presented and discussed. The findings from this research will enhance our ability to effectively culture lamprey.

BREEDING IN BOXES, IS IT ETHICAL?

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Pair breeding of zebrafish *Danio rerio* enables the generation of progeny with known genetic parentage and is essential for in vivo research. This practice is routinely carried out in small transparent breeding boxes with no water flow or filtration.

These breeding boxes typically contain less than one liter of water and this small volume increases the potential for fluctuations in water quality and the absence of filtration allows accumulation of toxic waste products from fish metabolism. This unstable environment compared to normal filtered systems has the potential to impact breeding performance, cause harm to the fish and also affect the quality of the science.

Factors such as timing of feeding before breeding and degradation of the water quality are poorly investigated, recommendations for best practice are highly variable and reliant on anecdotal information.

In this trial fish were split into 3 groups. Group A were fasted for 30 hours before being placed in to boxes. Group B were given their normal morning feed and then placed into boxes after 6 hours. Group C were fed to satiation 10 minutes prior to being placed into the boxes. The fish were subdivided into transparent or black breeding boxes, both static and on a water flow through system. The fish were left overnight and water quality tested at 18 hours, 22 hours and 26 hours post transfer. In addition to water quality, adult behavior and embryo quality and quantity were also assessed.

Table 1. Differences in water quality in transparent static breeding tanks

Test Time (Hours)	Test Group	Water Temperature (℃)	pH	Conductivity (µS)	Total Ammonia (mg/l)	Unionised Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Dissolved Oxygen (mg/l)
0	Recirc. System	27.7	7.64	478	0.15	0.0045	0.08	61	7.5
0.5	Empty Box	25.8	7.63	478	0.15	0.0038	0.09	62	7.5
18	Control	22.6	7.65	486	0.14	0.0029	0.08	59	4
	A	22.6	7.32	487	0.44	0.0044	0.08	62	3
	B	22.6	7.30	494	1.01	0.0096	0.16	61	3
	C	22.6	7.23	508	2.20	0.0178	0.34	60	2
22	Control	23.0	7.65	486	0.21	0.0045	0.09	59	4
	A	22.9	7.27	488	0.54	0.0049	0.09	60	3
	B	22.8	7.23	495	1.00	0.0082	0.15	57	3
	C	22.8	7.20	513	2.75	0.0211	0.77	55	2
26	Control	23.0	7.61	487	0.19	0.0038	0.08	58	4
	A	23.2	7.31	491	0.60	0.0061	0.09	58	3
	B	23.2	7.24	494	0.96	0.0083	0.11	59	3
	C	23.2	7.24	516	3.17	0.0275	0.91	56	1

AGE, GROWTH AND MORTALITY OF *Bagrus bayad* (FORSSKALL, 1775) FROM INLAND WATERS OF KEBBI STATE, NIGERIA

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The fish of Bagridae are generally referred to as naked catfish. The Bagridae family has 30 genera and 210 species, which include; *Auchenoglanis occidentalis, Chrysichthys nigrodigitatus, Clarotes lateceps, Bagrus docmac, and Oreochromis niloticus*. This study was aimed at providing information and estimating age, growth mortality, and rate of exploitation of *B. bayad* to assess stock status as there is presently no data available on their population dynamics from inland waters of Kebbi state. The information supplied could be helpful for fishery resource planning, conservation, preservation and management of this specie.

Age, growth, mortality and rate of exploitation of *Bagrus bayad* from Kebbi state inland waters were examined from January to December 2017. Monthly frequency data was assessed using FiSAT software to estimate population parameters, including asymptotic length (L ∝), growth coefficient (K) and recruitment pattern to determine stock status. Estimation of age was obtained by using Bhattacharya's method that is incorporated in FiSAT. The average length of the *Bagrus bayad* was of 11.65 cm, and 16.89 cm (Table 1), At the end of the first and second years respectively, with an average growth rate/year of 4.56 cm. The predicted extreme length (L_{max}) was found to be 45.03 cm Asymptotic length (L) and growth coefficient (K) were 45.03 cm and 0.47 (yr^{-1}), respectively. Total mortality (Z) by length-converted catch curve was estimated at 1.53 (yr^{-1}), fishing mortality (F) was at 0.56 (yr^{-1}), and natural mortality (M) was at 0.97 (yr^{-1}). Level of exploitation (E) was 0.37. The pattern of recruitment was constant, with two significant peak pulses per year. The results of the exploitation rate (0.37yr-1) indicate that *B. Bayad* was under fished by 26 percent. This implies that the fish stocks in the study area are underexploited as a subsistence fishery.

Table 1. Age group mean total length and index of *Bagrus bayad* in the inland waters of Kebbi state, Nigeria

Aged group	Mean TL (cm)	Growth rate (cm)	Index
1	11.65 ± 1.65	-	-
2	16.89 ± 2.10	5.24	2.22
3	22.54 ± 2.10	5.65	2.15
4	28.19 ± 2.19	5.65	2.11
5	34.08 ± 2.35	5.89	2.09
6	39.00 ± 1.80	4.92	2.04
Average growth rate/year		4.56	

DIQUAT INAD: AN UPDATE FROM ALABAMA

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From 2016 to 2019, the E.W. Shell Fisheries Center has participated in the Diquat INAD. We have used from 7 ppm to 28 ppm; 1, 2 or 3 doses; consecutive days and alternate days. What worked, what didn't and what did we learn along the way.

MECHANISMS GOVERNING HOST SUSCEPTIBILITY IN VIRULENT *Aeromonas hydrophila* INFECTIONS OF CHANNEL CATFISH (*Ictalurus punctatus*)

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An emerging pathotype of *Aeromonas hydrophila* (vAh) has been responsible for widespread farm losses in the US catfish industry over the last decade. While our genetic and biochemical understanding of vAh has been greatly enhanced in this time frame, our ability to reliably induce the disease in the laboratory has remained limited. Taking cues from observed farm conditions associated with outbreaks, here we perturbed iron scavenging dynamics and catfish feeding status. Addition of a xenosiderophore, deferoxamine mesylate (DFO), to vAh cultures prior to immersion challenge significantly increased virulence in several vAh isolates but not in a non-epidemic strain. DFO addition did not impact vAh growth dynamics or perturb iron-sensitive gene pathways, but did significantly enhance hemolysis of catfish blood. Furthermore, hours between last feeding and immersion challenge (postprandial status), was observed to be a critical determinant of catfish susceptibility. Fish with a full gastrointestinal tract had significantly lower survival than those in a fasted state, and this effect was cumulative with that of DFO-enhanced vAh virulence. Taken together, our results not only provide a more robust challenge model, they offer actionable insights into pond level host-pathogen-environmental interactions potentially underlying vAh pathogenesis. Utilizing these more robust challenge models, we are currently examining the practical efficacy of varying protective strategies for the industry including diet modification, vaccination, genetic selection, and modulation of the pond environment. Our latest results in this vein will also be presented.

USING SOUND TO IMPROVE HARVEST EFFICIENCY IN CHANNEL CATFISH, *Ictalurus punctatus*

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The once flourishing American catfish industry is now at risk because of high production costs and foreign competition. Seining is the most efficient way of harvesting channel catfish from ponds. Seining efficiency is poor when pond bottoms are soft and muddy with large depressions and elevations. The purpose of this study is to determine if seining efficiency could be increased with the help of sound. The research was conducted at Delta Western Research Center, Indianola, Mississippi, USA. Twenty-one experimental ponds were harvested with the seine and underwater speaker while thirty-nine control ponds were harvested with just the seine. The amount of catfish seined, in weight, was compared to the amount of catfish scrapped in all ponds. Data presented in this study demonstrates that the underwater speaker had no significant impact on the seining efficiency. The average percent seined from each pond with sound is 61.06% and the average percent seined from each pond without sound is 56.21%. This study suggests that using sound does not increase seining efficiency.

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.

ASSESSING CHALLENGES LIMITING TECHNOLOGICAL AND COMMERCIAL VIABILITY OF OFFSHORE AQUACULTURE IN THE AMERICAS

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Several commercially important species of tropical marine fish are or have been cultured in the US, Panama, Costa Rica, Mexico, Ecuador, Dominican Republic, Colombia, Brazil, Chile, Belize and the Bahamas. Some marine fish species whose aquaculture technologies have become or are becoming available are cobia (*Rachycentron canadum*), hamachi/kampachi (*Seriola rivoliana, S. lalandi*/*S. dorsalis*), pompanos (*Trachinotus carolinus*), snappers (*Lutjanus guttatus, L. peru and L. campechanus*), totoaba (*Totoaba macdonaldi*), red drum (*Sciaenops ocellatus*), barramundi (*Lates calcarifer*), snooks (*Centropomus spp*), mahi (*Coryphaena hippurus*), Nassau grouper (*Epinephelus striatus*) – among others. Modern hatcheries are capable of producing enough quantities of juveniles for stocking. Currently, steady supply of high-quality juveniles is still limited, but it is unlikely that it will remain an issue hampering industry expansion in the near future.

While land-based recirculating aquaculture systems (RAS) and traditional flow-through ponds, raceways and tanks are viable options, any significant contribution to the 30-40 MMT of seafood required in the next few decades will have to be produced in the offshore environment - where stronger currents and greater depths increase the carrying capacity of the sites. Raising fish in exposed, high-energy areas of the open ocean require advanced technologies that are automated, complex, and expensive to establish and operate. Thus, fish produced offshore must be sold at high prices to compensate the high capital and operating costs required, limiting their demand in a highly competitive white fish market.

Offshore aquaculture is expanding, yet the commercial viability of operations in the Americas remains elusive. The infrastructure and logistics are in place as well as a strong market demand. Technology continues to expand rapidly. Tools for site assessment and selection and environmental monitoring have been established. However, as with any relatively new industry, hurdles must be overcome before commercial viability can be secured. Some issues such as optimizing genetics, nutrition, and diseases control are inherent to all forms of aquaculture - whereas stocking, feeding, chemical treatments, net cleaning, predator avoidance, escapements, biomass estimates and crop management, mortalities collection and harvesting are exacerbated in offshore systems. We work with the industry to identify, address and resolve challenges limiting the technological and commercial viability of offshore aquaculture. These are presented and discussed along with potential solutions to assist producers and operators to overcome tethered drawbacks.

ADVANCES IN TUNA AQUACULTURE

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Much scientific research has been conducted and reported on tuna fisheries assessment and management, physiology, nutrition, trophic and foraging ecology, and data monitoring and modeling throughout all life stages of a number of tuna species. These were discussed, presented and reported during the 70th Annual Tuna Conference in May 20-23, 2019 in California, US. Conversely, recent progress in tuna aquaculture has not been as fast and productive as anticipated. Whilst tuna fattening and farming activities continue to expand in the last decades, progress towards large scale fully farmed tuna bred from eggs spawned in captivity and raised to market size remains limited. With the exception of Kinki University and affiliated Japanese companies that continue to steadily yet slowly expand close-cycle tuna farming since first reported in 2002 - most tuna farming activities today are ranching or fattening, still relying on the capture of juveniles at sea and fattening them in pens fed on small pelagics such as sardines, pilchards, mackerel, and other low value species considered “trash fish”.

The tuna fishery industry and management had to adapt to significant reductions in their fishery stocks worldwide as well as to the fact that over 50% of all juveniles and subadults are now caught for fattening and ranching purposes. Indeed, for these reasons, tuna fattening and ranching blur the line between fisheries and aquaculture and are intertwined to the point that it is difficult to analyze them separately.

Tuna ranching and fattening continue to improve efficiency. Despite criticisms, these activities can be considered value-added tuna fisheries from both ecological and economical perspectives. These activities are highly profitable, and improvements in farming practices have been driven by enforced TOCs limiting captures. Better management results in decreasing mortalities during capture, towing, transferring, and feeding in the cages. Tuna close-cycle aquaculture still faces important challenges. Closing their life cycle and the development of ecologically and economically efficient feeds that meet the specific nutritional requirements of tuna at the various developmental stages are required to ensure the future of tuna production and the conservation of tuna species.

Aquaculture and fisheries scientists continue to combine efforts and expertise to rely on science-based criteria and decisions to ensure the future of tuna fishery stocks and aquaculture production. Whilst much progress has been achieved at the experimental and research levels, technological feasibility and commercial viability of mass production of juveniles in hatcheries and the large scale production of practical diets for the ingrowing stages of farmed tuna remain largely elusive.

OVERVIEW OF THE RESEARCH ACTIVITIES, ACADEMIC TRACK, AND TECHNOLOGICAL ACHIEVEMENTS OF THE UNIVERSITY OF MIAMI AQUACULTURE PROGRAM

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The University of Miami Aquaculture Program has been playing a major role spearheading advanced aquaculture technologies. Our research and academic programs are centered on innovative approaches to ensure that seafood production is science-based, wholesome, environmentally sustainable, and economically viable. We are engaged in collaborations around the world and are recognized for our contributions to the field – particularly for the development of challenging and high-value species, technology transfer activities, and the education and training of top professionals at all levels.

The academic program encompasses an introductory undergraduate course and five graduate level courses – covering everything from basic concepts to science, environment, management, social and economic aspects of sustainable aquaculture as well as regulatory issues, business and production models, all the way to seafood market and marketing. The program thrives on innovative research and development concepts focusing on emerging technologies. It is supported by a renowned, Global GAP certified marine fish hatchery with state-of-the-art dry and wet laboratory facilities, numerous broodstock maturation systems for conditioning fish to spawn volitionally, a number of larval rearing and nursery tanks of various volumes for experimental trials and commercial-scale fingerling production, and a variety of replicated systems with tank volumes ranging in size from 1,000-L to 5,000-L for nutrition and nursery and on-growing trials.

Current and past species with which we have conducted R&D activities include: cobia, mahi-mahi, snapper, grouper, hogfish, red drum, sea trout, snook, tuna, goggle-eye, yellowtail jacks (*Seriola spp*), pompano and flounder –among others. Our nutrition program works closely with the industry to conduct practical research, such as digestibility trials, to improve the ecological and economic efficiency of aquafeeds. Besides probiotics, we are looking into batcheriophages, organic acids, essential oils, and trace minerals to improve fish health. We are also investigating using artificial intelligence (AI) to optimize live feeds and larval rearing protocols.

The research program is science-based and centered on advanced hatchery, land-based (RAS and flow-through), and open-ocean grow out technologies of marine fish. We have published over 200 scientific articles in aquaculture technology, production, reproduction, physiology, nutrition, environmental issues, toxicology, and systems operations and management. Our expertise and experience in the field has led to numerous collaborations with the private and public sector operations throughout the U.S., Latin America, Europe, Asia, Caribbean, Africa, Australia and the Middle East. As the industry continues to expand, the University of Miami Aquaculture Program will continue to assist in advancing aquaculture technologies globally.

REPLACEMENT OF FISHMEAL BY ANTARCTIC KRILL MEAL IN DIETS OF EUROPEAN SEA BASS *Dicentrarchus labrax*: EFFECTS ON GROWTH PERFORMANCE, FEED UTIZATION AND LIVER METABOLISM

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A sustainable growth of the aquaculture sector implies the use of sustainable novel raw materials as replacement of the traditional fish meal (FM) and fish oil (FO) ingredients. This fact will lead to the development of functional diets as part of a management strategy to reduce the effects on fish growth performance and health derived from low FM/FO dietary contents. In this sense, krill meal (*Euphausia superba*) may be a potential candidate to potentiate fish growth and health status.

European sea bass (*Dicentrarchus labrax*) were fed a commercial relevant diet with either a 15% fishmeal content (KM0) or the same diet substituted by 30% (KM5) or 50% Antarctic krill meal (KM7.5) for 12 weeks in triplicates. Diets were isoproteic (45%) and isolipidic (18%).

At the end of the feeding trial, growth performance, liver morphology, liver proximate composition and fatty acid profile, as well as liver lipid metabolism related genes were evaluated. After two months of supplementation, Krill meal-supplemented fish presented increased feed intake ($p<0.05$), regardless of the dietary level. However, feed conversion ratio (FCR) was only significantly lower ($p<0.05$) in fish fed the KM7.5 diet. At the end of the feeding trial, fish fed KM-based diets presented increased ($p<0.05$) final weight, final length, relative growth, specific growth rate (SGR) and improved FCR, irrespective of the KM dietary level. Livers of European sea bass fed the experimental diets presented similar ($p>0.05$) biochemical composition and fatty acid profile. Despite the similar content of liver lipids, fish fed KM diets presented a healthier liver morphological profile. Hepatocytes of KM fed fish presented lower vacuolization levels, better alignment of the hepatocyte nuclei along the sinusoidal lines, and in general lower sings of steatosis. Liver gene expression results revealed a down regulation of the *3*-hydroxy-3-methylglutaryl-coenzyme A reductase *(hmgcr)* and delta-6-desaturase (*fads2*) expression, when fish were fed the KM7.5 diet compared to fish fed the KM0 diet. Besides, a significant negative correlation between the gene expression levels of *hmgcr, fads2* and KM dietary levels were observed. On the other side, fatty acid binding protein 7 (*fabp7*) and KM were significantly positively correlated.

Altogether profiling KM as a potential growth and health promoter in European sea bass fed low fish meal and oil diets.

INSECT PROTEIN AS AN ADDITIVE IN FISH FEEDS: EFFECTS ON GROWTH AND IMMUNE PERFORMANCE IN RAINBOW TROUT *Oncorhynchus mykiss*

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Insect meal in fish feeds has been gaining attention over the last decades due to its high energy and protein content that can positively impact growth performance, gut microbiota, immune response and survival. Albeit insect protein is considered a valuable alternative as a partial or complete replacement of fishmeal in aquafeeds, large scale rearing for production is not yet cost-effective. However, research shows that using insect protein as an additive in fish feeds will also induce or influence the aforementioned effects. Therefore, two experiments were conducted to evaluate the specific effects of insect (black soldier fly) larval meal (Protein$^+$, a commercial product developed by Oreka Solutions) as an additive on growth performance and immune response in juvenile rainbow trout (*Oncorhynchus mykiss*).

Experiment 1 tested Protein$^+$ supplemented at a 7% in commercial diets and fed in triplicate to rainbow trout (1,800 fish, 3.0g initial weight) randomly distributed into 9, 250-L tanks for 21 weeks. Protein$^+$ fed group showed significantly higher growth and lower feed conversion ratio (FCR) than the control group. Gene expression of immune genes tumor necrosis factor beta (TNF-β), tumor necrosis factor alpha (TNF-α), interleukin 8 (IL-8) and interleukin 1 beta (IL-1β) supported the immune enhancement effect that was hypothesized from Protein$^+$.

Experiment 2 further investigated the role of Protein$^+$ in immune enhancement. The first 28 days consisted of a short-term growth performance trial in which a total of 600 rainbow trout (2.0 g) were randomly distributed into 12, 189-L tanks and fed three different diets in four replicate tanks to satiation level including a 0% (control), 4% and 8% Protein$^+$ supplemented diets. Consequently, a second 28-day pathogen challenge trial with *Flavobacterium psychrophilum* was conducted and fish remained on the same diets used in the growth performance component. For this part of the trial, 300 fish were randomly distributed into 12, 19-L tanks, four tanks per diet, where one of each 4 was mock-challenged. Spleen and kidney samples were collected pre- and post-challenge for gene expression analysis for the same proinflammatory cytokines from Experiment 1. Histological analysis of distal intestine was performed for both and no significant differences ($p \geq 0.05$) in morphology of lamina propria, thickness of connective tissue and vacuolization were observed among diets pre- and post-challenge. Results from this study revealed that supplementation of Protein$^+$ did not enhance the significant growth of fish during pre-challenge period and survival rate during challenge study ($p \geq 0.05$). However, 4% Protein$^+$ fed group exhibited numerically higher growth and survival rate than the control group. Results from gene expression analysis are underway and will help determine the potential role of Protein$^+$ in immune performance, post-feeding.

Conclusively, results from both experiments revealed that Protein$^+$ can be used as a feed additive. However, further research is warranted to investigate the long term feeding of 4% Protein$^+$ for sustainable salmonid production.

BUILDING EDUCATIONAL CAPACITY THROUGH COLLABORATIVE AQUAPONICS EXTENSION EDUCATION

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Recirculating aquaponics systems have been emerging as an alternative to mass food production in rural and urban landscapes. Significant work on fish breeding and vegetable production in aquaponics systems has been conducted at the Ohio State University (OSU) and other land grant universities. The success of aquaponics systems is sensitive to address socio-economic issues, changes in water availability and quality, and increased dissemination of unbiased science-based aquaponics knowledge is needed.

Outcomes of this project were to connect, develop and expand an 1890's institutional, Central State University (CSU) aquaponics research, education and outreach capacity collaboratively with an 1862's institute, OSU, by combining novel and holistic research, extension and demonstration approaches. OSU in its capacity as an 1862 doctoral granting institute has garnered in-depth research and extension capabilities at its South Centers Piketon campus (http://southcenters.osu.edu) in aquaculture, aquaponics and horticulture. The knowledge gained in these areas was extended from OSU to build the aquaponics research and education capacity at CSU. This capacity building was through development, expansion and transfer of existing science-based knowledge and educational materials to develop extension and academic curriculum and training in aquaponics education to build the educational capacity of CSU Extension Agents, students and faculty to teach rural and urban landowners' aquaponics production techniques.

Mirrored aquaponics research and demonstration production systems, used as the educational settings, were constructed at OSU and CSU research campuses, to collect research data, demonstrate, and transfer unbiased science-based aquaponics knowledge contributing to expansion of CSU's educational outreach capacity. Data results, a synopsis of the project, and a discussion will be presented.

EVALUATION OF A SMALL FLUIDIZED BED FILTER IN A LIVE HOLDING RECIRCULATING SYSTEM FOR FOOD SIZE HYBRID CATFISH

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Distributing locally grown food fish live may provide aquaculture producers in Kentucky a more profitable market opportunity than selling fish as a commodity. Holding fish live in recirculating systems will be required for this approach. Fluidized bed filters are expected to be a useful tool with regard to shock loading of these systems. In this study, investigators conducted experiments with hybrid catfish (channel x blue) held at 15 and 20°C with and without a small fluidized sand filter.

After simulated hauling for 3 hours, food size hybrid catfish were stocked at 40 kg/m^3 in recirculating aquaculture systems with an Aquadyne 17 L bead filter. An RK10 fluidized sand filter (15 cm x 180 cm) with 14 kg sand was installed inline in half of the tanks. Holding tank water was oxygenated, supplemented with salt at 4 ppt, and maintained at a temperature of 15 or 20°C in separate experiments for a period of 8 days each. Water quality for each holding tank was measured at stocking and daily thereafter (24, 48, 72, 96, 120, 144, 168, and 192 hours). Total ammonia nitrogen (TAN), unionized ammonia (UIA), nitrite, nitrate, temperature, dissolved oxygen, pH and conductivity was measured daily. Alkalinity, hardness, CO_2 and turbidity was measured every other day. Fish were not fed during the experiments.

At 15°C, TAN rose steadily to 10 mg/L in the control treatment and about 6 mg/l in the fluidized bed treatment. In the fluidized bed treatment, UIA stabilized at hour 120 at about 0.07 mg/L and slowly declined thereafter. Nitrite and Nitrate increased and pH decreased throughout the experiment. Mean weight loss was 1.78%.

At 20°C, TAN rose steadily to 5 mg/L in the control treatment and about 6.5 mg/L in the fluidized bed treatment. In the fluidized bed treatment, UIA stabilized at hour 72 and decreased rapidly after hour 96. Nitrite stabilized at hour 168 and began to decrease in both treatments. pH decreased throughout the experiment. Mean weight loss was 3.64%.

Addition of the fluidized bed filter did not significantly decrease TAN or UIA during the eight day holding period. Water temperature influenced the rate of nitrification more than installation of the small fluidized bed filter.

ESTIMATED PRODUCTION OF FISH (TILAPIA) AND PLANTS (BASILS) IN A LABORATORY-SCALE AQUAPONICS SYSTEM

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Experiments were conducted to estimate the total production of fish and plants in a laboratory-scale aquaponics system. For fish species, Nile tilapia were reared in a total of 300 gallon (divided into two groups- controlled and stressed, reach with two replicates) of water while basils were grown in two water beds (two species of basils- Holy and Thai) in a total area of 3.0 m^2. In six weeks, the total yield of tilapia was 40.18 pounds (23.69 pounds from control group + 16.49 pounds from stressed group), while in 20 weeks, the total plant yield was 44.44 pounds (23.35 pounds from Thai basil + 21.10 pounds from Holy basil). When examined, compared to fish reared in controlled environment, stressed fish had lower condition factor (length-weight relationship) and immune capacity (macrophage phagocytic capacity). If fish are given better husbandry, the total yields for fish will change and bring better profit to the farmers.

Figures. Basil plants (left) and tilapia (right) in a laboratory-scale aquaponics system.

BASILS AS NUTRACEUTICALS TO MODULATE STRESS OF TILAPIA IN AQUAPONICS SYSTEM

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Nile tilapia were cultured in a small-scale aquaponics system and checked for growth and disease resistance. Tilapia were divided into two groups- controlled and stressed, each group with two replicates, and were fed basil-supplemented commercial feed. Absolute feed intake, feed conversion rate, protein energy retention, protein production value, and condition factor were examined for physiological responses. Macrophage phagocytic capacity and lysozyme activity were examined for immunological responses. Fish were sampled frequently over a six-week long experimental period. Fish fed control diet and fish fed basil-supplemented diet had better growth parameters including overall condition factors compared to stressed fish groups. Fish fed basil-supplemented diets had also better lysozyme activity but not macrophage phagocytosis compared to stressed fish groups. These results confirmed the hypothesis that nutraceuticals are better supplementations to modulate stress in farming conditions and to prevent diseases.

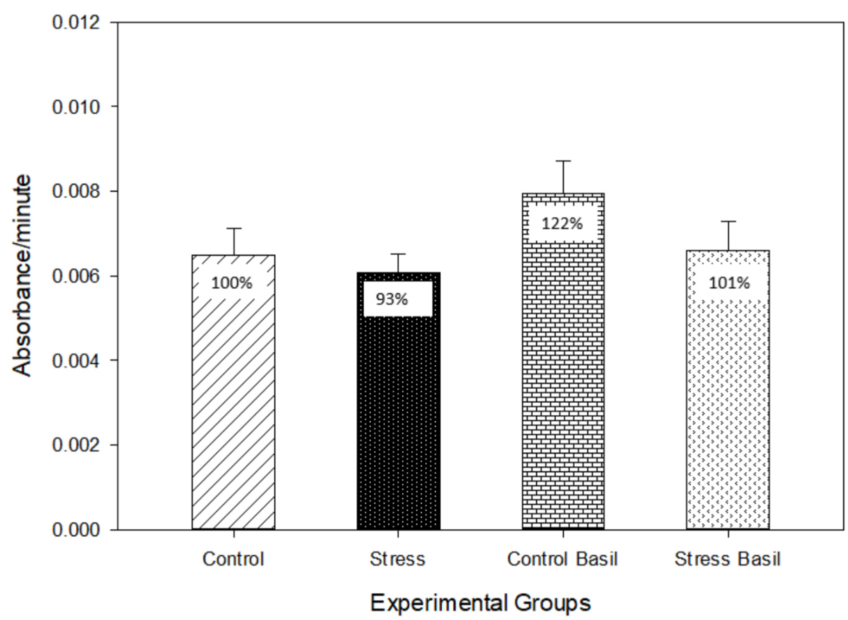

Figure. Lysozyme activity of four different fish groups reared in aquaponics system.

EFFECT OF pH ON CUCUMBER GROWTH AND NUTRIENT AVAILABILITY IN DE-COUPLED AQUAPONIC SYSTEM WITH MINIMAL SOLIDS REMOVAL

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A shift in aquaponic systems from coupled, recirculating systems to de-coupled systems has taken place due to the ability to adjust water quality parameters, namely pH, of each de-coupled unit to optimize production. In hydroponics and soil-based systems, pH plays a major role in nutrient availability. The role of pH in aquaponic systems is still not fully understood due to the many variations of aquaponic systems that exist today. Aquaponic systems are different from hydroponic systems in that, along with dissolved nutrients, solid particles remain behind in the irrigation water. Some aquaponic systems filter out solids while others do not. To determine the effect of pH in a de-coupled aquaponic system with minimal solids removal, a study was conducted using aquaculture effluent from tilapia culture tanks at 4 pH treatments: 5.0, 5.8, 6.5, and 7.0. Growth and yield parameters, nutrient content of the irrigation water, and nutrients incorporated into the plant tissue were collected over two growing seasons. pH did not have an effect on internode length or yield over the two growing seasons; however, for the Spring 2019 growing season a faster growth rate was achieved for the 7.0 treatment compared to the 5.0 treatment at 1.95 inches/day and 1.78 inches/day respectively. The difference in growth rate was not observed during the second growing season. Several individual nutrients were affected by pH at different time points (mid- vs. late-season), but there was no consistent change in the amounts of nutrients measured in the water and tissue samples for either growing season. Water and tissue measurements were compared to standards for hydroponic cucumber growing solution and cucumber tissues respectively. Comparisons revealed that, except for Zn and Cu, all nutrients in the aquaponic solution were below levels recommended for hydroponic solutions regardless of pH. However, tissue concentrations for all nutrients were within the range of normal, healthy cucumber tissue concentrations. While pH does not seem to have an effect on soluble nutrient availability in this aquaponic system, low nutrient levels in the water combined with non-deficient tissue in the plants indicate that there are other factors contributing to nutrient availability and uptake that were not captured in this study. Future studies to determine the nutrients contained in the suspended and dissolved solids in aquaculture effluent and their effect on plant growth are required to understand more fully the impact of pH in these systems.

Table 1. Growth and Yield vs. pH[z]

pH	Growth Rate		Internode Length		Yield	
	Inches ·day -1		Inches		kg ·plant -1	
	Spring	Summer	Spring	Summer	Spring	Summer
7	1.95a	3.86n.s	3.41.s.	3.74n.s.	6.59n.s.	8.39n.s.
6.5	1.85ab	3.83	3.40	3.82	6.01	8.24
5.8	1.84ab	3.80	3.38	3.80	6.63	8.84
5	1.78b	3.93	3.32	3.82	7.01	8.99

[z] Means with the same letters in a column are not significantly different at (P<0.05) as determined by analysis of variance and lsmeans using the GLIMMIX procedure and type III sum of squares in SAS. n.s.= not significant

DIFFERENCES IN CHANNEL AND HYBRID CATFISH FILLET TEXTURE

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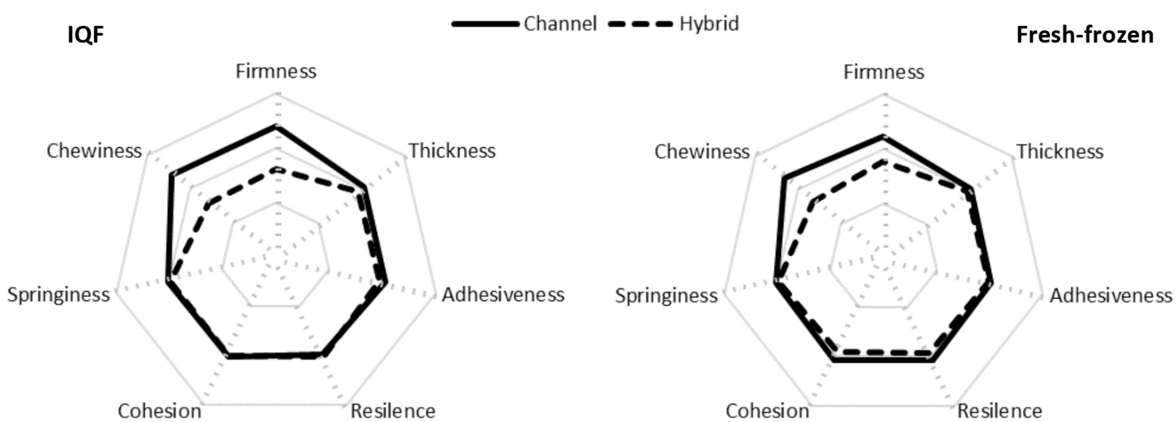
Hybrid (Channel ♀ x Blue ♂) catfish have recently surpassed the channel catfish as the major species being produced by US catfish processors. With this change, many questions arise on how texture quality and consistency of the catfish products may be affected, in both the cooked fillet by the consumer and the raw fillet by the processors. Differences in texture can result in rejection by the consumer and may create processing difficulties, with variable filleting requirements, to the processor. The purpose of this research was to determine the effect of the catfish species on fillet texture, for either raw or cooked fillets, in addition to effects from catfish gender or processing methods.

Fresh-frozen and individually quick frozen (IQF) catfish fillets were obtained from experimental ponds and processors. Instrumental texture analysis was performed using the texture profile analysis (TPA) protocol on a rectangular central section of the fillet. Fillets were cooked by baking, to an internal temperature of 165°F, cooled to 85°F, and the texture was measured by TPA at eight positions to give attributes, Firmness, Cohesiveness, Springiness, Chewiness, Resilience, and Adhesiveness, plus several additional attributes. In addition, cooked samples were analyzed by a trained descriptive sensory texture panel that determined the attributes, firmness, flakiness, moisture release, and moisture retention.

TPA methods determined that cooked channel catfish fillets were firmer and chewier than hybrid catfish, with a larger difference seen in the IQF fillets, as seen in Figure 1.

Figure 1. Texture profile attributes differences between Channel and Hybrid fillets.



REARING OF LARVAL FISH IN COCULTURE WITH COPEPODS

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Better aquarium technologies and more hobbyists have led to a higher demand for marine ornamentals. However, only a few species have been successfully reared in captivity, leading to increased pressure on coral reefs to meet the demand for ornamental fishes and invertebrates. In their native habitat, many larval fishes depend on copepods and other zooplankton as their primary food source. The challenge has been to develop culture technologies that produce enough copepods to sustain and meet the larval fish's dietary needs, especially at the commercial level. We have been using the clownfish (*Amphiprion ocellaris*) as a model to develop a protocol for raising the larvae in coculture with copepods (*Parvocalanus crassirostris*). The goal is to reduce the number of copepods needed from stock cultures by creating a self-sustaining copepod population in the rearing tanks, which serve as a continuous food source for the larvae.

In feeding studies, we found that young fish feed voraciously on the immature stages of the copepod but cannot capture the adults. Thus, fish larvae eat early nauplii and copepodites, while adult copepods reproduce. In our coculture larval rearing strategy, we establish a healthy copepod culture in the rearing tank one to two days before the fish eggs hatch.

In a pilot study, we started with 10 1-day-old clownfish larvae. At 14 days post hatch (dph), the remaining eight clownfish had developed their characteristic coloration and settled. Copepod abundances in the rearing tank remained high until 10 days post hatch (dph). At this time, the larvae were able to capture adult copepods and they decimated the copepod population. Fish were transitioned to *Artemia* nauplii and flake food reducing the need for copepods.

Feeding investment in terms of number of copepods was much lower than under the traditional daily feeding regime. A total of 13,800 copepods were invested from the stock cultures on three days (days: -1, 1 and 10; Fig. 1). In contrast, if larvae had been raised by daily addition of copepods, approximately 121,000 copepods would have been needed. After settling, larvae reared in coculture continued thrive reaching two centimeters at 35 dph (Fig. 2).

Fig. 1. Copepod Investment -14 days

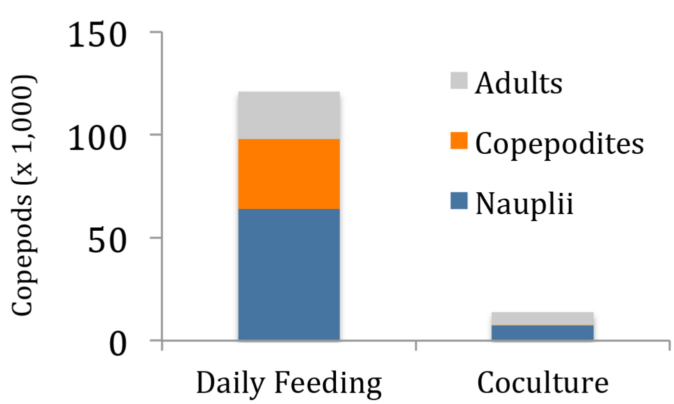
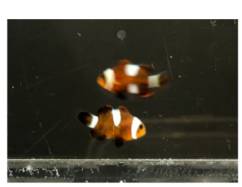


Fig. 2 Clownfish raised
in Coculture (35 dph)



SHRIMP GENETICS NOW AND IN THE FUTURE

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Historical global production of shrimp has seen enormous growth, with a typical cyclical pattern in production volumes. This cyclical pattern is mainly caused by increasing and varying disease pressures compensated with new developments in technology in husbandry, feeds and genetics. Worldwide shrimp farming is now heading towards controlled production and intensification, in line with historical development in longer established domesticated terrestrial and aquatic species to create stable production.

The shift to intensive controlled environments also requires breeding programs to change. We will demonstrate how genotypes interact with culture environment and how breeding programs can use this to optimise production in specific environments. To further improve the rate of change and to maximise production, large scale shrimp breeding programs should implement the latest genomic technology. Integration of genomic selection in breeding programs for terrestrial and other aquaculture species has enhanced the rate of genetic improvement. Similar changes in the rate of genetic improvement can be expected for shrimp. However, integration of the multiple “omics” resources now available (genome assemblies, transcriptomes, linkage maps, and proteomes) is likely to be critical to exploiting these currently isolated resources. At this stage, in shrimp this integration is at a much less advanced stage than other aquaculture species. Highlights and expectations of the recent advances in “omics” research, particularly for the implementation of genomic selection, will be given.

MAITAINING WATER QUALITY STANDARDS IN RECIRCULATING AQUACULTURE SYSTEMS: CRITICAL REVIEW OF AVAILABLE DISINFECTION TECHNOLOGIES

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Production in recirculating aquaculture systems (RAS) is increasingly sustainable with technical improvements promising zero exchange systems where water reuse and effluent management is essential. The technical background required to achieve water quality standards include the integration of mechanical filtration, biofiltration and disinfection methods. All these procedures are necessary to remove potentially dangerous accumulating waste and dissolved compounds as well as bacteria from the systems. Otherwise, water quality deterioration can affect production yields. Defining water quality standards is not straightforward as each species has different requirements. Ozone and UV are among the most widely used methods to guarantee best rearing conditions. Yet, not all bacteria in the system is dangerous or necessarily vulnerable to these methods. Moreover, safety issues and economic aspects motivate new evolving alternative technologies in aquaculture. In this study, three different disinfection approaches (ozone, hydrogen peroxide (H_2O_2) and ultrasound treatment) were tested and we compare the main available tools for disinfection in aquaculture.

A pilot study comparing the effects of ozone (3.5 g/h) and H_2O_2 (15.8 mg/L/h) in a 5 m^3 RAS rearing European seabass showed promising results in terms of oxygenation and disinfection (Fig.1, Table 1). Comparative results of an up-scaling assay in a 200 m^3 RAS rearing shrimps will be presented. In this system, 4-8 L/h H_2O_2 should supply or improve the disinfection effect commonly achieved by ozone (10 g/h). Additionally, we present the first results of a pilot research on ultrasound disinfection using a device especially conceived for aquaculture purposes and tested in our facilities. In this case, ozone (3.5 g/h) is tested against the disinfection potential of ultrasound (different frequencies / continuous vs. pulse) applied to a 5 m^3 RAS rearing European seabass. We analyse the impact of these three methods on the bacterial composition within the rearing tanks and list prospective benefits and drawbacks of their use as well as recommendations for application based on our practises.

Table 1. Bacterial burden (CFU) before and after 4h H_2O_2 application. Samples taken from tanks (TW), sump (SB), foam fractionator (ABS) and nitrification filter (BF).

Time	0	4h	Reduction
TW	604	160	74 %
SB	247	150	39 %
ABS	491	31	94 %
BF	396	47	88 %

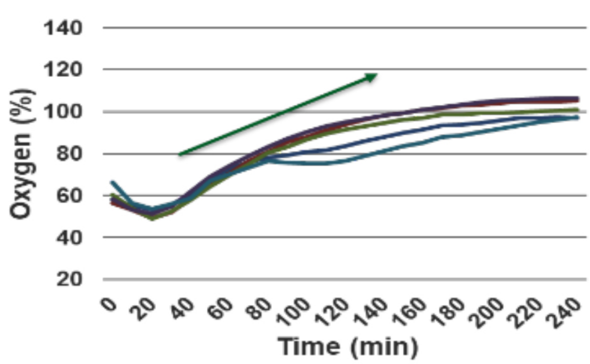

Fig.1 Effect of 15.8 mg/L/h H_2O_2 application on oxygen concentrations.

RAINBOW TROUT *Oncorhynchus mykiss* TRANSPORTATION – EFFECTS OF HANDLING PROCEDURES AND NEW TECHNOLOGICAL IMPROVEMENTS ON WATER QUALITY, STRESS MARKERS AND PRODUCT QUALITY

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Rainbow trout is one of the most frequently live-transported fish species in Europe. The health and stress response of the fish during and after transport and final product quality depend to a large degree on handling before and during the transport. Starvation periods and water changes before transport lower ammonia and faecal load in the transport water, but cannot prevent water quality deterioration during transport. An increase in toxic ammonium and a rapid increase in carbon dioxide concentration occur in transport water. Additional filter technology can optimise water conditions during transport. In the current study airlift degassing technologies and ammonium absorber filters were tested as were starvation practises before transport. Welfare and stress responses were measured as effects on blood markers, water parameters and product quality under laboratory and real conditions.

Tab.1: Fish and water parameters after transportation with airlift/zeolite technology (Treatment) and without (Control). Letters display significance.

Fish parameters	Treatment	Control
Blood lactate [mmol/l]	2.2 ± 0.8	2.1 ± 0.7
Blood pH	7.27 ± 0.11	7.25 ± 0.07
Plasma glucose [mg/dl]	50.6 ± 10.4^{a}	68.8 ± 22.8^{b}
Plasma NH_3 [µg/ml]	297.1 ± 91.7	350.8 ± 86.3
Plasma CO_2 [mmol/l]	8.1 ± 1.3^{a}	10.2 ± 0.8^{b}
Plasma cortsiol [ng/ml]	22.9 ± 9.0^{a}	46.6 ± 22.9^{b}
TBC filet day 1 [CFU/g]	64	63
TBC filet day 14 [CFU/g]	$9.8 * 10^{3}$	$1.9 * 10^{4}$
Water parameters	**Treatment**	**Control**
Temperature [°C]	11.6 ± 0.1^{a}	11.2 ± 0.0^{b}
pH	6.84 ± 0.03^{a}	6.71 ± 0.01^{b}
CO_2 [mg/l]	30.3 ± 1.4^{a}	39.6 ± 0.9^{b}
NH_4^+ - N [mg/l]	0.45 ± 0.00^{a}	0.48 ± 0.00^{b}
NO_2^- - N [mg/l]	0.001 ± 0.001^{a}	0.003 ± 0.001^{b}

ROLE OF CLCA GENE FAMILY MEMBERS IN FISH BACTERIAL DISEASES

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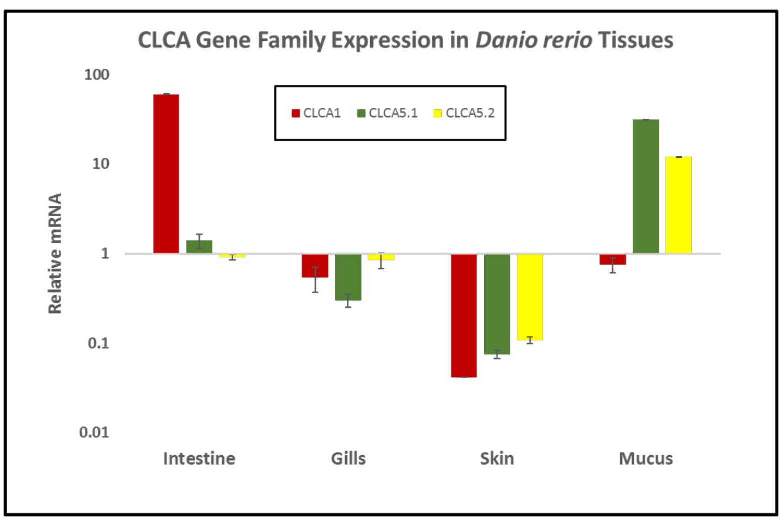
Bacterial diseases cause significant economic loss to the aquaculture industry. There is a need to find new solutions that can replace antibiotics. Epithelial tissues play a vital role in host defense mechanisms. One of them is a barrier function that protects inhibits pathogenesis. Epithelial cells 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. CLCA2 is expressed at the basolateral junctions of epithelial cells and is required for epithelial differentiation. Many studies have shown that dysregulation of CLCAs is significant in various pathologies including bacterial infections. 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. zCLCA1 is 903, zCLCA 5.1 is 888, and zCLCA 5.2 is 229 amino acids long. We found that the zCLCA1's highest expression is in the intestine when compared to gills, or skin; unlike zCLCA5.1 or zCLCA 5.2. There are ample studies showing the presence of proteases and metalloproteases in mucus that act as antibacterial peptides. CLCAs from other species are shown to be zn+2 metalloproteases. 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. zCLCA5.2 protein sequence analysis shows that it possibly comprises of only the ectodomain. We speculate that zCLCAs like other CLCAs are Zn^{+2} dependant metalloproteases expressed 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 are potential therapeutics for fish bacterial diseases.



SPECIES AND SYSTEM SELECTION FOR SUSTAINABLE AQUACULTURE IN THE REPUBLIC OF THE MARSHALL ISLANDS. SPAWNING AND LARVAL REARING OF THE STREAMLINED SPINEFOOT, *Siganus argenteus* (QUOY & GAIMARD, 1825)

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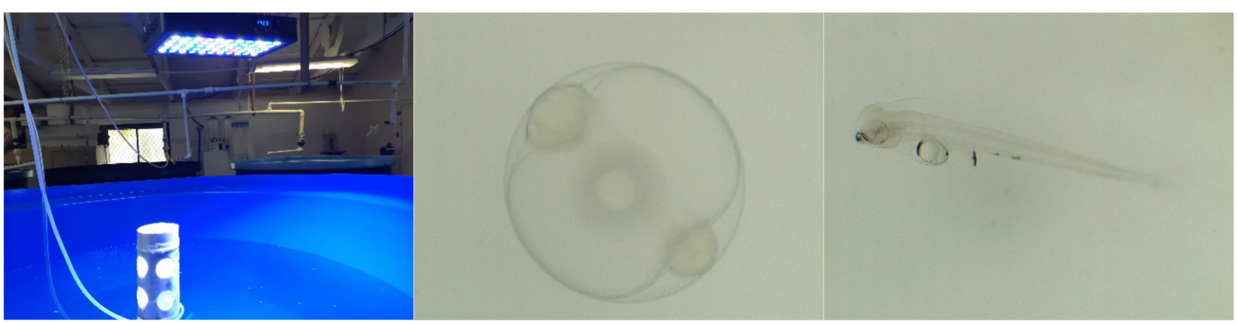
The Republic of the Marshall Islands, as other nations in the region are facing many severe upcoming challenges due to climate change. Coastal resources management are important issues in order to adapt to the increasing severity of this challenging changes. Aquaculture is a potential adaptation option to reduce the impacts on native populations.

The streamlined spinefoot, *Siganus argenteus* inhabits tropical coastal and inner reef slopes and lagoons, ranging from East Africa to French Polynesia. They are predominantly herbivorous, accepting a wide variety of food in captivity. They spawn pelagic eggs and the reproductive cycle is synchronized with lunar periods, producing eggs throughout the year. These characteristics along with the fact that they are much appreciated locally, make it a potential species for aquaculture.

The aim of the research is to develop the basic technology for culturing *S. argenteus* in order to meet demand for seafood, food security and stock enhancement, encouraging and helping farmers and fishing communities to improve their socioeconomic condition supporting healthy ocean populations and ecosystems.

Several attempts on the larval rearing of this species were made. Eggs were obtained from natural spawning after full moon were incubated in 500 liter plastic tanks with green water. The eggs are spherical (diameter 700 μm), transparent, contain a single lipid globule. Recent hatched larvae (TL= 1.5 mm) are transparent and after two days (TL= 2.5 mm) yolk was already absorbed at 30 °C. Different feeding schedules, illumination and temperatures were tested. Results showed that the seed production in captivity is feasible, the main factor to be considered is the size of the live prey at first feeding because of the small size of the mouth in the larvae of this species.



DOES FISH DIET AFFECT CONSUMER DECISIONS? A NUTRITIONAL AND SENSORY STUDY OF FARM-RAISED TROUT

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Although the aquaculture industry has successfully reduced its reliance on marine raw materials without impairing the growth performance or health of farmed fish, certain producers still raise their fish using diets containing high levels of fish meal and fish oil. The sustainability of the practice is a concern and therefore there is a need to determine how these dietary decisions are affecting fish nutritional value for consumers.

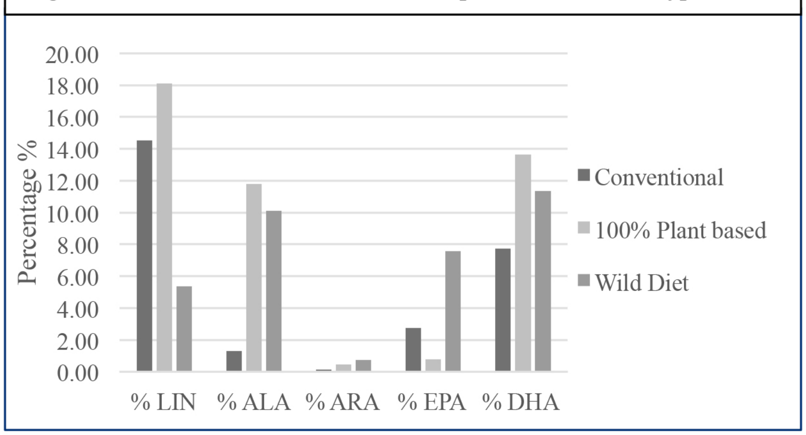
The focus of this study is twofold: 1) compare the nutritional value of farm-raised rainbow trout *Oncorhynchus mykiss* fed a conventional conservative fishmeal-based formulation, a 100% plant-based formulation, or a wild-diet. 2) conduct a sensory analysis test on both types of farm-raised trout products with a consumer panel focused on understanding their preferences and attitudes toward fish consumption.

The research objectives are as follows: 1) Produce a nutritional composition profile of fish edible portions (fillet, muscle) from the two different sources based on proximate analysis and fatty acid profile. 2) To conduct a consumer sensory analysis study to compare the two groups of farm-raised trout. 3) Survey a consumer panel discerning their attitudes, beliefs, and purchasing behaviors in regards to fish as food.

Preliminary Data: The omega n-6:n-3 fatty acid ratio was the highest (1.24:1) in trout fed with a 100% fishmeal-based diet compared to trout that received 100% plant-based diet (.71:1) or the wild diet (.21:1) (Fig 1.). Full sensory study data will be presented at the oral presentation.

Anticipated Outcomes: Industry and the public will be more informed on how aquaculture diets affect the fish from both nutritional and sensory standpoints. Differences in proximate composition and polyunsaturated fatty acids (PUFA) profile will be documented for this species across a range of diets to determine if diet has an effect on the flesh composition of this fish. The sensory study will tell us the consumers' preference for one regimen over another and provide more information on purchasing preferences and behaviors.

Figure 1: PUFA levels in trout filets sampled based on diet type



ACHIEVING RESTORATION SUCCESS UNDER CONFLICTING SOCIOECOLOGICAL OBJECTIVES

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Oyster ecosystems are subject to a litany of natural and unnatural perturbations, both of which are increasing in frequency and intensity, leading to more restorative management actions. Oyster restorations often have multiple objectives that may conflict in the short run, particularly in areas where oysters are harvested commercially. For example, setting a goal of increasing oyster abundance maybe difficult to achieve while also attempting to support socioeconomic recovery of coastal communities. Here we assess how restoration success may depend on spatial dynamics of oysters and their fishers, as well as the conditions under which additional fishery regulation may be necessary to achieve optimal outcomes. We developed a spatially explicit, socioecological model of a coastal oyster fishery that includes oyster larval and recruitment dynamics as well as dynamic fishing effort and spatial allocation. We then subjected the simulated population to different types of perturbations and potential restoration responses, with and without additional harvest regulations. What our results reveal is that under some perturbations and patterns of spatial connectivity, restoration actions alone could actually exacerbate population decline if fishing regulations are unchanged. However, limiting fishing effort during restoration certainly affected economic activity likely critical for human communities. We suggest that the most effective oyster restoration may need to be coupled with fishery regulations but also human community aid if the socioecological system function is to be restored.

ECONOMIC CONTRIBUTIONS OF FLORIDA'S AQUACULTURE INDUSTRIES

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Florida's aquaculture industry is extremely diverse, producing approximately 1,500 species or varieties of fish, plants, mollusks, crustaceans, corals, and reptiles for food and non-food markets. This industry is expected to grow due to a suite of factors, including increased demand for seafood products, and newly established recommendations by the state government to promote improved aquaculture development. Currently, Florida's aquaculture industry is dominated by ornamental fish aquaculture (valued at $34,506,000 in 2017) for non-food use, and molluscan aquaculture (valued at $17,291,000 in 2017) for food use. Activity in the aquaculture industry generates additional economic activity throughout Florida's economy via purchases of input goods and services and the re-spending of employee income. However, the estimation of total economic contributions for aquaculture is quite difficult due to the aggregate nature of economic data. Aquaculture is typically a subsector of a broader industry sector that covers all animal production, except cattle and poultry. Data from the 2017 Census of Agriculture are used to extract aquaculture production from the aggregated animal production sector. Economic contribution analysis is then implemented with IMPLAN© software to estimate the total economic contributions of the aquaculture industry in Florida, including indirect and induced activity supported in other industry sectors. In doing so, this provides a clearer understanding of the aquaculture industry's role in the state's economy and the broad scope of economic activities supported by the aquaculture industry in Florida.

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THE CASE FOR MANGROVE CONVERSION-FREE SHRIMP FARMS

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There was a drastic decline in global mangrove area during the 1980s and 19990s, but since 2000 the annual loss rate has declined to about 0.38% of global mangrove area. During the 1980s and 1990s, somewhere between 30% and 80% of mangrove loss could have been caused by shrimp farming. The actual percentage cannot be determined, because there are other causes of mangrove loss including conversion to fish ponds. The present-day loss of mangrove area to shrimp farming is less than in the past. In the five countries of this study, the entire loss of mangrove area since 2000 in Ecuador, India, and Vietnam could be attributed to shrimp farming if you assume no other driver of mangrove area loss – a dubious assumption. The loss of mangrove area in Thailand and Indonesia is much greater than the expansion of the shrimp farming area. In fact, in Thailand, the shrimp farming area has declined since 2000.

Most shrimp farming areas located in the tidal zone (where mangrove habitat is located) are for extensive production of *Penaeus monodon*. Mangrove areas are not well-suited for construction and operation of shrimp farms, and the income from extensive shrimp farming in mangrove areas is very low compared to the value of ecological services from mangrove areas. The annual income from intensive shrimp farming is typically less than 2,000 USD/ha/yr. Moreover, many crops fail and produce no income. The annual value of mangrove area for direct and indirect ecological services is usually several thousand USD/ha/yr. There seems no justification for national governments and NGOs encouraging small-holder shrimp farms to use mangrove areas for shrimp production even as a means of poverty alleviation.

It would seem reasonable for governments to give small-holder shrimp farmers an economic incentive to close their farms. The national value per hectare of mangrove areas for ecological services is many times greater than the income possible per hectare of extensive shrimp farms.

LAND USE EFFICIENCY IN SHRIMP AQUACULTURE

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Land use in shrimp aquaculture includes production and support area on shrimp farms. Agricultural land also is needed to produce shrimp feed ingredients and to provide carbohydrate sources for super-intensive culture ponds. The total shrimp farm area averaged 1.79 times production water surface area at intensive farms in Asia. Land use for shrimp feed ingredients averaged 0.310 ha/m.t. feed. Land use for carbohydrates often used in super-intensive culture was 0.133 ha/m.t. of sugar or molasses. The carbohydrate input may be 50-100% of the feed input. This would increase land use in super-intensive culture, but for now, most shrimp are produced intensively without carbohydrate addition. The total land use for shrimp culture in ponds decreased from around 4 ha/m.t. shrimp/yr in extensive culture to less than 0.5 ha/t in intensive culture. Ingredients alone require 0.25-0.4 ha/m.t. of shrimp feed, and the actual land use for feed is dependent upon the farm FCR. There is no appreciable reduction of land use per metric ton of shrimp at production intensity beyond 15-20 m.t./ha/yr. Thus, there is no land use justification for complex super-intensive production systems.

At present, shrimp farming has a global production area of about 2,440,000 ha and a total shrimp farm area of 3,660,000 ha. An additional 1,860,000 ha are cultivated for soybean and other plant meal feed ingredients. The average yield of shrimp is 2.12 m.t/ha production pond surface area/yr, 1.42 m.t./ha shrimp farm area/yr, or 0.94 m.t./ha total land area/yr. It requires an average of 1.06 ha of total land to produce 1 m.t. of shrimp per year. However, roughly 50% of the shrimp farm area is extensive and produces only 10% of shrimp. The efficiency of land use in shrimp aquaculture could be greatly improved by dissuading extensive shrimp production.

THE USE OF NON-DESTRUCTIVE HEALTH AND PATHOGEN SCREENING TECHNOLOGIES IN ATLANTIC SALMON (*Salmo salar* L.)

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Atlantic salmon (*Salmo salar* L.) are susceptible to several diseases which are caused by pathogens, parasites, husbandry, and environment, which significantly reduce productivity, sustainability and profitability of the industry. While resulting mortality and morbidity is highly variable, dependent on a number of factors, disease represents a sustainability issue for the industry. However, it is well established that disease impacts can be reduced significantly through regular health monitoring of stocks, with implementation of treatments, and disease management strategies.

The tools for diagnosing infection and disease in finfish aquaculture have grown significantly in recent years. Use of established diagnostic tools such as aetiological agent isolation, PCR and serology has dramatically increased. However, the manner in which these are most commonly carried out, as well as gold standard approaches for identifying clinical disease in gross pathology and histopathology are destructive. Destructive sampling has a number of drawbacks which centre around the need to remove individuals from the population, majorly this form of screening regimen is inhibitory to understanding herd health. As such, there is significant demand within the industry for non-destructive means of health assessment and pathogen screening.

This presentation aims to concisely describe the current utilization, challenges, and future potential of non-destructive technologies in Atlantic salmon culture. Specifically, environmental monitoring of conditions and pathogens and application of blood chemistryto infer health of stocks will be given focus.

INVESTIGATIONS ON THE DIFFICULTIES ASSOCIATED WITH DISEASE MODELS IN WHITELEG SHRIMP (*Litopenaeus vannamei*)

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Whiteleg shrimp (*Litopenaeus vannamei*) aquaculture is a major global industry which has undergone rapid growth in the previous decade. However, the industry's economic stability and sustainability is threatened by diseases caused by pathogens such as, White Spot Syndrome Virus (WSSV) and *Vibrio parahaemolyticus*. In order to reduce the impacts of these diseases there is significant requirement within the industry for advances in identifying pathogen presence, clinical disease, and practices and technologies. However, the development of such requires experimentation to validate test sensitivities and demonstrate efficacy. This study investigates the benefits and costs to different disease models used to do this. The role of variables such as size, pathogen dosing, infection route, housing set up, population densities, and environmental conditions will be shown.

TURNING AN INVASIVE CRAB SPECIES INTO A DELICACY: EXPLORING TECHNIQUES TO PRODUCE SOFT-SHELL GREEN CRAB IN NEW ENGLAND

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The European green crab (*Carcinus maenas*), an invasive species first detected in New England in the early 1800s have had a wide range of negative impacts on local ecosystems, including reducing native bivalve populations through predation, outcompeting native crustaceans for food and shelter, destruction of eel grass habitat and subsequent changes to fish community structure. Increases in green crab abundance and predation have also been linked to the recent decline of the soft-shell clam industry in New England. Compounding these issues is the link between increasing green crab abundance and increasing ocean temperature, which has had severe ecological and socio-economic consequences in the Gulf of Maine, which is warming faster than 99% of the world's other oceans.

Some New England states have either implemented mitigation measures to keep population levels at bay including bounty programs (MA) or developed techniques to diminish the effects of green crab predation on wild and hatchery reared juvenile soft-shell clams (ME). In Canada, the Department of Fisheries and Oceans suggests that a green crab fishery may be a viable way to control population abundance and derive value from this invasive species. Existing fisheries in the U.S. and Canada target green crabs for lobster and whelk bait, but a higher retail price is necessary to encourage expanded fisher effort and new participation. Therefore, as a way to mitigate green crab populations, engage and increase fisher participation, develop a potentially new aquaculture industry and create a high value, high quality, culinary product, we have been exploring techniques and market feasibility for soft-shell green crab in New England.

As with traditional soft-shell crab production- we collected green crabs in the 'pre-molt' phase of the molting cycle from the wild population during peak molting times. These 'pre-molt' crabs were identified by subtle color changes on the margin of the abdominal episternites of the crab (morphological indicators were determined independently from past research by the PI, researchers at the University of Prince Edward Island, Canada and Manomet, Inc., with assistance from Venetian fishermen). Pre-molt crabs were then held/stored individually until they molted or for 21 days in: a) Floating crates, b) Plastic containers in large flow through tanks, c) Wire mesh 'crab condos' in large flow through tanks or, d) Recirculating, modified salmon egg-tray incubators at different temperatures.

The results of this work over a three-year period, suggest that molt success is dependent on a combination of factors (e.g. temperature, salinity and how advanced in the molting cycle the crab is) and the most economic and time efficient method for soft-shell green crab production is wild harvest of advanced pre-molt crabs held in compartmentalized wire mesh 'crab condos' in large flow through tanks.

THERMAL TOLERANCE OF THE SEA VEGETABLE CROP *Alaria esculenta*

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Seaweed aquaculture is expanding rapidly along the Maine coast, but will be increasingly dependent on strains that are tolerant to coastal warming. We examined temperature tolerance of the edible kelp *Alaria esculenta* (L.) Greville with laboratory experiments, transcriptomic analyses, and grow-out of experimental strains on a sea farm.

We cultured gametophyte seedstocks from northern (Lubec) and southern (Two Lights) Maine. Replicates were maintained at 12 °C (controls) or slowly acclimated 1 °C/12 h to 22 °C, with gradual return to 12 °C three days later (heat acclimation treatment). Gametophyte health was affected by treatment, but there was no effect of source location (repeated measures MANOVA, treatment: $p = 0.002$, location: $p = 0.423$). Most (90%) gametophytes recovered from the heat acclimation. RNA was extracted from heat-acclimated and control treatments throughout the experiment to analyze gene expression. Preliminary analysis of transcript abundance profiles indicates that clusters of co-expressed genes in functional categories associated with temperature acclimation had distinct signatures between source populations.

Control and acclimated strains from both source locations were each blended within treatment, and seeded separately to produce juvenile sporophytes that grew in a common garden tank at our aquaculture center (CCAR) before transfer of seeded spools to a sea farm. Blade surface area was measured periodically during the growing season. Both treatment and source location of the gametophyte seedstocks significantly affected the next-generation sporophyte blade area (ANOVA, treatment: $p \leq 0.003$, location: $p \leq 0.010$). Thermal acclimation of gametophytes positively affected next-generation sporophytes. Acquisition now of wild strains and acclimation treatments in culture will help secure resilient *Alaria* aquaculture in warming waters.

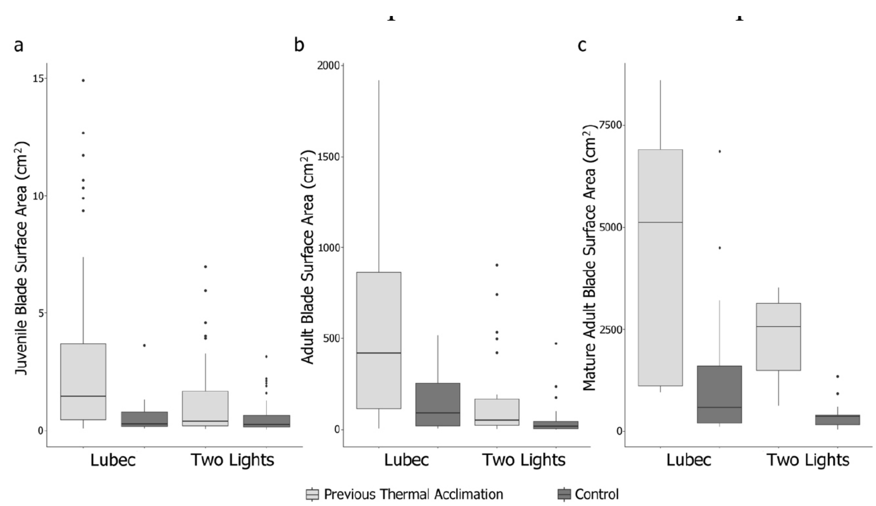

Figure: Blade surface areas of (a) juvenile, (b) young adults, and (c) harvest stage, by location and treatment.

USING BUSINESS AND FINANCIAL PLANNING TO IMPROVE SHELLFISH FARM RISK MANAGEMENT IN MAINE – A CASE STUDY

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Shellfish farming in Maine is in the process of transitioning from a number of hobby and side-job farms to an industry of full-time businesses. The industry, with its recent growth, is at a critical point. Back of the envelope calculations and “just wingin’ it” are no longer going to cut it. In order to strengthen Maine’s chances for success, the Maine Aquaculture Association has launched the development of a series of Maine aquaculture business support tools to address some of the industry’s most pressing issues: insufficient access to capital, inadequate business and production planning, and limited risk management and crop insurance options.

The first step of this process is to complete a series of benchmarking studies, which will provide lenders with standard metrics for assessing loan applications, and will allow farmers to compare their performance to industry standards. The benchmarking studies, in part, will inform the second component of the support tools: business and production planning. Results from the benchmarking studies, along with data-based shellfish growth models, are helping to shape separate, yet interlinked business and production plans. These plans will allow farmers to virtually plan, manage, and experiment with their business model, production cycles, and cash flow, while providing a platform to measure their actual performance. The benchmarking data and business-production plans will then be used to inform the Maine Aquaculture Association’s work with administrative officials on creating improved crop insurance options for shellfish farms in Maine, including an assessment on the feasibility of a Maine-specific crop insurance option. All of these data, tools, and insurance options are being used by the Maine Aquaculture Association to develop a best risk management practices guide for shellfish farms. The guide will be distributed across the industry, where farmers will be able to roll them out in conjunction with their new business planning tools and risk management strategies.

This presentation will begin by highlighting preliminary findings from the first-of-its-kind Maine shellfish benchmarking study. It will then explain how the study helped to inform the business-production plans, and outline the plans, pointing out how they were shaped through feedback from farmers. Last, the presentation will detail how these efforts, along with other meetings and cooperation, are helping to shape the future of shellfish best risk management practices and crop insurance in Maine.

NATIONAL MINORITY BUSINESS ENTERPRISE AQUACULTURE PROGRAM: YEAR 1 RESULTS

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Funded by a grant from the U.S. Department of Commerce's Minority Business Development Agency, the Minority Business Enterprise Aquaculture Program was operated by the Florida State Minority Supplier Development Council (FSMSDC) in partnership with the Southern Region Minority Supplier Development Council and the University of Miami Rosenstiel School of Marine and Atmospheric Science. The program's goal was to help minority-owned businesses around the nation engage and expand in the US aquaculture industry.

To be eligible for the MBE Aquaculture Program, a business must be 51% owned or controlled by African Americans, Hispanic Americans, American Asians or Pacific Islanders, Native Americans (including Alaska Natives, Alaska Native Corporations and Tribal entities), Asian Indian Americans, or Hasidic Jewish Americans.

First year results will be reported.

MODIFIED *Brassica carinata* SEED MEAL UTILIZATION IN HYBRID STRIPED BASS *Morone chrysops* ♀ x *M. saxatilis* ♂

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Glucosinolates (GLS), sinapine (SNP) and crude fiber (CF) are the most limiting antinutrients to including carinata meal (CM) in fish feeds. Manufacturing fish feeds by cook extrusion reduced GLS and SNP concentrations in CM by 48 and 57%, respectively. However, due to the high variation in GLS and SNP in different CM varieties, cook extrusion alone may not be sufficient to reduce GLS and SNP concentrations to tolerable levels by fish, without affecting performance.

In a GLS and SNP tolerance study on hybrid striped bass (HSB), diets containing ≥ 5.58 μmoles of GLS and 0.54 mg of SNP per gram, resulted in low feed consumption and thus low HSB growth. Therefore, CM had to be processed to reduce GLS and SNP concentrations, to enable increased CM inclusion levels in fish feeds. CM was subjected to aerobic microbial conversion (AC) followed with a single wash (ACCM) or merely double-washed (W) without AC (WCM). These two processing methods reduced GLS and SNP concentrations in ACCM and WCM by >70 and >83%, respectively. However, CF was increased in ACCM and WCM. Up to 30% of ACCM and 30% of WCM were included in HSB diets containing similar animal meal contents (20%) and fed for 12 weeks.

Growth increased with increasing processed CM in diets due to improved palatability and increased feed consumption. However, ACCM diets were poorly converted into fish biomass. HSB fed 30% WCM were in better condition and had healthier livers than HSB fed ACCM or the reference diet. Apparent digestibility coefficient (ADC) of protein decreased with increasing ACCM in diets. However, protein ADC of WCM and the reference diet were similar. In a time series study, all inclusions of ACCM or WCM increased serum and muscle methionine. High (30%) inclusions of processed CM increased serum tryptophan and valine but reduced serum arginine and leucine. High (30%) inclusions of processed CM increased muscle histidine but reduced muscle leucine and phenylalanine. Only 30% WCM increased muscle lysine and valine. The reference and 30% WCM diets resulted in the highest total essential amino acids (EAAs) in serum but release of total EAAs in serum of HSB fed 30% WCM was elevated continuously over a longer period. High inclusions of ACCM or WCM (30%) in diets resulted in more total EAAs in muscle over a longer period. Muscle EAA to lysine ratios showed that only histidine concentrations were adequate for muscle synthesis over the 36-hour period. Overall, the second wash in WCM improved meal utilization by increasing protein ADC when compared to single-washed ACCM. The specific mechanism(s) associated with process improvements is still under investigation.

INVESTIGATION OF GUT MICROBIOTA, PHYSIOLOGICAL STRESS RESPONSE, AND IMMUNE PARAMETERS IN CULTURED BURBOT *Lota lota maculosa*

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As aquaculture expands in the United States, there is a need to investigate novel fish species that may offer producers new products for the domestic market. Burbot (*Lota lota*), have shown strong potential as a new aquaculture species in the western states. Burbot share similar culture requirements to rainbow trout (*Oncorhynchus mykiss)* and have been shown to be relatively resistant to many salmonid pathogens, thus providing an excellent opportunity for diversification of trout production facilities. At the University of Idaho, there have been recent efforts to evaluate commercial diet selection, pathogen susceptibility, and life stage culture optimization for this species. Findings to date have provided insight for expanding burbot culture. The proposed research project will use recently published methodologies developed for other cultured species to investigate the ability of burbot to utilize plant-based feed ingredients and characterize some aspects of burbot immunity and health. This current study will develop and investigate areas of burbot nutrition, stress physiology and immunology related to the commercial development of this species. The specific objectives of this project will be to: 1) characterize the intestinal microbiota associated with feeding burbot plant-based diets, 2) evaluate the influence of rearing stressors in burbot culture, 3) investigate an array of burbot immune metrics and assess virulence of pathogenic bacteria isolated from commercial burbot operations and 4) assess the potential for killed vaccines to be used to prevent disease in this species. The results from this project will be of interest to commercial coldwater aquaculture producers, as well as agencies expanding burbot culture for management implications. Further, this project will provide novel findings on the burbot immune system and determine the feasibility and effects of vaccination efforts for this species. Increased production and stimulation within the domestic aquaculture sector will assist with curbing the current trade deficient and help achieve the necessary increase in global food production required by 2050.

GROWTH AND IMMUNE RESPONSES OF CULTURED BURBOT *Lota lota maculosa* FED VARIED INCLUSIONS OF SOY PROTEIN SOURCES

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Burbot (*Lota lota maculosa*) are the only freshwater member of the cod family (Gadidae) and are a new species for commercial aquaculture. Burbot culture methods and feeding trials at the University of Idaho are being optimized for commercial burbot production. This “freshwater cod” species could provide diversification for freshwater salmonid and other aquaculture operations, given its desirable fillet quality and growth potential. Burbot nutrition research is necessary to further define dietary requirements and the potential of this species to utilize more sustainable plant-based proteins. A 91-day feeding trial and subsequent 28-day pathogen challenge was completed and growth performance, survival, and immune parameters in juvenile burbot were evaluated. Dietary treatments included a fishmeal reference diet (FM), and 25% and 50% fishmeal replacements with soybean meal (SBM), soy protein concentrate (SPC) and bioprocessed soybean meal (BSBM). Growth results indicated a difference in relative growth (RG; P=0.01) and specific growth rate (SGR; P=0.02) among dietary treatments, with the fish fed FM and SBM-25% performing better than the SPC-50% and BSBM diets. Analysis of distal intestine samples discerned no differences in lamina propria thickness (P=0.489) or cellularity (P=0.979), and the amount of connective tissue of the submucosa was found to be similar (P=0.972), indicative of no diet-related complications. Immediately following the feeding trial, burbot were challenged (triplicate groups of 25 fish/tank) with a virulent strain of *Aeromonas* sp. while remaining on their respective dietary treatments. At day 28 post-challenge, no diet-related effects were found in cumulative percent mortality (P=0.17), and immune metrics post-challenge were not different for serum lysozyme (P=0.975) or respiratory burst activity of isolated head kidney-derived leukocytes (P=0.796). Overall, results indicate that low-level FM replacements with SBM and SPC are applicable for incorporation into burbot diets and long-term feeding of soy-based proteins does not impair disease resistance or innate immune function. Utilizing soy-based protein sources for commercial burbot culture will expand the use of soy ingredients in aquaculture and provide potential producers with a more cost-effective alternative to high fishmeal diets.

CROSS-PROTECTION OF A LIVE-ATTENUATED COLDWATER DISEASE IMMERSION VACCINE AGAINST NOVEL *Flavobacterium* spp. AND *Chryseobacterium* spp.

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Flavobacterium spp. infections remain a major aquaculture industry issue and can cause major losses to fish stocks and economic implications. For salmonid producers, a common threat within this genus is *F. psychrophilum*, the causative agent of bacterial coldwater disease (BCWD). Recent advancements in BCWD disease management have included a live-attenuated vaccine that provides cross-protection from an array of *F. psychrophilum* strains. Emerging family *Flavobacteriaceae* isolates have also been identified in global fish health diagnostic cases and include novel, virulent *Flavobacterium* spp., and *Chryseobacterium* species. The cross-protective ability of this live-attenuated *F. psychrophilum* vaccine against novel *Flavobacteriaceae* was evaluated in juvenile rainbow trout. Fish developed high *F. psychrophilum*-specific antibody titers 8 weeks post-vaccination, with the vaccinated fish showing a greater response than mock-vaccinates (P<0.001) as expected. Fish were then challenged with two *Chryseobacterium* spp. (S25 and T28), a *Flavobacterium* spp. (S21), a mixed combination of S21:S25:T28 strains, and a standard virulent *F. psychrophilum* CSF259-93 strain. With respect to vaccination, the 259-93 V group had a relative percent survival (RPS) of 94.44% compared to the control (259-93 C) fish (P<0.001). Interestingly, vaccinated fish also had a high RPS (85.18%) following the mixed *Flavobacteriaceae* infection. Protection was not observed following challenge with the single novel *Flavobacteriaceae* strains. Analysis of whole-cell lysates of bacterial strains revealed differences in protein profiles (SDS-PAGE), but antigenic regions were observed in S25 and T28 when probed with post-challenge sera from surviving fish the mixed S21:S25:T28 and CSF259-93 groups in a western blot. Results indicate that the live-attenuated *F. psychrophilum* vaccine provides indirect protection against mixed infections with novel *Flavobacteriaceae,* but further work is needed to determine the synergistic virulence mechanisms for these emerging isolates.

AUTOTROPHIC AND HETEROTROPIC TREATMENT IN SEMI-INTENSIVE, INTENSIVE AND SUPER-INTENSIVE FISH AND SHRIMP CULTURE

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Aquaculture productivity has progressively increased over the last 50 years. In the 1960's un-aerated aquaculture ponds, referred to as extensive systems, yielded 1,000-2,000 lbs/ac of fish or shrimp in freshwater or marine systems. Feed rates averaged 10-20 lbs/ac-d, the limiting factor being wind-driven re-aeration rates. Algal photosynthesis suppresses ammonia levels at algal productivities of 0.5-1.0 g-C/m^2-d at water column volatile suspended solids (VSS) levels of 10-20 mg/l. Beginning in the 1980's farmers added 1-2 hp/ac supplemental aeration to marine shrimp and freshwater fish-ponds, pushing yields to 4,000-7,000 lbs/ac, at average feed rates of 50 lbs/ac-d and maximum of 100 lbs./ac-d. At this loading, algal photosynthesis of 2-3 g-C/m^2-d matches ammonia generation rate. Exceeding this level of production requires enhanced algal productivity, or pond flushing with discharge of ammonia and VSS to the environment.

In Asian marine systems, flushing of ammonia-laden water and increased pond aeration to 10-20 hp/ac allowed shrimp production in excess of 10,000 lb/ac-cycle. In the U.S. discharge of water was increasingly prohibited; beginning in the 1990's U.S. aquaculturists turned to enhanced photosynthetic systems such as partitioned aquaculture systems, split-ponds, in-pond raceways, or intensively aerated ponds to increase carrying capacity. Fish yields of 10,000-19,000 lbs/ac were achievable with feed loadings of 100-250 lbs/ac-d. Under these conditions, algal photosynthesis peaks at levels of 6-12 g-C/m^2-d.

To further increase production to "super-intensive" levels, farmers turned to alternative biological treatments techniques, fixed-film nitrifying reactor (trickling filters), or suspended-culture microbial reactors (biofloc). Trickling filters are economically favored in most finfish culture because of potential for higher volumetric density in fish as opposed to shrimp culture. In general, biofloc treatment is economically favored in shrimp culture, using nitrifying systems (NS) or heterotrophic systems (HS). HS require external organic supplementation in addition to feed application. Optimal microbial levels in the range of 300-400 mg/l keep pace with needed ammonia removal while not imposing excessive oxygen demand. Shrimp production in excess of 40,000 lbs/ac-cycle is achievable. NS require aeration power of 50-60 hp/ac, whereas HS aeration can exceed 60-80 hp/ac. The major advantage of HS culture is rapid growth rate of heterotrophic microbes providing nearly instantaneous control of ammonia levels. HS culture requires organic supplementation of 40-60% of feed and removal of as much as 10% of microbial mass per day. Microbial solids production from NS is typically 10% that of HS, and no additional organic supplementation is required. Enterprise budgets suggest shrimp production costs in NS and HS at 40,000 lbs/ac-120d are similar at $8.00/kg-shrimp. An added advantage of super-intensive aquaculture is the potential to provide "zero-discharge" systems, eliminating water and sludge discharge by converting microbial biomass production to useful byproducts.

SYSTEM	Yield lb/ac	Feed lb/ac	Aeration hp/ac	Type (g-C/m^2)	VSS mg/l	Timeline
Extensive	1,000-2,000	10-20	wind	Algal (0.5-1)	10-20	1960
Semi- Intensive	4,000-7,000	50 -100	1-2	Algal (2-3)	50-100	1980
Intensive-Pond	10,000-12,000	100-180	6-10	Mixed (3-4)	100+	1990
PAS/SP	12,000-19,000	190 -250	6-10	Algal (6-12)	50-100	2000
Super-Nitrifying	40,000+	800	50-60	Nitrification (40+)	300-400	2006
Super-Heterotrophic	40,000+	800/600	60-80	Heterotrophic (60+)	300 -400	2006

SALES OF LIVE CATFISH IN AFRICAN MARKETS

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Small scale fish producers in the state of Kentucky face both production and marketing challenges. Also, pond-based aquaculture can be an expensive enterprise, specifically in Kentucky where pond construction costs can be three times as expensive as other states due to widespread karst systems and rocky soil conditions. Indoor fish farming is very capital intensive with most enterprises requiring insulated building with road access, drainage, lighting, and temperature control. These high costs of entry and high levels of risk mean that it is very important for small-scale producers to have markets that can support their enterprise.

Ethnic markets for fish, particularly among African and Hispanic consumers, might pay premium prices creating an avenue for selling their products profitably. Although research has been done on Hispanic markets, very little research has been done around African markets.

This project looks at how small-scale fish farmers can sell live/whole catfish among African consumers in urban areas of Kentucky. A Becker–DeGroot–Marschak model auction was used in order to determine the willingness-to-pay for live catfish. The average willingness-to-pay for live catfish was $7.70/kg. Using these prices and consumer demand estimates, one could calculate the return on investment of a small-scale fish operation in the suburban regions of Kentucky. The results showed that direct-to-consumer sales in ethnic markets, particularly African markets, are one of the few viable options for small-scale fish producers.

ADVANCES IN HATCHERY TECHNOLOGY OF RED SNAPPER *Lutjanus campechanus*, CANDIDATE SPECIES FOR MARINE AQUACULTURE IN THE CARIBBEAN

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A summary of techniques for capture, transportation, handling, sampling, sexing, spawning, larval rearing and weaning of red snapper (*Lutjanus campechanus*) is presented. Wild broodstock were captured during the beginning of their reproductive season in May 2018 in waters of Port Canaveral, Florida. All fish were tagged and treated prophylactically for parasites with fresh water, hydrogen peroxide and Formalin baths. After acclimation, seven female and five male fish averaging 5.5kg, were maintained in a 60 m^3 tank equipped with RAS and temperature control at a stocking density of 1.2 kg/m^3. Periodic prophylactic treatments and controlled diet were administrated throughout the season. In order to stimulate the maturation of the fish, temperature was gradually increased each month, starting at 22 °C in January and reaching 26 °C in April, in the beginning of the spawning season. Prophylactic treatments were administrated every 2 months and consisted in transferring the fish to a quarantine tank, treating them with a 3 minutes fresh water bath, 150 ppm of hydrogen peroxide for 3 minutes, and 100 ppm of Formalin for 1 hour. Fish were sampled to observe stage of maturation of gametes. The system was cleaned and disinfected for 24 hrs. Diet of the broodstock was maintained 3% of the biomass and consisted of squid, sardines and a vitamin and mineral supplement. The broodstock diet was enhanced with mackerel, shrimp, and polychaetes as the spawning season approached.

Continuous volitional spawns were obtained in the first months of the season. The first high quality spawns were obtained in August, when 3,135,050 eggs were collected, of which over 1,500,000 eggs were stocked in tanks ranging 400-L to 2,400-L. Spawns had a fertility rate >65% and maximum density of incubation of 500 eggs L^{-1}. Incubation of the eggs last 24hrs at 26 °C, and hatch rate averaged 85%. After hatching, larvae were passive transferred to tanks of same volume and stocked at densities ranging from 40-100 larvae L^{-1}. Temperature in the larval rearing ranged from 24-26 °C. Algae in paste were used as green water. S-strain rotifers, *Brachionus rotundiformis*, were used as exclusive first feed. Enriched *Artemia* were gradually introduced as feed to the larvae and co-feeding of both prays occurred until the beginning of metamorphosis. During and after metamorphosis *Artemia* were gradually replaced by dry feed. Survival rates from yolk-sac larvae to post-metamorphic, early juvenile averaged 5% in the first batch. Broodstock are conditioned and continue to regularly spawn volitionally and various experimental trials currently are being performed aiming the development of an ideal larval rearing protocol for mass production of red snapper fingerlings.

GENOMIC SELECTION IN SHRIMP

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The availability of SPF (Specific Pathogen Free) shrimp broodstock has had a significant impact on the aquaculture of shrimp; from reducing the incidence of disease to facilitating more traditional family-based breeding for shrimp. From a breeding perspective, one of the shortcomings of the SPF system is that for biosecurity reasons, it does not allow for genetics from the growing ponds to be incorporated into the breeding program. Similarly, selection for other traits such as disease resistance and carcass quality prevent broodstock on which a trait was measured from returning and contributing to the breeding nucleus. Thus, the only way to use this information for genetic improvement is to rely on family and sib-ship information. This approach is not ideal as the accuracy of selection is limited because within family genetic effects are not captured. Mass selection approaches suffer from the same short comings in biosecurity, lose potential increases in genetic gain to reduced accuracy, and suffer from the risk of inbreeding depression. Genomic selection was developed to increase the accuracy of selection, accelerate genetic gain, and consequently increase genetic gain per generation while simultaneously allowing for the control of inbreeding on a whole-genome level. It relies on the measurement of genomic similarity to predict breeding values, rather than a sib-ship relationship. This allows 1) increases in selection accuracy, 2) selection of breeding candidates from different genetic backgrounds that are more likely to perform well, 3) for the control of inbreeding (relatedness) in a genome wide fashion, 4) for selection on phenotypes that cannot be measured on the breeding candidates without depending solely on family information. This last point can have a great impact on shrimp breeding, as it would allow the accurate incorporation of genetic data from ponds without increasing biosecurity risks. Application of genomic selection is feasible when combining an economically efficient method for scanning the genome of broodstock for SNP (single nucleotide polymorphism) markers, and genomic imputation to reduce the overall burden of genotyping costs. With the development of genetic tools and understanding of breeding strategies, the economic costs for incorporating genomic selection to accelerate shrimp breeding programs are no longer barriers to implementation.

DIFFERENTIAL REGULATION OF HYPOXIA RESPONSIVE GENES IN ATLANTIC SALMON SUBJECTED TO LOW OXYGEN STRESS DURING EARLY DEVELOPMENT

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During the on-growth phase in sea cages, Atlantic salmon are subjected to a large variability in dissolved oxygen (DO) concentration. Low DO concentrations are known to limit food utilization, growth, production performance and welfare, and may contribute to some of the mortalities observed at sea. The environment (e.g. temperature, oxygen, photoperiod) during an organism's embryonic development can shape its phenotype at later life stages, through modifications in the epigenetic machinery. The aim of the present study was to determine the effects of different DO levels during early ontogeny on the transcriptomic and methylomic profiles in later life stages.

Until start feeding, salmon eggs and larvae were subjected to 30%, 60% and 100% DO levels. Development was monitored based on embryonic stages. Whole embryos and larvae were sampled at consecutive time points to investigate differential gene expression and DNA methylation using qPCR and RNA-sequencing, and pyrosequencing and Reduced Representative Bisulfite Sequencing (RRBS), respectively.

Growth was affected especially in the 30% DO group, such that the occurrence of start feeding was delayed ca. 8 days compared with the 60% and 100% DO groups. The 60% and 100% showed no clear differences with regard to development and growth, suggesting that 60% DO may not visibly affect larval development. However, on the molecular level clear differences were observed between all groups. Preliminary data shows that between the three groups, 69 genes were differentially expressed, and had biological functions relating to, for example, oxygen transport (e.g. haemoglobin subunits), and major energetic metabolic processes (e.g. cytochrome c oxidase, p450 subunits).

Further, the expression levels of *prolyl hydrogenase-domain* (PHD) genes, which are essential regulators in maintaining oxygen homeostasis, were significantly increased with decreasing oxygen levels. Moreover, two putative CpG sites in the promoter of PHD3 were found differentially methylated and showed a negative correlation with its expression. It is hypothesized that through the inhibitory effects of these PHD genes on hypoxia inducible factor (HIF) the potentially harmful effects of hypoxia may be reduced during early development.

EVALUATION OF NOVEL NON-GMO SOYBEAN CULTIVARS FOR NUTRIENT AVAILABILITY, REDUCING METABOLIC WASTE AND INCREASED ENERGY EFFICIENCY IN ATLANTIC SALMON (*Salmo salar*)

Gary Burr, Brian Peterson, Rick Barrows, Alejandro Buentello

Soybean meal has had limited inclusion in aquafeeds for marine carnivorous finfish due to the presence of anti-nutritional factors and effects on the GI tract. The chosen varieties were selected based on relatively high protein levels, low quantities of anti-nutritional factors (ANFs), and agronomic performance. For these studies, candidate soy varieties were selected based on two preliminary feeding trials conducted for 6 weeks on first feeding Atlantic salmon, *Salmo salar*. In the fry study, final average weight (1.18g-1.29g) and percent increase (225%-343%) did not significantly vary, however survival was greater for some varieties compared to others (60%-95%). The best preforming cultivars were evaluated in a 16-week smolt feeding trial. Most performance indicators were not significantly different (weight gain, specific growth rate, final average weight). Only hepatosomatic index was significantly $P < 0.05$ different (1.00 to 1.45). Based on results from this study the assertion can be made that these soy cultivars can be incorporated into the salmon diet up to 20% without significant performance differences compared to a fishmeal-based diet.

Performance data from the smolt growth trial.

Diet		Fillet yield (%)	HSI	intestinal length/ fork length	Final Avg weight (g)	Weight gain (g)	Final FE (g fed/g gain)	Percent Increase	SGR (%/day)
	1	48.68	1.45^{A}	0.36	235.98	119.73	1.04	99.35	1.07
	2	47.52	1.44^{A}	0.36	229.05	107.95	1.12	85.25	0.96
	3	51.16	1.27^{AB}	0.41	242.17	109.49	1.26	84.30	0.94
	4	43.79	1.05^{B}	0.36	238.33	96.13	1.38	68.92	0.81
	5	50.39	1.09^{B}	0.42	240.49	114.60	1.10	91.12	1.01
	6	49.93	1.21^{AB}	0.37	245.97	115.26	1.41	88.28	0.99
	7	49.49	1.00^{B}	0.35	243.78	105.81	1.17	76.72	0.89
	8	47.56	1.12^{B}	0.34	267.70	130.90	0.98	96.45	1.05
Overall average		48.57	1.20	0.37	242.93	112.49	1.18	86.30	0.97
P value		0.08	0.01	0.24	0.46	0.54	0.78	0.44	0.40

BENEFITS OF LOW INCLUSIONS OF KRILL MEAL IN FISH MEAL-CHALLENGED SHRIMP DIETS

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To reach economic success in shrimp farming, maximal feed consumption with minimal feed waste is essential. High feed intake and growth depend on a diet that is nutritionally suitable and stimulates special sensory cells of shrimp. Water-soluble, low molecular weight compounds, free amino acids, nucleotides, nucleosides, quaternary ammonium compounds, phospholipids, biogenic amines and monosaccharides have all been identified as feeding effectors that improve attractability and palatability of diets. Increased attractability of diets reduces the feeding response time and thereby limits nutrient leaching and feed waste. This not only helps with nutrient-overloaded pond issues, but also lowers the energy a shrimp has to spend to find the feed and cost of feed that can make up to 50% of the total farming costs.

Intensive shrimp farming has extensively used fishmeal for its nutrient composition and high attractability, but stagnant supplies and increasing prices have led to the use of alternative protein sources such as plant and rendered animal byproducts. While these changes have the advantages of reducing cost and increasing sustainability of aquaculture, they can also severely impact shrimp growth. Reasons may include the absence of essential nutrients, lower attractability and palatability and antinutritional factors that can suppress feeding stimulus and reduce nutrient bioavailability. A good feed attractant may help to overcome these issues, but there may be differences in effectiveness, when different sources such as fish, krill, mollusks, shrimp or squid are compared.

This poster reviews the latest studies with krill that were performed in Brazil, Thailand and India where growth performance, feed preference, hepatopancreas condition and feed formulation costs were in focus.

FATTER. FASTER WITH BUGS: HOW MULTI-STRAIN PROBIOTICS IMPROVED GROWTH, INTESTINAL HEALTH, AND MORTALITY IN RED DRUM (*Sciaenops ocellatus*) JUVENILES

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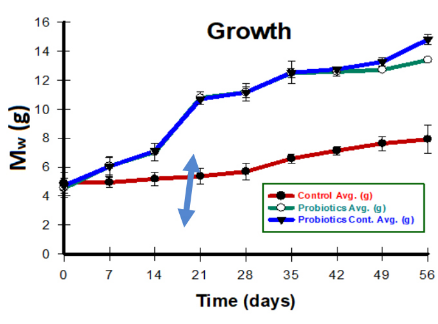
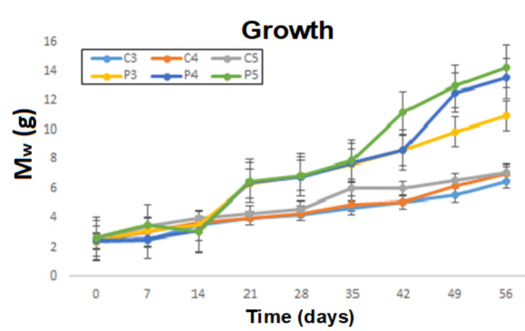
Probiotics are live microorganisms, which confer health benefits to the host when administered in adequate amounts. They have been shown to affect fish health through improved gut health and stabilizing gut flora, improved growth and immunity in some fishes, and increased survivorship of others. In the present study, analyses of growth performance, intestinal morphology, mortality rates and feed conversion efficiency (FCE; mass gained (g) /mass of feed (g)) were compared for juvenile red drum (*Sciaenops ocellatus*) treated with the multi-strain probiotics using two methods of administration: 1) added to water and, 2) added to feed.

For the water-soluble probiotic trials (WSP) (n = 3) a total number of 1,080 juvenile red drum (*S. ocellatus*) were used. For the Probiotic Enhanced Starter Feed (PESF) trial (n =1) a total number of 120 juvenile red drum (*S. ocellatus*) were used. The water-based, multi-bacteria probiotic, PrimaLac® approved for the aquaculture industry, containing *Lactobacillus acidophilus, Lactobacillus casei, Bifido bacterium bifidium, Enterococcus faecium* used in this study, obtained from Star Labs© (Missouri, USA). The feed used in this study was formulated at Rangen Inc, Idaho for Ekstrom Aquaculture LLC (Copper Shoals Red Drum and Ekstrom Enterprises) [44% crude protein, 15% crude fat, 3% crude fiber, 1% phosphorus, and 12% ash].

Overall, results show that with the use of the water probiotics there was a growth increase by 21 days of 32 percent ($p<.05$) and with the feed treatment (PESF), though not as great an increase at 22% by 21 days, it was still quite significant ($p<.05$). Even though the growth was not as much when using only the feed administration method, the growth for both groups was greater than that of the controls from both administration methods. The mortality was also decreased significantly in the first 21 days over controls. This shows that not only were the red drum growing more rapidly they were also coping with stressful tank conditions and adapting to the changes with the use of probiotics. This was partly explained by increased surface area of gut epithelial linings and increased goblet cell production in the distal intestine, which likely led to enhanced nutrient absorption, improved growth performance as well as reduced waste (digesta). These results have clear benefits to the sustainable aquaculture industry, and show that you can create fish “fatter, faster with bugs.”



The Mean Growth vs Time (days) for juvenile red drum treated with no probiotics (controls- red), probiotics until 28 days (green), and probiotics for 56 days (blue).



The Mean Growth vs Time (days) for juvenile red drum treated with no probiotics at 3 feed levels (controls – light blue, orange, gray) and PESF probiotics at 3 feed levels (yellow, dark blue, green).

CHANGING PERCEPTION THROUGH THE EYES OF AN INFLUENCER- FULL CIRCLE- JOURNEY OF WATERMAN

Submitted by Jennifer Bushman

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Where we begin this "Journey of the Waterman" was with an idea at the most elemental level. Align two communities - Aquaculture and the Surf Community. A group at odds since nearly the inception of Marine Based Aquaculture. But with the power to change perception if educated.

In 2017, the search for the champion began in earnest. I interviewed nearly a dozen surfers both male and female in search of the person whose values, life cycle and sense of adventure would align with the mission to champion aquaculture in this most influential community. I was looking for someone that wanted to engage in more than just a sticker on the surf. Jamie Mitchell checked all of the boxes. He was a true champion with over 10 world champion paddle boarding titles and a winning surfer on the current World Surf League Big Wave Tour. He is also a family man, with a new wife and two young daughters.

Once we had several discussions with Jamie, he agreed to come on this journey with us. The earliest reference that I can find to the present day wording for the best phrase to describe our journey is in Samuel Purchas's Pilgrimage, 1613: "The Arabians out of the deserts are as Fishes out of the Water." Jamie, was on his way to Patagonia and was literally a fish out of water. By the time we were on the boat, we realized some important things. Jamie had NO idea what he had signed on for. Even with all of our preparation he really had signed on to this for simply "The sticker on the board". He faced protest, social media outrage and threatened to leave us. But once he got to the farm, everything began to change. His journey continued to Pacifico Aquaculture and to Pipe masters in Honolulu.

He realized that he had to embrace the journey and find the impacts for himself. The water that surrounded him also began to soften his skin, both literally and figuratively. At each farm he visited, listening, learning and keeping his eyes wide open. He tasted the feed, toured the hatcheries, the farms, processing plants interviewing farmers and absorbing the entire journey. Jamie saw the entire ecosystem. He did countless interviews in his nearly 3 weeks on the farms and in the region speaking with workers, farmers and others in the industry. What role did it play and how could things improve. He called friends in the surf community, vetting the entire process and confirming that he had not has the wool pulled over his eyes in any way.

We are all trapped in our own way of thinking. Trapped in our own way of relating to what we think we know. He had gotten used to seeing the ocean in his own way and it had become one dimensional. His views have changed on this journey. It has made him more deeply aware of the fact that the ocean healed him as a child, his constant companion as he traveled the world that had served him as his passion and career needed his help and aquaculture was a solution. If nature sustains itself through a continuous give-and-take process and human interaction with nature throughout history has been mostly taking then it is time for that relationship to evolve. He believed that sustainable aquaculture can bring balance and that his voice could bring meaningful change.

Jamie's voice proved to be powerful. To date, the film has had over 1.2 million views and the campaign has had over 40 million impressions. We did a series of premieres, lectures with Quicksilver and much more. We learned that there was a lot of misinformation within this group but that as active ocean lovers, they were easy to convince when given the correct facts through the voice of someone they admired so much. It was a successful campaign that gave us an understanding of how to begin a delicate dialogue and bring a solution based dialogue to the forefront.

IMPROVING HYBRID CATFISH PRODUCTION: IMPACTS OF PARENTAL EFFECTS AND GAMETE INTERACTIONS

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Catfish farming accounts for nearly 70% of total U.S. freshwater aquaculture production, where the channel catfish, *Ictalurus punctatus* ♀ × blue catfish, *I. furcatus* ♂ hybrid constitutes >50% of the harvest. Although the catfish industry has seen sustained growth there are still challenges associated with fertility, broodstock selection, and inconsistent production of hybrid catfish fry. In order to address these challenges, several experiments were conducted with the following objectives: 1) determine optimal sperm density required to fertilize eggs using assisted reproductive techniques, 2) assess sperm performance by incorporating female ovarian fluid into the sperm activation environment, 3) identify mechanisms of sexual selection and gamete interactions, and 4) examine gene expression profiles between low vs. high quality eggs at different stages of embryonic development.

Preliminary results demonstrate that previous sperm densities required for fertilization were overestimated (1.25×10^5:1 to 1.2×10^7:1 sperm per egg) and that a ratio of 1×10^4:1 sperm per egg maximizes hatch success of hybrid fry (Fig. 1A). Secondly, our results confirmed ovarian fluid can play a critical role in the fertilization environment by increasing sperm motility, velocity (Fig. 1B), and longevity. Significant sperm-ovarian fluid effects were also evident that caused differential sperm performance and sexual selection at the gametic level. Gene expression analyses are currently underway to decipher biomarkers for egg/embryo quality at "critical" developmental stages. Overall, our research results show the importance of sperm density, individual male-female parental crosses, and sperm-ovarian fluid interactions for hybrid catfish production. Future research will be discussed in light of these findings.

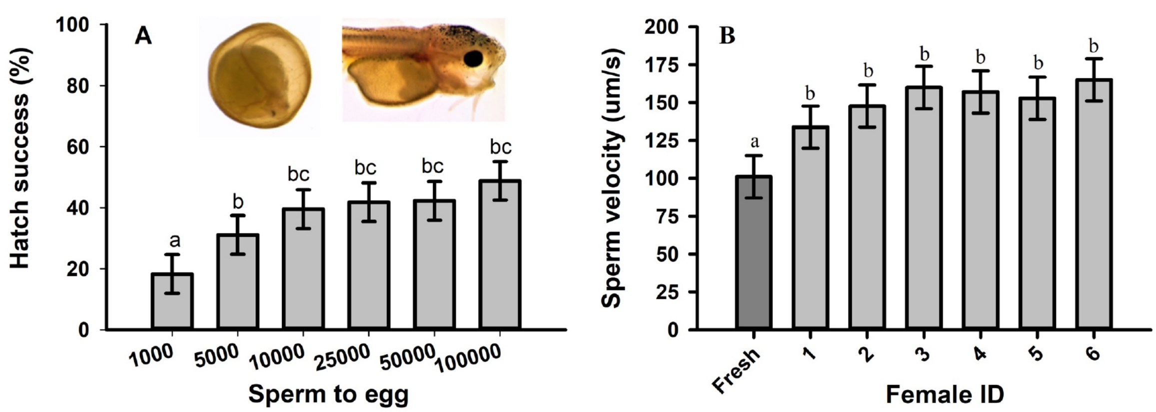

Fig 1. (A) Hatch success (%) of hybrid catfish fry across a gradient of sperm to egg ratios. (B) Blue catfish sperm velocity (VCL) at 10 s when activated without ovarian fluid (fresh) and with ovarian fluid from six different channel catfish females.

UNLEASH AQUACULTURE GROWTH POTENTIAL IN LATIN AMERICA AND THE CARIBBEAN

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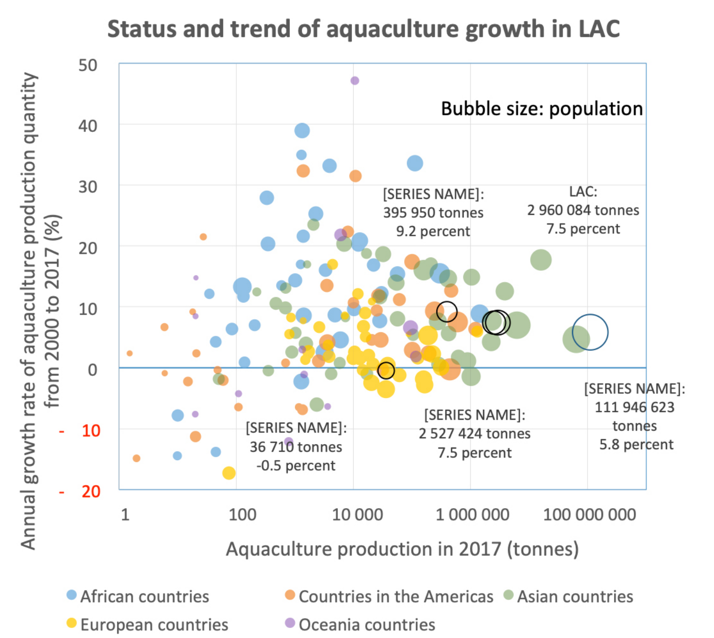
Aquaculture production in Latin America and the Caribbean (LAC) increased from 872 516 tonnes in 2000 to 2 960 084 tonnes in 2017; the 7.5 percent annual growth was faster than the 5.8 percent world aquaculture production growth, yet the growth varied in LAC sub-regions (see the chart on the right). LAC's 2.6 percent share of world aquaculture production was much lower than its share in world population (8.4 percent), world GDP (6.9 percent), world land area (including inland water surface; 15.2 percent), world inland water surface area (8.9 percent), and world renewable water resources (35.1 percent). LAC's 10 kg per capita fish consumption in the early 2010s was only half of the world average, and the region's population is expected to exceed 700 million in 2030, with 84 percent of the population living in urban areas. These supply- and demand-side factors bestow a great aquaculture growth potential in LAC. This paper examines the status and trends of aquaculture development in LAC (as well as sub-regions and major fish farming countries in LAC) against various socioeconomic and technical backdrops (e.g. geo-location, natural resources, population, income, food security, nutrition, health, fish consumption, fish trade and capture fisheries). The aquaculture growth potential in LAC is assessed from both the demand- and supply-side perspectives at the regional, sub-regional and national levels. Salient issues and potential way forward for unleasing aquaculture potential in LAC are discussed based on country experiences.



Data source: FAO. 2019. Fishery and Aquaculture Statistics. Global fisheries commodities production and trade 1976–2017 (FishStatJ).

SOCIAL AND ECONOMIC PERFORMANCE OF TILAPIA FARMING IN MEXICO

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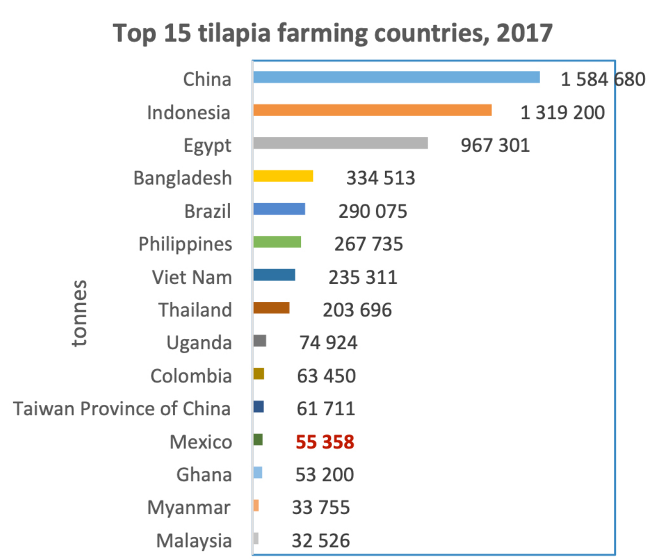
Tilapias are the 4th largest species group by both quantity and value in global aquaculture 2017 (18 tilapia species items farmed in 127 countries/territories with 5.9 million tonnes, USD 11 billion farmgate value of total production).[1] Tilapias are also a major international seafood commodity with nearly half million tonnes of global trade volume and USD 1.4 billion trade value in 2017. Global tilapia aquaculture production grew 11 percent annually (or 13 percent in terms of farmgate value) over the past three decades, from 0.3 million tonnes (USD 304 million) in 1987 to 5.9 million tonnes (USD 11 billion) in 2017. Aquaculture production in Mexico increased 9.3 percent annually (or 12 percent in terms of farmgate value), from 53 918 tonnes (USD 226 million) in 2000 to 243 307 tonnes (USD 847 million) in 2017. Tilapias are the 2nd largest aquaculture species group in Mexico (next to marine shrimps and prawns), contributing to 23 percent of the country's total aquaculture tonnage in 2017. Mexico was the 12th largest tilapia farming countries/territories in 2017 (see the chart above) with 55 358 tonnes of farmed tilapia production accounting for 0.9 percent of the world total. This paper assesses tilapia aquaculture and the value chain in Mexico by examining tilapia farming technology and practices, dissecting the tilapia value chain, evaluating the sector's social and economic performance, discussing the importance of proper governance to the sector development, and highlighting potentials, issues, constraints and challenges in the development of tilapia aquaculture in Mexico.

http://www.fao.org/3/ca5224en/CA5224EN.pdf



Data source: FAO. 2019. Fishery and Aquaculture Statistics. Global fisheries commodities production and trade 1976–2017 (FishStatJ).

HOALA LOKO IA: A STREAMLINED PERMITTING PROCESS FOR THE RESTORATION OF TRADITIONAL HAWAIIAN FISHPOND SYSTEMS

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The Hoala Loko Ia program was designed to streamline the state permitting process for the restoration of traditional Hawaiian fishpond systems (loko ia). The program was born in response to the advocacy of practitioners, who reported that it could take years, and sometimes decades, to secure all the necessary state and federal permits to repair the ponds.

A partnership of regulatory agencies, funders, and non-profit organizations was formed in 2012 to design a better process. With input from participants in Hui Malama Loko Ia, a statewide network of fishpond stewardship organizations, this effort addressed nearly three decades of concern and advocacy around this issue.

The completion of the Hoala Loko Ia involved extensive consultations with practitioners, scientists, resource agencies, and government officials. The program was implemented in 2015, and has resulted in 18 applications and approved projects, with an average processing time reduced from many years to under one month. Practitioners are now covered by an environmental assessment that provided scientific backing to the notion that traditional cultural practices have a positive effect on the environment, and a change in State law that acknowledged that the restoration of loko ia has a beneficial effect on water quality.

This presentation will share the process of multi-agency and public consultation to develop this system. It will explore how culture, science, and regulators worked together to overcome the many bureaucratic hurdles that stood in the way of the program's success. There are still regulatory burdens which impact fishpond restoration work, and we offer lessons learned and remaining areas of opportunity to ensure these significant sites continue feeding Hawaii communities long into the future.

YELLOW TANG (*Zebrasoma flavescens*) AQUACULTURE IN HAWAII; AN UPDATE ON COMMERCIALIZATION PROGRESS AT OCEANIC INSTITUTE

Chatham K. Callan* and Tom Bowling

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Yellow Tang (Zebrasoma flavescens) is the most heavily collected reef species from Hawaii with nearly 300,000 fish being removed from reefs annually for the aquarium trade. Recent legislation in Hawaii has temporally suspended the collection of aquarium species, pending the completion of a comprehensive environmental impact study. Therefore, this highly popular, and iconic, species will (at least for the foreseeable future) need to be obtained from aquacultured sources. In 2015, Oceanic Institute of Hawaii Pacific University (OI) was successful in overcoming the tremendous challenges culturing this important species. For the first time, the culture of Yellow Tang was shown to be technically possible, and this achievement provided significant hope that many other reef species might also be able to be cultured using similar methods. Over the past several years, this has indeed been shown to be the case, with dozens of new species being cultured by facilities around the world owing in large part to the technical achievements (the barriers being broken down) by OI.

The recent successful culture of several coral reef species for the marine aquarium trade has sparked renewed excitement and enthusiasm for this alternative, and perhaps more sustainable, supply of marine ornamental organisms. However, despite these exciting successes, commercial adoption of production methods remains low due to the limited efficiency of current production methods (low numbers of juveniles being produced at relatively high costs compared to wild supplies). This project seeks to address these challenges by building upon prior successes and in collaboration with Biota Aquariums, LLC aims to significantly improve the yield of Yellow Tang production. Improving the yield (egg to juvenile) will have the most profound effect on lowering total cost of production thereby greatly improving commercialization potential for this, and likely other Acanthuridae, species.

This presentation will provide an overview of current production methods in use (at OI) and highlight the current state of commercialization potential for this species. Specifically, we will review the current mean production yield experienced after one year of commercial-scale culture effort and review opportunities for improved production. A separate presentation will cover (in more detail) the specific areas of research we are pursuing in order to improve survival over several critical periods.

INCREASING SURVIVAL AND GROWTH IN LARVAL LEOPARD CORAL GROUPER (*Plectropomus leopardus*) USING INTENSIVELY CULTURED COPEPOD NAUPLII

Aurora I. Burgess, Chatham K. Callan* & Renee Touse

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Leopard Coral Grouper (*Plectropomus leopardus*) are a heavily exploited, high value fish commonly found in the Asian live reef food fish trade. In past decades, many attempts at mass culture of various grouper species have been undertaken, however their small mouth gape at first feed has resulted in very low survival when using traditional live feeds such as rotifers. Use of wild caught or extensively cultured copepods has yielded potentially promising increases in survival and growth, but overall survival to the juvenile stage remains low making mass culture currently impractical.

The current study sought to build on past developments in grouper culture and recent advancements in copepod culture technology by observing how growth and survival were influenced by the addition of intensively cultured copepods to the early diet of *P. leopardus* larvae. Six tanks of larvae (n=3), were fed either eggs and nauplii of the calanoid copepod *Parvocalanus crassirostris*, at a starting density of 5 mL-1, and the rotifer *Brachionus rotundiformis*, at a starting density of 10 mL-1, or were fed only *B. rotundiformis*, at a density of 15 mL-1, starting on the evening of 2 days post hatch and continuing until 9 days post hatch. After this initial period, all larvae were fed the same diet of rotifers, *Artemia*, and dry feed until the cessation of the trial at 21 days post hatch. Larvae fed *P. crassirostris* in addition to rotifers had significantly higher survival, 9.9% vs. 0.5%, than those fed only rotifers. Growth was also significantly enhanced in larvae offered copepods. Larvae fed rotifers were on average 1.5 mm shorter at 21 days post hatch than those that had been fed copepods. More rapid development and earlier onset of flexion were also noted in larvae that were offered copepods in their diet.

The use of intensively cultured copepods, in this study, increased survival tenfold over previous studies with *P. leopardus* larvae fed wild caught copepods. The application of intensively cultured copepods to the early diet of *P. leopardus* along with future research to evaluate late stage mortality issues may facilitate commercial production of this species.

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ENGINEERING PARAMETERS OF OYSTER AQUACULTURE SYSTEMS

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Previous models for filtration of oyster beds were useful in their simplicity of gathering data in the field but completely disregard any other effects that could be attributed to turbulent diffusion. This would explain some of the previous discrepancies in hydrodynamic effects to oyster filtration noted by others (Grizzle et al. , 2008; Grizzle et al., 1992; Harsh and Luckenbach, 1999; Lenihan et al., 1996). Forsyth (2014) noted in her numerical mass balance model that the inclusion of diffusion had a significant impact on the profile of seston concentration across an oyster reef. This result validated previous observations of particle gradients and food limitation across reefs (Lenihan, 1999).

Upweller aquaculture systems are particularly influenced by flow that regulates food, oxygen, and water quality within the bed of oysters being cultured. Upweller systems have reported superficial velocities that range from 0.5 to 7.1 cm s-1. In practice, higher current velocities are desirable because they increase delivery of food to the oysters, improve water quality, and enhance dispersal of biodeposits. This presentation summarizes the findings of previous studies and provides preliminary results of hydrodynamic model considering effects on feeding of juvenile oysters within a packed bed. This model relates axial diffusion and packed bed reactor theory to the oyster aquaculture system.

A series of experiments were conducted using upflow tubes constructed from 2-inch (5 cm) polycarbonate tubes packed with juvenile oysters (1-2 cm) that were subjected to 6 superficial velocities (0.5, 1, 2, 4, 8, 16 cm s^{-1}). The upflow tubes were designed to create a predictable and repeatable flow within a porous packed bed of solid material. The tubes had sampling ports along the porous bed zone which double as ports for measurement (i.e. differential pressure). The results from the physical and physical-biological experiments were used to calibrate axial dispersion models developed from packed bed reactor theory. The development of the axial dispersion parameters will allow for the design and optimization of upweller aquaculture systems. This presentation summarizes the findings of previous studies, details experiment methodology, model development and calibration, and provides results of engineering parameter calibration for juvenile oysters within a packed bed.

INSECT MEAL: A POTENTIAL ALTERNATIVE FOR AQUACULTURE FEEDS

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Feeds still represent the largest expense for many aquaculture operations. At the same time, they are a critical component of the production as it has the potential to improve feed and economic conversion ratio, growth, survival, egg quality, size variation and other key parameters. Wild populations of forage fish will not be able to satisfy the growing demand for fish meal, a main ingredient in feeds, and thus novel alternative meals are being researched, developed and implemented to reduce fish meal inclusion while maintaining optimum production performances. Insect meal is gaining a considerable amount of attention as a potential alternative protein source in feeds. The insect that is of interest in this proposal is the black soldier fly (*Hermetia illucens)*. Already being commercially produced in the USA, France, Germany and China to name a few, the larvae of this insect allows for greater protein production per area than other protein systems, requires little not no added water, feeds on a wide variety of organic materials ranging from plant to protein waste and trimmings and animal manure, and can be produced in a closed and indoor system with complete control over its life stages. Research on black soldier fly larvae meal inclusion in feeds has been carried out for tilapia (*Oreochromis spp.)*, catfish (*Ictalurus punctatus & Pelteobagrus fulvidraco*), Atlantic salmon (*Salmo salar*)*,* Pacific white shrimp (*Litopenaeus vannamei)*, turbot (*Psetta maxima)*, European seabass (*Dicentrarchus labrax)*, barramundi (*Lates calcarifer)*, rainbow trout (*Oncorhynchus mykiss)*, omega perch (*Therapon barcoo*), Jian carp (*Cyprinus carpio)* and gilthead seabream (*Sparus aurata)*. Additional key research has been done to increase the levels of omega-3 fatty acids in the meal by feeding fish offal (*Oncorhynchus mykiss)* and brown algae (*Ascophyllum nodosum)* to the larvae. In this paper we intend to summarize the current status of black soldier fly larvae (*Hermetia illucens)* meal inclusion in aquaculture feeds and its effects on production performances.

TESTING PERFORMANCE OF FISH FREE RABBITFISH *Siganus lineatus* FEED IN PALAU

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Aquaculture is quickly becoming more widely accepted and developed as a means of efficiently and sustainably producing protein for a growing population. Some countries already have a strong aquaculture industry and are investing in fine tuning and perfecting their techniques and practices; other countries have a young industry which is still in development with considerable potential. Island countries like Palau have limited resources to produce food on the islands and reach a stable level of food security. This is why aquaculture is currently being developed in Palau through a joint effort between The Nature Conservancy and the Bureau of Marine Resources. Golden lined rabbitfish (*Siganus lineatus*) are currently being farmed in Palau in floating ocean cages and ponds due to high demand for human consumption which has led to dwindling wild stocks. The feed that is being used for rabbitfish in Palau is actually intended for milkfish (*Chanos chanos*) and it contains fish meal. As aquaculture adopts more sustainable practices, alternative sources of protein are being researched and implemented into feeds to reduce the overall environmental impact. At the same time, the fish nutrition sector is also adapting by developing feeds specialized for specific species rather than general fish categorizations. This paper will investigate the feed performance of four experimental diets for rabbitfish that do not contain any fish meal but rather alternative sources of proteins. Key performance indicators like growth, feed conversion ratio and protein efficiency ratio will be presented comparing these four diets with the commercially used milkfish feed. In the first trial, the two experimental diets that were tested had similar feed conversion ratios than the control, while in the second trial the other two experimental diets had a better performance across all treatments. These results show that a feed for rabbitfish could be produced without the use of fish meal while at the same time increasing growth performances.

CULTURE OF LOGPERCH *Percina caprodes* FOR MUSSEL CONSERVATION PROPAGATION

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Interest in logperch culture has recently increased due to the fish's use as a host species for propagation of the endangered snuffbox mussel (*Epioblasma triquetra*). Logperch are widely distributed throughout both the Mississippi River and Great Lakes basins. Collection of wild-caught fish is an alternative to propagation but could negatively impact logperch populations and introduce disease concerns to the propagation facility. Although an abundance of information is available on the natural spawning habits and life cycle of logperch, little information is present on logperch propagation.

We conducted various experiments to propagate this species in both indoor laboratory and earthen pond environments. Experiments performed at the Columbia Environmental Research Center examined pond culture, feed preferences, spawning techniques, out-of-season spawning manipulations, egg incubation, and larval grow-out trials.
Successful propagation of larvae occurred in both indoor and pond settings. Indoor culture utilized induced spawning via temperature manipulations. This method allowed for multiple spawning events and greater control to produce abundant, high quality, and known-age embryos and larvae. However, larval transition to exogenous feed sources indoors proved to be a bottleneck in producing large numbers of juveniles.

In contrast, pond culture offered a successful method for raising larvae into adulthood, either through natural parental spawning or by stocking swim-up fry that were first propagated indoors. Besides the reduced amount of labor, pond propagation promoted maturation of embryos to reproductive adult in one year. Additionally, pond-raised adults could easily be brought into the labs and acclimated to prepared diets prior to being used for mussel glochidia inoculations. A downside of pond culture is reduced ability to track fish quantity and the potential to transfer invasive species or pathogens into the hatchery environment from the pond.

In summary, the ability to propagate logperch in a controlled environment provides a high-quality source of healthy host fish that are essential for successful mussel propagation.

EFFECT OF EMAMECTIN BENZOATE ON EXPRESSION AND TISSUE DISTRIBUTION OF MULTIDRUG RESISTANCE PROTEINS IN THE DIGESTIVE TRACT OF *Caligus rogercresseyi*

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Caligus rogercresseyi ("*Caligus*"), is a copepod ectoparasite that strongly affects massive salmon farming of Chile, and like the northern hemisphere sea lice, *L. salmonis*, currently causes millionaire losses in this industry. Pharmacological treatments against this infestation are based on the use of insecticides, such as emamectin benzoate (EMB), which is delivery orally in food. Due to the increasing drug resistance developed by the parasite since 2007, the effectiveness of EMB has decreased significantly. The aim of this study was to characterize the effect of treatment with EMB on protein expression levels and tissue distribution of two proteins implicated in drug resistance processes, P-glycoprotein (Pgp) and Multidrug resistance-associated protein 1 (MRP1), in the digestive tract of *Caligus*.

Specimens of *Caligus* were collected from rainbow trout (*O. mykiss*) grown on farms in southern Chile, and transported to the laboratory. After 24 h, culture plates with 10 male or female lice, were exposed to seawater (control) or 300 ng/mL EMB in seawater by 24 h. The survivor specimens were fixed in p-formaldehyde for the immunohistochemistry (IHC) and immunofluorescence (IF) analysis, or submerged in RIPA buffer and frozen at -20 ºC for Western blot analysis.

Figure 1A shows the tissue distribution of Pgp and MRP1, both in female (top) and male (below), but with higher protein expression levels in females (Fig. 1A). Treatment with EMB caused an increase in the expression levels of both proteins, in a relatively proportional way, maintaining the highest response observed in females (Fig. 1B).

These data support the notion that drug resistance proteins, such as Pgp and MRP1, could be involved in the drug resistance of *Caligus* to antiparasitic drugs, by affecting the intestinal absorption of these drugs. This information generates fresh perspectives in the search for new strategies to combat this deleterious parasite.

Funding: Fondecyt 1150934 & FONDAP 15110027, Chile.

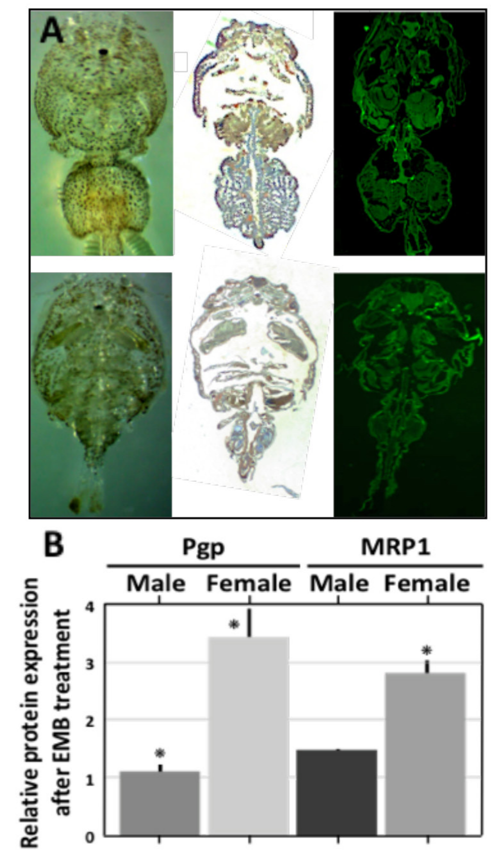

Fig 1. Effect of EMB on the expression of P-gp and MRP1 in *Caligus rogercresseyi*, measured by IHC and IF (A); Western blot (B).

THE APPLICATION OF PROBIOTICS IN FEED AND TANK WATER TO IMPROVE THE HEALTH AND SURVIVABILITY OF *Litopanaeus Vannamei* POST LARVAE IN HATCHERY PRODUCTION

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The shrimp industry has historically depended on hatcheries for quality post larvae (PL) that are disease free to achieve successful production. As stocking densities increase and growing conditions become more challenging, there is a growing need for PL that are also faster growing with increased resistance to unpredictable environmental stressors such as; temperature, salinity, and pH changes. Further, since shrimp do not grow in a sterile environment, problematic pathogens are always present in pond systems representing continual disease potential. This leads to concern that some of the disease challenges faced during production may also be the result of PL being more susceptible to disease due to weakened immune systems caused by these stressors.

Several proprietary probiotics are showing benefits improving the health and vigor of both PL and shrimp during production, increasing their survivability and resistance to environmental stressors. Commercial hatchery testing in Vietnam and China has been completed using strains of lactobacilli and bacilli as feed and water treatment additives that show PL with improved health/vigor and survivability (Table 1). These PL also score significantly higher ($p<0.05$) on the FAO (Food and Agriculture Organization of the United Nations) criteria for 1) Hepatopancreas (HP) Lipid count, 2) Intestinal content, 3) HP color, 4) Muscle to gut ratio, 5) HP condition, and 6) Size variation.

TABLE 1. PL Survivability Results from four Vietnam commercial hatcheries using probiotics.

Hatchery	Experimental Detail	% Survivability Control Treatment	% Survivability Probiotic Treatment
VN-SV	• $7m^3$ tank; 9 tanks • 0.5 million nauplii per tank • Harvested at PL10	61%	71%
VN-SL	• $5m^3$ tank; 4 tanks • 1 million nauplii per tank; • Harvested at PL5	54%	87%
VN-TH	• $6m^3$ tank; 10 tanks • 2 million nauplii per tank; • Harvested at PL7-12	52%	61%
VN-NMT	• $4m^3$ trial tank; 10 tanks • 0.8 million nauplii per tank • Harvested at PL10-12	54%	65%

THE USE OF PROBIOTICS IN FEED AND POND WATER TO SUPPRESS THE GROWTH OR ACCUMULATION OF *Vibrio Parahaemolyticus* AND OTHER *Vibrio* SPECIES IN *Litopanaeus Vannamei* SHRIMP

Richard Carpenter*, Jo Ella Barnes, Jack K. Crockett, Sim Sih Yang, Loc H. Tran, Josh Ison, Chris Kitts, Addison Lawrence

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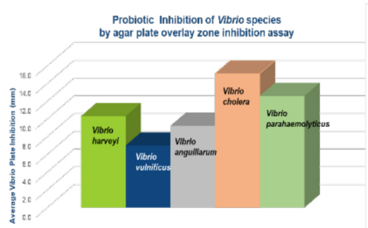
Disease pressure in all stages of shrimp production continues to be an escalating threat to the industry, particularly in Asia. *Vibrio parahaemolyticus* is responsible for Early Mortality Syndrome / Acute Hepatopancreatic Necrosis Disease (EMS/AHPND), but other species of *Vibrio* also represent problematic pathogens contributing to various conditions related to "vibriosis". As shrimp production is not conducted in a sterile environment, these pathogens are always present, though not always virulent. Pathogenicity is connected to their ability to concentrate in key organs, and once reaching critical levels, become virulent. Antibiotics have been used historically to try and control these effects. While it is debatable whether antibiotic use applied to pond systems is an effective treatment, there is growing recognition that their benefits are outweighed by concerns to eliminate their use.

Probiotics are being evaluated as an alternative mode of action to antibiotic use. Research is now showing that the use of select probiotics in aquaculture can suppress *Vibrio* growth in pond water, reduce their accumulation in the gut track and exoskeleton, and prevent problematic species reaching critical virulent concentrations. Laboratory testing using proprietary blends of lactic acid bacteria and bacilli show that select strains of these species can broadly suppress *Vibrio* growth (Figure 1). Growth experiments confirm the lack of accumulation of *Vibrio* in both PL (PL10) and juvenile shrimp (0.5-1.5 g). These results are supported by repeated *Vibrio* challenge studies. These studies show that shrimp raised on a diet that includes these probiotics in feed and applied to pond water improves their survivability after exposure to lethal levels (10 days) of *Vibrio parahaemolyticus* (Figure 2) in both juvenile shrimp and hatchery produced PL10. (Figure 3).



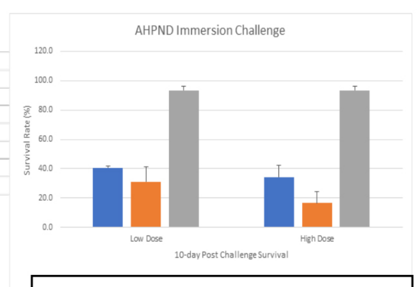


Figure 2: Percent survival of shrimp (O.5-1.5g) immersed in lethal doses of *V. parahaemolyticus* at a low dose ($3x10^5$ cfu/mL) and a high dose ($8x10^5$ cfu/mL) for 10 days.

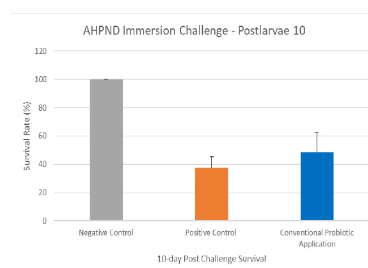


Figure 3: Percent survival of Post Larvae (PL10) shrimp immersed in lethal doses of *V. parahaemolyticus* ($3x10^5$ cfu/mL) for 10 days.

AQUACULTURE IN THE CLASSROOM: THE TROPICAL AQUACULTURE LABORATORY'S YOUTH EDUCATION PROGRAMS

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The University of Florida's Tropical Aquaculture Laboratory (TAL) is dedicated to helping K-12 schools teach aquaculture in their classrooms. This is done through a multitude of avenues including system design and construction, procuring supplies and aquatic animals, curriculum building, test evaluation, and more. The goals of these programs is to provide access to aquaculture for those individuals that desire it as well as exposing students to the realities (and myths) of aquaculture production. The Florida FFA Career Development Event (CDE) and the Florida Aquaculture Association's (FAA) Aquaculture Certification are two programs we are involved with that help fulfill that mission.

The TAL hosts the Florida FFA CDE. Middle and high school FFA aquaculture teams participate in a pre-test to qualify for this state competition held each spring. Four member teams from the top-scoring schools in the state travel to TAL for the competition. Teams rotate through three practicums and a written exam before making a team presentation on a given topic. The purpose of the Aquaculture CDE is to stimulate student interest in the aquaculture industry, encourage aquaculture instruction in the agricultural education curriculum, and to provide recognition for those who have demonstrated skills and competencies in the area of aquaculture management.

The FAA Aquaculture Certification program is designed to grant entry level certification into the industry and increase the use of aquaculture curriculums taught in high schools. The TAL works closely with FAA to ensure that the test adequately addresses aquaculture issues that are important to Florida and the US and annually validates the accuracy of the test itself. In addition, TAL helps schools prepare for issues that will help them do well on the certification test and when they segue into the aquaculture industry.

This talk will discuss details associated with both of these programs as well as cover other activities that help TAL expand K-12 aquaculture knowledge within Florida.

NMR-BASED METABOLOMIC EVALUATION OF A FORMULATED FEED IN JUVENILE YELLOWFIN TUNA *Thunnus albacares*

Fabio Casu*, Aaron Watson, Alejandro Buentello, Daniel W. Bearden, Juan Sierra, Daniella Acevedo and Michael R. Denson

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Nuclear Magnetic Resonance (NMR)-based metabolomics is an emerging analytical technology that allows for the evaluation of potential differences at the molecular level induced by various environmental factors including diet in an organism's tissues and biofluids. Currently, metabolomic studies on tuna are extremely sparse, which limits our understanding of physiological responses of this species to alternative feeds in tuna ranching. A 13-week feeding trial with juvenile yellowfin tuna (YFT) was conducted at the Achotines Laboratory in Panama in two concrete tanks arranged as a semi-closed recirculating system. The effects of an alternative diet on YFT metabolite profiles were evaluated at the conclusion of the trial.

The formulated feed contained ~ 22 % total crude protein and ~ 17 % total crude lipid. A diet composed of baitfish (squid, *Loligo opalescens*, and Pacific thread herring, *Opisthonema spp*; 50:50) was used as a control.

Tissues (liver, muscle, intestine) and plasma samples were collected at the end of the feeding trial to evaluate possible differences in metabolite profiles between the experimental group (n=6) and the control group (n = 3). Polar extracts of each tissue were analyzed by NMR spectroscopy. Results from the NMR-based metabolomic analysis of these samples (Fig. 1) indicate a high similarity in metabolite profiles between tuna fed baitfish and those fed the compound (CPD) diet.

Differences between treatments included amino acids, organic acids, TCA cycle intermediates, biogenic amines, metabolites associated with oxidative stress, and other metabolites including feed additives. Some of these metabolites were clearly of dietary origin, since they were also detected at significant levels in feed extracts. However, non-dietary metabolites were also shown to vary according to the specific dietary treatment, possibly indicating significant effects on YFT metabolism.

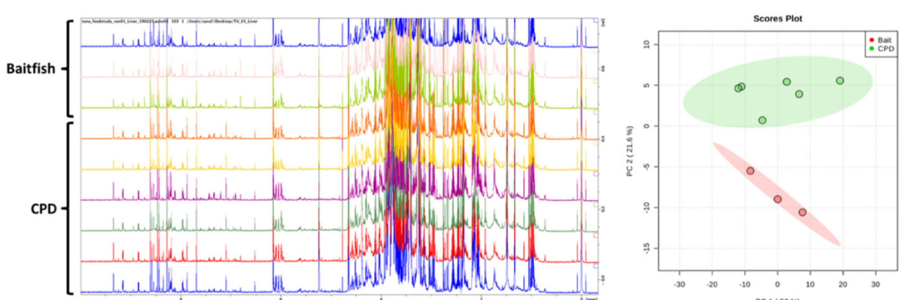

Figure 1. ^{1}H-NMR spectra of liver tissue extracts (polar extracts) and Principal Component Analysis (PCA) score plot for the different dietary treatments (baitfish and CPD diets). Bait: n=3; CPD: n=6.

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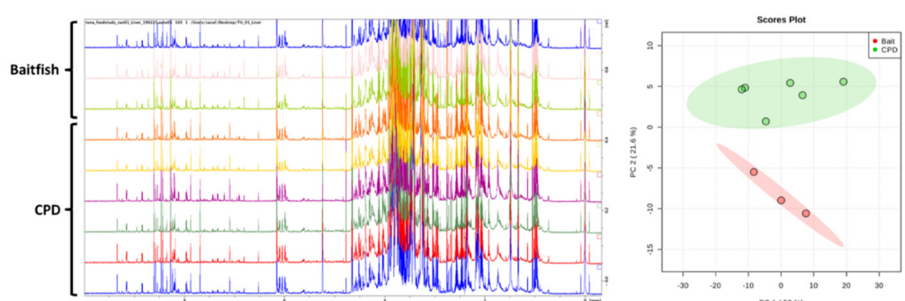

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ESTROGENIC CHEMICAL POLLUTANTS AFFECT GROWTH AND REPRODUCTION-RELATED GENES IN MALE MOZAMBIQUE TILAPIA *Oreochromis mossambicus*

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Accumulation of waste products and chemical pollution in aquatic environments is detrimental to humans and aquatic life. Many of these pollutants, termed endocrine disrupting chemicals (EDCs), often affect the endocrine system of organisms that become exposed. EDCs released into the environment through anthropogenic activities can have short-term and/or long-term sustained developmental and physiological effects on fish. While tilapia species have been widely studied with respect to how environmental conditions impact growth and reproduction, little is known on how tilapia and other tropical fish species respond to EDC exposure. Our present study aims to determine the long-term physiological effects of early-life exposure of tilapia fry to nonylphenol (NP), an environmentally pervasive byproduct of plastic production that mimics the actions of estrogens.

We exposed Mozambique tilapia fry to waterborne NP (10 and 100 μg/L) for 21 days. 17**β**-estradiol (E2; 0.1 and 1.0 μg/L) was used as a control. After the exposure period, juveniles were reared for an additional 112 days without chemicals until males were sampled. We found that hepatosomatic index was decreased by exposure to NP (100 μg/L), indicating an adverse effect on liver metabolism. Gonadosomatic index was elevated in fish exposed to E2 (0.1 μg/L). E2 and NP stimulated hepatic estrogen receptor (*erα* and *erβ*) mRNA expression but did not affect the expression of *vtg* transcripts (Figure 1). Furthermore, the somatotropic axis of adult tilapia was impacted by early-life exposure to NP and E2. Hepatic mRNA levels of growth hormone receptor (*ghr*), insulin-like growth factor 1 (*igf1),* and igf-binding proteins (*igfbps)* were affected by exposure to E2 and NP. We conclude that tilapia exposed to E2 and NP as yolk-sac fry exhibit subsequent changes in the endocrine systems that control growth and reproduction during their adult life.

[Supported by HATCH (#HAW02051-H), NOAA/ UH-Sea Grant (#NA14OAR4170071, R/SB-18), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01) and NSF (IOS-1755016 and -1755131)]

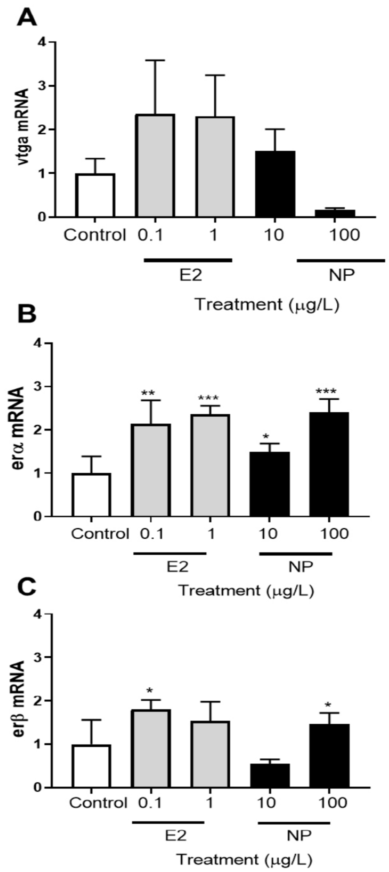

Fig. 1. Hepatic *vtga* (A), *erα* (B), *erβ* (C) mRNA levels in Mozambique tilapia adults 112 days after exposure as fry to water containing 0 (control), 0.1 µg/L or 10 µg/L E2 or 10 µg/L or 100 µg/L NP. mRNA levels are presented as fold-change relative to the control group. Asterisk indicates significant difference between treatment and control group.

SEX, SALINITY, AND SAMPLING PERIOD MODULATE GROWTH HORMONE EXPRESSION IN MOZAMBIQUE TILAPIA *Oreochromis mossambicus*

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Tilapia has become a widely farmed finfish, in part due to its tolerance to a wide range of environmental conditions and its sexually dimorphic growth. Growth in vertebrates, including teleosts, is largely controlled by growth hormone (GH). In tilapia, environmental salinity directly modulates growth through the activation of GH and associated growth factors. In our previous study, we found that tilapia reared in a tidally-changing salinity regime (TR) grew faster than fish reared in steady-state fresh water (FW) or seawater (SW). Moreover, pituitary *gh* mRNA expression was found to be a better indicator of body weight than circulating GH and insulin-like growth factor 1 (IGF-1). It is unknown, however how salinity regime may influence the sex-specific regulation of growth. The objective of this study was to determine the integrated effects of salinity regimen and daylight period on pituitary *gh* mRNA expression in male and female Mozambique tilapia.

Tilapia adults were reared in FW, SW and TR, which is characterized by salinities that change between FW and SW every six hours, over a 24 h period. *gh* expression was greater in fish reared in SW and TR compared with those in FW, in both sexes. Pituitary *gh* expression was also higher in fish sampled during dark hours, compared with those sampled in daylight hours. Sex-specific differences in pituitary *gh* expression was only observed in fish reared in SW and TR. These results indicate that sex-specific patterns in *gh* expression is modulated by salinity regime and daylight period in Mozambique tilapia. [Supported by HATCH (#HAW02051-H), NOAA/ UH-Sea Grant (#NA14OAR4170071, R/SS-12), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01) and NSF (IOS-1755016)]

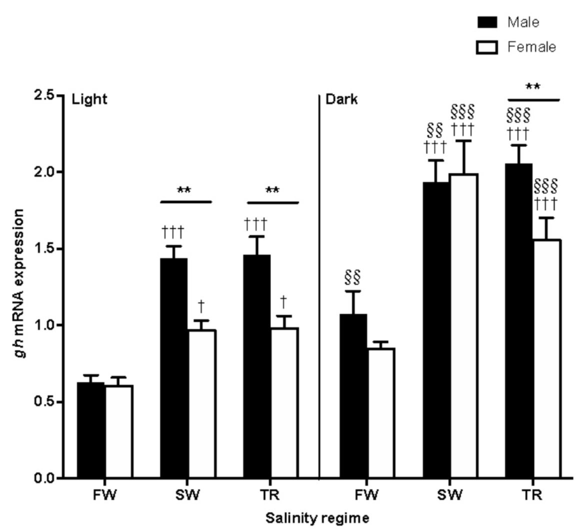

Fig.1. Pituitary gene expression of *gh* in male (black bars) and female (white bars) Mozambique tilapia reared in freshwater (FW), seawater (SW) and tidally-changing salinities (TR), and sampled during light and dark hours. **Significantly different from males at $P < 0.01$. †, ††† Significantly different from FW period at $P < 0.05$ and 0.001, respectively. §§, §§§ Significantly different from the daylight sampling.

A NOVEL ZEBRAFISH MODELLING FOR EVALUATING THE TOXICITY OF FOOD ADDITIVES

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As known that food additives have been using for extending the shelf life of foods, for aroma and colour enhancer, to improve the structure and cooking properties of food, and to maintain and improve nutritional value. More than 10.000 chemicals are allowed to be added to food and food contact materials including fish food. Although an estimated 1.000 chemicals are used under a GRAS designation process, the others are only available on the basis of the Acceptable Daily Intake (ADI) values. The authorities/commissions authorized to determine the ADI value have continuously been opening the calls for toxicological data to re-evaluate the values and have published many re-evaluation reports. Some of these reports contain advice regarding the ADI values which should be re-evaluate and re-determinate continuously with new technological and current methods. The reports containing such recommendations raise the question of whether these values, which could affect the health of millions of people, are actually determined at the right limits. Here, telling about a new zebrafish modelling for evaluating the toxicity of food additives. Zebrafish embryos use only eggs with reserves of fat droplets in the yolk as a food source during the first five days of embryonic development. These animals, which are forced to live dependent on yolk, can be defined as "closed systems" in which food intake is not influenced by outside. During that time, adding any additive to yolk sac (to food) via microinjection, make these animals be a good model for detailed evaluating side effects or ADI limits of additives.

THE NEW THREAT FOR AQUATIC LIFE: NANO PLASTICS

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Plastics have low density, longevity, excellent barrier properties and relatively low cost, due to these features they are ideal materials for a wide range of manufacturing and packaging applications. According to records, a continuously increasing global annual production of plastics have around 300 million t and this total continues to grow at about 4 % per year. Increasing use of plastics comes together with them the problems of waste management and recycling practices. It is suggested that uncontrolled disposal of litter along with poor waste management and recycling practices is the main reason for the accumulation of environment. On the other hand, studies have shown that the main sources of nano-sized plastics are (i) degradation process of plastic which is the mass source, (ii) personal care and cosmetic products and (iii) producing process of engineered plastics. Due to the chemically linked by weak secondary bonds or by physical interaction of polymers, eventually, nano-sized particles from any size of the plastic would spread to the transient environment. The worrying part is that 'smaller particles are generally more toxic than the corresponding bulk material at the same mass concentration'. Furthermore, mechanistic parts of some studies have shown that nano-sized plastic particles can transport through the food web, translocate between organs in the body and transfer from mothers to offspring. In vivo studies have shown that nano-sized plastic exposure has resulted in bioaccumulation within the body even brain (from our recent studies), leading to oxidative DNA damage in the brain regions where it bioaccumulates (from our recent studies), compromising immune responses, induction liver lesions and ultimately affecting behavior, physiology and metabolism. Here, based on current publications in literature and our recent studies, I would try to introduce danger dimensions of plastic wastes and nano-sized particles released from the wastes and other sources.

OPPORTUNITIES FOR MULT-TROPHIC AQUACUTLURE IN HAWAII AND THE PACIFIC

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Integrated multi-trophic aquaculture (IMTA) systems have been explored and demonstrated on land and at sea. The basic concept mimics that of the food chain where a waste product from a higher trophic species such as fish, is bioextracted and assimilated by lower trophic species including bivalves and marine plants.

The University of New Hampshire (UNH) has been evaluating a marine IMTA raft that has brought steelhead trout, kelp, and mussels to the marketplace (Fig. 1). A nitrogen (N) mass balance model was utilized for the project. Nitrogen input via fish food was calculated and compared to N retained in the fish tissue at harvest and N extracted by mussels and kelp cultured around the fish net. The results suggested a 1:3 ratio of higher trophic to lower trophic species for a N neutral balance when the organisms are harvested and removed from the environment.

More recently, UNH was funded by Sea Grant to build a second generation, commercial scale IMTA platform called the AquaFort (AF). The two-year program will recruit fishermen and farmers from ME, NH and MA to participate in workshops and daily operations of the AF farm with production capacity of 20,000 kg. The goal is to increase safe and responsible seafood production in the US.

This concept will be explored for Hawaiian waters with local finfish, bivalve and macroalgae species. Permitting, site selection, culture structure, farm operations and ecotourism will be discussed as a potential business model for Pacific Islands.

Figure 1. Prototype integrated multi-trophic aquaculture raft developed by the University of New Hampshire to culture, steelhead trout, blue mussels and sugar kelp.

ADVANCING US MARINE AQUACULTURE AT THE UNIVERSITY OF NEW HAMPSHIRE

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The University of New Hampshire (UNH), through programs like the School of Marine Science and Ocean Engineering, NH Sea Grant, and the College of Life Sciences and Agriculture, has a long-standing history of innovative marine aquaculture research, demonstration sites (both at research and commercial scales), and working with industry partners. These activities mostly have occurred at the Judd Gregg Marine Facility Complex in New Castle, NH which consists of the Coastal Marine Laboratory (CML) and the Marine Research Pier (Pier), as well as at nearshore and exposed, offshore demonstration aquaculture farms in the Gulf of Maine.

The CML is a marine laboratory facility with both wet and dry lab infrastructure for faculty, graduate, and undergraduate student research projects. The wet lab, with full strength seawater capabilities, ultra violet filtration systems, and a variety of supporting infrastructure, contains tanks from 3 L to 7,570 L, both on flow-through and re-circulating systems. Ambient water temperature can range from 0 to 20˚C. More than 30 cold water, marine species have been cultured at the CML. Current aquaculture research in the CML is focused on producing and promoting lumpfish as biological delousers in US salmonid farms.

UNH is a leader in aquaculture research in high-energy, offshore environments. The Pier is the staging operations center for the UNH vessels and open ocean aquaculture projects, including the steelhead trout farm (AquaFort), offshore kelp farms in NH and ME, as well as *in-situ* experimental cage systems and nursery pens beneath the pier. Our successes are due, in part, to the wide range of experts at UNH, including ocean engineers, marine biologists, ecologists, and oceanographers, who collaborate and participate in public-private partnerships. Results of the projects are communicated through extension and outreach activities. In addition, UNH provides guidance and assistance in acquiring state and federal permits necessary to conduct offshore research and operations in the Gulf of Maine.

BUILDING PATHS TO ADDRESS LOOMING RETIREMENTS IN THE AQUACULTURE EXTENSION WORKFORCE THROUGH EDUCATION PARTNERSHIPS

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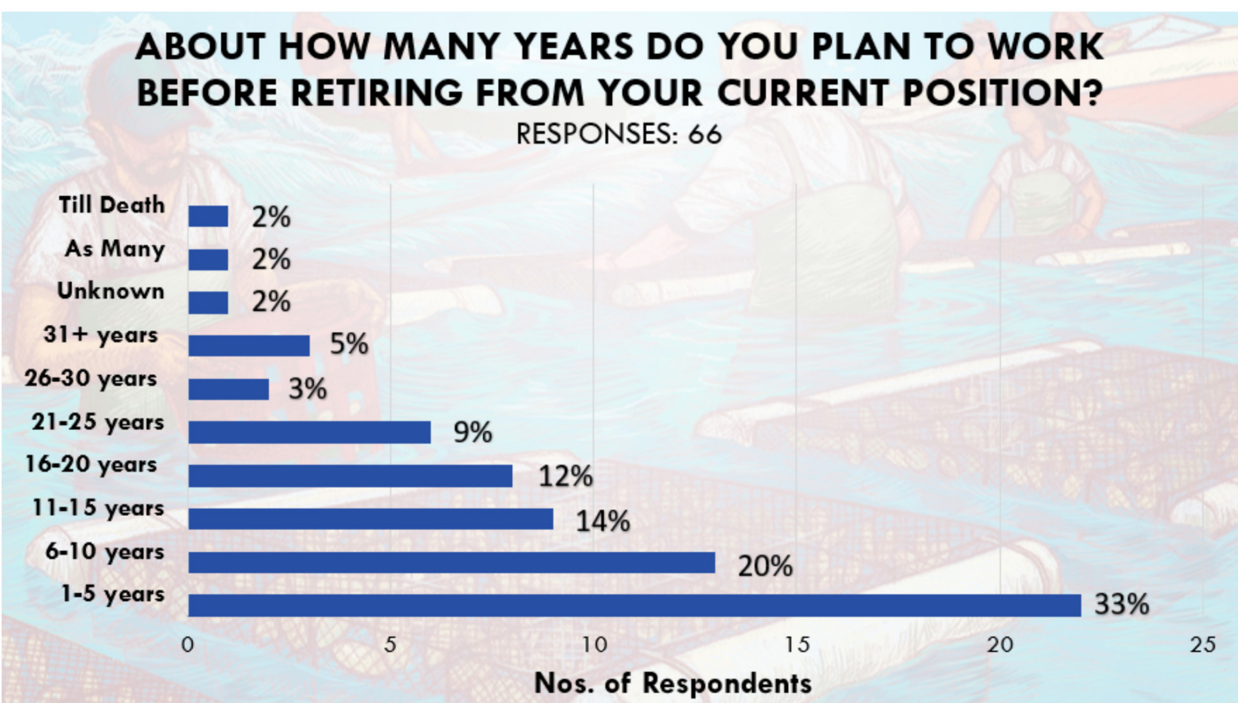
A 2019 survey of Land and Sea Grant Aquaculture Extension professionals by Faculty with Oregon Sea Grant, Kentucky State University and the University of Idaho revealed that a generation of aquaculture extension professionals (specialists, educators, agents and administrators) in their 50s to 70s (nearly half the estimated current workforce) plan to retire in the next 10 years. Their departure could leave a skills gap if not addressed before this becomes a crisis. The projected departure of over half of the surveyed aquaculture professionals creates an urgency to retain, increase professional development opportunities and attract people into the sector, particularly when the US government is boosting research and partnerships funding to help increase USA capacity to culture, process and market farm fish, shellfish and seaweed. The survey found that a nearly a third of the aquaculture Extension workforce are middle-aged (35-50 years-old) but, only have five or fewer years of experience in the field. Twenty-five percent of the survey respondents are women, with the majority self-reporting as between 35 - 50 years-old. Survey participants emphasized a need to define aquaculture as a career that goes beyond traditional seafood farming. That aquaculture is increasingly complex and integrates the sciences, the art of seafood culture and human behaviors. The survey revealed that aquaculture extension professionals are increasingly asked to develop programs that aid in navigating regulations, planning, business and understanding human perceptions. Therefore, Extension professionals are needed from other fields to serve as specialists and as part of integrated teams. To attract new hires, the aquaculture profession needs to emphasize the variety of skills and employment options that a career in aquaculture affords beyond traditional oyster and fish farming. For example, law, business, planning, engineering, research, water resources, restoration ecology, education, social science, food science, pharmaceutical development, sustainability specialist, those versed multitrophic food systems, environmental science, social media and occupational health are part of a growing field of skills and professionals needed in aquaculture. This requires the integration of many disciplines and thus affords options for a breadth of aquaculture- based job opportunities.



(Continued on next page)

Increasingly, Sea Grant and Land Grant Educators around the nation are being asked by schools, science centers, agencies and Extension to develop partnerships in learning about and from aquaculture. A joint effort between Sea Grant (SG) and Land Grant (LG) Education and Extension to leverage one another's expertise can address some of the critical pending workforce demographic needs and skill gaps revealed in the 2019 survey. This joint effort includes educating, training, problem solving and mentoring a workforce better prepared to enter and be successful in aquaculture and other marine related fields. Extension has built long standing trust through science-based aquaculture learning programs for stakeholders. Aquaculture extension specialists bring keen awareness of stakeholder needs, including traditional and emerging knowledge and skill requirements for entry-level to mid-career and senior positions. Educators' bring expertise in curriculum correlation and development, innovative tailored learning and the ability to scaffold learning to ensure STEM foundations such as science, mathematics and communication skills. Educators also have strong relationships with aquariums and informal learning centers who work together in providing lifelong learning addressing aquaculture messaging and experiences. Thus, Educators can offer powerful partnerships with aquaculture Extension specialists. SG and LG Extension and Education can use their partnership building opportunities in schools, industry, public agencies and NGOs. These partnerships also present opportunities to: 1) collaborate in learning and addressing negative public perceptions on some aspects of aquaculture and 2) use aquaculture-based learning as a mechanism to better serve and increase the role of those underserved and underrepresented population in STEM fields.

Aquaculture Education and Extension can and will need to intersect to foster the exchange of knowledge, especially in the view of the high percentage of retirement from the workforce. As the National Research Council's, 2000 report indicates, "Although employment opportunities in academic research institutions are decreasing in many scientific fields, the demand for scientists in government, industry, and teaching is outpacing the availability of qualified individuals." This seems to be specifically relevant to aquaculture and brings to the forefront the question of how we ensure that the pathways for aquaculture Extension and Education workforce professional development and students to pursue aquaculture are available and inviting.

PRESENCE OF MULTIPLE COPIES OF THE VITELLOGENIN GENE IN *Fennerpenaeus merguiensis*: EVIDENCE FOR GENE EXPANSION AND FUNCTIONAL DIVERSIFICATION IN SHRIMP

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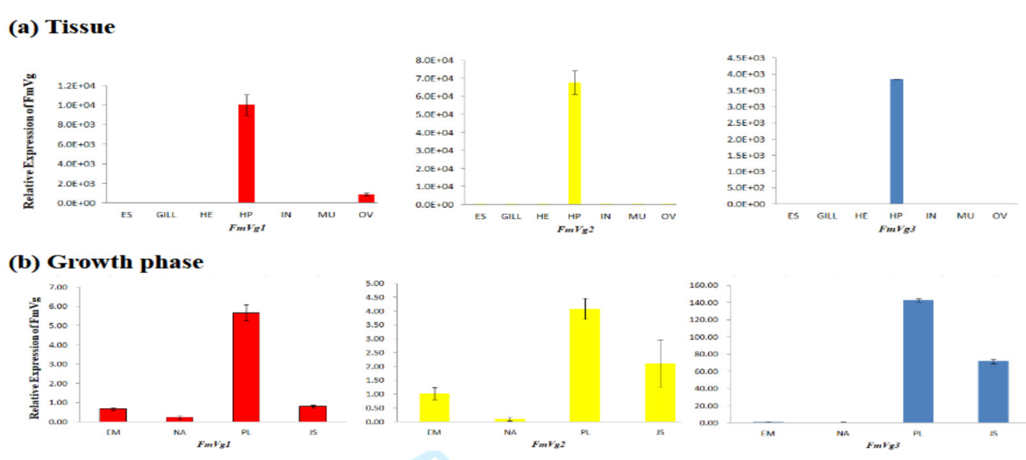
Abstract: Vitellogenin (Vg) is the precursor of egg yolk proteins providing nutrients during early embryonic development. Information related to shrimp vitellogenesis is important for us to understanding the mechanism of reproduction in shrimp aquaculture. In this study, 3 Vg genes (FmVg1, FmVg2, FmVg3) were identified from the banana shrimp *Fennerpenaeus merguiensis*. The cDNA of FmVg1, FmVg2 and FmVg3 ranged from 7758-7764 bp, and the encoded protein consisted of 2553-2585 amino acids. The genomic structure of FmVg1, FmVg2 and FmVg3 were highly 35conserved, containing 15 exons and 14 introns. Protein sequence alignment showed that the identity between FmVg1-FmVg2, FmVg1-FmVg3 and FmVg2-FmVg3 was 52.25%, 60.77% and 3756.45%, respectively. Phylogenetic analysis further revealed that 'FmVg1' class vitellogenins seemed to be more conservative among shrimp, as these genes were clustered in a small branch separated from the 'FmVg2 and FmVg3' class vitellogenins. The expression patterns of FmVg1, FmVg2 and FmVg3 were similar in the hepatopancreas at different ovarian developmental stages, while the expression patterns between FmVg1 and FmVg2/3 in the ovary were remarkably different. During ontogenic development, expression of these Vg genes all reached the highest levels at the post larvae stage. The three Vg genes also exhibited dynamic expression changes at different molting stages, with the highest expression level detected at the inter-molt stage. Taken together, our study provided evidence that at least three copies of the Vg gene are present in the banana shrimp. These Vg genes are potentially involved in ovary maturation and larval development, and the divergence in their sequences, structures and expression dynamics may reflects their functional diversification in shrimp.



REGULATION OF GROWTH AND REPRODUCTIVE GENES BY PITUITARY HORMONES IN MALE AND FEMALE MOZAMBIQUE TILAPIA *Oreochromis mossambicus*

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Male tilapia grow faster than female tilapia, a characteristic that is widely employed in aquaculture production. Differences in the allocation of energy towards somatic growth and reproductive functions may underlie sex-specific growth rates. Sexual dimorphism in tilapia is largely governed by the endocrine system, which, through the coordinated actions of hormones, regulates a range of physiological processes including growth and reproduction. Secreted by the pituitary gland, growth hormone (GH) regulates growth and development both directly through growth hormone receptors (GHR) and indirectly through the stimulation of hepatic insulin-like growth factor (IGF). Also secreted by the pituitary gland, gonadotropins regulate gonadal development through the stimulation of sex steroid hormones. Our objective was to characterize the mechanisms associated with the endocrine control of growth and reproduction that may underlie sexual dimorphism.

To address this objective, we surgically removed the pituitary gland (hypophysectomy) of Mozambique tilapia and replaced GH and gonadotropins by intraperitoneal injections with ovine GH (oGH) and ovine luteinizing hormone (oLH). We found that hypophysectomy decreases gonadosomatic index (GSI) by ten days in both males and females; combined injections of LH and GH restore GSI to control levels (Figure 1). These results suggest that combined actions of GH and LH play a role in maintaining gonadal development in both male and female adult tilapia. Additional analysis of hepatic and gonadal expression of GHR, IGFs, and sex steroid hormone receptors will further our understanding of the sexual differences in the endocrine control of hepatic expression of GH/IGF system genes in tilapia.
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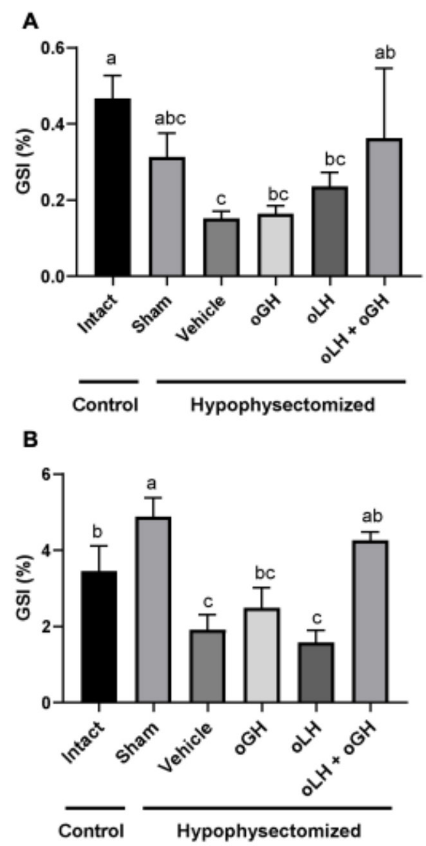

Fig. 1. Effects of GH, and LH injections in male (A) and female (B) tilapia on GSI.

EVALUATION OF OvaLH TO INDUCE SPAWN CHANNEL CATFISH, *Ictalurus punctatus* TO PRODUCE CHANNEL X BLUE HYBRID CATFISH IN HATCHERIES

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Channel x Blue hybrid catfish accounts for over 75% of US farm-raised catfish production because of its production superiority and suitability for intensive production systems. Mammalian luteinizing hormone releasing hormone analog (mLHRHa) is the most widely used ovulating agent to induce spawn Channel Catfish in commercial hatcheries. Hatchery production involves broodfish preparation, hormone-induced spawning, fertilization protocols, hatching and rearing conditions. Production protocols have to be optimized and simplified to minimize losses for consistent and increased hatchery fry production.

The purpose of the study is to compare the efficacy of OvaLH (liquid formulation of mLHRHa in sterile conditions) to commonly used mLHRHa (lyophilized powder dissolved in non-sterile saline) to induce ovulation of gravid Channel Catfish to produce hybrid catfish fry in early, peak and late portions of the spawning season. Liquid formulation simplifies the hormone preparation process in hatchery, reduces wastage and improves shelf-life of the product. Three spawning trials were conducted to compare OvaLH and mLHRHa administered at 100 ug/Kg BW. In each trial, fish were randomly assigned to OvaLH, mLHRHa, and Saline (control) formulations. Hormone-injected fish were suspended in soft-mesh nylon bags in a 10,000 L raceway during latency. Ovulating fish were identified, stripped eggs were fertilized with pooled blue catfish sperm, and hatched in flow-through hatching troughs following standard protocols.

Saline injections did not induce ovulation in Channel Catfish. Mean percent ovulation, relative fecundity, egg density, percent hatch and fry produced per Kg BW did not differ ($P < 0.05$) between OvaLH or mLHRHa formulations (Table 1). However, differences ($P < 0.05$) were evident among the seasons. Results of OvaLH were comparable to mLHRHa in this study and similar such results were also observed in four commercial hybrid catfish hatcheries.

OvaLH has a potential to replace mLHRHa to produce hybrid catfish fry in hatcheries.

Table 1. Reproductive parameters (Mean±SE)) of Channel Catfish induced to spawn with OvaLH (liquid formulation of mLHRHa) or mLHRHa to produce Channel x blue hybrid catfish fry in three trials. LSmeans did not differ (P>0.05) between the two ovulating agents

Formulation	Ovulation (%)	Latency (h)	R. Fecundity (Eggs/Kg BW)	Hatch (%)	Fry/Kg BW (#)
OvaLH	83.3 ± 2.2	*57.2 ± 1.3*	*6269 ± 423*	*22.4 ± 2.4*	*1294 ± 164*
mLHRHa	*88.8 ± 4.4*	*55.6 ± 1.2*	*6702 ± 358*	*22.9 ± 2.2*	*1509 ± 155*
P-Value	*0.44*	*0.39*	*0.40*	*0.87*	*0.35*

SCREENING FOR PROTEINS THAT CAN INTERACT WITH GROUPER NERVOUS NECROSIS VIRUS (NNV) COAT PROTEIN

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Nervous necrosis virus (NNV) is a prominent pathogen that infects a variety of cultured marine fish, leading to nerve necrosis and a high mortality rate. At present, there are many studies on the genetics and structure of nervous necrosis virus, but its pathogenic mechanism remains unclear. To investigate this mechanism, in this study native NNV coat protein (NNVCP) was used to screen for proteins that bind to NNVCP in grouper tissues by immunoprecipitation (IP) assay. The initial results identified various proteins that can interact with NNVCP and may function as putative receptor or co-receptor, cytoskeleton, glucose metabolism and ATP generation, immunity, mitochondrial ion regulation and ribosomal proteins. The putative receptor or co-receptor can help virus to invade the host cell and then complete the replication process. Cytoskeleton contributes to the spread of the virus within the cell. The identified proteins participating in sugar metabolic pathways and calcium signalling may affect the ATP energy balance and may also cause host cell apoptosis and necrosis. Further experiments are needed to verify the role and authenticity of the proteins found to interact with nervous necrosis virus in this study.

EVALUATION OF ENHANCED TORULA YEAST WITH HYBRID STRIPED BASS (*Morone chrysops×M. saxatilis*)

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Two separate trials were conducted to evaluate graded levels of enhanced torula yeast (SylPro®, Arbiom) in the diets of hybrid striped bass and the digestibility of various nutrients and energy in this ingredient. In trial 1, a basal diet was formulated to contain 43% crude protein and 11% lipid, and four incremental levels of enhanced torula yeast (5, 10, 20, and 30%) were added to the basal diet in place of dehulled soybean meal on an equal-protein basis. A total of 10 fish (18.6±0.21g) were assigned to 15, 110-L glass aquaria connected as closed-recirculation system with each diet fed to triplicate groups of fish for 9 weeks. In trial 2, digestibility of a reference diet consisting of practical ingredients and a test diet consisting of 80% of reference diet and 20% of the enhanced torula yeast ingredient was determined. A total of 15 fish (452.5±42g) were assigned to each of six, 1200-L fiberglass tanks connected as closed-recirculation system with each diet fed to three replicate tanks for 21 days after which fecal samples were collected using the stripping technique. In both trials, fish were fed twice daily at levels approaching apparent satiation.

In trial 1, a non-significant increasing tendency was found in weight gain, feed efficiency, and hepatosomatic index when fish fed with yeast-supplemented diets, except for those fed the diet containing 30% of yeast. Compared to the fish fed with basal diet, fish fed the yeast-supplemented diets had higher whole-body lipid content and a significant difference was noticed between fish fed the diet containing 30% of yeast and basal diet. In addition, according to the quadratic regression relationship between dietary yeast levels and weight gain and feed efficiency, 10.2 to 11.5% of yeast, respectively, in place of dietary soybean meal improved growth performance of hybrid striped bass. In trial 2, digestibility coefficients for protein, lipid, organic matter, and energy were obtained for the yeast product as shown in Table 1. Availability of various amino acids also was determined. In conclusion, according to the digestibility results, enhanced torula yeast is well utilized by hybrid striped bass, and can substituted for soybean meal up to 10 or 11% of diet without reducing growth performance.

Table1 Protein, lipid, organic matter, and energy digestibility of the yeast ingredient by hybrid striped bass

	Protein	Lipid	Organic matter	Energy
Digestibility %	97.4	98.0	75.5	75.6

EFFECTS OF ASTAXANTHIN DEPOSIT ON PENAEUS VANNAMEI WHEN FED NATURAL XANTHOPHYLL

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Under intensive commercial shrimp-culture, abnormal body color happens frequently because lack of carotenoid food sources. Natural xanthophyll (extracted from marigold) is a natural pigment obtained from marigold flowers, and its main pigment components are lutein and zeaxanthin. The long-term application practices in animal feed have verified its safety and effectiveness. Studies have shown that adding natural xanthophyll in shrimp feed can effectively enhance shrimp body color. In comparision with astaxanthin, natural xanthophyll is relatively inexpensive, also its safety has been affirmed. There has a great potential for its application in shrimp feed. The present study was conducted to test the pigmentation efficiency of Leader Golden 5% (natural xanthophyll product) adding to feed of Penaeus vannamei, and to compare astaxanthin product.

An 8-week feeding trial was conducted in an indoor shrimp-culture system in Hainan prov ince of China. The shrimp is juvenile Penaeus vannamei with initial mean weight of 0.29 g. Indi vidual units were contained within fiberglass tanks with a density of 40 shrimp per tank. Each tank is independent and has the same size of 500L. Water quality was operated well during the trial by daily exchange, water temperature was 23-26 ° C , salinity was 33-35 % , dissolved oxygen was 6-7 ppm, pH was 8-8.2 ppm, ammo nia was below 0.1 ppm, nitrite was below 0.01 ppm. The goal of this study was to examine the effects of astaxanthin deposit on juvenile Penaeus vannamei when fed tl1ree practical diets with or without natural xanthophyll and astaxanthin.

Other practical ingredients included fish meal, soybean meal, peanut meal, wheat flour, vitamin and mineral premix, etc (Table 1).

After 8 weeks trial, Penaeus vannamei fed a practical diet with xanthophyll (Diet 2) or astaxanthin(Diet 3) had significantly ($P < 0.05$) higher final body astaxanthin content when compared to the control group (Table 2). In addition, there is no significant differences between the two experiment group. The results indicate that natural xanthophyll can efficiently convert into astaxanthin by Penaeus vannamei itself and deposit on its body.

TABLE1. Formulation of experimental diets fed to Penaeus vannamei

	Diet		
	1	2	3
Fish meal	250	250	250
Soybean meal	270	270	270
Peanut meal	120	120	120
Wheat flour	237	237	237
Natural Xanthophyll(5%)	00	1.6	00
Astaxanthm(10%)	00	00	0.8
Other	123	123	123

TABLE 2. Final bodyastaxanthin content (mg/kg)comparision after 8 weeks trial

	Diet		
	1(Control)	2(Xanthophyll)	3(Astaxanthin)
Final body astaxanthin content	24.22 ± 3.60^a	48.35 ± 1.44^b	50.12 ± 4.20^b

PATHWAY TOWARDS BECOMING AN INDEPENDENT RESEARCHER: LESSONS FROM CELLULAR SIGNALING

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Cellular signaling is a process by which all living cells communicate and is essential for physiological responses such as proliferation, differentiation, secretion, fertilization and apoptosis. This network of information transfer is often multi-step, and involves several proteins, ions, enzymes, and transcription factors located in the plasma membrane and cytoplasm. Furthermore, defects in cellular signaling can lead to cancer, diabetes, autoimmune disorders and many other diseases. Although cellular signaling systems have been extensively studied in mammalian cells, there is a significant lack of knowledge in aquatic species. The fact that aquatic species (e.g. zebrafish) have now emerged as a dominant alternative to mammalian models for biomedical research makes it even more relevant to understand how cellular signaling takes place in these animals. These findings are critical for drug development and disease treatment; hence they rely on our understanding of how different cell types communicate. This presentation will address some important requirements for establishing a research program in the field of cellular signaling. Topics include academic and postdoctoral training; types of techniques and equipment utilized; setting up an independent research program; application in aquaculture.

HEAT SHOCK PROTEIN 70 GENE EXPRESSION AND STRESS RESPONSES OF RED-SPOTTED *Epinephelus akaara* AND HYBRID *E. akaara* FEMALE ×*E. lanceolatus* MALE GROUPERS TO HEAT AND COLD SHOCK EXPOSURE

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Temperature changes, such as those involved in heat and cold shocks, can induce heat shock protein 70 (HSP70) gene expression and stress responses in fish. This study observed the expression regulation of the *HSP70* gene and the changes in plasma cortisol and glucose levels as a stress response in red-spotted (*Epinephelus akaara*) and hybrid (*E. akaara* female ×*E. lanceolatus* male) groupers during exposure to heat and cold shock. In red-spotted groupers, heat shock exposure did not induce upregulated expression of the *HSP70* gene. This compares to a seven-fold increase in *HSP70* gene expression, but it did trigger gradual increases in plasma cortisol and glucose as a stress response in both types of groupers. During cold shock exposure, *HSP70* expression in red-spotted groupers almost doubled, but it was lower than that of the hybrid groupers. The hybrids exhibited gradual increases in *HSP70* expression, nine times greater than that of the control group. *HSP70* expression patterns of hybrid groupers were completely reflective of thermal stress responses of the fish. Although the effects of temperature stress on *HSP70* expression in fish requires further study, the results of this study suggest that variations in *HSP70* expression can serve as a sensitive biomarker of adaptation to temperature stress in specific fish.

SUBSTITUTION EFFECT OF VARIOUS ANIMAL PROTEIN SOURCES FOR FISH MEAL IN DIET ON GROWTH PERFORMANCE AND FEED EFFICIENCY OF JUVENILE OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Dietary substitution effect of various animal protein sources for fish meal on growth and feed efficiency of olive flounder (*Paralichthys olivaceus*) was investigated. The 65% fish meal was included in the control (Con) diet. Thirty percent fish meal in the Con diet was substituted with tuna byproduct meal, chicken byproduct meal, hydrolyzed chicken offal meal, meat meal, meat and bone meal and blood meal, referred to as the TBM, CBM, HCOM, MM, MBM and BM diets, respectively. The greatest weight gain and specific growth rate were obtained in fish fed the TBM diet, followed by the HCOM, CBM, Con, MBM, MM and BM diets, in that order. The greatest feed efficiency was obtained in fish fed the TBM diet. Thirty percent fish meal could be replaced with tuna byproduct meal, chicken byproduct meal and hydrolyzed chicken offal meal in juvenile olive flounder diet without retardation of growth performance.

Dietary substitution effect of various animal protein sources for fish meal on growth and feed efficiency of juvenile olive flounder was investigated. Sixty five percent fish meal was included in the control (Con) diet. Thirty percent fish meal in the Con diet was replaced with tuna byproduct meal, chicken byproduct meal, hydrolyzed chicken offal meal, meat meal, meat and bone meal and blood meal, referred to as the TBM, CBM, HCOM, MM, MBM and BM diets, respectively. Fish were fed with one of the experimental diets twice a day for 8 weeks.

Greatest weight gain and SGR were obtained in fish fed the TBM diet, followed by the HCOM, CBM, Con, MBM, MM and BM diets, in that order (Table 1). The greatest feed efficiency (FE) was obtained in fish fed the TBM diet. The greatest growth and FE were obtained in fish fed the TBM diet, followed by the HCOM and CBM diets, in that order.

Table 1. Growth performance of fish in the 8-week feeding trial

Diets	Weight gain (g/fish)	SGR (%/day)	FE
Con	40.6 ± 0.35^{bc}	2.36 ± 0.011^{bc}	1.02 ± 0.008^{b}
TBM	43.0 ± 0.22^{a}	2.44 ± 0.007^{a}	1.07 ± 0.007^{a}
CBM	40.7 ± 0.21^{bc}	2.37 ± 0.007^{bc}	1.02 ± 0.005^{bc}
HCOM	41.0 ± 0.37^{b}	2.38 ± 0.012^{b}	1.02 ± 0.009^{bc}
MM	39.3 ± 0.49^{d}	2.32 ± 0.016^{d}	0.99 ± 0.015^{cd}
MBM	39.8 ± 0.42^{cd}	2.34 ± 0.013^{cd}	1.01 ± 0.011^{bcd}
BM	39.0 ± 0.48^{d}	2.31 ± 0.015^{d}	0.98 ± 0.012^{cd}

DIETARY SUBSTITUTION EFFECT OF TUNA BY-PRODUCT MEAL (TBM) FOR FISH MEAL ON GROWTH PERFORMANCE AND FEED EFFICIENCY OF JUVENILE OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Dietary substitution effect of tuna by-product meal (TBM) for fish meal on growth and feed efficiency of juvenile olive flounder was investigated. Sixty five percent fish meal was included in the control (Con) diet. Ten, 20, 30, 40 and 50% fish meal in the Con diet were substituted with tuna byproduct meal, referred to as the TBM10, TBM20, TBM30, TBM40 and TBM50 diets, respectively. Fish were fed with one of the experimental diets twice a day for 8 weeks. The greatest weight gain and specific growth rate (SGR) were obtained in fish fed the TBM40 diet, followed by the TBM50, TBM30, TBM20, Con and TBM10 diets in that order. Weight gain, SGR and feed efficiency (FE) of fish fed the TBM40, TBM50 and TBM30 diets were greater than those of fish fed the Con diet. Fifty percent fish meal could be replaced with TBM in juvenile olive flounder when 65% fish meal was included in diet. The greatest growth performance and FE were obtained in fish fed the TBM40 diet substituting 40% fish meal with TBM.

Dietary substitution effect of tuna by-product meal (TBM) for fish meal on growth and feed efficiency of juvenile olive flounder was investigated. Sixty five percent fish meal was included in the control (Con) diet. Ten, 20, 30, 40 and 50% fish meal were substituted with TBM, referred to as the TBM10, TBM20, TBM30, TBM40 and TBM50, respectively. Fish were fed with one of the experimental diets for 8 weeks.

After 8-week feeding trial, the greatest weight gain, specific growth rate and feed efficiency (FE) were obtained in fish fed the TBM40 diet, followed by the TBM50, TBM30, TBM20, Con and TBM10 diets, in that order (Table 1). In conclusion, fish meal up to 50% could be substituted with TBM in juvenile olive flounder feed when 65% fish meal was included. The greatest improvement in growth performance and FE were obtained in fish fed the TBM40 diet substituting 40% fish meal with TBM.

Table 1. Performance of olive flounder fed the experimental diets for 8 weeks

Diets	Weight gain (g/fish)	SGR (%/day)	FE
Con	40.6±0.35^{c}	2.36±0.011^{c}	1.02±0.008^{c}
TBM10	40.5±0.42^{c}	2.36±0.014^{c}	1.01±0.010^{c}
TBM20	41.5±0.42^{c}	2.39±0.014^{c}	1.03±0.011^{bc}
TBM30	43.0±0.22^{b}	2.44±0.007^{b}	1.07±0.007^{b}
TBM40	45.1±0.44^{a}	2.50±0.013^{a}	1.11±0.011^{a}
TBM50	44.9±0.35^{a}	2.50±0.010^{a}	1.10±0.009^{a}

SUBSTITUTION EFFECT OF CABBAGE EXTRACT BY-PRODUCT (CEB) FOR MACROALGAE (*Saccharina japonica*) IN DIET ON GROWTH OF JUVENILE ABALONE (*Haliotis discus*)

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Dietary substitution effect of cabbage extract by-product (CEB) for macroalgae (*Saccharina japonica*) on growth of juvenile abalone (*Haliotis discus*) was investigated. Twenty percent *S. japonica* was included in the control (Con) diet. Twenty five, 50, 75 and 100% *S. japonica* in the Con diet were substituted with CEB, referred to as the CEB25, CEB50, CEB75 and CEB100 diets, respectively. Finally, the dry *S. japonica* was prepared to compare the effect of formulated diets on growth performance of abalone. Juvenile abalone were fed with one of the experimental diets once a day for 16 weeks. Weight gain and specific growth rate (SGR) of abalone improved with an increased amount of substitution of CEB with *S. japonica* in the diets. Weight gain and SGR of abalone fed all formulated diets were greater than those of abalone fed the *S. japonica*. CEB is a promising alternative source for *S. japonica* in abalone feed.

Dietary substitution effect of cabbage extract by-product (CEB) for macroalgae (*Saccharina japonica*) on growth performance of juvenile abalone (*Haliotis discus*) was investigated. Twenty percent *S. japonica* was included in the control (Con) diet. Twenty five, 50, 75 and 100% *S. japonica* were substituted with equal amount of CEB, referred to as the CEB25, CEB50, CEB75 and CEB100 diets, respectively. Finally, the dry *S. japonica* was prepared to compare the effects of formulated diets on abalone. Abalone were fed with one of the experimental diets for 16 weeks.

After the 16-week feeding trail, survival of abalone was not different among the diets (Table 1). However, weight gain and specific growth rate (SGR) of abalone improved with an increased amount of substitution of CEB with *S. japonica* in the diets. All formulated diets achieved superior weight gain and SGR to those of abalone fed *S. japonica*. Therefore, cabbage extract by-product (CEB) seems to be a promising alternative feed ingredient for *S. japonica* in abalone feed.

Table 1. Performance of abalone fed the experimental diets for 16 weeks

Diets	Survival (%)	Weight gain (g/abalone)	SGR (%/day)
Con	92.9±2.47^{a}	3.46±0.236^{c}	0.93±0.040^{c}
CEB25	91.0±1.72^{a}	3.58±0.073^{bc}	0.96±0.013^{bc}
CEB50	92.4±0.48^{a}	3.77± .144^{abc}	0.98±0.022^{abc}
CEB75	90.5±2.08^{a}	4.10±0.127^{ab}	1.04±0.019^{ab}
CEB100	91.4±3.78^{a}	4.21±0.299^{a}	1.06±0.046^{a}
S. japonica	91.0±1.26^{a}	2.15±0.032^{d}	0.68±0.008^{d}

SUBSTITUTION EFFECT OF BEET LEAF BY-PRODUCT (BLB) FOR MACROALGAE (*Saccharina japonica*) IN DIET ON GROWTH OF JUVENILE ABALONE (*Haliotis discus*)

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Dietary substitution effect of beet leaf by-product (BLB) for macroalgae (*Saccharina japonica*) on growth of juvenile abalone (*Haliotis discus*) was investigated. Twenty percent *S. japonica* was included in the control (Con) diet. Twenty five, 50, 75 and 100% *S. japonica* in the Con diet were substituted with BLB, referred to as the BLB25, BLB50, BLB75 and BLB100 diets, respectively. Finally, the dry *S. japonica* was prepared to compare the effect of formulated diets on growth performance of abalone. Juvenile abalone were fed with one of the experimental diets once a day for 16 weeks. Weight gain and specific growth rate (SGR) of abalone fed the BLB25, BLB50 and BLB75 diets were greater those of abalone fed the Con diet and *S. japonica*. Weight gain and SGR of abalone fed all formulated diets were greater than those of abalone fed the *S. japonica*. BLB is a promising alternative source for *S. japonica* in abalone feed.

Dietary substitution effect of beet leaf by-product (BLB) for macroalgae (*Saccharina japonica*) on growth performance of juvenile abalone (*Haliotis discus*) was investigated. Twenty percent *S. japonica* was included in the control (Con) diet. Twenty five, 50, 75 and 100% *S. japonica* were substituted with equal amount of BLB, referred to as the BLB25, BLB50, BLB75 and BLB100 diets, respectively. Finally, the dry *S. japonica* was prepared to compare the effects of formulated diets on abalone. Abalone were fed with one of the experimental diets for 16 weeks.

After the 16-week feeding trail, survival of abalone was not different among the diets (Table 1). However, weight gain and specific growth rate (SGR) of abalone fed the BLB25, BLB50 and BLB75 diets were greater than those of abalone fed the Con diet and *S. japonica*. All formulated diets achieved superior weight gain and SGR to those of abalone fed the *S. japonica*. Therefore, beet leaf by-product (BLB) seems to be a promising alternative source for *S. japonica* in abalone feed.

Table 1. Performance of abalone fed the experimental diets for 16 weeks

Diets	Survival (%)	Weight gain (g/abalone)	SGR (%/day)
Con	92.9±2.47^{a}	3.46±0.236^{b}	0.93±0.040^{b}
BLB25	92.9±1.43^{a}	4.42±0.194^{a}	1.08±0.028^{a}
BLB50	94.3±0.82^{a}	4.38±0.074^{a}	1.08±0.010^{a}
BLB75	92.9±0.82^{a}	4.28±0.153^{a}	1.07±0.023^{a}
BLB100	91.9±1.26^{a}	3.94±0.209^{ab}	1.01±0.036^{ab}
S. japonica	91.0±1.26^{a}	2.15±0.032^{c}	0.68±0.008^{c}

EFFECTS OF DIFFERENT LEVELS OF DIETARY γ-Aminobutyric acid (GABA) SUPPLEMENTATION ON JUVENILE OLIVE FLOUNDER *Paralichthys olivaceus* REARED AT TWO STOCKING DENSITIES

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Following a recent unpublished trial which determined the optimal dietary supplemental γ-Aminobutyric acid (GABA) level to be 145 mg kg^{-1}, an 8-week feeding trial was conducted to determine the effects of GABA approximating optimal supplementation at four different levels on juvenile olive flounder reared at both optimal and high stocking densities. A total of 1,080 fish with an average weight of 5.66 ± 0.02 g(mean±SD) were randomly stocked at two densities, 30 fish and 60 fish into 24, 40 L, 0.153 m^2 seawater tanks which resulted in 1.11 and 2.22 kg/m^2 respectively. Each tank at both densities were assigned to triplicates of four dietary treatment groups; a basal diet without supplemental GABA (CON) and three other diets prepared by adding 150 (GAB_{150}), 200 (GAB_{200}), and 250 mg kg^{-1} (GAB_{250}) GABA to the CON diet. Parameters including growth performance, non-specific immune responses, disease resistance, enzyme activity, histology, and genetic expression are currently under analysis. The final results of this trial will be reported in conference.

CREATING FREE CUSTOMIZABLE MARKETING RESOURCES TO CONNECT PRODUCERS TO END USERS

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The complexities surrounding seafood production, the short shelf life, and the diversity of species make seafood preparation daunting for most home cooks. In the United States, consumers rely heavily on culinary professionals to select and prepare their seafood for them. This transfers the burden of sourcing safe and sustainable seafood onto the culinary professionals and affords them a great deal of power over consumption patterns in the U.S. As trendsetters, innovators, and influencers it is important to ensure that culinary professionals are well educated and knowledgeable about the safety, nutrition, sustainability, and production of seafood. To complicate the task of sourcing seafood, the modern day consumer is more in tune with the foods they consume and are more attentive to where and how the foods they eat are produced. To assist in bridging the gaps between producers and end users New York Sea Grant has been working with the National Aquaculture Association to create marketing resources that will provide producers and chefs with affordable tools to educate their buyers/patrons.

Marketing resource cards were created to provide foodservice providers with information about the origins and sustainability of targeted species. They can be used by producers to inform and educate buyers about the species and products they sell. They can also be used by the foodservice industry to market their dishes to consumers by sharing where and how the seafood they serve is produced and prepared.

Image 1 depicts one of 17 species cards that have been created to assist in marketing farmed seafood. Each card is fully customizable, allowing users to help promote their brand and adjust as needs and resources change. The cards will be available for free online as fillable PDF's, making them affordable and easy to print at home or in the office. A guide to using these resources will be presented along with the species available. Feedback on additional species resources will be encouraged and the web platform housing them will be officially launched.

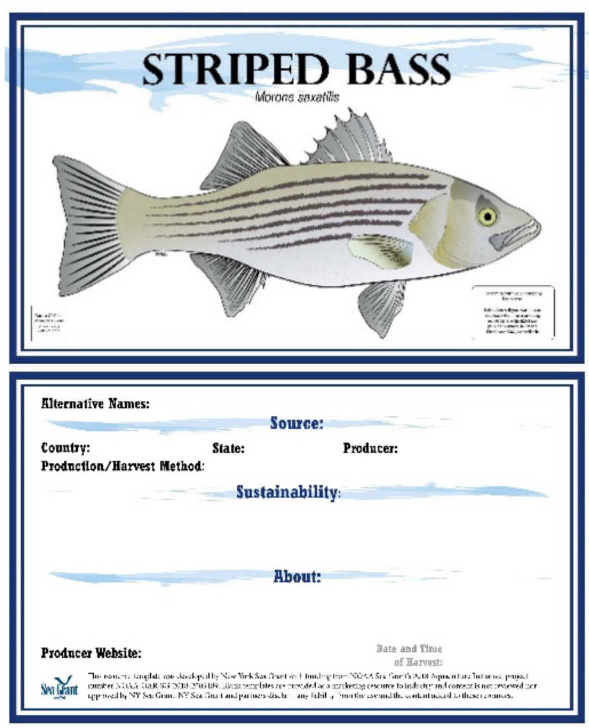

Image 1: Seafood marketing resource card for Striped Bass.

AQUACULTURE PRODUCTS AND RESOURCE GUIDE FOR CULINARY PROFESSIONALS AND SEAFOOD PRODUCERS

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Increased efforts to educate culinary professionals about the value and sustainability of farm raised seafood often fall short when chefs start asking where they can access such products. While aquaculture production is on the rise in the U.S., access to such products can be limited, especially when looking to source "locally." To support the growth of a domestic aquaculture industry, an effective education program that highlights the need for farmed U.S. seafood to complement wild capture fisheries and directs chefs to where they can find those products is necessary. In addition, small scale start-up operations often struggle to effectively market their products affordably. Building a platform to connect these farmers with potential buyers locally and nationally can reduce this burden.

New York Sea Grant in collaboration with the National Aquaculture Association has been collecting data on farmed products that are commercially available and are drafting a resource to direct chefs to U.S. farmed products to incorporate into their menus. The guide will also include links to additional resources about aquacultured products and various culinary and aquaculture organizations to assist in creating linkages between chefs and seafood growers. Partnerships with chefs will be used to highlight the various uses for currently available seafood products and showcase recipes utilizing U.S. farmed seafood.

This session will discuss progress and engage attendees to identify additional resources, operations, and topics that would be relevant and important for inclusion in such a resource. Farmers are encouraged to share their information to be included in the final resource developed.

Table 1: Overview of "Aquaculture Products and Resource Guide" proposed topical sections.

#	Topic	Notes
1	Aquaculture Products	Sorted by region and indexed by species.
2	Aquaculture Industry Groups	Various professional organizations across the country.
3	Culinary Groups and Organizations	Various professional organizations across the country.
4	Aquaculture Resources	Main resource sites for general aquaculture information.
5	Seafood Resources	Main resources sites for general seafood information.

EXAMINING OYSTER SPAT NUTRITIONAL QUALITY IN ALASKA

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One of the major challenges faced by oyster farmers in Alaska is consistent supply of larval/juvenile (seed) oysters. Further, shellfish growers in Alaska have indicated that inconsistent and or poor seed quality leads to poor juvenile survival. Growth and survival in oyster spat has been found to be dependent on the amount of lipid reserves in the larvae at the time of settlement, as well as on post larval diet. We propose to secure seed from all suppliers available to Alaskan farmers and to conduct seed quality and subsequent grow-out experiments at Auke Bay Labs. If large differences in somatic growth are observed between suppliers, a genetic component will be subsequently added. This will be necessary to address differences in growth due to heritable effects and condition effects.

AGILE OCEAN OBSERVATION SYSTEMS FOR AQUACULTURE

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Understanding variations in ocean conditions and how they relate to feeding patterns, mortality rate, and overall health of the fish population in aquaculture pens. Traditional ocean instrumentation is often expensive, difficult to deploy and operate, and lack real-time data transmission. As a result, long-dwell and real-time multi-sensor ocean observation systems are not yet widely deployed across the industry, which hampers our ability to adequately respond to ocean environmental events (e.g. algae blooms) and limits our understanding of how the ocean environment affects fish feeding patterns and health.

Here we present the development and deployment of an agile sensor system based on the Sofar Spotter buoy (Fig. 1) at aquaculture sites in Baja California (Mexico), Nova Scotia (Canada), and Helgeland (Norway). The solar-powered surface buoy is two-way connected through the Iridium satellite network to a web-based dashboard and API (Fig. 1). The dashboard allows the user to see live data, change settings on the device, and access features such as geofence and system health. The buoy is designed for plug-and-play simplicity, allowing for easy use, and simple integration with existing and future environmental sensors.

We will present the deployment of a number of buoys, each with strings of 20 temperature sensors to resolve the vertical variability of water temperature, along with collocated, wave, wind, and temperature data. We will present data from each of the sites and discuss how the temperature variations and stratification dynamics may affect fish behavior and feeding patterns. This work will be a first demonstration of how easy-to-use ocean observing systems, combined with user-friendly data transmission and access, enable remote-monitoring of ocean data for investigating fish behavior and survivability.

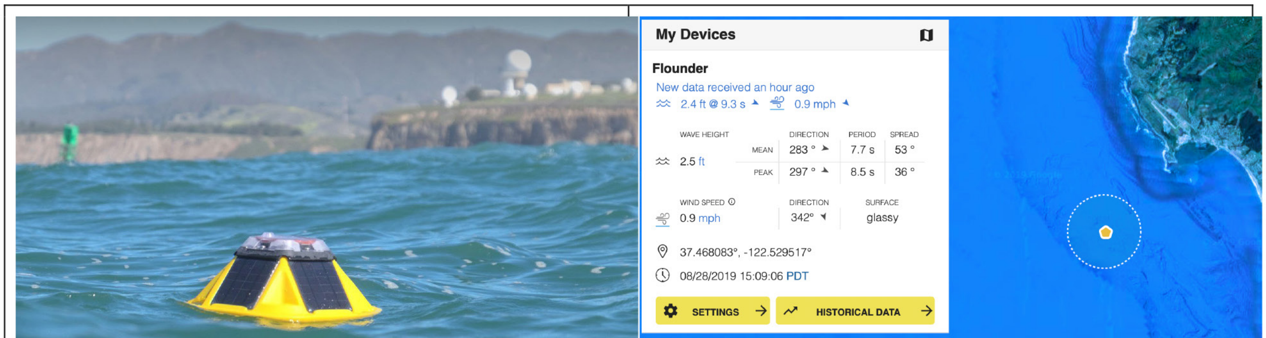

Figure 1 Left panel: Spotter buoy deployed near Half Moon Bay, California. Right panel: screenshot of user Dashboard showing real-time weather data.

OPTIMIZING EGG COLLECTION METHODS FOR FATHEAD MINNOWS *Pimephales promelas*

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Fathead minnows (*Pimephales promelas*) are commonly used for laboratory research studies of reproductive fecundity, quantified as egg number, embryo viability, and larval feeding success. However, the methods of egg collection can impact these outcomes, independent of experimental conditions. Therefore, we tested different methods of initial egg collection to determine best practices in support of fathead minnow reproductive studies.

In culture, adult fathead minnow females lay their eggs on the underside of "spawning huts" that are coated with sand to provide a rough surface that facilitates egg adherence during spawning. Males then clean and guard the eggs through hatching, which takes approximately four days at 25 °C (Figure 1).

However, for research, eggs are usually collected within a few hours of fertilization, a process that prematurely separates eggs from the hut using manual or chemical means. Manual egg removal involves rubbing the eggs off the hut with your fingers and can mechanically damage eggs, but with the advantage that water quality is not affected. Chemical egg removal employs sodium sulfite to dissolve the adhesive that attaches eggs to the hut. Sodium sulfite avoids mechanical egg damage but can have the side effect of depriving eggs of oxygen, which may impact embryo survival and larval development and feeding. To determine which of these methods best supports fish reproductive studies, we tested the effects of manual and chemical egg collection on the percentage of feeding larvae obtained from spawning pairs of fathead minnows and compared these data to control larval numbers obtained when eggs are naturally raised to hatching by the male fathead minnow.

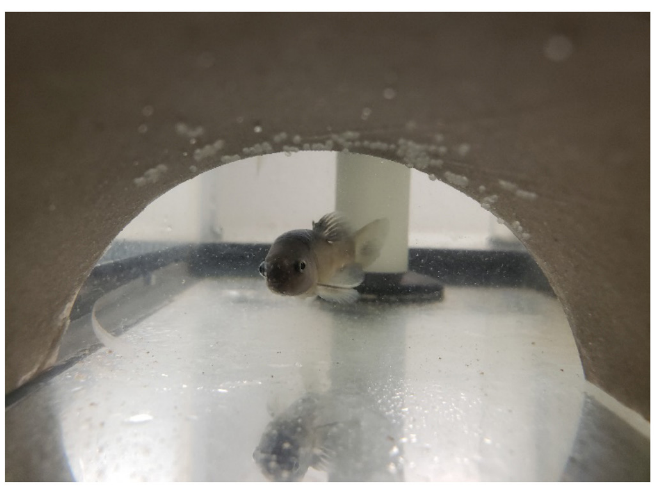

FIGURE 1. Male fathead minnow guarding eggs.

AQUAPONICS: CONNECTING STUDENTS AND SCIENCE

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Schools across the country have been integrating Aquaculture and Aquaponics to teach numerous academic subjects. Aquaponics is an outstanding cross-curricular teaching platform to engage student minds and hands in a living laboratory that brings to life principles described in textbooks. We have now trained over 500 teachers from 23 states how to build and utilize small aquaponic systems to teach, biology, chemistry, math, physics and other less obvious subjects. Each teacher has approximately 200 students per year we are exposing 100,000 students a year to the exciting world of aquaculture and aquaponics perhaps reaching the next generation of aquaculturists. We have numerous accounts from teachers that these systems provide a meaningful experience for less academically inclined students and for some students it is the only reason they come to school.

SEA URCHIN HATCHERY UPDATE: IMPROVED SURVIVORSHIP, PEST REDUCTION AND COMMUNITY INVOLVEMENT

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Tripneustes gratilla is raised in the hatchery at the Department of Land and Natural Resources' (DLNR) Anuenue Fisheries Research Center (AFRC) in Honolulu, as a biocontrol to mitigate the effects of invasive seaweeds in Kaneohe Bay, Hawaii. Post-larval settlement and survivorship have been production bottlenecks. Larvae of the nuisance marine midge, *Kiefferulus longilobus* interfered with biofilm cultivation in settlement tanks and competed with larval urchins for resources. Hatchery temperature ranges tend toward the upper end of this urchin's thermal tolerance, resulting in post-larval mortality. The use of marine adapted poeciliids are among improvements made to defeat the life cycle of *K. longilobus*. Improvements to biofilm cultivation, temperature controls and urchin settlement systems have improved metamorphosis and survivorship. These improvements have resulted in intermittent increases in labor demand. In response to these labor spikes, the urchin hatchery hosts regular volunteer events that promote involvement and interest in the community.

QUALITY DIFFERENTIATION IN THE ATLANTIC SALMON INDUSTRY: BARRIERS AND OPPORTUNITIES

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The value chain for farmed salmon has experienced tremendous growth over the past decades as a result of innovation in production technology, logistics, distribution and marketing. The high level of control over the production environment in principle makes it possible for Atlantic salmon to be tailored on a number of dimensions in response to requirements from different customer groups in the supply chain. However, when compared to meat production, differentiation in farmed salmon is in its infancy. Based on interviews with Norwegian producers, we outline the character and degree of differentiation at each stage in the value chain today and explore opportunities and barriers for further differentiation. Harvesting and primary processing of farmed Atlantic salmon generally result in a relatively homogeneous product. Differentiation is to a degree based on physical properties, which limits the range of product development and differentiation towards consumers, concomitantly impacting the possibility for upstream producers to increase their value creation. Opportunities for differentiation are greater in B2B marketing than in B2C marketing, as the former allows salmon companies to offer differentiated value to customers on several dimensions, such as contracts, certifications, traceability and guarantees related to e.g. volumes, timing of deliveries, feed ingredients, production practices and environmental impact. Salmon is also sold on provenance, but being largely uniform, the claims of uniqueness may be difficult to substantiate. This study presents an overview of the prominent differentiation strategies today, as well as a clarification of limitations and prospects for further value creation through differentiation. Throughout, we offer a means for ranking quality dimensions and propose an index of the degree of differentiation at each step in the Atlantic salmon value chain.

BIOTECH-BASED ALTERNATIVES TO FISH OIL IN RAINBOW TROUT *Oncorhynchus mykiss* DIETS

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Lipid alternatives to fish oil (FO) to date have been mainly plant- or animal-based products, with much effort focused on novel sources that contain fatty acid profiles that are close to that of FO. Without long chain polyunsaturated fatty acids in these sources, completely replacing FO has remained unattainable. Recent efforts in producing new lipid sources using biotechnology have opened the doors to the potential of completely replacing dietary FO in aquafeeds. Genetic engineering (GE) of oilseed crops has been an effective method to produce dietary lipids with beneficial fatty acid profiles for aquaculture.

In the first experiment, we tested GE camelina oil to replace FO in diets for Rainbow Trout (~50 g/fish initial weight), at low level camelina oil inclusion (LCO) and high inclusion (HCO) for 12 weeks. The GE camelina oil contained relatively high levels of EPA; 20:5n-3 (9.5%) and DHA; 22:6n-3 (7.8%), as well as LNA; 18:2n-6 (21%) and ALA; 18:3n-3 (12%). Compound specific stable isotope analysis was completed to distinguish the origin of EPA and DHA in the muscle tissue of fish fed the LCO and HCO treatments and to calculate the contribution of DHA from GE camelina oil into muscle tissue. Relative quantitative expression of desaturase and elongase transcripts in muscle and liver were used to relate to tissue fatty acid content and CSIA data to determine level of DHA synthesis.

In the second experiment, we tested microalgae oil to replace FO in diets for rainbow trout (~10 g/fish initial weight), at low level microalgae inclusion (LMO) and high inclusion (HMO) for 9 weeks. The microalgae oil contained high levels of DHA (40%), low levels of ALA (0.09%), LNA (0.25%), and only trace EPA. The oil mainly consists of saturated (43%) and polyunsaturated fatty acids (47%). Compound specific isotope analysis was used to determine the origin of EPA that was stored in muscle tissue, in the absence of dietary EPA, to answer the question whether ALA was synthesized to EPA, or DHA was retro-converted to EPA. Relative quantitative expression of lipid metabolism related genes, including desaturase and elongase transcripts were used

Based on growth performance and tissue fatty acid content alone, GE camelina and microalgae oils are both promising dietary lipid sources that could completely replace the use of wild-sourced fish oil in Rainbow Trout feeds. The presence of EPA in GE camelina may have a slight advantage if synthesis of EPA becomes energetically expensive for trout fed microalgae oil.

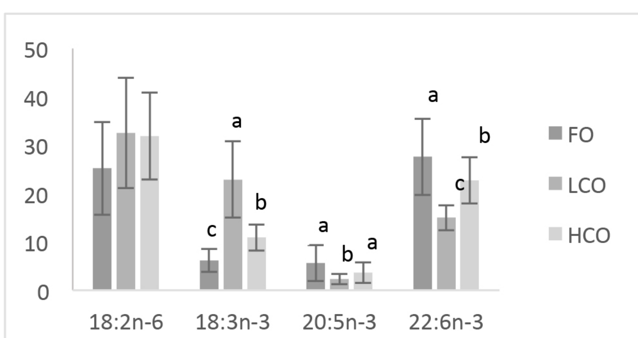

Fig. 1. Muscle tissue fatty acid content (µg/mg dry weight) in rainbow trout fed FO, LCO, HCO treatments

CORRECTION OF METABOLIC PARAMETERS AND UNIT PROCESS PERFORMANCE DATA

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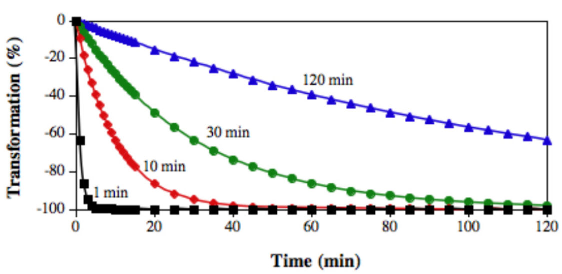
In the design of aquatic culture and holding systems, the metabolic characteristics of the culture animal must be known as a function of feeding and other important operational parameters. This includes consumption of dissolved oxygen, production of carbon dioxide, total ammonia nitrogen, and fecal solids. For laboratory work, the preferred method for determining metabolic rates is the intermittent-flow respirometer. For large commercial systems, the use of intermittent-flow techniques is physically impossible because the flow cannot be stopped.

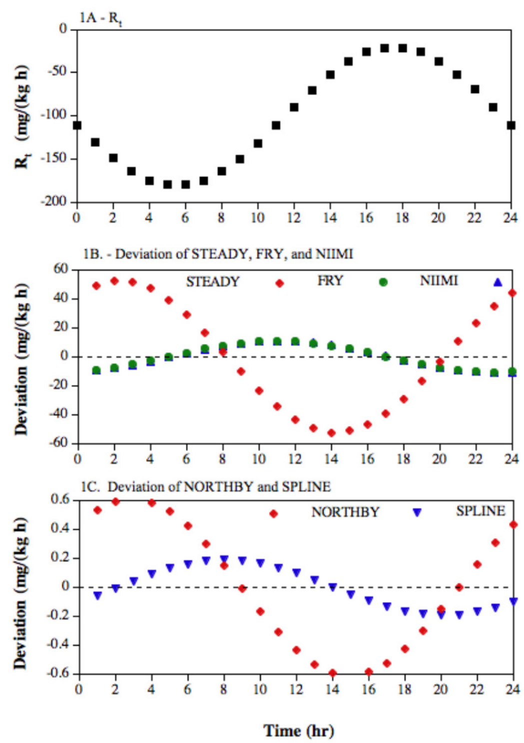
The source of error in measurement of metabolic parameters in a flow-through system is due to a lag in system response. The system response depends strongly of Q/Vol or Vol/Q for a negative step change at t = 0:



For a simple flow-through system, the oxygen consumption rate is equal to:

$$R_t = -\left[\frac{Q}{M}\right](C_t^{in} - C_t) + \left[\frac{Vol}{M}\right]\left[\frac{dC_t}{dt}\right]$$

Where Q =flowrate, M = biomass, Vol is rearing volume, C_t^{in} =influent DO, and C_t = effluent DO.



under some conditions may be quite misleading. While a number of diverse procedures have been developed to correct these parameters for the lag times between input water and effluent water, their accuracy has not been documented. Six correction approaches (Steady-state, Fry, Northby, Niimi, Spline, and Poly) and three analytical

approaches (Point, Mean, and Detailed) were evaluated. For an assumed sine response of R_t and the Mean analytical approach, the variation of (a) R_t with time, and (b) deviation of Steady, Fry, Northby, Spline, and Niimi are shown below:

In terms of the smallest deviation, the ranking of the correction approaches are: Spline and Northby (best), Fry and Niimi, and Steady (worst).The Detailed analytical approach had the smallest error, followed by Mean and Point.

A REPORT ON THE SPECIES COMPOSITION OF A RESTORED HAWAIIAN FISHPOND

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Hukilau - by Jack Owens (Copyright 1948 Owens-Kemp Music Co.) https://www.huapala.org/Hu/Hukilau.html

“Oh we’re going to a hukilau
A huki huki huki huki hukilau
Everybody loves the hukilau
Where the laulau is the kaukau at the luau
We throw our nets out into the sea
And all the amaama come a swimming to me
Oh, we’re going to a hukilau
A huki huki huki hukilau...”

This song was written about the hukilau experience of song writer/performer Jack Owens. It was popularized by many singers in the 1940’s-1950’s, introducing a romanticized Hawaii to the world. The hukilau was not a tourist experience. It was and continues to be an important traditional tool that involves the community in harvesting and sharing the productivity of Hawaii’s nearshore fisheries. We describe the results of a hukilau sampling to assess the fish population in a traditional Hawaiian fishpond.

Waikalua Loko Ia is a 400 year old historic fishpond located in Kaneohe ahupuaa on the windward coast. Physical restoration of the pond has been taking place since 1995. With restoration nearly complete the community came together on June 22, 2019 for paepae hukilau to gather and assess the existing fish population in order to plan future efforts to restore fish populations. A fine meshed net was used to encircle a portion of the pond. All introduced species as well as major predators were weighed and removed from the pond. The native species were weighed, measured and released back into the pond.

Fish Species of Waikalua Loko I‘a, 22 June 2019

Name	Species	Total Weight (g)	Total (lbs)	% by Numbers	% by Weight
<u>Native Species</u>					
Kaku (barracuda)	*Sphyraena barracuda*	11,215.4	24.7	7.62	9.96
O‘io (bonefish)	*Albula glossodonta*	4,421.7	9.7	29.40	3.93
Awa‘awa (ladyfish)	*Elops hawaiensis*	3,005.9	6.6	9.17	2.67
Pualu (surgeonfish)	*Acanthurus blochii*	1,205.8	2.7	0.95	1.07
‘Ulae (lizardfish)	*Synodus* spp.	290.9	0.6	1.31	0.26
‘O‘opu hue (puffer)	*Arothron hispidus*	226.8	0.5	0.24	0.20
‘O‘opu (goby)	eleotrid/goby species	55.4	0.1	2.02	0.05
Lai (leatherback)	*Scomeroides lysan*	28.7	0.1	1.19	0.03
Pa‘opa‘o (golden trevally)	*Gnathodon speciosus*	9.4	0.0	0.36	0.01
‘Ama‘ama (mullet)	*Mugil cephalus*	8.3	0.0	0.36	0.01
<u>Introduced Species</u>					
Tilapia	*Sarotherodon melanotheron*	91,577.5	201.9	39.05	81.31
Summer Mullet	*Osteomugil engeli*	579.5	1.3	8.33	0.51
TOTAL WEIGHT:		112,625.3	248.3		

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

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Seaweed aquaculture is an emerging industry in the United States and several states are actively cultivating seaweed at commercial or research/investigative scale. Although there are many challenges still preventing the industry from becoming commercially viable, the following four main challenges have been identified:

1. Lack of identified and established diverse markets for domestic seaweed;
2. Lack of commercial-scale post-harvest processing, storage, and transportation infrastructure, and associated policies and regulations;
3. Increasing food safety concerns and lack of guidelines and standards for handling, processing, storage, and distribution for different domestic seaweed products, species, and production methods which may be impacted by regional and/or state differences with regard to oversight responsibility; and
4. Lack of clarification in the permitting process for seaweed aquaculture.

There is a need to compile the available science-based, non-proprietary, practical resources related to previous and current research and outreach efforts into a mechanism that is easily accessible by everyone. A national effort led by Sea Grant is underway to address these challenges and look for viable opportunities for domestically produced seaweed through several processes:

1. A thorough needs assessment of all seaweed stakeholders in seaweed producing states;
2. The first National Seaweed Symposium will be convened to disseminate results from the needs assessment and refine challenges and opportunities to be addressed;
3. Stakeholder-driven work groups will be established to address challenges and opportunities identified from the needs assessment and Seaweed Symposium;
4. A website dedicated to this effort will be established providing up-to-date information and status of on-going efforts including work group projects and outputs.

Through this nationally-focused and collaborative effort, the Sea Grant established Seaweed Hub will provide current and prospective seaweed stakeholders information on the status of the domestic seaweed industry in order to make more informed decisions as well as provide a chance to participate in addressing challenges and pursuing viable opportunities.

GENERATIONAL INHERITANCE, GROWTH AND DISEASE RESISTANCE OF MYOSTATIN GENE-EDITED CHANNEL CATFISH *Ictalurus punctuatus* AND CHANNEL CATFISH X BLUE CATFISH *Ictalurus furcatus* HYBRIDS

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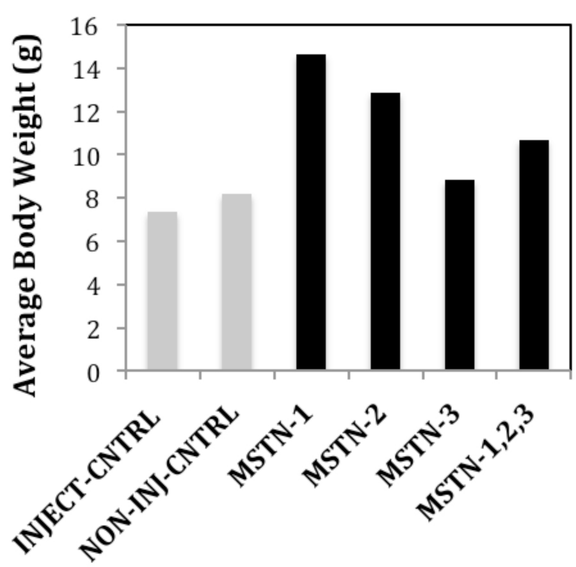
Myostatin (MSTN) is a member of the transforming growth factor β (TGF-β) superfamily and important in the regulation of skeletal muscle growth in all vertebrates. Individuals with mutations in their myostatin-coding region have displayed significantly enhanced muscle growth, as seen in Belgian Blue and Piedmontese cattle. For this reason, myostatin is a potentially and economically important gene in food production. This study measured the effects of gene knockout of the myostatin gene, using CRISPR/Cas9, on growth and disease resistance in P1 channel catfish, *Ictalurus punctatus*, P1 channel catfish x blue catfish *Ictalurus furcatus* hybrids and F1 channel catfish.

Four year classes of P1 mutated channel catfish and channel catfish x blue catfish hybrids were produced along with the first F1 generation of mutant channel catfish. Mean body weight was 30%, 10%, and 38% larger in P1 mutants in fry, fingerling and food fish stages respectively when compared to controls ($p<0.05$). Mean body weight was 63% larger in F1 mutants when compared to controls at 130 days post hatch ($p<0.05$). Mutants showed significantly increased muscle development. The mutation had a high rate of inheritance in F1 channel catfish.

TABLE 1. Mean body weight of channel catfish 100 days post hatch. Mutated individuals from three separate target sites and all combined target sites are in black and controls in grey.



NEW PROCESS FOR MICROALGAE CULTIVATION FOR HATCHERY/NURSERY APPLICATION

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Microalgae production is essential in shellfish hatchery/nursery. Among species that are cultivated, *Tetraselmis* and *Isochrysis* are common strains in shellfish structures. The aim of the study was to test ultrafiltered seawater to cultivate/protect those microalgae at industrial scale and compare the growth obtained with classical seawater

Two microalgae species, *Isochrysis lutea* (T-iso) and *Tetraselmis suecica* (Tetra) were cultivated in 300 L tanks in two water qualities: seawater with common treatments (prefiltration, UV, filtration 5 μm, 0.22 μm and a second UV) and ultrafiltered seawater. This last water was produced with an ultrafiltration unit able to treat 20 $m^3.d^{-1}$, equipped with PES hollow fibre membranes (pore size = 0.02 μm).

Every day, a volume (between 50 and 150 L) was extracted from tanks to feed oysters and cultures and were completed to 300 L with targeted water.

Except water treatments, the 4 cultures were grown in the same conditions (temperature, light, culture medium and volume of microalgae extracted). Physico-parameters of cultures and waters were controlled and microalgae concentrations were measured using spectrophotometer method. Tests were realized for 3 months with 2 cultures of Tetra and 4 cultures of T-iso.

In the case of T-iso, experiments highlighted an impact of water quality on cell density (Figure 1). Indeed, in ultrafiltered water, microalgae concentrations were higher for 3 tests from 6 to 30 %. For Tetra, no significative difference in concentration was observed. However, ultrafiltration led to a better protection of cultures towards parasites because no predator was observed with tetra microalgae contrary to control cultures where ciliates (Figure 2), for instance, where detected.

To conclude, the study showed that ultrafiltered seawater was adapted for microalgae culture. Moreover, advantages of this process compared to common treatments were put in light: (i) for T-iso, ultrafiltration lead to a higher growth, (ii) a better removal of parasites is obtained and (iii) a reduction of water treatment steps is realized (from 5 to 2 steps). Now, microalgae are only cultivated in ultrafiltered seawater and the same conclusions were made for another microalgae specie, a diatom, *Thalassosira weissflogii*: high protection and better production.

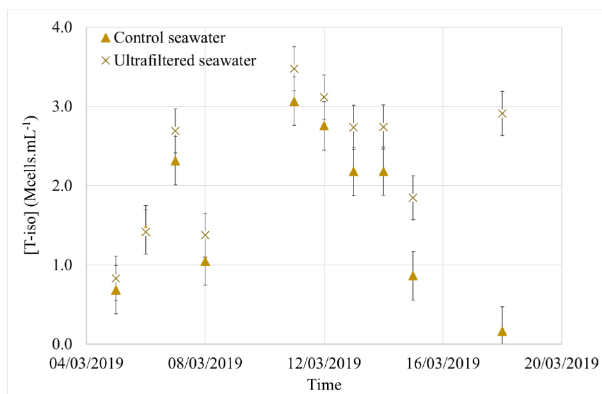

Figure 1: Evolution of T-Iso concentration vs. time

Figure 2: Ciliate observed in control cultures of tetra

ULTRAFILTRATION TO TREAT GAMETE EFFLUENTS FROM HATCHERIES

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Hatcheries might produce non endemic species, such as polyploid oysters, that induces the risk of gametes or larvae propagation in the environment. To protect marine biodiversity, effluents from these structures must be treated but conventional processes present disadvantages: an efficiency dependent on water quality (UV treatment) or a byproducts generation (chemical oxidation). The objective of this work was to evaluate the efficiency of ultrafiltration for gametes removal from shellfish hatchery effluents. The retention by ultrafiltration membranes was determined and the oyster gametes viability was evaluated after treatment for chronic and accidental pollutions.

Tests were carried out with a pilot able to treat 20 $m^3.d^{-1}$, continuously fed with seawater. This automated filtration unit, equipped with hollow fibre membrane with a pore size of 0.02 μm, included filtration, backwash and cleaning steps. First, sustainable conditions for which a moderate degree of fouling occurs, have been determined with seawater filtration under different conditions of flux and cleaning frequency. Then, two types of effluents were treated by addition of spermatozoa (spz) or oocytes from oyster *Crassostrea gigas*, to simulate (a) a chronic pollution: low concentrated effluents during a long time and (b) an accidental pollution: highly concentrated effluents during a short time to reproduce an accidental release of biological material during oyster maturation process. Flow cytometry analyses were performed to determine gametes concentrations and assess their integrity before and after ultrafiltration.

Whatever the gamete treated or their concentration, retention rate was 100 % with a removal superior to 3 log. For instance, spermatozoa concentration measured in permeate was lower than detection limit (Figure 1). Hydraulic performances, continuously monitored on the period of the tests (>6 months), remained stable confirming that the process is adapted for this application. Furthermore, flow cytometry analyses and microscopic observations highlighted an impact on integrity of oocytes and spermatozoa in the case of backwash and more especially when membrane was first drained with air. In the case of spermatozoa, a drastic reduction of the number of these species was observed, from 50545 to 10340 $spz.mL^{-1}$, reflecting a loss of integrity (Figure 1).

To conclude, ultrafiltration is an efficient process to protect environment biodiversity towards biological contamination because (i) a total retention is obtained whatever the concentrations (i.e. type of pollution) and type of gametes (ii) an impact on integrity of these contaminants was highlighted for particular cleaning procedure of membranes and (iii) the sustainability of the process was demonstrated.

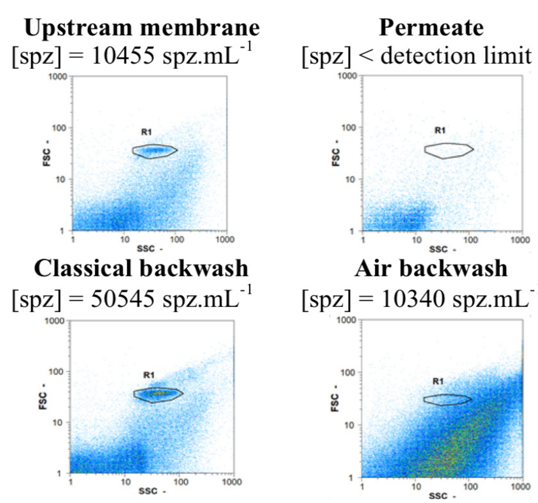

Figure 1: Cytometry analyses – Treatment of spermatozoa

A SOCIAL-ECOLOGICAL APPROACH TO THE DEVELOPMENT OF INTEGRATED OFFSHORE AQUACULTURE/WIND ENERGY SYSTEMS

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For over 30 years there have been numerous studies on the feasibility of integrating offshore aquaculture with non-renewable (oil, gas) and renewable (wind, wave, tidal, etc.) energy systems in the USA. Opportunities/constraints have been reviewed, debated, revisited, re-reviewed, and debated again with no significant developments in offshore aquaculture, while offshore aquaculture speeds ahead internationally. The USA has the best conditions in the world for the expansion of offshore aquaculture; Kapetsky et al. (2013) found the US EEZ to have >5,800 km^2 space for economically viable offshore production. Based upon Statistics Norway (2017) and Liabø (2011), biosecure salmonid aquaculture alone in this area could produce 8-18 MMT live weight; other analytical predictions are higher when full consideration of the full panoply of the available marine species is given due to the wide range of temperatures available such as cobia, yellowtail, mussels/oysters, kelps, etc. FAO (2018) data ranked the USA 16th in total aquaculture production in 2016 just below Ecuador, producing just 0.4 MMT; however, the USA produces high value products worth ~$1.2 billion or ~$3,100/T. The USA imports more seafood than any other nation, spending an estimated $20.5 billion in 2016. Taking this high value estimate, it could take only ~7 MMT of new offshore aquaculture production to equal the total value of all US imports and virtually erase the horrendous US seafood trade deficit. Development US offshore aquaculture would turn the USA into one of the world's largest mariculture nations. *Why is there no offshore aquaculture combined with energy production in the USA?* We conclude that the economic and legal/regulatory structures of offshore oil/gas leasing systems have not and will not change, and that offshore aquaculture will not develop to any significant degree in the fossil fuel industry or in their lease areas. This is conclusion should be nothing new to anyone. However, what *is* new is the coming "blue wave" of offshore windpower, especially in the NE USA (~17 GW by 2035). In De. 2018 a "frenzied two-day auction" was run by the Bureau of Ocean Energy Management for windpower development sites off Massachusetts. Winners were experienced European companies who paid over $400 mil for just three lease areas. *How can offshore aquaculture develop rapidly in windpower lease sites?* The experiences of Deepwater Wind who installed the first US offshore wind energy facility (~30 MW off Block Island, RI) are relevant especially in needs for accelerated offshore engineering capabilities, state-based port upgrades and manufacturing enhancements, and especially for Jones Act compliant vessels to eliminate imports of major parts and vessels from Europe. But the business case has to be combined with thoughtful, respectful, participatory, transdisciplinary, University/Sea Grant extension processes that have the ability to convene hundreds of stakeholders over an extended time period to meet the multiple requests for information. The Rhode Island experience points the way forward not only to secure the business case but also for enhanced federal-state investments in offshore aquaculture in the windpower leases.

PREDICTING THE IMPACTS OF LARGE SCALE SEAWEED MARICULTURE ON WILD FISHERIES

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Globally, the seaweed market is projected to more than double from approximately $4 billion in 2017 to more than $9 billion by 2024 (Allied Market Research 2018). This is in part due to the diversity of products derived from seaweed, which span biofuels, food, hydrocolloids, and bioplastics. Seaweed farming has occurred for centuries in locations with strong cultural connection to algal products, mostly in Asia. To date, however, domestic seaweed culture operations have been relatively small or land-based, due to market and regulatory limitations. In addition to a growing culture behind seaweed products in the U.S., The Department of Energy is exploring large-scale offshore seaweed mariculture for biofuel production (DOE ARPA-E program). The United States' extensive coastline and nutrient rich waters make it ideal for this type of mariculture, and several applications for offshore seaweed farms are currently under review. As we work to make offshore culture a reality there are still many unknowns about how these massive farms will impact wild fisheries and the fishing communities that depend on them.

While the ability of wild fisheries to supply society's demand for healthy marine-based protein has grown insufficient, as global wild fisheries yields have remained relatively stagnant over the past few decades, fisheries do still provide a substantial portion of marine derived protein to human diets. Therefore, as we push farming out into the wild ocean, it is important to understand how these farms will impact our wild fish resources. Seaweed farming tends to evade the detrimental pollution and eutrophication we've seen from fed mariculture species. So could moderate levels of nutrients and increased structure aggregate or provide refuge for commercial species? Can we anticipate how wild fisheries will react to a mariculture farm in order to better plan and manage new farms? We constructed a framework to predict the responses of wild fisheries to different seaweed mariculture designs and management strategies. Applying lessons learned from the implementation of marine protected areas and resulting chages to population dynamics, this multi-species bioeconomic model estimates the total benefits from fishing and farming relative to a baseline yields in the absence of a farm.

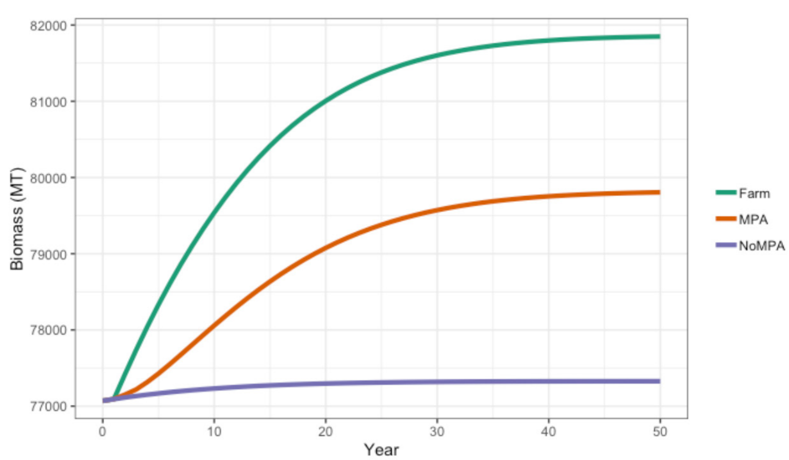

Figure 1: Increase in wild fish biomass with a farm can exceed that of and MPA due to the attractive properties of a farm

EFFECT OF DIETARY CARBOHYDRATE TYPE AND INCLUSION RATE ON GROWTH AND FEED EFFICIENCY IN LARGEMOUTH BASS, *Micropterus salmoides*

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Carnivorous fish are generally considered to be glucose intolerant, showing persistent hyperglycemia when ingesting carbohydrate (CHO)-rich diets. In largemouth bass (LMB), *Micropterus salmoides*, excessive dietary CHO (>20%) leads to increased glycogen deposition in the hepatocytes and may lead to liver dysfunction, reduced growth, and an increase in mortality. To produce the floating feeds preferred by fish farmers, diets require CHO concentrations ≥20%. Corn flour (CF) and wheat flour (WF) are the most commonly used CHO sources in practical diets for fish, whereas dextrinized corn starch (DCS) is a purified source of hydrolyzed starch, which can be more easily digested by fish.

A 12-week feeding trial was conducted to compare the effects of different CHO sources and inclusion rates on LMB growth and feed utilization is ongoing. Nine experimental diets were formulated to be isonitrogenous and isocaloric. These diets include three CHO types (WF, CF, and DCS) at two inclusion rates (20% or 30%). Fifteen fingerling LMBs (~5.1 g) were stocked into each 76-L acrylic aquaria in a shared recirculating aquaculture system. With four replicates per diet, a total of 24 aquaria were randomly assigned to one of the diets. Fish were fed once daily to apparent satiation.

After 12 weeks, fish fed 20% DCS diet (37.5g) were larger ($P<0.05$) than fish receiving 20% WF (32.9g), 20% CF (31.7g) and 30% CF (28.7g) but not different from those receiving 30% DCS (34.1g) or 30% WF (33.8g). Fish fed 30% DCS were larger than fish fed 30% CF but not different from other diets. Fish fed both 20% and 30% WF were larger than fish fed 30% CF. Similarly, feed efficiency was also improved in fish fed DCS (FCR, 1.0) compared to fish fed WF (1.1) or CF (1.2). These results indicate that DCS may be a better carbohydrate source for use in diets for LMB based on improved growth and feed conversion efficiency.

PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) FED PRACTICAL DIETS CONTAINING INCREASING LEVELS OF BREWERS GRAINS WITH OR WITHOUT AN ADDED ENZYME COMPLEX

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Brewers Grains (BG) are the solid residue left after the processing of cereal grains to produce beer. Due to poor digestibility in monogastric animals like fish BG are primarily fed to ruminant livestock. Inclusion rates of BG in diets for fish rarely exceed 30% of the formulation due to poor utilization. Allzyme™ is a proprietary digestive enzyme complex containing live bacteria cultures to aid in gastric fermentation of poorly digestible feedstuffs which may improve utilization of BG in diets for fish.

A feeding trial was conducted to evaluate the effects of adding Allzyme™ to diets containing increasing levels of BG in diets fed to Nile tilapia (*Oreochromis niloticus*). Twenty juvenile tilapia (average weight 5.0g) were stocked into each of thirty-six 76 L aquaria with four aquaria per dietary treatment. Fish were fed three times daily one of nine randomly assigned experimental diets with basal formulations containing either 0% BG, 27% BG or 54% BG and with Allzyme™ added at either the manufacturers recommended rate (MRR) or at two times the MRR to each basal diet. The 0% BG diet served as a control and was formulated after a traditional commercial tilapia diet. All diets contained 8% fish meal and were formulated to be isocaloric and isonitrogenous. Added BG replaced conventional soybean meal and wheat flour in the formulations.

Water quality variables remained acceptable for tilapia production throughout the trial. Feed acceptance and overall growth was good for all diets with an average of >800% weight gain in six weeks. There was no significant difference (P>0.05) in survival or FCR among treatments which averaged 90% and 1.4 overall, respectively. The average weight (g) and SGR of fish fed the control diet 0% BG (54.1 and 1.1, respectively) was statistically greater than for fish fed the 27% BG diet without enzyme addition (46.4 and 0.93, respectively) and for fish fed all the 54% BG diets which averaged 44.2 g and SGR of 0.88 overall. The 27% BG diets containing added Allzyme™ achieved similar growth performance as the control diet. Statistically, addition of Allzyme™ did not significantly improve fish performance over the performance of the basal formulations; however, the trend was a slight increase in growth performance of fish when Allzyme™ was added at the MRR, which did not increase further when the inclusion rate was doubled. Addition of Allzyme™ may provide a growth benefit to feed formulations which include alternative low nutrient density feed ingredients such as BG; however, under the conditions of this study the magnitude of the benefit was slight. To further evaluate the potential benefit of adding Allzyme™ in diets for fish; additional studies should evaluate different species and longer durations of feeding.

A CLOSER LOOK AT INTEGRATED MANGROVE AQUACULTURE

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The world's population is projected to increase to an estimated 10 billion by 2050 (FAO 2017), with demand for animal protein expected to increase at a far greater rate due to the increased affluence in the developing world (FAO 2017). Globally, 3.2 billion people rely on seafood for 20% of their animal protein (FAO 2018). In tropical and subtropical coastal areas, aquaculture has been associated with widespread mangrove forest loss, with 38% of global historic mangrove loss being attributed to shrimp culture (UNEP 2014).

Intact mangrove forests provide an extremely valuable range of ecosystem functions and services and are principally found in intertidal zones along the coast—the same areas used for most shrimp aquaculture. The land required to raise shrimp, and the impacts on mangrove habitats and ecosystem services use vary by production type and location. Integrated Mangrove Aquaculture (IMA), or "silvofisheries", is a form of low-density shrimp and fish aquaculture where mangrove trees are incorporated into the farm system. While there have been studies on the socio-economic impacts of such systems, comprehensive studies on the ecological dimensions are lacking.

I have taken a closer look at IMA systems within the broader context of global shrimp aquaculture production, aggregated available information relating to the environmental benefits and ecological considerations of IMA systems compared to intact mangrove forests, and projected potential future implications of continued use and expansion of IMA systems to meet an ever growing global demand for shrimp.

ECONOMIC COST OF "BIG FISH" ON THE ALABAMA CATFISH INDUSTRY

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Catfish processing plants in west Alabama desire catfish to weigh between 1-4 pounds so they can be easily processed on mechanized lines. Catfish weighing above this range are considered "Big Fish" and can result in a reduced price or no compensation to farmers due to the added expense of hand filleting these fish and lack of a market for this size. Catfish farmers in west Alabama have an issue with oversized catfish in their production ponds due to harvest inefficiencies and the use of old ponds with uneven bottoms. The majority of the ponds in west Alabama have not been renovated since they were built decades ago with only 741 total acres renovated since 2000 (currently 17,450 acres in production). This can lead to pond erosion which can allow catfish to escape the seine net during harvest by burrowing in the mud or swimming under the net. These "Big Fish" remain in the pond for multiple production cycles costing farmers money by consuming additional feed. In 2018, it was estimated that 20% of all pond inventories in west Alabama consisted of "Big Fish" that can have zero value to the farmers. In spring 2019, a survey was mailed to catfish producers in west Alabama to determine the production factors leading to large "Big Fish" inventories and provide details to develop base enterprise budgets. Additionally, the catfish industry respondents provided insight into their strategies to manage and mitigate the oversized fish issue on their farms. From the returned survey, it was estimated that 11.2 million and 14.1 million pounds of "Big Fish" were harvested in 2017 and 2018, respectively. Total production of catfish in Alabama during those years was 81.4 and 79.7 million pounds, indicating that "Big Fish" represented 14 and 18 percent of all production. This equates to a total loss of $4.6 and $6.1 million in potential sales revenue or $99,584 and $133,144 loss per farm in 2017 and 2018, respectively. The most common method farmers use to control "Big Fish" is to hire a custom seiner to re-seine their ponds multiple times after harvest. This is an additional expense incurred by farmers to clean out ponds before starting a new production cycle. For producers that use this approach this has become a necessary expense to ensure all fish are harvested out of the ponds on time so fish do not become "Big Fish." Data analysis is ongoing to develop economically viable scenarios to reduce the impact of "Big Fish" to help increase profitability on commercial catfish farms.

DETERMINATION OF AGE OF HYBRID CATFISH (*Ictalurus punctatus* female x *Ictalurus furcatus* male) AND CHANNEL CATFISH (*Ictalurus punctatus*) FROM COMMERCIAL FISH FARMS TO BETTER UNDERSTAND THE "BIG FISH" PROBLEM IN WEST ALABAMA

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In 2018, the Alabama catfish industry was facing an oversupply issue, as evidenced by a falling fish price paid to producers. Adding to this problem was an over-abundance of "Big Fish" on farm inventories. These farms typically utilize watershed ponds that are deeper than levee ponds and can be difficult to partially harvest effectively, particularly when ponds are irregular in shape and pond bottoms have not been re-worked for long periods of time. Multiple batch production systems are the norm and require frequent partial harvests to increase yields and reduce detrimental issues, such as catfish escaping the seine and growing into the "Big Fish" size category. Catfish processors have defined "Big Fish" as catfish greater than 4 pounds. Once this threshold weight is reached, catfish are too large for the mechanized cutting lines at the processing plants and must be hand filleted at the expense of more time, labor and removing line workers from their regular work. In 2018, catfish processors paid varying amounts for 4-6 pound "Big Fish" and the price range varied from zero to $0.50-$0.60 per pound as processors have limited markets for this larger size. Farmers often do not receive compensation for fish larger than 6 pounds, which are deemed unacceptable and logged as "weigh backs". Much of the "Big Fish" problem can be linked to inefficiencies related to seining and seining frequency but there are other important factors such as market demand conditions, pond type, and pond renovation schedules. Despite widespread adoption of hybrid catfish, there is scant scientific documentation of age as it relates to growth within the catfish production system, with large hybrids being reported by farmers to exceed 50 lbs in some instances.

Therefore, we undertook this study to determine ways to reduce "Big Fish" in pond inventories and have begun by determining the age of "Big Fish", how long fish have been in the pond and the economic cost to the industry. To age catfish, we collected 50 fish from each harvest event to determine the age of different size classes (range: 1.5 – 34.0 lb) in commercial ponds to provide information on catfish age at harvest. We documented catfish length, weight, sex, and collected otoliths from each individual fish sampled to determine their weight and respective age (years). Otoliths were extracted following the methods of Buckmeier et al (2002) and read by two independent readers to determine age (Maceina and Sammons 2006). Mean length-weight at age was compared between ponds and species using an analysis of variance (ANOVA) and growth analysis using an Analysis of Covariance (ANOCOVA) method to compare the slopes of total length to log10 age regressions (Sammons et al. In Press). Collectively, the information garnered in this study has provided needed information on quantities and percentages of "Big Fish" that remain in pond inventories, size variation among the long-term catfish that continually escape harvest, fish ages, and harvest efficiency. This information will be used to develop helpful, economical scenarios to mitigate this issue.

GIANT RED SEA CUCUMBER *Parastichopus californicus* AQUACULTURE AS A TOOL FOR CONSERVATION

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The giant red sea cucumber, *Parastichopus californicus,* is an emerging aquaculture species with substantial economic and ecological value for the North American West Coast. In Washington State, *P. californicus* wild stocks have continually declined since their harvest peak in the early 1990s. As a result, fishery closures and reduced quotas have been enforced despite increasing demand and value. Furthermore, poaching concerns are rising as in other parts of the world because sea cucumbers are easy to locate and harvest. Due to all of these factors, *P. californicus* is a good candidate for aquaculture.

P. californicus aquaculture could have multiple economic and ecological benefits through (1) generating revenue for coastal communities, (2) relieving pressure on wild stocks, and because sea cucumbers feed on waste products of other species, if integrated into existing aquaculture systems they may (3) alleviate some of the negative environmental impacts associated with existing aquaculture systems.

Aquaculture research and development for this species has been in progress since 2016 at NOAA's Kenneth K. Chew Center for Shellfish Research and Restoration. Research has focused on larval and early juvenile development. Several experiments have been conducted to optimize rearing conditions (e.g., temperature and diet) for larval and juvenile culture. The results of these experiments along with anecdotal information on broodstock handling, spawning techniques, and general animal husbandry will be discussed.

Photo 1. Broodstock sea cucumbers spawning in the hatchery.

FISH MORTALITY FROM BIOFILM EXPOSURE IN A RESEARCH AQUACULTURE FACILITY: A CASE REPORT ON CAUSE AND MITIGATION TO PREVENT FUTURE PROBLEMS

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We report an episode of black biofilm particulates that dislodged, darkening the water and obscuring visibility to the point that we could not see the fish in flow-through aquaria systems. The particles originated from both the chilled and heated water recirculating loops. Some juvenile channel catfish died with open mouths and flared gills.

Fish housing systems in the aquaculture research facility are routinely disinfected between fish batches. However, the facility has filtered water recirculating through 3" PVC pipes which continuously flow through two sets of pipes (loops), one for chilled water and one for heated water. These loops supply water to individual fish systems and had not been decommissioned for cleaning.

During the episode, water quality parameters (dissolved oxygen, temperature, ammonia, nitrite, and pH) were within normal ranges. A sample of the black particulates was evaluated using Raman spectroscopy, confirming the material was organic and not carbon particles from the water filter tank. The gross appearance of the fish (open mouths, flared gills) suggested chlorine exposure; however, chlorine tests were negative. We also investigated hydrogen sulfide, which can be formed from degradation of biofilm, as the toxicity causes similar responses and signs as chlorine exposure. We were unable to sample H_2S levels during the event; it is unknown what levels were present in the tanks at that time. Hydrogen sulfide concentrations from affected aquaria were 0.03 mg/L and 0.11 mg/L in the loop sampled 11 days after the episode. It has been reported that channel catfish exposed to acute levels, 0.05 mg/L, of H_2S for 6.5 min caused opercular ventilation to cease (Torrans and Clemens 1982).

We collected tissue for histology from five moribund fish. In 4 of 5 gill samples, we noted epithelial injury and hypertrophy likely due to irritant effect of the particulate material causing respiratory compromise and hypoxia. Necrosis was observed in 3 of 5 livers which is compatible with hypoxia. We also cultured posterior kidney and spleen from 12 fish, moribund, dead, and healthy. Bacteria were isolated from 4 moribund fish; two had isolates of *P. shigelloides* from the posterior kidney and *A. veronii* from the spleen. DNA was extracted from four biofilm samples collected from the chilled and heated water loops for metagenomic analysis.

A consultant recommended physical cleaning of the water loops with a rotating spray nozzle attached to a high-pressure pump followed by a secondary treatment of 10-15 ppm free chlorine. Thereafter, the loops will be placed on a regular cleaning schedule.

STREAMLINING OFFSHORE FINFISH MARICULTURE SITING AND PERMITTING IN FLORIDA STATE WATERS OF THE GULF OF MEXICO

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The limited development of food fish mariculture in the United States can be largely attributed to a lack of a coherent and unified permitting and leasing process at the federal level. Florida, however, is uniquely positioned to provide a streamlined and environmentally sustainable regulatory climate for fin fish mariculture in state waters. A singular state agency, the Florida Department of Agriculture and Consumer Services, Division of Aquaculture (FDACS), has the authority to regulate and authorize all aquaculture activities in the state, including granting sovereignty submerged lands leases through a streamlined process (Figure 1) and comprehensive marine net pen regulations.

To spur develop this industry, FDACS has concurrent projects underway that will provide a pathway for fin fish mariculture in state waters. A technical advisory committee has been formed, including aquaculture experts, engineers and stakeholders, to identify operation and production requirements which will ensure the development of feasible, realistic and environmentally sustainable permits and regulations. A marine spatial planning project has been conducted for state waters (3-9 nm) along the western coastline of Florida to identify suitable areas for mariculture development. FDACS is engaging the U.S. Environmental Protection Agency and the U.S. Army Corps of Engineers to obtain programmatic general permits that will substantially streamline permit requirements and timelines. FDACS is also planning to conduct baseline environmental surveys and pre-permit a mariculture use zone(s) in state waters off the Florida panhandle.

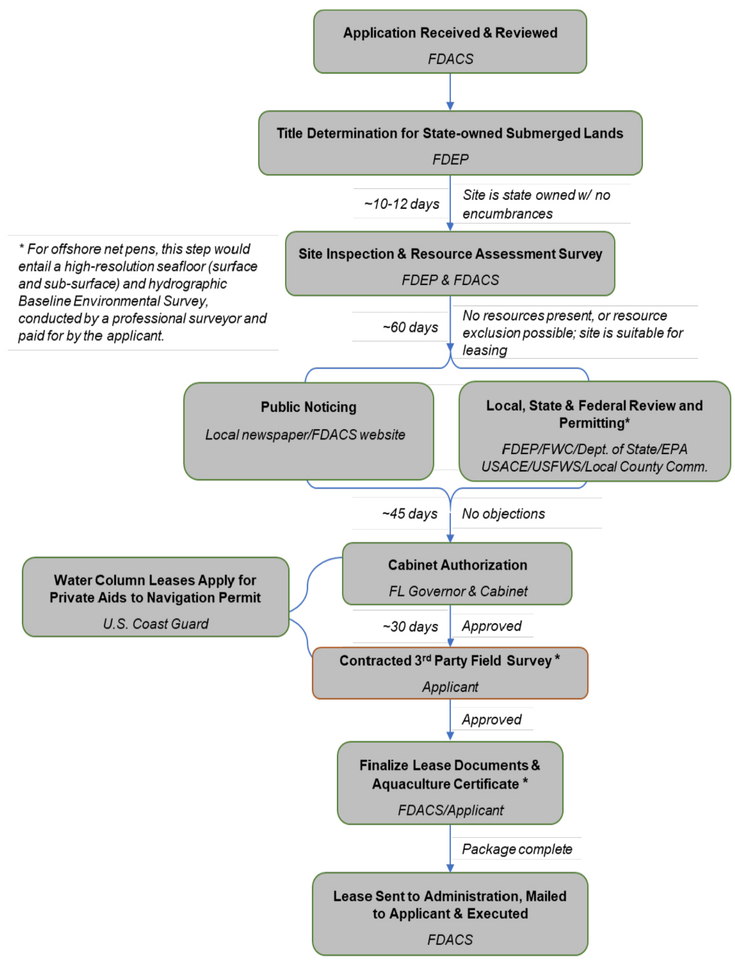

Figure 1. Florida state submerged land leasing process for state waters.

AQUACULTURE EDUCATION PROGRAMS IN FLORIDA

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With more than 1,000 certified commercial aquaculture farms and ~$100 million annual farm-gate value, Florida produces the greatest variety of aquaculture products in the nation and continues to grow and diversify. As the industry expands, so does the need for workforce development and student exposure to this important industry. Aquaculture demands knowledge and skills that are not typical of more traditional forms of agriculture, and teachers interested in covering aquaculture curriculum lack the information and training to confidently teach the subject and maintain systems for hands-on activities. A state-wide survey of all agriculture and science teachers in Florida found that less than ten percent of Florida students are exposed to aquaculture curricula, although ninety-three percent of teachers reported that they are interested in covering aquaculture. To meet this demand and increase aquaculture education in Florida, a diverse group of academic and regulatory agencies and the Florida Aquaculture Association (FAA) have collaborated for years to enhance aquaculture education in Florida.

Harbor Branch Oceanographic Institute and the University of Florida Institute of Food and Agricultural Science, Tropical Aquaculture Laboratory (UF/IFAS) developed a variety of curriculum and lesson plans, *Teach Aquaculture*, that is closely tied to National Science Standards and has been utilized by hundreds of teachers since development. UF/IFAS hosts an annual Future Farmers of America aquaculture competition where student teams showcase both academic and technical aquaculture knowledge and partnered last year with a local school in Cedar Key to provide a one-year aquaculture education program, *Shark Aquaculture Life Training*. UF/IFAS and the FAA has also developed an Aquaculture Industry Certification program which has now been completed by more than 100 students. This program provides real-world skill training and results in a technical certification that improves graduate employment opportunities in the aquaculture industry.

FDACS has completed numerous aquaculture education projects in the past, and future projects are in development. FDACS has published two books that provide an overview of Florida aquaculture for elementary and middle school students and hosts educator resources and networking websites. FDACS staff attend science and agriculture education conferences annually to promote resources. In 2019, FDACS will host an aquaponic system workshop, which provides a desktop aquaponic system to participating teachers and a variety of lesson plans and activities. FDACS and UF/IFAS have a large three-day workshop planned for summer of 2020 which will provide participants with a recirculating aquaculture system and two full days of hands-on training and curriculum review. FDACS is now developing a full aquaculture education program that can be incorporated into high-school agriculture courses. A regional pilot version of this program is expected to launch in the fall of 2020, and provide participating schools with a greenhouse, recirculating aquaculture system, and fully developed lesson plans, curriculum, industry volunteers and a Florida Aquaculture guide book and video series.

AQUACULTURE GEAR MANAGEMENT IN FLORIDA

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Marine debris is a growing problem worldwide that has recently commanded interest and urgency within scientific and consumer communities alike. In addition to being an aesthetic nuisance, marine debris can complicate navigation, entangle and kill marine life, harbor communities of pathogenic bacteria and leach harmful chemicals into the environment. By far the greatest contributor of plastic debris to the marine environment is trash from land-based sources. Yet, commercial fishery and aquaculture industries have received considerable criticism for loss or abandonment of gear made with synthetic materials. The types of gear that are sometimes lost to the environment include cages, bags, ropes, pipes and cover netting. In Florida, clam cover net is by far the most abundant and problematic aquaculture debris. Since lost aquaculture and fishing gear is highly visible to the public and may cause fish, bird, and marine mammal deaths, solutions to alleviate this problem will support public acceptance of aquaculture practices and enhance sustainability of the industry.

The Florida Department of Agriculture and Consumer Service, Division of Aquaculture (FDACS) has taken a proactive role in developing a streamlined permitting system and regulations that will promote the growth of the industry, while also ensuring public safety and environmental sustainability. Florida is one of few states in the nation to have developed mandatory regulations for aquaculture gear management which require compliance with a variety of sustainable farming practices and include language directly addressing shellfish aquaculture gear loss and retrieval. FDACS' annual shellfish harvester training, required for all registered shellfish aquaculturists and commercial shellfish harvesters in Florida, also includes marine debris prevention and removal training.

Beyond regulations, FDACS conducts a variety of activities to provide oversight, education and novel prevention strategies for the shellfish industry. As a majority of gear loss occurs due to severe weather events, FDACS and the University of Florida have hosted aquaculture gear management and hurricane preparation workshops with the industry for several years. In areas of the state with an abundance of aquaculture leases, FDACS conducts routine and post-storm debris surveys to quantify the abundance of derelict gear and provide spatial maps which are then used to guide industry led cleanup events. To provide practical and accessible disposal options to farmers, FDACS and local shellfish associations have funded dumpsters placed near commercial boat launches and processing plants for year, and these dumpsters are still heavily utilized by the industry today. Increasing access to disposal options in rural areas has been very successful in improving industry compliance. Research is also underway to investigate a biodegradable alternative to plastic clam cover nets which would provide a cost-effective yet environmentally friendly gear option. As shellfish aquaculture activities are highly visible to the public, solutions to environmental and perception issues will play a key role in the future development of the shellfish aquaculture industry in the United States.

COMMERCIAL ROCK SCALLOP CULTURE: WHAT DOES IT HINGE ON?

Carolynn Culver*, Jonathan Davis, Brent Vadopalas, Molly Jackson, Andrew Kim, Art Seavey, and Paul Olin

More than 40 years ago, the purple-hinge rock scallop, *Crassadoma gigantea,* was identified as a candidate for marine aquaculture. Since that time rock scallops have been the focus of research along much of the North American West Coast. Growers too have been experimenting with rock scallop culture either on their own when natural sets of seed have been available and/or in collaboration with researchers. Despite all the interest and effort, rock scallop culture has eluded commercial production leaving some to question whether its culture is just a fantasy.

To help move rock scallop culture closer to commercialization, we addressed two remaining bottlenecks: lack of commercial quantities of seed and the development of cost effective grow-out techniques. Specifically, we conducted trials to improve diploid production and develop triploid induction protocols. During this process, we found that scallops recently collected from the wild could be readily spawned using serotonin, but some that had been held in the laboratory for more than 3-4 weeks could not. Further, those that readily spawned did not always produce viable eggs as determined from a lack of successful fertilization or development. As a result, there was only one fairly robust spawning event, with about 12,000 diploid seed produced. These seed were settled on artificial seaweed with a diffuser that provided aeration and increased water flow. While triploid seed production was not achieved, several techniques were tested informing future work.

Factors associated with the rock scallop cementing stage, when scallops permanently attach to substrates, were also examined. Cementation can hinder commercial growout when scallops cement to the culturing gear, often damaging both the product and gear during harvest. We expanded our previous work on this issue and confirmed that: 1) the cementing behavior is primarily exhibited over a certain size range, 2) cementation can be inhibited through occasional disturbance in some locations, 3) shell growth and adductor muscle size is enhanced when scallops are attached, and 4) continuous attachment is not required for good adductor muscle growth. This means promoting or inhibiting (when possible) attachment may result in good growth and survival, and the manipulation may be required for only a small window of time thereby reducing costs associated with it. However, whether and how to manipulate the cementing stage will depend on the culture system used and related environmental conditions.

Based on this work, remaining issues with rock scallop culture include: broodstock conditioning to achieve reliable gametogenesis, identification of suitable conditions for metamorphosis, and, to a lesser extent, refinement of grow-out techniques. Overcoming regulatory hurdles with use of diploid seed in the vicinity of wild populations (possible use of triploids) and harvesting scallops during harmful algal blooms also require additional attention in some areas. Careful experimentation in these research areas will undoubtedly solve the remaining hurdles of commercial scale rock scallop culture, such that it will become a reality.

BEYOND MAPPING: CAPTURING THE COMPLEXITIES OF FISHERIES SPACE USE TO BETTER INTEGRATE AQUACULTURE WITH OTHER OCEAN USES

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In recent years, federal agencies have taken steps to encourage the expansion of ocean aquaculture in the United States. The State of California likewise has sought to advance development of this ocean use. However, project proposals have faced challenges related to potential or real conflict, most notably with existing commercial and recreational fishing activities. These experiences have highlighted the need for a better process for obtaining, appropriately interpreting and integrating information about ongoing fishery uses, and for engaging affected groups, in the aquaculture development process.

We investigated the information needs related to aquaculture development decision-making, with a particular focus on fishery and aquaculture ocean space use needs. Using California's Santa Barbara Channel as a test case, we interviewed commercial and recreational fishery participants, aquaculturists, and relevant state and federal agency staff. We also observed public meetings and other activities related to proposed aquaculture projects. We found that efforts for siting aquaculture projects have relied heavily on the use of geographic information systems (GIS), using data typically collected for other purposes, to map space use. This approach has proven useful, especially when considering fixed space uses such as offshore oil and gas production and aquaculture projects. However, these efforts have fallen short in being able to adequately portray the dynamic and variable nature of fishery space use. For example, fisheries landings data have limited utility for aquaculture siting. Further, efforts to obtain more useful data through engagement with fishery participants has had notable shortcomings, in part due to a fixation on particular *places* identifiable on a map as opposed to the valued *characteristics* of the places. Trust in the information and how it is portrayed and integrated into decision-making processes also have been common concerns.

Potential solutions for obtaining and using local, fine-scale fisheries information include 1) engaging knowledgeable, local third parties and/or a liaison to work with and account for needs and values of both sectors, and 2) developing materials that fully and accurately identify and describe regional fisheries along with key contacts who can provide reliable, valid information and facilitate communication with the larger fishing community. Together these can support a process that recognizes and better accommodates the space needs of both groups while providing agencies with the information necessary for evaluating aquaculture projects and integrating the two activities.

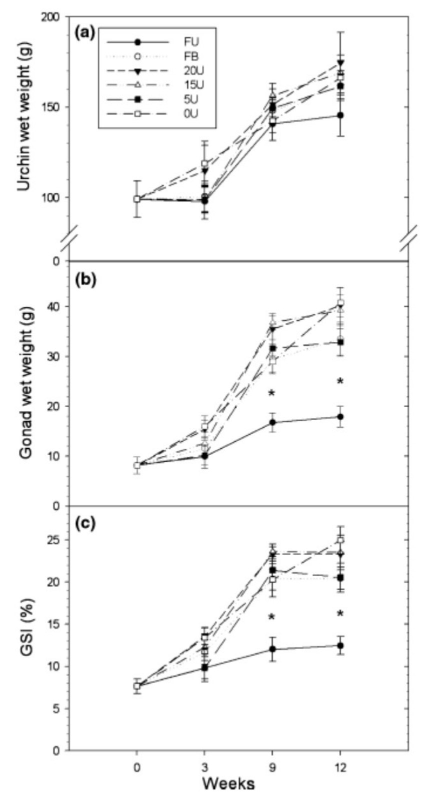

Figure 2 Mean (a) urchin wet weight, (b) gonad wet weight and (c) gonad somatic index (GSI) of *Tripneustes gratilla* fed with four artificial diets, fresh *Ulva* or a mixed diet consisting of fresh *Ulva* and a basal feed over a 12-week grow-out period in recirculating seawater systems. Data represents mean ± SE of eight replicates per treatment group. *($P < 0.05$, Tukey test) represents a significant difference in the means of urchins fed the artificial or mixed diets from the means of urchins fed fresh *Ulva*. Treatment groups: FU = fresh *Ulva*; FB = fresh *Ulva* and the basal diet; 20U = 20% *Ulva*; 15U = 15% *Ulva*; 5U = 5% *Ulva*; 0U = 0% *Ulva*.

ECHINOCULTURE DEVELOPMENT IN SOUTH AFRICA: AN OVERVIEW

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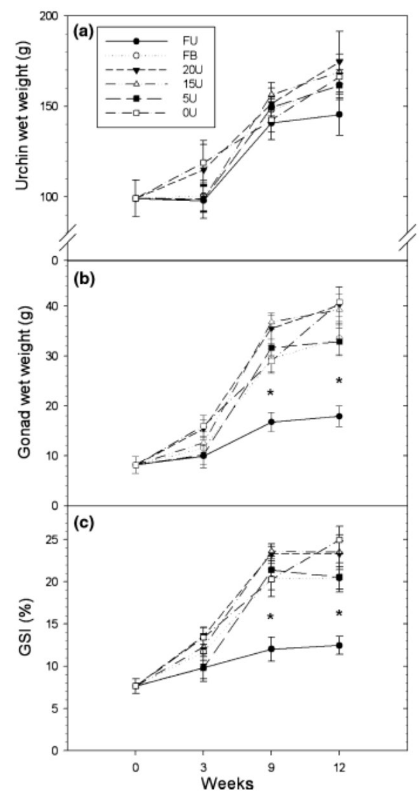
Tripneustes gratilla has been proposed as a viable candidate for aquaculture in South Africa due to its fast growth rate, early maturation and high gonadal production. A number of key areas have been identified that require research in order to successfully culture *Tripneustes* on a commercial scale worldwide, these include: (1) production of cost-effective diets; (2) development of protocols for uniform conditioning of adults prior to harvest; (3) improving larval survival, settlement and post-settlement survival.

Over the past few years our research group has managed to address each of the three critical areas mentioned above, and this presentation will provide an overview of some of this work. One of our main research focuses thus far has been the development of an artificial diet supplemented with macroalgae and the optimization of a feeding regime to produce high quality gonads. We have demonstrated that a formulated feed supplemented with 20% dried *Ulva* (20U diet) produced high quality gonads in terms of both size and colour. Moreover, dietary *Ulva* supplementation significantly increased protein digestibility, the chemosensory properties of the formulated feed and feed consumption rates. Growth studies on hatchery reared urchins to optimize feeding regimes indicated that somatic growth was similar for urchins fed fresh *Ulva* (FU) or the formulated feed (20U). However, gonad growth of urchins fed with FU was significantly less compared with urchins fed 20U. Feeding urchins that were previously fed FU for 20 weeks with the 20U diet for an additional 6 weeks successfully produced gonads of a similar size compared to urchins continually fed 20U. Currently, our research group is investigating the effects of stocking density on a number of growth indicators and water quality parameters for commercial production. One of the most critical factors affecting stocking density is basket depth. Urchins show a preference for occupying the side walls of baskets. However, when fed the urchins migrate to the bottom of the baskets and collide with one another causing spine loss. Spine regeneration adversely affects resource allocation, resulting in stunted somatic and reproductive growth, thereby adversely affecting the economic potential of a commercial sea urchin operation. The combined effects of basket depth and feed type are also being investigated. Our research has also examined the effects of a range of inductive substrates and chemicals (including *Ulva* extracts) on larval settlement and metamorphosis as well as post-settlement survival and growth of the sea urchin *Tripneustes gratilla,* all of which have helped to reduce rearing times and improve larval survival and growth rates. These results will have important implications for the development of a cost-effective and successful echinoculture industry in South Africa.



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COMPARISON OF GROWTH AND MORPHOLOGICAL CHARACTERISTICS OF KOI CARP AND HYBRIDS WITH GOLDFISH OR SILVER CARP MALES

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In the most comprehensive review on distant hybridization in cyprinid fishes, Wang et al. (2019, Sci.China 62:22-45) provides evidence of multiple crosses of species with 100 maternal chromosomes and 48 paternal chromosomes (reciprocal hybrids are not viable) where progeny is composed of diploids (2n=100), triploids (2n=124) and tetraploids (2n=148). The unique feature of F1 hybrids is the formation of unreduced, diploid eggs and sperm (Gomelsky et al. 1988. Dokl.Akad.Nauk.SSSR 301:1210). However, survival, growth potential, and fertility of polyploids was not sufficiently scrutinized. We propagated maternal half-siblings of koi carp (*Cyprinus carpio*; 100 chromosomes, Cc) with goldfish (*Carassius auratus*; 100 chromosomes; Ca), silver carp (*Hypophthalmichthys molitrix*; 48 chromosomes, Hm) and koi males. Larval-juvenile rearing stage (21 days) involved replicated small static containers with live feeds (rotifers and *Artemia*), algal turbidity, salinity and constant light adopted from the zebrafish protocol (Dabrowski, Miller, 2018.Zebrafish 15: 295).

Fish were then moved to formulated feed (Otohime B1 and 2) and larger tanks with continuous water flow. Survival during larval-juvenile stage (at 26 dpf) amounted to 94.5 ± 10.7 (Ca), 66 ± 21 (Hm), and 87.5 ± 6.3% (Cc). Red blood cells fluorocytometric analysis of each offspring cross (n=5-18) revealed c-values (pg) of 1.74 ± 0.01, 1.36 ± 0.02, and 1.69 ± 0.05 for Ca, Hm and Cc, respectively.

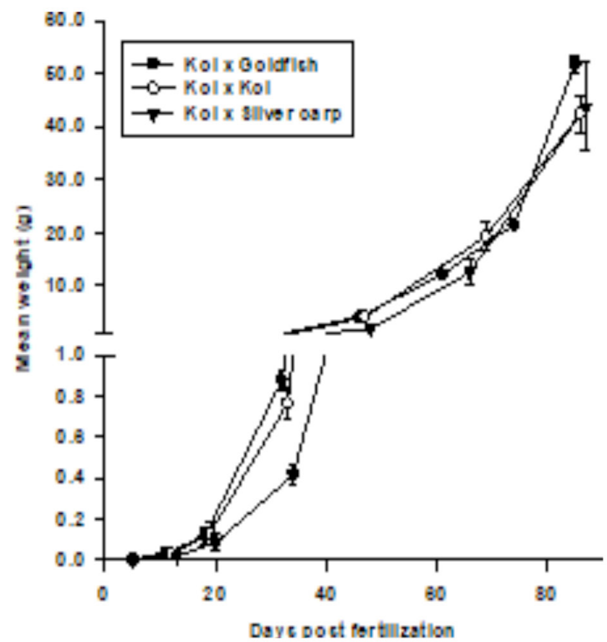

Figure 1. Growth of 3 linages.

Figure 2. Koi-silver carp hybrid, 146 g at 86 days post-fertilization (dpf).

REPRODUCTION, DEVELOPMENT, GROWTH AND EFFECT OF STEROID MASCULINIZATION IN TRIHYBRID CICHLID ON POSSIBLE RESTORATION OF MALE FERTILITY UPSTREAM OF GENETIC SEX DIFFERENTIATION

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We already have evidence that estradiol treatment of zebrafish (polygenic fish species) triploids (all males) prior and during morphological sex differentiation does not act on restoring feminization (Delomas and Dabrowski, 2018. Mol.Reprod.Dev. 85:612), however, the mechanism of action of testosterone in other fish species might be different. In the present study the original trihybrid (*Vieja melanura x Cichlasoma urophthalmus* (female) were fertilized by 4 different males of *Amphilophus citrinellum*) and initially raised on formulated feeds (Otohime B1 and B2) or live *Artemia* nauplii (control). The third experimental treatment were fish exposed for 21 days to Otohime diet containing methyltestosterone (30 ppm). Progenies from all pairs of trihybrids in replicated groups were then fed control Otohime B2 diet. In the following period of 42 days significant differences in mean weight were found among treatments when different progenies, that were fed the same diet, were combined. Fish treated with MT continue to grow slower, 12 $\pm$ 0.8 g in comparison to fish initially offered live food, 17.5 $\pm$ 2.5 g, but not different from dry food (control, no MT), 14.8 $\pm$ 2.1 g. Fish were then PIT-tagged and grown in "common garden" set up until 235 days post fertilization (dpf) (Fig. 1). At the time of submission only 12 fish out of 91 tagged had identifiable sizable "nuchial hump" (Fig. 2).

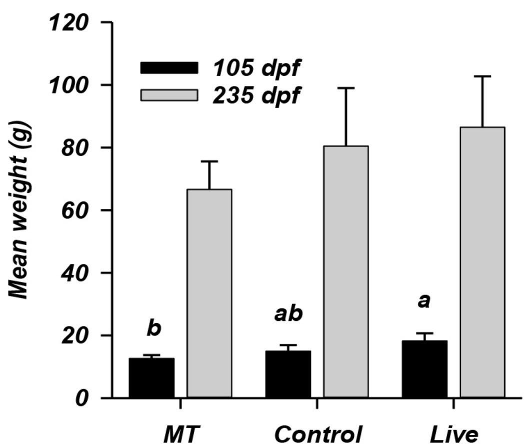

Figure 1. Mean weight of fish from measured 3-5 replicate tanks (105 dpf) and "common garden" rearing (235 dpf).

Figure 2. Enlarged "nuchial hump" in precocious male trihybrid at the size of 110g.

PRODUCTION OF THE GYNOGENETIC FEMALE WALLEYE (*Sander vitreus*) AND INDUCTION OF SEX REVERSAL WITH METHYLTESTOSTERONE

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Three meiotic gynogen families of walleye (*Sander vitreus*) were obtained via pressure shock in April 2018. We attempted hormonal sex reversal to produce "neomales" via feeding methyltestosterone (MT)-containing diet to both gynogenetic and control fish. The final objective of the project is to produce all-female sterile triploid fish. Fish from each family were fin-clipped and combined to "common garden" design (four 400L tanks) where they were fed MT- or Control-diets for 50 days and then PIT-tagged and fed the same commercial diets (Skretting USA). Survival and growth have been recorded throughout rearing. Fish were measured and PIT-tagged in September 2018 (140-147 dpf) and then measured in January 2019 (294 dpf). In October 2019 (544 dpf), all surviving fish were measured and combined to a single 400L tank. Mean weights of each treatment group across the three families, are presented in Table 1. Resulting sex ratios of MT-treated gynogen and control fish will be examined histologically at the advanced stage of gametogenesis at age-1+. Preliminary data indicate sexual maturation of females preceding that of males (Figure 1).

Table 1: Mean ± SD individual mass (g) of fish from each treatment group at four measuring points .

Treatment Group	September 2018	January 2019	October 2019	
Control, Control diet	11.2 ± 2.4	39.3 ± 9.2	109.5 ± 48.8	n = 2
Control, MT-diet	9.2 ± 2.4	31.4 ± 7.8	113.0 ± 36.8	n = 2
Gynogen, Control diet	10.3 ± 2.9	32.4 ± 7.7	130.6 ± 39.4	n = 30
Gynogen, MT-diet	9.7 ± 2.7	29.3 ± 7.7	114.3 ± 52.4	n = 13

Figure 1: Female walleye sacrificed at 560 dpf (total weight 155g; 1.4 GSI %, in formalin).

ON-FARM EVALUATION OF SURVIVAL AND GROWTH OF LARGE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN LOW SALINITY WATER OF WEST ALABAMA

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Farmers in west Alabama raising Pacific white shrimp, *Litopenaeus vannamei*, in low salinity water have reported higher than usual mortality of shrimp late in the production season. Typically mortality is observed in shrimp larger than 20 g in size and occurs in the final months of culture (August, September, October). Commercial shrimp producers have referred to this poor survival of larger shrimp as "late term mortality". Throughout the last few years of production, this chronic late term mortality has resulted in reduced profits for commercial producers raising shrimp in semi-intensive pond production systems in Alabama and other states. In order to evaluate survival of large shrimp late in the production season, an experiment was carried out in an on-levee tank system installed adjacent to a shrimp production pond on a commercial farm in west Alabama. The tank system consisted of 12, 800-L tanks supplied continuously with low salinity (2 g/L) pond water via a regulated water pump. Aeration was provided to each tank via two air stones and a regenerative blower. Baby Belt automatic feeders were installed to supply feed to each tank with a commercial feed (32% protein) ration divided to provide 4 feedings over a 24-hour period. The tank system was stocked with shrimp (16.1 ± 0.47 g mean initial weight) at densities of 20, 25, 30, and 35 shrimp per tank (3 replicates per treatment). Experimental shrimp were captured by cast net from a production pond on the commercial farm in which the trial was carried out. Throughout the trial shrimp are being sampled every two weeks to track growth rates. Dissolved oxygen and temperature are being monitored daily, while pH, salinity, total ammonia nitrogen, and nitrite nitrogen are being measured weekly. The production trial is currently ongoing and will be harvested in October 2019. Shrimp survival, final weight, yield, and food conversion ratio will be determined at the end of the trial. Results will be used to evaluate survival of large shrimp late in the production season.

INVESTIGATION OF COPEPODS AS A LIVE FEED FOR LARVAL SHRIMP CULTURE

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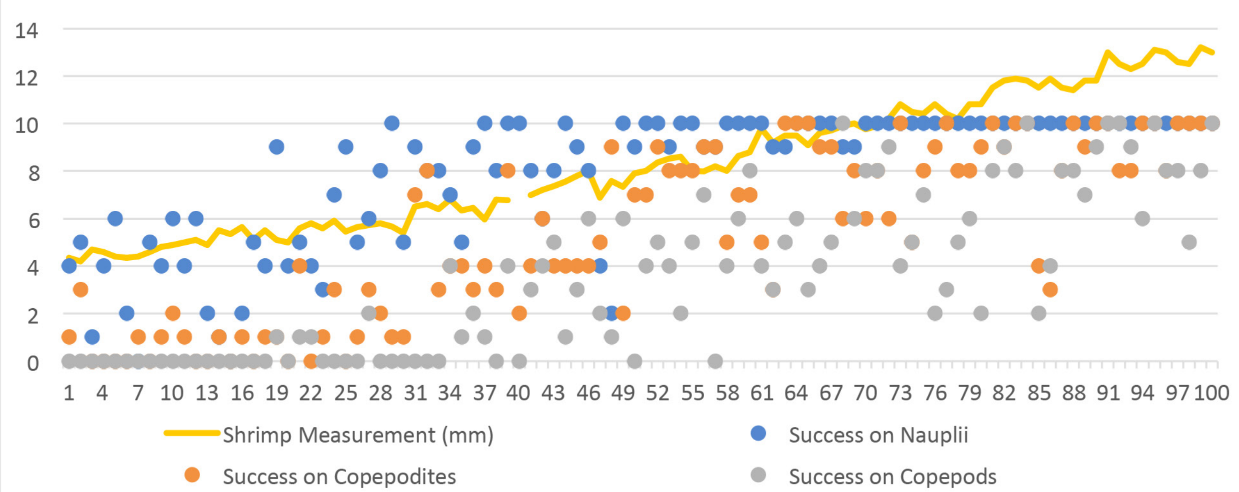
Hawaiian Specific Pathogen-Free or SPF shrimp breeding has been an important genetic resource of seedstock for vannamei broodstock business and world-wide shrimp production. we are interested in developing innovative technologies for quality broodstock breeding and PL production. During larval rearing in aquaculture the survival rate can plummet at a couple key stages of larval development. In production, keeping the survival rate high in all stages is economically important. Previous studies have shown that copepods as a live feed for shrimp larvae increases the survivability and growth rates. The technology is not widely implemented due to the difficulty in keeping steady copepod cultures. Through evaluations of the predator-prey interactions a case for the efficacy of implementing more high value live feeds such as copepods in the larval culture in Hawaii can be made.

An investigation for the optimal algal diets of the copepod *E. acutifrons* and the cultivation of locally sourced phytoplankton has provided further information to be used in production protocols and provides copepods as a viable source of live feed. Evaluation of the capture success rates and preference of *Litopenaeus vannamei* on the feeding of three copepod developmental stages also provides needed data for optimal protocol development.

Utilizing observational study, the findings of this study confirms that *E. acutifrons* is a high potential species for shrimp larviculture due to the continued capture success and preferences by *L. vannamei*. The *E. acutifrons* also had high culture productivity, adding to its advantages as a live feed.



Capture Success throughout Shrimp Growth

AN AQUACULTURE DEVELOPMENT FUND IN THE CARIBBEAN – A FAO/UM/CEI INITIATIVE

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Globally, aquaculture has been growing at a fast, exponential rate. However, the Caribbean region has failed to experience a similar level of growth over the past several decades despite the region's astounding potential. Compared to global averages, the development of aquaculture in the Caribbean has experienced slower growth, often plagued with more barriers to entry than other regions – including inadequate logistics and infrastructure, absence of enabling policy and legal framework, lack of funding, inappropriate farm management, global market shifts, and the presence of inexpensive fish imports, among numerous others. As of 2014, the Caribbean only constituted ~0.05 percent of the world's total aquaculture production. While these matters may seem discouraging, this region has immense aquaculture potential and it only further demonstrates the necessity for the development and implementation of a strategic approach for the successful expansion of this sector in the Caribbean region. As in many areas of the world, wild capture fisheries have reached and, in some cases, exceeded their maximum sustainable yield. Additionally, Caribbean Small Island Developing States (SIDS) boast some of the highest fish consumption rates globally, ranging from 10 to 35 kg/capita - with many nations exhibiting consumption rates greater than the global average. Aquaculture development is vital to fulfill the seafood demand and will not only improve food security, but will drive economic development, provide local, regional and international trade opportunities, and support alternative livelihoods.

The development and implementation of a Caribbean Aquaculture Development Fund presented is needed in order to ensure the successful and sustainable growth of the industry in this region. The Fund will be the first of its kind, with the aim of providing aquaculture development funding as incentives to organizations that wish to drive aquaculture projects and development in the Caribbean region. The objectives of the fund address four main areas; financing, capacity building, market development, policy development and improvement and technical support access. The establishment of the Caribbean Aquaculture Development Fund will provide opportunities for the region to access the necessary capital to provide improved access to imports (feed, seed, etc.), delivery of technical support, improvement of managerial and workforce capacity, development of markets, development of certification schemes and improved access to fundamental training opportunities. To this effect, the Caribbean Aquaculture Development Fund will be a necessary tool to mobilize the financial resources required to enable sustainable aquaculture to reach its potential while supporting the achievement of the Sustainable Development Goals (SDGs). The development and implementation of the Caribbean Aquaculture Development Fund will be vital for the successful growth of the aquaculture industry; contributing to many country priorities along with the achievement of multiple Sustainable Development Goals.

BRINGING AQUACULTURE TO LIMITED-RESOURCE BEGINNING FARMERS: THE CASE OF TILAPIA

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The United States Department of Agriculture (USDA) funds beginning farmer training through a competitive grant program. The program's goal is to provide knowledge and technical support to beginning farmers, sufficient for them to start farming that will become profitable within a three year period. Typically, aquaculture is excluded from beginning farmer training because of 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 and poultry.

Despite the challenges associated with aquaculture, it should not be totally forsaken by beginning farmers. Many of these farmers are refugees who have traditional consumption habits of live/fresh fish, which could lead to a significant marketing advantage within their communities. 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 production and marketing of tilapia in outdoor tanks during the warm season in Kentucky. The young fish were purchased from commercial hatcheries, grown for 120 to 150 days, and auctioned to ethnic consumers in urban areas of the state following the Becker–DeGroot–Marschak model. This experimental auction revealed a range of willingness-to-pay for the live fish. Using production and marketing data from this project, business feasibility and profitability measures were determined, such as initial investment, labor requirements, a range of the cost of production, expected annual profit, and a predicted returns on investment. The results showed significant risk, i.e., considerable chances of having both profit and loss; additionally, direct-to-consumer sales in ethnic markets was the only avenue for small-scale, outdoor, tilapia production to be profitable.

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.

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.

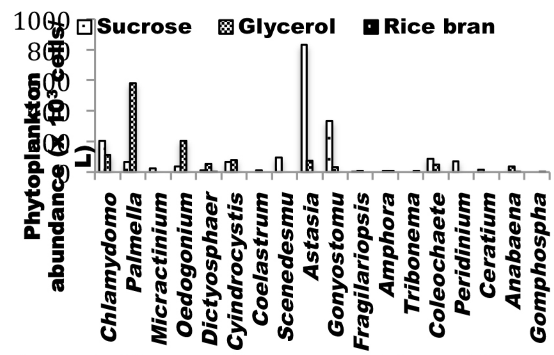

Figure 1: Phytoplantkon composition of the bioflocs

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.

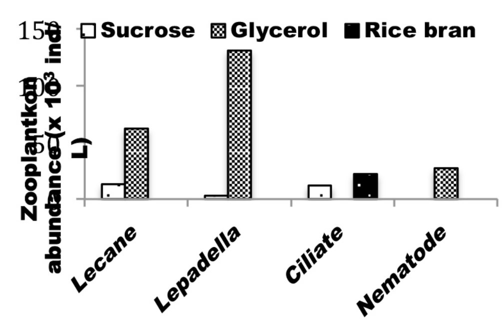

Figure 2: Zooplantkon composition of the bioflocs

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.

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

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

These profiles enabled to evaluate vertical patterns of thermocline, dissolved oxygen, chlorophyll and phycocyanin. Objectives of this work was to understand the occurrence of thermocline associated with bottom anoxia and the vertical distribution of phytoplankton in reservoirs. Three aquaculture areas were monitored from 2014 to 2016, sampling inside each farm, upstream and downstream. Relationship of climate and water outflow upon stratification were also evaluated, as well as the applicability of *in situ* fluorometry for monitoring cyanobacteria. Results showed the predominance of stratification conditions with bottom anoxia in all sampling seasons for at least one sampling site. Maximum values of pigments in subsurface layers were common, indicating underestimation by surface water samplings, traditionally used for the trophic state evaluation of aquatic environments, as chlorophyll maximum frequently was registered in subsurface layers. The stratification strength is variable and it was not evidenced that hydrological regime, air temperature and precipitation had no significant influence on the thermal stratification and bottom anoxia. Thus, the use of real time water monitoring technology proved to be a useful and important tool to follow the changes in the vertical structure of reservoirs. Sites with high oxygen in surface frequently had bottom anoxia extended several meters up in the water column, posing severe risks to cage aquaculture. In addition, it became evident the need to access vertical distribution of oxygen and phytoplankton to proper evaluations of water quality and environmental risks for cage aquaculture in large hydroelectrical reservoirs.

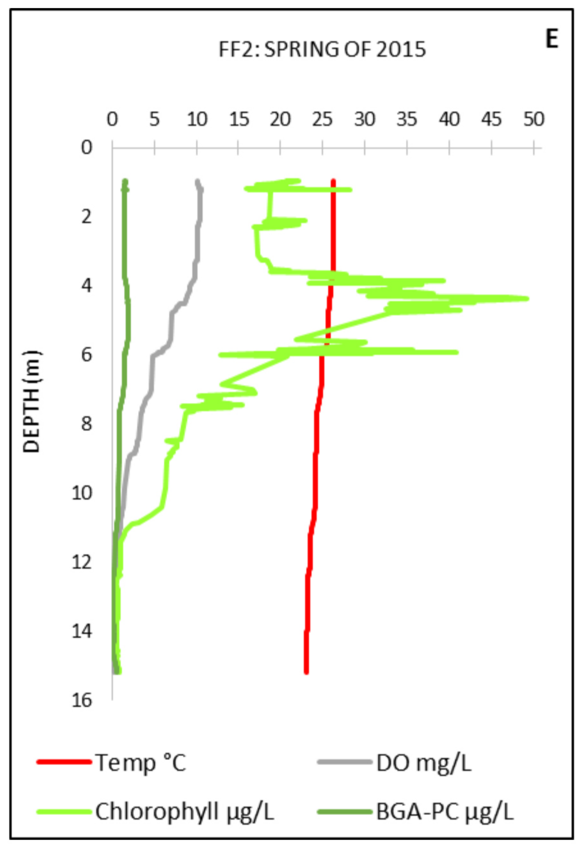

Figure 1: Vertical structure profiles of Temperature (Temp °C), Dissolved Oxygen (DO mg/l), Chlorophyll (µg / L) and Phycocyanin (BGA – PC µg / L).

Support: FAPESP 2013/50504-5

RESEARCH AND DEVELOPMENT OF STANDARD OPERATING PROCEDURES TO OPTIMIZE DEPURATION OF GEOSMIN FROM RAS-PRODUCED ATLANTIC SALMON *Salmo Salar*

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Common off-flavor compounds, including geosmin, are produced by specific bacterial populations that persist within microbial biofilms in recirculation aquaculture systems (RAS). Waterborne off-flavors released by these bacteria rapidly bioaccumulate in the flesh of RAS-produced fish resulting in unpalatable fillets that are objectional to consumers. To effectively remediate off-flavor from market-ready products, most RAS facilities relocate fish from grow-out to separate depuration systems prior to harvest. Facility-specific standard operating procedures to remediate off-flavor from fish flesh are generally established; however, certain aspects of the depuration process have not been well defined and significant room remains for improvement and refinement of this process, particularly for market-size Atlantic salmon *Salmo salar* produced in RAS.

As such, two research trials were carried out evaluating the depuration kinetics of geosmin from Atlantic salmon ($\geq$ 5 kg) originally cultured in a semi-commercial scale RAS. Study 1 evaluated the effects of water flushing rates and associated depuration system hydraulic retention time on the kinetics of geosmin remediation. Study 2 assessed the effects of water rotational velocity, related fish swimming speed, and dissolved oxygen concentrations (metrics that affect fish metabolism) on geosmin reduction kinetics. Each research trial was carried out using twelve replicate partial reuse aquaculture systems (PRAS) each consisting of a 5 m^3 culture tank, a gas conditioning column, and a low-head oxygenator. One day prior to each study, 320 Atlantic salmon were relocated from the semi-commercial scale RAS and stocked in an 18 m^3 production tank within a separate PRAS. The next day, salmon were exposed to a concentrated geosmin solution (Sigma Aldrich) to boost concentrations of this off-flavor in the flesh. Salmon harvested from onsite RAS generally have fillet geosmin levels that are below the detection limit of the human pallet; therefore, geosmin dosing was necessary for this research. Time series water samples were collected during the four-hour geosmin dosing period to evaluate off-flavor uptake by the fish. Immediately following geosmin dosing, 26 salmon were moved from the holding tank into each PRAS to begin the respective trials. Fish (fillet) and water samples were collected on Days 0, 3, 6, and 10 for subsequent solid phase micro-extraction analysis of geosmin concentrations. Geosmin levels in water and fish flesh will be compared between treatments using ANOVA analyses, and practical considerations for cost, energy, and time savings will be evaluated as these factors relate to the study results.

At the time of the abstract submission deadline, data collection and processing for these research trials was still underway. Complete results will be available at the conference.

RAS PRODUCTION OF MARKET-SIZED ATLANTIC SALMON *Salmo salar* AT THE FRESHWATER INSTITUTE: HISTORICAL PERFORMANCE OF VARIOUS COHORTS

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The Conservation Fund's Freshwater Institute (FI) has been culturing Atlantic salmon *Salmo salar* to market-size (≥ 4 kg) in a semi-commercial recirculation aquaculture system (RAS) for nearly a decade to assess the feasibility of this fish production method at relevant scale. During that period, FI has cultured salmon cohorts with various genetics and life history, sex distribution (mixed sex and all-female), and ploidy (diploid and triploid), from egg suppliers in North America and Europe. A wealth of fish performance data has been gleaned from these grow-out trials. Atlantic salmon typically grow to 4-5 kg in FI's semi-commercial scale RAS in 23-26 months post-hatch while maintaining feed conversion ratios of 1.1-1.3. However, most cohorts have demonstrated a propensity for early maturation under conditions common to FI's research facility (freshwater, 14-16 °C, hard water, around-the clock feeding, etc.). Early maturation is generally undesirable to farmers, because fillet market-value is reduced due to pale flesh color, reduced product yield, and diminished lipid content. Thus, identification and genetic selection for strains that balance rapid growth with a reduced inclination for early maturation is a necessity for the growing land-based Atlantic salmon industry.

A recent grow-out trial evaluating all-female diploid and triploid Atlantic salmon from a European provider indicated further progress towards development of RAS-suited stocks. Growth performance of all-female diploid salmon was comparable to the fastest growing strains (North American) cultured onsite, and percent maturation for the all-female diploid and triploid groups was 13.6 and 0.0%, respectively. Overall, early maturation was substantially diminished in these cohorts compared to that of previous mixed sex populations (17 - ≥ 50% maturity). All-female triploid salmon exhibited an increased prevalence of deformities, i.e. 16%, while no deformities were noted for sampled fish from the all-female diploid population. Positive and negative characteristics for all-female diploid and triploid Atlantic salmon cohorts were realized, providing information that will guide RAS producers in weighing the risk/reward of these options.

A full summary of Atlantic salmon growth performance, maturation, and associated fish production conditions will be provided for cohorts produced at FI over the last ten years. Overall, FI's research has demonstrated that land-based production of market-size Atlantic salmon in RAS is both biologically and technologically feasible.

A DESCRIPTION OF COPEPOD CULTURE TECHNOLOGY IN PRACTICE AT THE OCEANIC INSTITUTE

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Marine finfish aquaculture relies on the use of live feeds to provide first feeding fish larvae with living prey of the appropriate size and nutritional profile. Calanoid copepod nauplii, particularly *Parvocalanus crassirostris*, are the natural prey of many first-feeding fish larvae in the wild and are smaller and more nutritious than the conventional live feeds of rotifers and *Artemia*. The use of copepod nauplii has enabled the culture of a growing number of difficult to rear fish species such as coral grouper, blue-fin trevally, red snapper, flame angelfish and yellow tang (to name a few). Additionally, fish species that don't traditionally require copepods (such as pacific threadfin and mullet) have shown increased survival when their rearing protocol is supplemented with copepod nauplii. Despite significant improvements in growth and larval survival in many marine fish species, the inherent challenges of producing copepod nauplii in sufficient quantities has limited their use in the commercial sector.

The Oceanic Institute has overcome some of the challenges of large scale, intensive copepod production and has developed a system capable of producing over 100 million nauplii per day. The system consists of three 1500L tanks stocked with ~2 adult copepods/mL and fed two live algae species: *Tisochrysis lutea* and *Chaetoceros muelleri*. Nauplii are harvested daily, and adult concentrations are adjusted daily. A separate maturation system consisting of ten 500L tanks produces mature adult copepods to supplement the production system.

The current system's daily nauplii production is highly variable. As of the submission of this abstract, the total nauplii production for the year 2019 averaged 51,812,099 per day (SD = ± 16,921,412). The highest daily total nauplii production was 103.29 million and the lowest was 8.19 million. The highest daily nauplii production from a single 1500L tank was 39.09 million on May 18, 2019.

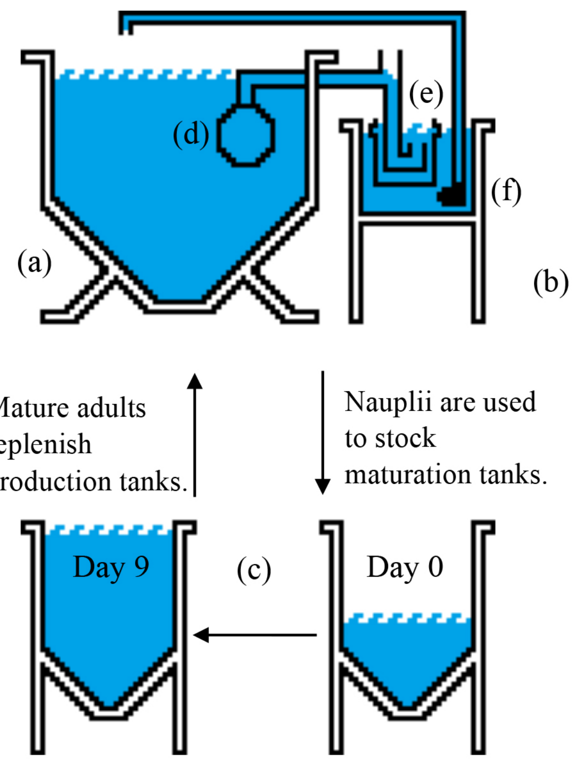

Figure 1. Diagram of copepod production and maturation system. (a) 1500L production tank, (b) 200L harvest tank, (c) 500L maturation tanks, (d) 125µm banjo

THE CAN WE SAVE LAND THROUGH PRODUCITION INTENSITY IN AQUACULTURE? A META-ANALYTIC APPROACH WITH SHRIMP FARMS

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Our demand for seafood is increasing, and harvests from the world's ocean have plateaued. This means that any future growth must be accomplished via aquaculture. Shrimp aquaculture is one of the fastest growing sectors in aquaculture, at approximately 5 million metric tons of production annually, and has a considerable land footprint. Here, we explored the impact of utilizing different production methods (extensive vs intensive) for expanding aquaculture production on cumulative land footprint of shrimp aquaculture.

A meta-analytic approach was utilized to simultaneously estimate model coefficients to create global models to explore three relationships: production intensity and total land burden (model 1), production intensity and the proportion of the burden at the farm level (model 2), and production intensity and the farm land burden (model 3). Structured literature searches were conducted in relevant databases and studies included had to meet the following criteria: i) published in English ii) contain multiple farms iii) not include experimental feed trials iv) report total farm area, total pond area, production or production intensity and Food Conversion Ratio (FCR) vi) include intensive farms vii) report on farms producing Whiteleg shrimp *Penaeus vannamei* or Black Tiger Shrimp *Penaeus monodon* viii) were published after 2003.

These models were used to generate projections of land use in shrimp aquaculture under different scenarios. The most land intensive projections involved using only extensive production to increase production when compared to a business as usual scenario. The least land intensive scenario involved utilizing intensive production. The average production intensities needed to meet a zero farm expansion target were explored. Scenarios where farm land were not expanded used 23% less land and 34% less land to produce 7.5 and 10 million mt of shrimp, respectively, when compared to business as usual scenarios. These estimates are limited by uncertainty of ingredient composition in shrimp feeds, but demonstrate the effect of different land uses in shrimp aquaculture on the overall land footprint.

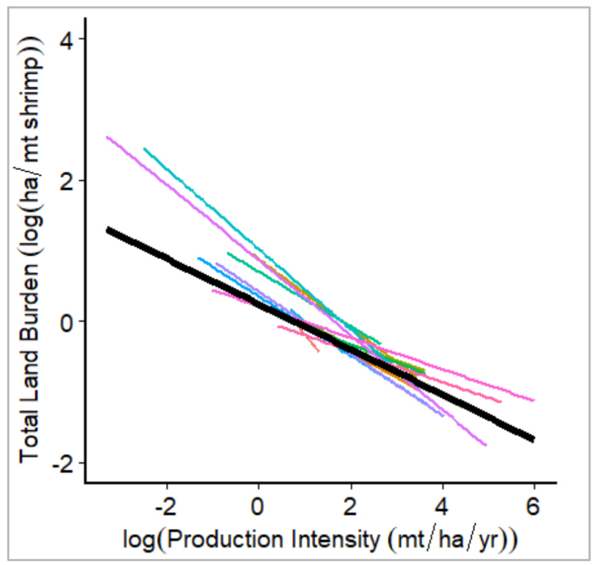

Figure 1. The meta-analysis results of model 1: total land use and production intensity. The global model is y = 0.2447 – 0.3206(x) where y is the total land burden of shrimp production and the x is production intensity of a farm. The Black line represents the global average of the data included

DEVELOPMENT OF A FISHERMEN OPERATED PILOT-SCALE QUEEN CONCH *Lobatus gigas* HATCHERY AND NURSERY FACILITY FOR SUSTAINABLE SEAFOOD SUPPLY AND RESTORATION OF WILD POPULATIONS IN PUERTO RICO

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The queen conch *Lobatus (Strombus) gigas* is an important fisheries species in the Caribbean that has been overfished. In the U.S. Caribbean, The Queen Conch Resources Fishery Management Plan established a program to help rebuild conch populations in Puerto Rico and U.S. Virgin Is. The majority of the conch "Carrucho" fished in Puerto Rico are consumed locally with little export. With the decline in conch populations in Puerto Rico's state and federal waters, closed seasons, and disruption of conch habitats from hurricanes such as Maria, conch is a prime candidate to be cultured in a pilot-scale hatchery in Puerto Rico. The goal of the 2-yr project (S-K NOAA Award NA10NMF4270029) is to assist with restoration of queen conch fisheries in Puerto Rico by producing conch in a fishermen-operated conch aquaculture facility.

The conch hatchery is being built at the Fishermen's Association in Naguabo, Puerto Rico. The first hatchery season will be the summer of 2020. Hatchery-reared juvenile conch will be released into the Luis Peña Channel Natural Reserve no take MPA near Isla de Culebra. The hatchery will be open to fishermen, community, students and visitors to learn about conch aquaculture, biology, conservation and fisheries. A live webcam will broadcast hatchery activities and a conch aquaculture Spanish training manual will be completed. This project will serve as a model, which can be transferred to other fishing communities in Puerto Rico and elsewhere. The project will aid sustainable fisheries practices through aquaculture by working with the fishermen, using the commercial Fishermen's Association's working waterfront, helping provide diversified incomes for the fishery communities, promoting aquaculture practices, and ensuring the conch population is available for future fishing and food security through aquaculture and restoration.

CHOLESTEROL REQUIREMENT IN SEMI-PURIFIED DIETS OF JUVENILE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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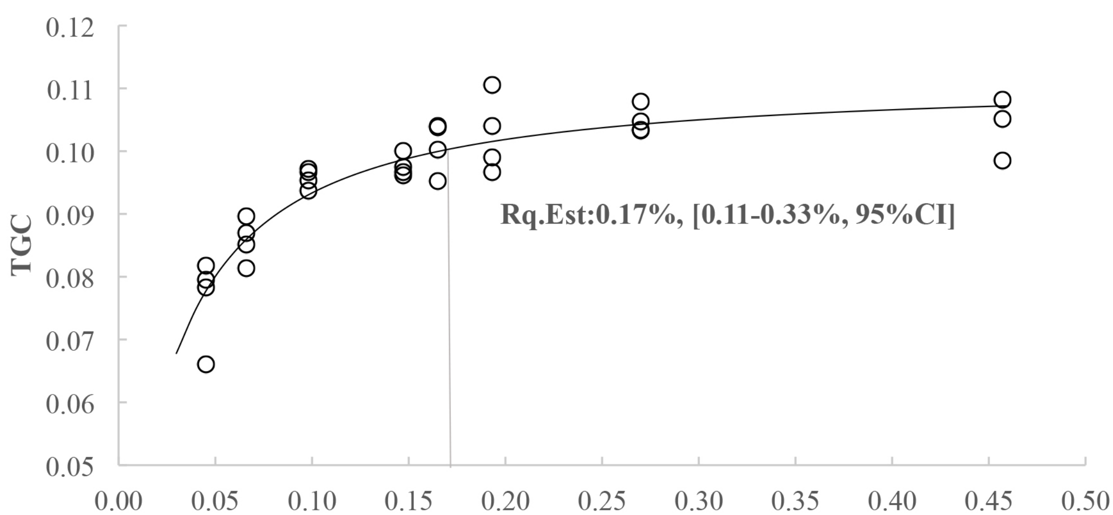
Cholesterol cannot be synthesized by crustaceans; hence, it is an essential nutrient which is critical for the synthesis of numerous compounds. Studies pertaining to cholesterol requirement are limited based on the levels' evaluated as well as statistical analysis. Hence, the objective of the study was to confirm and refine the cholesterol requirement and to provide preliminary data on the efficacy of phytosterol(s) as a substitute for cholesterol in Pacific white shrimp *Litopenaeus vannamei* feeds. The basal diet was supplemented graded levels of cholesterol (0, 0.04, 0.08, and 0.12%) analysis confirmed cholesterol ranged from 0.048 to 0.185%. A 6-week period using shrimp with initial weight of 0.24g reaching a final weight of 3.68 to 6.86 g or percent weight gain from 1522 to 2640% (n=5). The growth response was very linear with no clear plateau in growth. Hence, a follow up trial was conducted to refine the response. Eight diets were designed with an increased range of cholesterol level from 0.045 to 0.457% of diets. In addition, two levels of un-esterified phytosterol supplements (0.06, 0.12%) were also evaluated. Each diet was fed to 4 replicate groups of juvenile shrimp (0.38g initial weight) for 6 weeks reaching a final weight of (4.31-7.43g) or percent weight gain from 1014-1874%. Saturation kinetic model, broken line models with linear or quadratic ascending portions, were used to evaluate dose-response relationships of feed efficiency (FE), thermal-unit growth coefficient (TGC), body cholesterol (BC), body cholesterol deposition (BCD) and body cholesterol deposition efficiency (BCDE) against dietary cholesterol. The cholesterol requirement of juvenile shrimp was estimated at 0.19% [0.11-0.43%], 0.17% [0.11-0.33%], 0.25% [0.23-0.29%], 0.27% [0.24-0.31%] and 0.17% [0.14-0.21%] of shrimp diet for FE, TGC, BC, BCD and BCDE, respectively. Those results with phytosterol also indicated reasonable biological availability which may allow partial replacement for cholesterol.

Figure 1. thermal-unit growth coefficient (TGC) against dietary cholesterol level of shrimp (0.38g initial weight) after 6 weeks. Each point represents one replicate of a treatment. The solid line represents the best fit 4-saturation kinetic model analysis. CI: confidence interval.



THE EFFECT OF SALINITY ON POPULATION DEMOGRAPHICS OF THE COPEPODS *Acartia tonsa* AND *Parvocalanus crassirostris*

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Copepods are considered the best initial live food item for the initial feeding of many marine fish. Their small size is compatible with the mouth gape of small fish larvae and their high nutritional value leads to improved growth rate, improved survival, and reduced rates of deformities compared to rotifers. In fact, many commercially important marine fish species that have small larvae including many grouper, snapper and marine ornamentals have been successfully reared past their larval stage only by using a diet of live copepods. However, the culture of copepods on a large scale has been problematic due to the high variability of productivity of cultures and the relatively low density that can be applied in culture. Understanding the impact that different environmental conditions have on copepod population demographics and production characteristics will facilitate the optimization of copepod culture methods.

In this study, the commonly cultured calanoid copepods *Acartia tonsa* and *Parvocalanus crassirostris* were reared at four salinities (20, 25, 30, 35 ppt). After acclimation to the treatment salinities for a least three generations, replicated experiments were conducted to assess the impact of culture salinity on sex ratio, egg production, egg hatching rate, and mortality post hatch. The temperature was maintained at 25°C for *A. tonsa* and 27.5°C for *P. crassirostris*, and live *Tisochrysis lutea* was fed twice daily to maintain food availability above estimated carbon saturation densities for the two species (1,500 µg C L^{-1} for *A. tonsa* and 1,000 µg C L^{-1} for *P. crassirostris*).

For *A. tonsa*, the percentage of females varied significantly and inversely (p=0.025) with salinity from 68.24% ± 2.13% [SD] at 20 ppt to 62.53% ± 3.01% at 35 ppt. For *P. crassirostris* the percentage of females did not differ significantly among salinity treatments and averaged 50.69% ± 6.21%. Survival from initial stocking of early nauplii (N1-N2) to the adult stage (*A. tonsa* - day 8, *P. crassirostris* - day 5) was not affected significantly by salinity in either species (58.41% ± 10.48% for *A. tonsa*, 70.51% ± 10.67% for *P. crassirostris*). For both species, daily egg production by individual females significantly decreased (p=<0.001) over 7 days. For *A. tonsa*, mean egg production decreased from 36.28 ± 13.83 on day 1 to 23.58 ± 13.54 on day 7. Likewise, egg production in *P. crassirostris* decreased from 41.84 ± 8.69 on day 1 to 23.58 ± 13.54 on day 7. Fecundity significantly increased (p=0.02) in 30 ppt for *A. tonsa*; salinity had no effect on fecundity in *P. crassirostris*. Egg hatching rate (mean of 67.13% ± 32.68% for *A. tonsa*, 91.79% ± 15.69% for *P. crassirostris*) was not impacted by salinity or age for either species. The optimal salinity regime for production may be specific to individual species or culture methods, but this study suggests that production by *A. tonsa* may be optimized at 30 ppt whereas the production by *P. crassirostris* is minimally impacted within the salinity range tested.

ECONOMIC AND PRODUCTIVE EVALUATION OF EXTRUDED AND PELLETED FISH FEED IN BIOFLOC SYSTEM WITH TILAPIA *Oreochromis niloticus* FINGERLING

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In comparison with traditional flow through or partial water change systems, biofloc technology (BFT) combines the reduction of water exchange with the removal of nutrients using heterotrophic bacteria, which can serve as complementary food by aquatic organisms, allowing better utilization of fish feed. Manufactured feeds, depending on its physical or chemical characteristics, can affect water quality, an in BFT it can increase turbidity or suspended solids volume, affecting visibility and diet consumption by fish, reducing growth. The present study was conducted to compare the economic and productive parameters of extruded and pelleted feeds in biofloc and a partial water change system with tilapia fingerling to identify the one that provides the greatest economic and productive benefits at this stage of growth.

An 8-week feeding trial was conducted in 0.35m^3 concrete tanks with tilapia fingerlings (mean weight 25,06 ± 0,73 g). Fish were fed with extruded (ED) and pelleted (PD) diets containing 40% gross protein at 6% up to 3.5% daily feeding rate. The PD was obtained from the reprocessing (pelleted) the ED to keep the same nutritional value but different physical characteristics of the feed. Biofloc (BFT) had a C:N ratio of 15, using molasses (40% carbon) as carbon source, while partial water change system (PWC) consisted in daily 10-50% water renewals, both systems were kept with heaters (average temperature 27±1°C) and constant aeration. Productive (PP), water quality (WQP) and partial cost effectiveness parameters (PCE) were evaluated in a 2x2 (water system x feed) in a factorial design with three replications. The PCE were calculated based on the resources used and its relation to the biomass produced (Kg.m^{-3}).

The results showed no statistically significant differences in the specific growth rates and food conversion rates of tilapia fingerlings between treatments (Table 1), although BFT/PD and PWC/ED reached the best values. The analysis of the PCE at the experimental level not only showed that BFT were up to 24% cheaper than PWC with both types of feeds, but also that BFT/PD was 7% cheaper than BFT/ED. The culture of tilapia fingerling improved in BFT even with the use of rapid sinking feed (pelleted feed), but with high water stability as was the case of PD.

TABLE 1. Specific growth rate, food conversion rate and partial cost effectiveness (mean± standard deviation) in tilapia fingerling trial (n=12). Different letters indicate significant differences (P<0.05).

PARAMETER	UNITS	TREATMENT (WATER SYSTEM/ FEED)			
		PWC/ED	PWC/PD	BFT/ED	BFT/PD
Specific growth rate	%.day^{-1}	1.91 ± 0.12^a	1.81 ± 0.10^a	1.73 ± 0.06^a	1.80 ± 0.23^a
Food conversion rate		1.56 ± 0.08^a	1.71 ± 0.07^a	1.84 ± 0.04^a	1.69 ± 0.32^a
Partial cost effectiveness	US$. Kg^{-1}	7.03±0.30^a	7.62±0.22^a	5.63±0.19^b	5.21±0.33^b

PCE= ΣUS$ (Food + Water + Heating energy + Aeration energy + molasses*+ Na bicarbonate*). Kg^{-1} (*only in BFT) (1US$= 3.3 PEN)

CONSUMERS' ACCEPTANCE AND PREFERENCE FOR CONVENIENT CATFISH PRODUCTS: EVIDENCE FROM EXPERIMENTAL AUCTIONS

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The Aquaculture and Fisheries Center of the University of Arkansas at Pine Bluff (UAPB) in collaboration with the Food Processing and Sensory Quality Research Unit of the USDA-ARS Southern Regional Research Center in New Orleans, Louisiana and Texas State University have successfully developed five convenient (ready-to-cook) catfish products. These are: Panko Breaded Standard Strips (PBSS), Panko Breaded Standard Fillet (PBSF), Panko Breaded Delacata Fillet (PBDF), Sriracha Marinated Delacata Fillet (SMDF) and Sesame-Ginger Marinated Delacata Fillet (SGMDF). This study has analyzed the level of acceptance and preference for these products among the potential consumers in the United States. Relevant data for this study was generated through experimental auctions where 121 catfish consumers from Arkansas participated in 18 sessions. All the participants were provided information about the products and completed a short training about the procedures to be followed in the auctions. Then they saw a video about the catfish products, looked and tasted the products. Participants provided their assessment about the products and bided for each of the products in separate sealed envelopes. Consumers' acceptance for various product attributes was measured through hedonic scale (Likert scale) and just about right (JAR) scale. Consumers preferences for the products and their attributes were analyzed through two-way internal preference mapping (Principal Component Analysis) and three-way internal preference mapping (Parallel Factor Analysis—PARAFAC). The study revealed that acceptance level for panko breaded products was higher than marinated products. Consumers preferred breaded products than marinated products. Two-way preference mapping indicated that Panko breaded products (PBSS, PBSF and PBDF) were preferred by the participants over the marinated products (SMDF and SGMDF) in terms of appearance and color. Three-way preference mapping revealed that two products (PBSS and PBDF) were primarily preferred while PBSF was secondarily preferred. Two other products (SMDF and SGMDF) were not preferred by the consumers. We can conclude that all the newly developed catfish products are likely to be accepted by American consumers and panko breaded products are likely to succeed more as future seafood products in the United States.

METABOLOMIC AND NUTRITIONAL RESPONSES IN YELLOW PERCH (*Perca flavescens*) FINGERLINGS FED DIFFERENT DIETARY STARCHES

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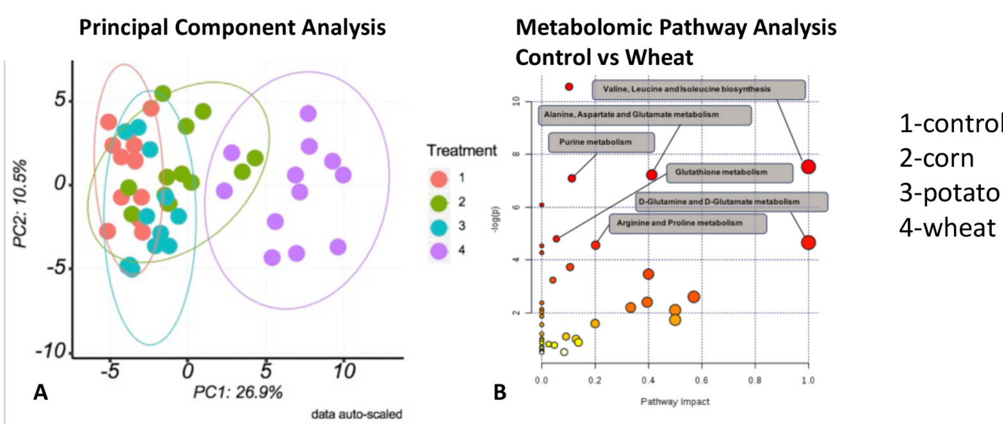
Yellow perch (*Perca flavescens*) are an important food fish and ecological species in the Midwestern United States. Fatty liver and over accumulation of visceral lipid is commonly observed in yellow perch fed commercial feeds. The objectives of this study were to determine whether accumulated liver and visceral lipid in yellow perch can be alleviated by feeding an appropriate starch source and investigate the underlying mechanism in relation to dietary carbohydrate utilization in yellow perch.

Yellow perch (initial body weight, 13.1±1.6 g) were fed a control diet (no added starch; protein, 54%; lipid, 12.5%) and three diets with corn, potato or wheat starch (200 g/kg diet; 41%; lipid, 12.4%)) to replace an equal proportion of fishmeal in the control diet. The feeding trial was conducted in an indoor system run with flow-through water at 22 °C. At the end of 8-week feeding, all fish fed the test diets had similar growth rates and feed conversion ratio ($p > 0.05$). However, the wheat starch diet resulted in significantly enlarged livers and increased accumulation of lipid in the liver, viscera, and whole body ($p < 0.05$). Wheat starch diet also caused significantly higher levels of fasting glucose, aspartate aminotransferase and alkaline phosphatase activities in serum than the control diet. Liver cell diameters were larger, and Kupffer cell numbers were lower in the fish fed the wheat starch diet than those fed the control diet. Potato or corn starch had less impact on fish health.

Results based on metabolomic pathway analysis showed that the branched-chain amino acids as well as proline were lower in fish fed the starch-based diets when compared to the control diet. Principle component analysis (PCA) score plots for liver extracts showed a clear separation between the fish fed the control diet and wheat starch diet based on liver metabolomes. The levels of alanine, glutamate and glutamine were higher in yellow perch fed the wheat starch diet compared with fish fed the control diet. The wheat starch diet also significantly affected purine and glutathione metabolism in yellow perch. The current study suggested that potato or corn starch had less impact on fish health and metabolic profile and is considered to be an appropriate carbohydrate sources for yellow perch under the current testing conditions.



REPRODUCTIVE PHYSIOLOGY OF LUMPFISH, *Cyclopterus lumpus*

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Lumpfish, *Cyclopterus lumpus* L. 1758, is widely distributed across a large area on both sides of the North Atlantic Ocean. This species is a determinate batch spawner, with extended ovary development period (at least 8 months) and a relatively long spawning season (over a 4-month period, from March to July).

Lumpfish are increasingly used as a cleaner fish for removal of sea lice from Atlantic salmon, *Salmo salar,* as a greener and more sustainable alternative to chemical delousing. Interest in use of hatchery-reared lumpfish has therefore increased rapidly; hence, there is a need for year-round production of lumpfish juveniles. A requirement to achieve this objective is a deeper basic understanding of the Brain-Pituitary-Gonad (BPG) axis of the lumpfish. This endocrine cascade consists of a brain-to-pituitary communication through gonadotropin-releasing hormones (GnRHs) and their receptors, relayed by a pituitary-to-gonad signaling through two gonadotropins (LH and FSH) and their respective receptors. In turn, the gonads produce sexual steroid hormones, leading to the final maturation process.

We have now established the lumpfish genomic repertoire for the GnRHs, gonadotropins and their receptors, by performing *in silico* analyses on the recently released, non-annotated draft genome. The presence of multiple paralogues of our genes of interest was addressed by phylogenetic analyses and synteny verification. We have documented the presence of three GnRHs, four GnRH receptors, one LH- and one FSH-β subunit, and one FSH receptor.

To investigate dynamic changes in transcriptional activity of these BPG genes, we monitored initially immature lumpfish individuals for 10 months (end of September to beginning of August) in a facility in Bergen (Norway), under simulated natural light conditions. We have characterized the molecular, morphological histological and endocrine changes associated with the maturation process. Plasma was analyzed for 11-keto-testosterone (11-KT) and 2-estradiol (E2) of males and females, respectively.

In females, we observed a gradual increase in GSI peaking at the vernal equinox, correlating with a peak in LH-β mRNA in the pituitary and in E2, sharply decreasing thereafter. This LH-β/E2/GSI peak was preceded by a peak of GnRHRn2 expression in the pituitary in the end of February, with an increase detectable already from one month after the winter solstice. The GnRHRn2 peak correlated with a sharp increase in FSH-β mRNA in the pituitary. In males, the GSI increase was more subtle, but a sharp increase in 11-KT was detected around the vernal equinox, with a peak at the beginning of May, a bit later than in females, followed by a clear drop. In contrast to females, this peak was correlated with a peak in LH-β but also FSH-β and GnRHRn2 expression. The mRNA levels of LH-β and FSH-β increased at the end of February, while the increase in GnRHRn2 was more continuous.

These results indicate a different regulation in males and in females, and give insights into the temporal sequence of signaling that regulates maturation. Knowing the identity and temporal expression pattern of GnRHRn2 receptor is key on the way to establishing a protocol to try to artificially stimulate maturation in this species at a very upstream level. This research bears a great potential for improving the efficiency and sustainability of Atlantic salmon aquaculture, in addition to providing basic knowledge on lumpfish biology.

AQUACULTURE AT THE MARINE RESOURCES RESEARCH INSTITUTE-WADDELL MARICULTURE CENTER, SOUTH CAROLINA, USA

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The South Carolina Department of Natural Resources' Marine Resources Research Institute MRRI) has conducted aquaculture research for almost 50 years. The MRRI is 25,000 sq ft research facility designed to provide essential life history and environmental data for management of the State's living marine resources. The building is equipped with two wet labs that contain eight individual broodstock maturation systems, algal culture laboratory, three environmental rooms with photothermal control, replicate RAS systems, as well as flow-through extensive tank systems. The MRRI is situated on Charleston Harbor, a high salinity estuary, and the facility is equipped with settled, polished, flow-through seawater and 8 g/L well water.

The Waddell Mariculture Center (WMC) is part of the MRRI and is in the Southern part of South Carolina in Bluffton, SC. It is equipped with (12) 0.10 ha, (9) 0.25 ha, and (3) 0.50 ha lined seawater ponds with water control structures. In addition, the Center has a newly renovated laboratory with seven RAS systems for broodstock maturation, larval culture, and juvenile grow-out. WMC is on the Colleton River, another high salinity estuary.

The Hollings Marine Laboratory is a consortium facility shared between SCDNR, NOAA, NIST, College of Charleston and Medical University of South Carolina. The facility contains the MRRI Genetics laboratory, an aquaculture facility with algal room, live feeds production, hatchery, three broodstock maturation rooms, and a RAS system with (24) 6 ft dimeter tanks. Adjacent to the animal production wing is the chemistry wing with two Nuclear Magnetic Spectrometers and numerous Mass Spectrometers.

Scientists from all three facilities and numerous outside partners work cooperatively on questions regarding nutrition, extensive and intensive production, genetic impacts of escapement, and emerging species life history. We are currently working with red drum, seatrout, cobia and tripletail.

BUILDING AND MAINTAINING PUBLIC AND POLITICAL SUPPORT FOR SHELLFISH AQUACULTURE IN THE PACIFIC NORTHWEST

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While shellfish farming has been an integral part of the rural character and economy and in Western Washington State for over a century it is not without its detractors. Changes in industry farming practices involving ropes, plastics, structures on the beaches or floating on the surface have drawn opposition from adjacent shoreline home owners. Some of the concerns raised are legitimate and others are fabricated to attempt curtail farming operations or get permits denied. The conflicts have increased in recent decades as adjacent shoreline properties have evolved from summer cabins and local residents who value the working waterfront and enjoy seeing the farming activities to year-round residences with new comers to the region who may or may not share those same values.

As the largest shellfish farming business in Washington State, Taylor Shellfish Farms has had it share of detractors. The company has a public affairs team that works to educate the public, various stakeholder groups and policy makers about shellfish aquaculture. Much of this work is coordinated with the Pacific Coast Shellfish Growers Association and other shellfish growers.

Taylor Shellfish Farms employs a multifaceted approach to building social license. The company used to sell 95% of their products wholesale outside the state. Enlightened by public opinion polling that showed them that people who eat shellfish like them more they now have three retail stores and six oyster bars under the company name, they sell their products to a couple hundred local restaurants and have the company logo and shellfish themed truck wraps on all their delivery trucks and semi-trailers. They have transitioned a historic waterfront processing plant into one of their oyster bars. At that location they have incorporated a demonstration plot and educational kiosks where customers get to experience a working shellfish farm. These public facing facilities are profitable but more importantly that have dramatically increased public and political support for our business. Other company and industry efforts include tours, beach clean up events, shellfish themed festivals, Hill walks in Washington DC and Washington State, political fundraisers and product donations for ENGOs who support our efforts to protect water quality and shoreline environmental health.

Finally, Taylor Shellfish Farms strives to be a good neighbor. We are active in the communities where we farm and encourage our managers and employees to engage with neighbors and the community.

FIELD VALIDATION FOR A HYDRODYNAMIC FINITE ELEMENT MODEL OF A MACROALGAE LONGLINE AT AN EXPOSED SITE

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Successful design and operation of a macroalgae farm depends on an accurate understanding of the system's behavior in waves and currents. Dynamic finite element modeling techniques can be used to quantify the response of aquaculture farms to operational and extreme environmental conditions. In this study, a dynamic finite element model of an exposed kelp line was developed in Hydro-FE. This model used previously measured mechanical properties of *Saccharina latissima*. It also employed hydrodynamic properties previously derived from tank tests of a physical model of a 1-m section of a kelp longline with 3-m long kelp fronds, including normal and tangential drag coefficients and added mass coefficients. The project team deployed load-cells on each mooring leg of the exposed kelp line to measure mooring tensions under environmental loading. The environmental conditions, including waves, currents, and tidal elevation were measured with two Acoustic Wave and Current (AWAC) sensors each deployed near the load cells. These loadcases were replicated in the dynamic finite element model. Power-spectral densities and maximum values for mooring line tension were calculated for the field data and the model outputs for the same environmental forcing conditions. Root-mean-squared error was quantified between the model predictions and the field observations.

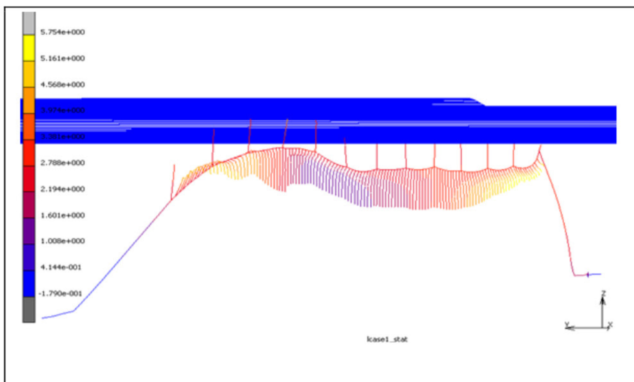

Figure 1. Hydrodynamic Finite Element model of exposed kelp line in Hydro-FE

Figure 2. Authors installing load cells in mooring line to measure tensions during wave and current forcing

ENGINEERING ANALYSIS OF A MUSSEL BACKBONE SYSTEM FOR AN OPEN-OCEAN SITE

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The sustainability of open-ocean shellfish farms depends on an accurate understanding of the system's behavior in waves and currents. Several engineering designs were tested using numerical models for farming systems proposed for the Ventura Shellfish Enterprise (VSE) project to be located in federal waters outside Ventura Harbor, California. This modeling was conducted to address regulatory concerns about the suitability of farm engineering to withstand storms.

Extreme wave, wind, and current values were quantified for 100-year and 20-year return periods at the selected project location by fitting Gumbel distributions to historical data for the site. The dynamic behavior of the system was quantified under the extreme storm conditions using a hydro-/structural dynamic finite element approach. The engineering approach considered four factors: Survival (minimum required capacities of lines and anchors); operations (force required to lift the backbone for maintenance and harvesting, installability, and navigability); performance (RMS accelerations of mussel ropes as a proxy for mussel drop-off; ability to facilitate predation avoidance); and budget (minimum required component sizes and availability of components). Multiple design alternatives were compared based on those four factors. Additionally, a theoretical limit for the percentage of mussel weight that should be supported by submerged buoyancy was established as a function of incident current speed. This limit is based on the force balance between the wet weight of the mussel ropes and the vertical component of normal drag on the mussel ropes as they lay back at an angle due to the incident current. For the maximum expected mussel growth considered in this application, it was found that the maximum submerged buoyancy should be limited to two-thirds of the wet weight of the mussel droppers.

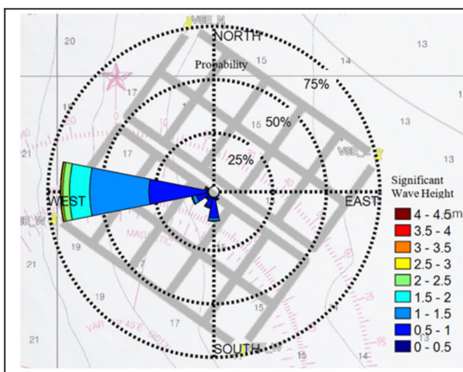

Figure 1. Probability distribution of sea-states by wave direction and significant wave height.

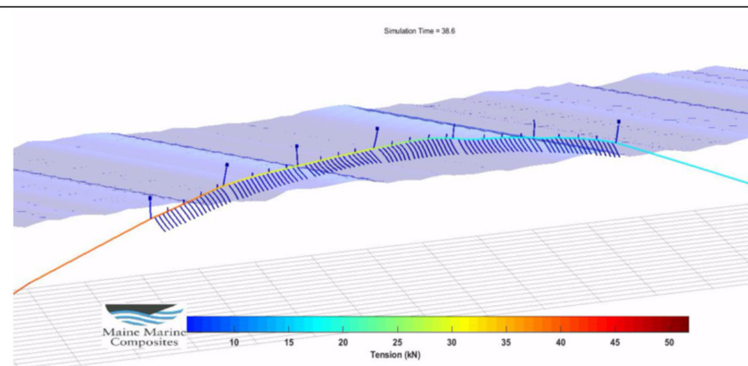

Figure 2. Backbone deformation and distribution of tensions in a 100-year current event with 10-year return period waves and wind speed.

ASSESSING GENETIC RELATEDNESS OF INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS (IHHNV) ISOLATES RECENTLY DETECTED IN THE US

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Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) is an OIE-listed viral pathogen of penaeid shrimp and is prevalent in the Americas, Asia and Australia. The virus has caused large-scale mortalities in blue shrimp (*Penaeus stylirostris*) and is known to cause growth retardation in Pacific white shrimp (*P. vannamei*) and black tiger (*P. monodon*) shrimp. IHHNV is a non-enveloped, single-stranded DNA containing virus belonging to the family *Parvoviridae*. During June of 2019, *P. vannamei* samples (broodstock and post-larvae originating in shrimp facilities in Texas and Florida) were submitted to Aquaculture Pathology Laboratory for routine screening of a panel of OIE-listed and non-listed viral, bacterial and fungal pathogens by two commercial facilities in the US. DNA and RNA were isolated using a Maxwell 16 cell LEV DNA Purification Kit (Promega, Wisconsin) and a Maxwell 16 cell LEV RNA Purification Kit (Promega, Wisconsin), respectively. The screening of samples were done by polymerase chain reaction (PCR) and following OIE-recommended protocols for the OIE-listed pathogens. For the OIE non-listed pathogens, PCR was performed following published protocols for the corresponding pathogens. For both commercial operations, samples were tested negative for all the pathogens screened except for the IHHNV. IHHNV PCR screening was done using the primer sets, 389F/R and 309F/R that amplifies two different regions in the non-structural gene, NS1 in the IHHNV genome. Both amplicons (309 bp and 389 bp) were sequenced and NCBI database search showed 99-100% similarity to IHHNV isolates from Asia and Latin America. Subsequently, IHHNV capsid protein (CP) gene (990 bp) was amplified by PCR and sequenced. The CP gene of the US isolates showed >99% similarity to Type II infectious forms of IHHNV isolates from Ecuador. Phylogenetic analyses using Neighbor-Joining and Maximum Likelihood methods showed that the IHHNV isolates from the US clustered with homologous isolates from Ecuador. The detection of an OIE-listed pathogen in commercial facilities in the US highlights the need to follow biosecurity in the hatcheries and grow-out ponds to mitigate economic losses.

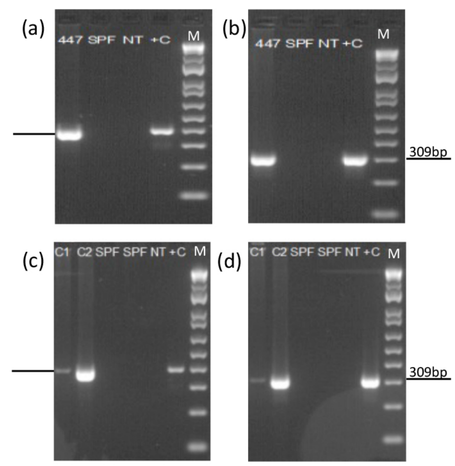

Figure 1. Detection of IHHNV by conventional PCR in *Penaeus vannamei* samples originated in the US. 447= *P. vannamei* broodstock sample originating in Texas, C1 and C2= *P. vannamei* post-larvae samples originating in Florida. SPF= Specific Pathogen Free *P. vannamei* shrimp, NT= No template control, +C= Positive control for PCR. M= 100 bp molecular weight marker from New England Biolab.

TRANSCRIPTIONAL NETWORK REGULATION BY 17BETA-ESTRADIOL IN MALE RADIAL GLIA OF *Halichoeres melanurus* A PROTOGYNOUS MARINE ORNAMENTAL SPECIES

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Teleost fishes have evolved impressive strategies to maximize reproductive fitness. One strategy is to change sex, which involves the "re-modeling" of sexual dimorphic brain regions that control sex-specific reproductive behaviors. The teleost brain shows tremendous plasticity and, unlike mammals, synthesizes high levels of aromatase to control this transformation. *Halichoeres melanurus* or tail-spot wrasse is a species of wrasse endemic to the western Pacific from Japan to Samoa and south to the Great Barrier Reef. Larval fish first develop into smaller females, and transition into larger males. Thus, this species is sexually dimorphic and phenotypic sex is discernable, although little is known about the transition state during female to male sex change. We employed SMRT sequencing (PacBio IsoSeq) to obtain a reference transcriptome for gene mapping and *de novo* sequence assembly for *H. melanurus*. Primary cell cultures for radial glia cells (RGCs) were isolated from male wrasse, and subsequently treated with 17beta-estradiol at 1 μM for 24 hours in culture to identify estrogen-responsive transcripts in RGCs. This is important because radial glial cells express aromatase (AroB), the enzyme that converts testosterone (T) into estradiol (E2) and one, which is considered to be one of the master regulators for sex change. E2 altered the expression of 55 genes in the RGCs and down-regulated spectrin alpha, non-erythrocytic and 1 3-phosphoinositide dependent protein kinase 1b while up-regulating ATPase H+ transporting V1 subunit C1 and calcium binding protein 39. Our transcriptome network analysis revealed that E2 induced gene networks associated with plasticity of neurons (e.g. synaptic transmission, nervous system activation, and axonal guidance) suggesting that E2 mediates glial derived synaptic patterning in the CNS. Other major processes that were increased with E2 treatment in the wrasse RGCs were those associated with immune system development and function, as well as the inflammatory and innate immune response. These investigations are expected to shed novel insight into the neuroplasticity of sex in teleost fishes.

DEVELOPMENT OF AQUACULTURE PROTOCOLS FOR BLACKBANDED SUNFISH *Enneacanthus chaetodon* AND FLAGFIN SHINER *Pteronotropis signipinnis* TWO FLORIDA NATIVE ORNAMENTAL SPECIES

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Interest from domestic and foreign markets in native North American fishes for ornamental use has provided a unique opportunity for expansion in the ornamental aquaculture industry. Developing aquaculture techniques for these animals not only supports novel economic aquaculture opportunities, but also provides a valuable foundation for potential restoration aquaculture endeavors for imperiled species. Two candidate species for ornamental aquaculture are the blackbanded sunfish *Enneacanthus chaetodon* and the flagfin shiner *Pteronotropis signipinnis*. *E. chaetodon* is a small, imperiled centrarchid native to the Eastern US, while *P. signipinnis* is an active, schooling cyprinid ranging throughout the gulf states. Both are currently traded as wild collected individuals in the ornamental fish industry. Here, we develop techniques for spawning, embryo incubation, embryo disinfection, larval rearing, as well as characterize larval development.

For *P. signipinnis*, spawning substrate preference was determined experimentally by providing three different types of spawning media to six replicate tanks stocked with eight individuals: gravel, a floating yarn mop to mimic floating vegetation, and a bottle brush to mimic submerged structure. Brood fish were allowed to volitionally spawn for 3 weeks, with eggs being collected every other day. *P. signipinnis* preferentially spawned on floating yarn mops, followed by bottle brushes, while showing the least preference for gravel. Evaluation of incubation techniques revealed that static and upwelling incubation are both viable strategies ($P = 0.314$), with an overall mean hatch rate of 81.6 ± 10%. Embryo disinfection was safe for multiple concentrations of formalin, hydrogen peroxide, and iodine, with hatch rates following disinfection being similar to the control in each group (P 0.104). *P. signipinnis* larvae survived best following 14 days of feeding with Otohime and Ziegler AP-100 microfeeds (57.5 ± 6.8% and 52.5 ± 3.9%, respectively) when compared to Golden Pearls and live *Artemia* nauplii (27.5 ± 4.9% and 17.5 ± 5.3%, respectively, $P < 0.001$).

E. chaetodon volitionally spawned in dense, submerged vegetation and resulting larvae only survived on *Artemia* nauplii with no larvae surviving following 14 days of feeding with microparticulate diets. Embryos were successfully incubated *in situ* in brood aquaria. Larval growth was characterized from hatch until 34 dph. In addition, gastrointestinal ontogenetic development was characterized using histology, histochemistry and digestive enzyme activity assays. In total, the results of these investigations provide preliminary culture techniques that will support commercial and restoration aquaculture endeavors for these native North American fishes.

EXPECTED VS ACTUAL DIVERSITY OF THE KELP *Alaria marginata* ZOOSPORES IN MARICULTURE SEEDING

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Alaria marginata is a kelp species in the mid to low intertidal and is of commercial importance in the growing Alaskan mariculture industry. Understanding how sporophyte parent origin affects microscopic kelp survival, growth, and reproduction is important for informing regulators to understand how seeding string in a hatchery may affect wild gene flow. Do gametophytes from one sporophyte parent collected directly next to another outperform its neighbor?

In this study we investigated differences in the characteristics of gametophytes of *A. marginata* from sporophytes collected from a microscale (1-10 m) and on larger scales (>10 km) within the Juneau, AK area. Fertile sporophytes were collected from two sites (AUK and SUN). Spore release was conducted and zoospore densities were adjusted to the same concentration. Nine petri dishes were seeded from each sporophyte parent and placed in a lit incubator at 12° C.

Dishes were monitored daily, with weekly media changes over a 20-day period. Dishes were monitored for gametophyte length and settlement density.

Our preliminary results suggest that site as well as sporophyte parent nested within site were significant factors in growth and density of gametophytes measured. Results from an upcoming second round of sporophyte parent effects on growth is underway.

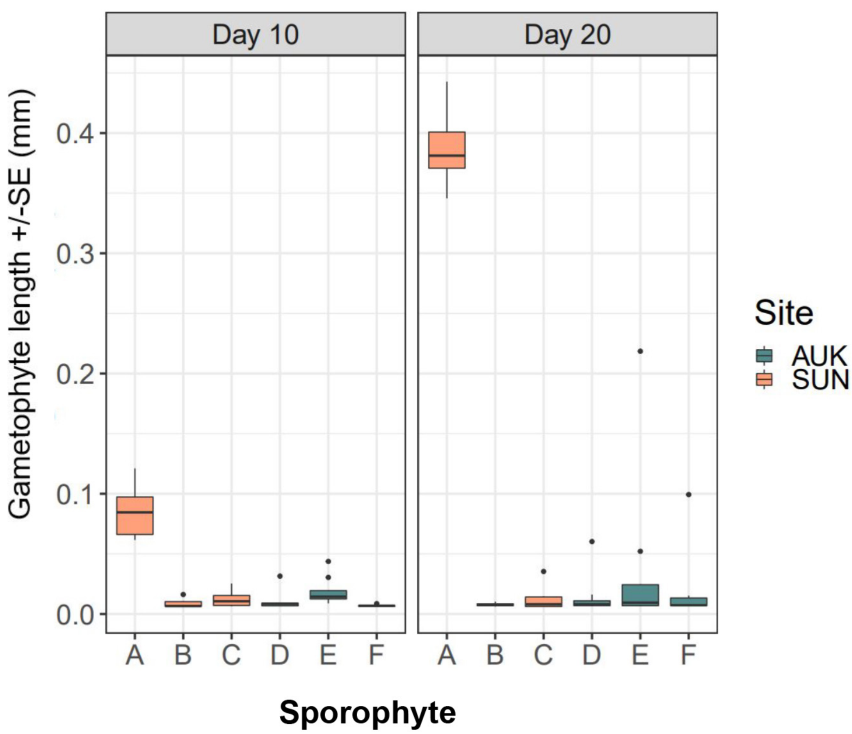

Figure 1. Average gametophyte length at day 10 and 20 by sporophyte parent (A-F) within site (AUK and SUN).

NEW DIRECTIONS IN THE GENETIC IMPROVEMENT OF OYSTERS ON THE US WEST COAST

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Two objectives of the Pacific oyster (*Crassostrea gigas*) industry on the U.S. West Coast are currently (i) resistance to OsHV-1, a deadly oyster virus that is currently present in Tomales Bay and San Diego Bay, and (ii) robust triploid oysters, which are more palatable due to their inability to develop mature gonads in the summer months. The Molluscan Broodstock Program (MBP) is a Pacific oyster breeding program that works with industry stakeholders to reach these objectives. In 2018, field and laboratory trials conducted on MBP broodstock families showed that between 50% to 70% of the between-family variation in OsHV-1 survival was heritable. After one generation of genetic selection, we estimate that OsHV-1 field survival in the MBP increased by 30% using survival data collected in a laboratory trial, although a field trial is still in progress.

Triploid oysters, which are derived by crossing diploids and tetraploids, have a tendency to be sensitive to environmental fluctuations on the West Coast, and thus experience greater mortalities than diploids. One hypothesis attributes this sensitivity to the small genetic base of tetraploid Pacific oysters used by hatcheries; therefore, developing new stocks of tetraploids would allow this hypothesis to be tested. We developed a new method of selecting tetraploid larvae after polyploidy induction from diploids. The method was tested on one-day-old Kumamoto oyster (*C. sikamea*) larvae. After selection, larvae were returned to the hatchery system for further growth. Larval size at day five did not significantly differ between diploid (mean: 80 µm, sd: 11 µm) and tetraploid (mean: 74 µm, sd: 14 µm) larvae. This method holds promise as it skips the intermediary triploid stage toward tetraploid induction, its applicability to any shellfish species with a larval stage similar to *Crassostrea* spp., and its ability to sidestep use of hazardous chemicals in the isolation of polyploid oysters.

EVOLVING SOCIAL LICENSE CHALLENGES AND OPPORTUNITIES FOR SEA FARMING. A MAINE PERSPECTIVE

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Maine issued its first marine sea farm lease 46 years ago. Since that first mussel farm lease, 28 other species have been farmed in our state waters, and our understanding of biology, ecology, and farm site technology and operations have improved dramatically. As innovation moved forward, so did the regulatory framework that codifies Maine farmer's social license to operate.

Through the first four decades of Maine's experience, opposition to leasing sites on the ocean for farming has been ever present. Riparian owners, commercial fisherman, recreational boaters, conservation organizations, and community members concerned about the environment have voiced opposition to permitting farm sites during and after the leasing process.

Over the last decade farming intensity has increased. Today there are 815 lease sites; 165 standard and 650 limited production sites in Maine waters. In addition, 80 new standard site applications are being processed by the Maine Depart of Marine Resources. As the number of sea farm sites has grown, opponents to farming have adopted sophisticated approaches in their opposition to lease applications. But this is not the only change. Former sea farm opponents have begun farming, and in the bays they farm, opposition is lessening, and social license to operate is improving. This presentation will address what has changed and how lessons learned over the past decade may be employed in other regions.

MARINE FINFISH AQUACULTURE RESOURCES IN SOUTHERN CALIFORNIA

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Hubbs-SeaWorld Research Institute (HSWRI) is a 501-(c)(3) nonprofit research organization that was established in 1963 to “Return to the sea some measure of the benefits derived from it”. Marine fish aquaculture has been a core area of research at HSWRI since the late ‘70s. We operate a Marine Research Laboratory in San Diego that has a flow-through seawater capacity of 350 gpm and a Fish Hatchery in Carlsbad that has a seawater capacity of 1200 gpm. Within each facility are specialized systems (flow through and reuse) from experimental to commercial-production scale. Currently, we are spawning white seabass (*Atractoscion nobilis*), California yellowtail (*Seriola dorsalis*), and California halibut (*Paralichthys californicus*). Reliable protocols for commercial-scale production of seabass and yellowtail have been developed and halibut are close behind. In addition to land-based facilities, HSWRI owns and operates three coastal cage systems that are permitted for rearing (and releasing) white seabass. The cages are located in San Diego Bay, Agua Hedionda Lagoon, and Catalina Harbor. Collectively, these land and sea-based facilities are very unique and would be very difficult and expensive to permit in California today.

The core of our aquaculture staff have decades of experience and are expert in the production of marine fish and the live feeds required to support production. Additionally, we currently have in-house expertise in fish health and systems design. Expertise in other required disciplines (e.g. nutrition, genetics, and physiology) is filled with postdoctoral positions when funding is available but more often we collaborate with others given the challenges of funding these positions on soft money. These collaborations have been highly fruitful and include working with NOAA and USDA, as well as universities in the United States and Mexico. We have developed an excellent working relationship with state and federal agencies, ocean user groups, and the aquaculture industry through decades of interaction and participation on various boards and advisory panels.

EFFECTS OF HEAT KILLED *Lactobacillus plantarum* L-137 (HK L-137) SUPPLEMENTAL DIETS ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF JUVENILE STRIPED CATFISH (*Pangasianodon hypophthalmus*)

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The present study was conducted to determine the effects of dietary heat killed *Lactobacillus plantarum* (HK-L137) on growth, survival, and immune response of juvenile striped catfish (*Pangasianodon hypophthalmus*). A 60-day feeding trial was carried out and four replicates groups of experimental fish (initial wt. 0.06 g) were fed with respective test diets containing four different concentrations of HK L-137 (0 ppm as control diet, 10 ppm, 20 ppm and 50 ppm). At the end of the feeding trial, results showed that the dietary supplementation of HK L-137 significantly enhanced the final weight (W_f), weight gain (WG) and specific growth rate (SGR) of juvenile striped catfish fed the diets containing 20 ppm and 50 ppm HK L-137 ($p<0.05$). Simultaneously, the survival rate of experimental fish fed the diet contains 50 ppm HK L-137 was significantly higher than the HK L-137 free group of fish fed with basal diet ($p<0.05$). In addition, the highest protein efficiency ratios (PER) were also recorded in HK L-137 supplemented groups. Moreover, feed conversion ratio (FCR) of HK L-137 supplemented groups were also significantly lower than the control group. Furthermore, the dietary supplementation of HK L-137 improved and augmented the immune parameters including lysozyme activity, the number of RBC and WBC of juvenile striped catfish. Thereafter, a 14-day bacterial challenge experiment was also carried out and the highest mortality rate (100%) was recorded in the positive-control group of fish fed with basal diet. Consequently, post challenge survival rates (PCSR) of HK L-137 supplemented groups were higher than the control group. Present study demonstrated that the dietary supplementation of HK L-137 has the tremendous positive-effects on SR, FCR, WG, SGR, FI, PER, immune response and disease resistance of juvenile striped catfish.

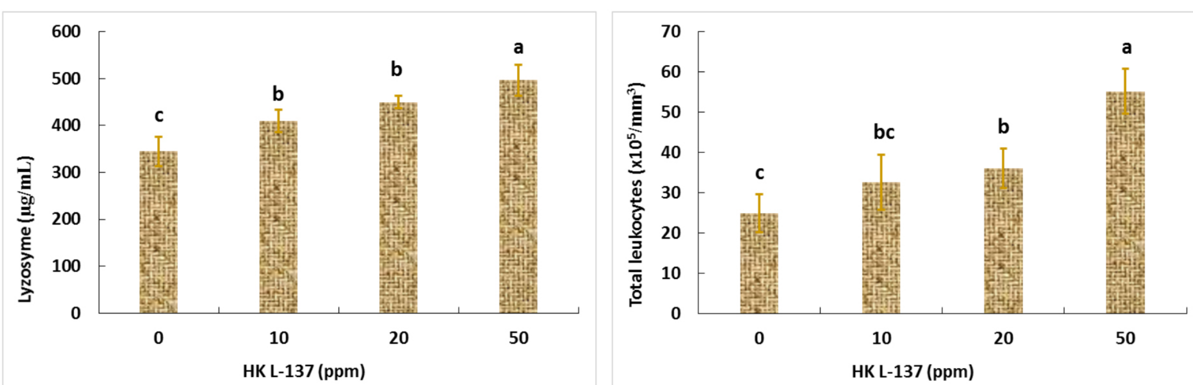

Figure 1. Lysozyme activity and number of WBC of juvenile striped catfish, each bar represents the mean value for each group with standard error of mean.

SEA URCHIN BIOCONTROL OF INVASIVE MACROALGAE: AQUACULTURE INNOVATION FOR SUCCESSFUL REEF RESTORATION

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In the 1974 several algae species were introduced to Kāne`ohe Bay as part of study to promote a carrageenan industry. Over the next 25 years coral reef habitats in the bay became increasingly dominated by invasive algae. In 2002 an advisory group consisting of the State of Hawai'i Division of Aquatic Resources (DAR), The Nature Conservancy and the Hawai'i Institute of Marine Biology (HIMB) proposed a two-step solution for invasive algae eradication: mechanical removal and urchin biocontrol.

Seaweed was mechanically removed by means of a diver operated suction pump known as the 'Super Sucker'; an urchin hatchery was developed to provide native collector sea urchins for biocontrol. Native patch reefs throughout Kāne`ohe Bay were surveyed and a set of statistical analyses were developed to rank, rate, and prioritize the reefs based on invasive algae cover.

In 2018 all priority target patch reefs determined to be over 90% free from invasive algae. Due to the lower invasive algae levels on the patch reefs, the Super Sucker is not currently used. The native corals are recovering and urchins are used to spot treat invasive algae hotspots.

FATTY ACID DEPOSITION IN FILLETS OF POST-SMOLT ATLANTIC SALMON *Salmo salar* FED DIETS SUPPLEMENTED WITH DHA-RICH OIL EXTRACTED FROM *Schizochytrium* spp.

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The increasing demand for natural sources of omega-3 fatty acids combined with the pressure to improve sustainability of aquaculture operations are conducive to developing innovative solutions for the salmon producers. The objective of this study was to assess the effect of a novel oil (DHA NaturTM) extracted from the microalgae *Schizochytrium* spp. on weight gain, feed intake, feed conversion, proximate (dry matter, crude protein, crude lipid, ash) and fatty acid composition, and polyunsaturated fatty acid retention efficiency of Atlantic salmon (*Salmo salar*). Salmon (initial body weight: 552.0±13.7 g) were fed one Control diet (Diet A: 10% South American fish oil + 18% canola oil + 0% DHA NaturTM) and four diets containing graded levels of DHA NaturTM (Diet B: 0.677%; Diet C: 1.354%; Diet D: 2.031%; Diet E: 2.708%) for 112 days. The dietary content of DHA+EPA was 2.62 % (as-is) across diets. Dietary fish oil to vegetable oil ratios varied from 0.36 (Diet A) to 0.20 (Diet E). The vegetable oils used in the feed formulas were canola oil and flaxseed oil (degummed). Each diet was randomly allocated to three 800-liter circular tanks at 25 salmon per tank and maintained in saltwater (25 ppt) at 13.9±0.6°C in a recirculating aquaculture system.

Growth, feed intake, feed conversion (FCR; feed:gain) and proximate composition of skinless fillets were not significantly affected by dietary treatments (P>0.05). Although not significant, fillet DHA (% as-is in fillet) showed upward trends in all experimental diets, with those containing higher levels of DHA Natur™ inclusion presenting with an overall greater rate of total fillet DHA content after 112 days. When fillet DHA content (% as-is) was plotted against time (days), the positive regression slopes indicated diets with higher dietary DHA Natur™ inclusion generally had higher rates of DHA deposition: Diet A (Control): 0.0008 %/day, Diet B: 0.0011%/day, Diet C: 00012%/day, Diet D: 0.0010%/day, Diet E: 0.0016%/day. Fillet DHA deposition rate (mg/°C*day) did not differ significantly between experimental diets (P=0.9401) and ranged from 1.01 (Diet A) – 1.15 (Diet D). However, there was a significant negative linear relationship between EPA deposition rate (mg/°C*day) and DHA Natur™ inclusion (P=0.0046) as a result of the low content of this fatty acid in *Schizochytrium* spp. EPA retention efficiency did not differ significantly between treatments, which suggested the potential EPA requirement was met at all inclusion levels of DHA Natur™.

In conclusion, the oil extracted from *Schizochytrium* spp. (DHA Natur™) was a nutritious and beneficial source of available DHA for post-smolt Atlantic salmon. The results in this study indicated DHA from this novel ingredient was as available and efficient as that from high quality South American fish oil.

EVALUATING THE POTENTIAL OF USING FRESHWATER PRAWN (*Macrobrachium rosenbergii*) AS A BIOLOGICAL CONTROL FOR SOLIDS ACCUMULATION IN HYDROPONIC TROUGHS OF A DWC AQUAPONICS SYSTEM

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Solids accumulation within hydroponic troughs of aquaponic systems is a problem due to the suffocation of plant roots and oxygen depletion within the system. The freshwater prawn, *Macrobrachium rosenbergii*, is a commercially important decapod crustacean which may have potential for use as a biological control of solids accumulation within hydroponic troughs due to their detritivore feeding behavior as well as their potential to disrupt settled solids from the trough bottom through movement and constant grazing.

Two four-week trials were designed to determine the effects of freshwater prawn as a solids control agent within aquaponics systems. A biomass of 2 kg of GIFT strain Nile tilapia (*Oreochromis niloticus*) with an average weight of 59g were stocked into each of six replicate DWC aquaponics systems and fed 100g of 34% protein feed/day. The 1800L/systems consisted of one 415-L fish tank, one 190-L clarifier, one 115-L mineralization tank, two 450-L hydroponic troughs and a 180-L sump. Three systems were stocked with 5 prawn/trough and three systems without prawns (control). Prawn were placed under floating rafts containing Bibb Lettuce (*Lactuca sativa var. capitate*) (66 plants/system). Based on preliminary data, root guards were used to isolate the prawn from the plant roots to limit prawn from foraging on roots. All systems were supplied with root guards to maintain consistency between treatment and controls.

Water quality variables remained acceptable for fish, prawn and plant production throughout both trials for treatment and controls. There was a 69% decrease ($P<0.05$) in solids accumulation within the troughs of systems with prawn compared to systems without prawn. This led to an overall reduction in total settleable solids within the systems with prawn compared to systems without prawn. There was a slight increase in nitrate in systems with prawn compared to systems without prawn, although it was not statistically significant. Average fresh plant weight was significantly lower for systems with prawn (76.5g) compared to systems without prawn (84.6g). Leaf nutrient concentrations of K, S, and Na were significantly higher for plants from systems with prawn than without prawn.

The results showed that prawn can be used to decrease the biological solid accumulation within hydroponic troughs of a DWC aquaponics system. The results also confirm the need for root protection if prawns are to be used for this purpose within the hydroponic troughs due to the consumption of plant roots. The root protection devices implemented in this study were partially successful at protecting the roots; however, due to the prawn's tendencies to escape the enclosures plant growth was decreased in treatments containing prawns due to root consumption.

SATELLITE MAPPING OF THE DEVELOPMENT OF SHRIMP AQUACULTURE, AND ITS IMPACTS ON MANGROVES AND OTHER COASTAL WETLANDS

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Given the explosive development of shrimp aquaculture in recent decades, Clark Labs has developed a semi-automated mapping program based on Landsat satellite imagery to facilitate monitoring. Working in conjunction with the Gordon and Betty Moore Foundation, and its partners such as the World Wildlife Fund, mapping began in 2014 and continues on an ongoing basis.

To date, 8 countries have been mapped for 1999, 2014 and 2018: Bangladesh, Cambodia, Ecuador, India, Indonesia, Myanmar, Thailand and Vietnam. Landsat 8 OLI imagery is processed to yield 15m resolution imagery over 7 spectral reflectance bands over the visible, near- and middle-infrared wavelengths. A team of analysts identify representative examples of the land cover classes of interest in the imagery, after which machine learning computer algorithms complete the classification. The key classes include pond aquaculture, mangroves and other coastal wetlands, from which a variety of change maps can be produced (Figure 1).

The mapped data are made publicly available through an interactive web map which also provides the ability to download the data as georeferenced layers for Geographic Information Systems (GIS). The results indicate a rapidly growing industry. For example, just between 2014 and 2018 there has been a net gain of 1665 km^2 of pond aquaculture across the 8 countries (92% of which came from Indonesia and India). While 41% of this came from mangrove, there has actually been a net gain in mangrove as a result of restoration and new colonization (2238 km^2).

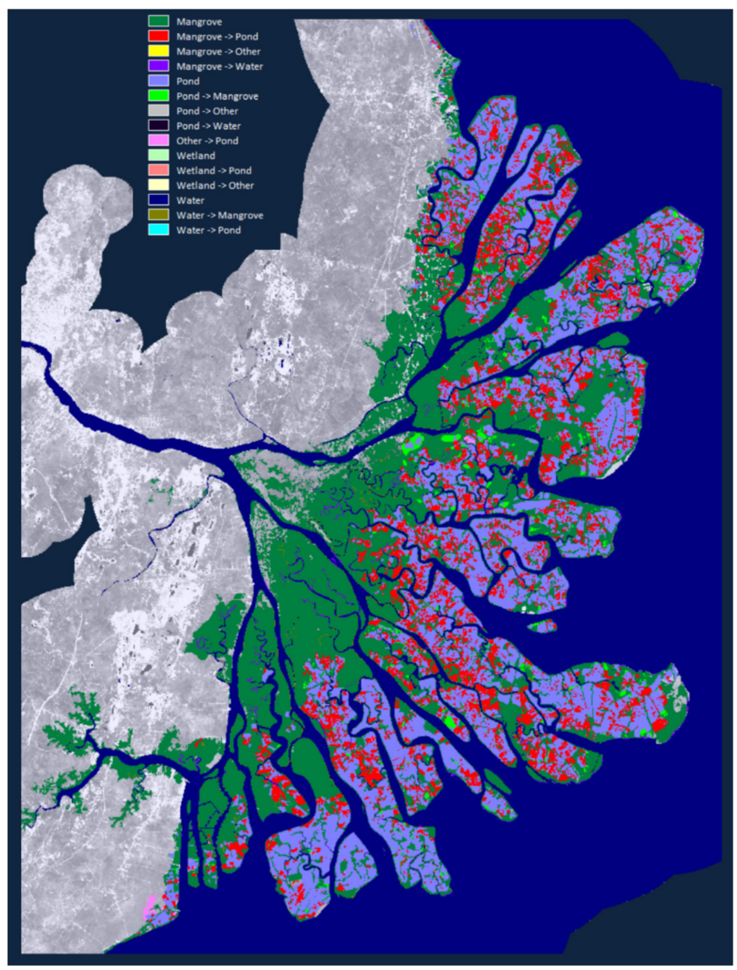

Figure 1. Example of the change map from 2014 to 2018 in the Mahakam Delta, East Kalimantan, Indonesia. The red areas indicate mangroves that were converted to pond aquaculture over this period. Dark green areas are remaining mangrove while light blue areas are pond aquaculture in both 2014 and 2018. Gray areas are other land covers.

UNTIL ONLINE TRAINING FOR ENTRY-LEVEL AQUACULTURE WORKERS: TAKING A SUCCESSFUL IN-PERSON MODEL IN RHODE ISLAND AND GOING DIGITAL

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Rhode Island (like much of the East Coast) has been enjoying geometric growth in shellfish farming. Aquaculture business owners struggle to find and retain skilled, entry-level workers seeking long-term career paths in the industry. New hires typically wash out after a few days on the job and the injury rates among new employees are unacceptably high.

To respond to training needs, the Aquaculture Training Partnership was created in 2015 with financial assistance from the Rhode Island Department of Labor and Training. The partnership offers a training curriculum based upon industry-identified employment needs, targeting entry-level workers. The program fosters linkages amongst the government, private sector, academic and community interests working to expand aquaculture employment opportunities in the 21st century.

The classroom content includes soft-skills, basic boating skills, boating safety, shellfish biology, dealer-shipper skills, ServSafe Kitchen Manager certification, Captain's OUPV 6 Pack class & license and critical hands-on learning through a two-week internship program. The program also is a Nationally Registered Apprenticeship program with Veterans Affairs Approval, enhancing the reach and services. The Partnership started with five employer industry partners in 2015 and has grown to 46 industry partners and 30 strategic partners. In the spring of 2017, the Partnership had an 80% job placement rate, with numerous former training participants in management positions on Rhode Island farms.

While successful by all measures, the in-person training program is expensive, time-intensive (on participants and trainers), and future funds are uncertain. In response and through a grant from NOAA's National Sea Grant program in 2018, the project team is designing a comprehensive online training program that will be shared across the country and available for free to interested workers. The team plans to offer trainings for Sea Grant programs and others in states interested in using/adapting the Rhode Island online program in their state. The platform will be through Teachables.com and feature the current training topics, in addition to emerging topics of import like farm safety, new products, and eco-tours.

Aquaculture Jobs
Training Program
Building Skills for Entry-Level Workers

PLANARIA CULTURE SYSTEMS AT STOWERS INSTITUTE FOR MEDICAL RESEARCH

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The Aquatics team at the Stowers Institute for Medical Research (SIMR) maintain multiple planaria species, including *Schmidtea mediterranea*, *Schmidtea polychroa*, *Dugesia japonica*, *Dugesia sanchezi*, *Dugesia dorotocephala*, *Phagocata gracillis*, *Phagocata velata*, *Girardia tigrina*, and *Girardia guanajuatiensis*. The most widely used species at SIMR is *Schmidtea mediterranea*.

Traditionally, the husbandry and care for planarians in a laboratory setting has been provided using a static culture methodology which has the capacity to consume a considerable amount of resources including space, supplies, time, and manual labor. This is particularly true for large planarian colonies. Here we present alternative culture methodologies to improve efficiency, maximize space usage, and reduce labor. Using recirculating and flow-through aquaculture systems, feeding can be intensified, and good water quality can be maintained for significantly longer periods of time. Therefore, these systems reduce the constraints commonly encountered when managing static cultures. We will review multiple designs of recirculating and flow-through systems that have been tailored to optimize growth and reproduction of both the asexual and sexual *Schmidtea mediterranea*.

VIRULENCE VARIATION OF *Flavobacterium columnare* IN RAINBOW TROUT EYED EGGS AND SAC FRY

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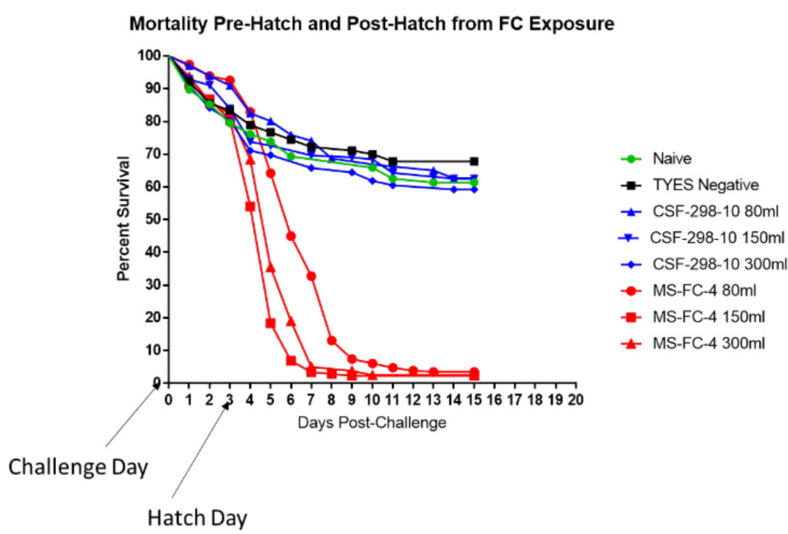
Flavobacterium columnare (Fc) is the causative agent for columnaris disease (CD) and considered an emerging problem in the Rainbow trout industry. Herein, we characterize the virulence phenotype of two Fc isolates against rainbow trout at the eyed egg and sac fry life stages. Both Fc isolates, CSF-298-10 and MS-FC-4, were isolated from gill tissue of diseased fish and are virulent to juvenile fish >1g in size. The virulence phenotype between these two isolates differed when used for immersion challenges against eyed eggs and sac fry. When sac fry were immersion challenged the CSF-298-10 isolate did resulted in 10 to 38% mortality, depending on the dose used, starting on day 6 post hatch. A total loss of viable sac fry was not recorded till challenged on day 8 post hatch with the CSF-298-10 isolate. The MS-FC-4 produced 80% or greater mortality by day 3 post hatch, and could produce 100% mortality when exposure occurred on day 4 post hatch. When sac fry were immersion challenged with the Fc isolate CSF-298-10 on day 3 post hatch no increase mortality was recorded above mock challenged controls. Similarly, when eyed eggs were exposed to the CSF-298-10 Fc isolate, 3 days pre-hatch, by immersion no increase in mortality was observed over controls. Conversely, immersion exposure of eyed eggs with the MS-FC-4 isolate produced 90% or greater mortality by day 6 post exposure. Mortalities did not start occurring till day 1 post hatch and resulted in greater than 95% mortality regardless of the dose tested. These two Fc isolates present stark differences in virulence phenotypes to both eyed eggs and sac fry and present an interesting model system for virulence kinetics and potentially alternative pathways. Importantly, these results demonstrate the potential problem Fc can have on early life stage rainbow trout production, associated with issues concerning vertical transmission and biosecurity.



UNDERSTANDING THE VALUES AND PERSPECTIVES ASSOCIATED WITH FISHING AND SHELLFISH AQUACULTURE IN NORTH CAROLINA, USA

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Fishing as a productive activity has long been integral to the identities, livelihoods, and economies of many coastal North Carolina (USA) communities. The nature of seafood production is changing, however, as commercial fisheries evolve, community demographics shift, and new production techniques like shellfish aquaculture grow. Over the past five years, interest in expanding shellfish aquaculture has risen dramatically among state policymakers, regulators, and prospective farmers, mirroring growth in adjacent states and at the US federal level. A shift toward shellfish aquaculture presents both opportunities and challenges for coastal communities, linked to changing livelihoods, re-allocation of coastal spaces, and prospects for new forms of community and economic development. In this paper, we use Q method to understand local values associated with these changing patterns of seafood production, and how they group together to form broad perspectives.

By combining interviews and quantitative data based on the sorting of value statements, Q method offers an approach to identifying a broad range values associated with seafood production, how those values tend to group together into perspectives, and specific areas of greatest agreement and disagreement. Complemented by document review, we report on these findings and discuss their implications for coastal community wellbeing, aquaculture expansion, and the future of North Carolina seafood production. We find that four distinct perspectives exist in our study area about shellfish aquaculture, fisheries, and associated topics like coastal communities and environments. These perspectives represent thinking on seafood production that goes beyond commonly accepted dichotomies like pro/anti-aquaculture or pro/anti-regulation. The results illustrate a need to attend to nuance in policy debates over shifting seafood production, and suggest opportunities for consensus across perspectives and stakeholders. This project also informs emerging work that expands our Q method beyond this case study context to develop a common comparative tool across geographies and communities undergoing transitions in the seafood sector toward aquaculture.

THE STATUS OF LUMPFISH CULTURE IN THE US

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Lumpfish, *Cyclopterus lumpus,* a species endemic to the northern Atlantic Ocean, has become the focus of the salmonid cleanerfish industry only since 2013. Commercial or large-scale research lumpfish production now occurs in many countries including Norway, UK, Iceland, and Canada. In the US, while no commercial production exists yet, there is a history of lumpfish research, including physiology and aquaculture studies, many of which occurred at the University of New Hampshire (UNH). Currently, researchers at UNH and the USDA National Cold Water Marine Aquaculture Center are working towards the goal of domestic production of lumpfish for use as a cleaner fish to control sea lice in Atlantic salmon and steelhead trout net pens. In addition, a newly formed US Lumpfish Consortium, made up of additional research institutions and aquaculture businesses, is working to address some of the barriers that limit cleanerfish use, in general, and to transfer all known technology to the US to stimulate growth in this aquaculture sector. The major goals of the US Lumpfish Consortium are to: advance the use of cleanerfish in the US by stimulating transdisciplinary research groups to tackle barriers limiting US commercialization of lumpfish; demonstrate commercial-scale production protocols and analyze costs-and-return and scale of growing lumpfish to support industry needs; integration and bioeconomic modeling of lumpfish in salmonid farms; promote lumpfish as cleanerfish by transferring knowledge and technology gained through the Consortium through educational activities, farm tours, manuals and outreach materials, workshops, online media, and presentations.

UNDERSTANDING FACTORS INFLUENCING SOCIAL LICENCE IN PLANNING FOR SUSTAINABLE AQUACULTURE IN COASTAL COMMUNITIES

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Aquaculture is an economic driver for coastal and Indigenous communities on the East and West coasts of Canada and an area of opportunity for the Northern Territories. While the industry has the potential to expand significantly, thereby providing the basis for new employment opportunities and enhanced production techniques in Atlantic Canada, the aquaculture sector faces challenges that can only be successfully addressed by taking an integrated and interdisciplinary approach that connects innovations in the technical and operational aspects of fish farming with society's willingness to fully embrace the sector.

The challenges linked to aquaculture production are widely recognized. They include: infectious diseases, environmental and health impacts on workers and consumers, and ecosystemic interactions. From a societal perspective, the seemingly intractable challenge for the sector has been the difficulty of gaining full acceptance of aquaculture as a complementary valued activity along with other more established uses of the coastal and marine environment. Despite its promise, aquaculture has not been overly successful at achieving and sustaining the social licence needed to fully obtain the benefits that it can provide. We argue that the future development of sustainable aquaculture requires ground-breaking, interdisciplinary 'outside the box' research and development that tightly couple science, innovation and societal needs in a manner that allows for the local and global opportunities within aquaculture to be realized through a societally endorsed, sustainable aquaculture.

Our research takes a holistic and integrative approach to addressing the challenges confronting the sector by linking researchers in the natural sciences with experts in sustainable community development, occupational health and safety, marine management and marine spatial planning. We focus on understanding social licence by explicitly linking knowledge generated with underlying value systems, behaviour and policy change. To do this, our research sheds light on: (i) advancing mechanisms to enhance global leadership in the theory and practice of societally endorsed sustainable aquaculture; (ii) anticipating and addressing occupational health and safety hazards in marine and coastal work associated with aquaculture and its interactions with other marine uses; (iii) improving understanding of other areas of concern that affect social licence such as farmed escapes and waste generation; (iv) filling a significant gap in understanding ecological and social carrying capacity for finfish aquaculture to better predict feasibility of siting locations; and (v) mobilizing knowledge on the processes, parameters, and implementation tools essential for an adaptive, dynamic community-based marine spatial planning system for a societally endorsed, sustainable aquaculture.

VISUALIZING THE SOCIAL EFFECTS OF AQUACULTURE ACROSS GEOGRAPHIC SCALES

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Until very recently, governments of many countries, as well as their supporting organizations, have primarily addressed the biological and, technical and economic aspects of aquaculture. In contrast, social and cultural aspects of aquaculture production have taken a backseat. Drawing on the observation that aquaculture development in Western Societies has largely failed to address these social effects across different scales and contexts, this paper offers a new way of capturing and visualizing the diverse social dimensions of aquaculture. It does so by testing the ability to operationalize a set of social dimensions based on categories and indicators put forward by the United Nations, using several case studies across the North Atlantic.

Local/regional stakeholder knowledge realms are combined with scientific expert knowledge to assess aquaculture operations against these indicators. Several key issues have emerged from this exercise: First, by providing definitions and descriptions to guide the assessment, we make qualitative research data rigorous whilst ensuring generalizability and transferability to other settings. Second, this approach indicates that one needs to have a minimum farm size in order to have an impact of a visible scale for the different social dimension categories. Third, while finfish aquaculture seems to be more social impactful than rope mussel farming, the latter can hold important cultural values and contribute to place-based understanding, connecting people with place and identity, thus playing a vital role in maintaining the working waterfront. Fourth, aquaculture boosts a potential significant pull-factor to incentivize people to remain in the area, keeping coastal communities viable. Fifth, by visualizing the social effects of aquaculture, a door may be opened for new narratives on the sustainability of aquaculture that render social license and social acceptability more positive.

SUMMARY OF A FOUR-YEAR PROJECT EVALUATING AN IN-POND RACEWAY SYSTEM

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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 (4.9 m x 10.7 m x 1.2 m deep) IPRS units place into them, and ponds B3 and B4 had 45 m^3 (3.1 m x 12.2 m x 1.22 m) 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. First-year production of hybrid catfish (channel catfish, *Ictalurus punctatus*, □ x blue catfish, *I. furcatus*, □) used 41 g fingerlings stocked in March 2016 and were fed 35% and 32% CP. In year 2, the same IPRS units were used, and to improve the system an additional 1.0 HP aerator was added to each pond and Channel catfish (*Ictalurus punctatus*) fingerlings (44 g) were stocked into IPRS units in April 2017 and fed a 35% and 32% CP. In year three, an additional "stocker generator", a smaller floating IPRS unit 14 m^3 (1.8 m x 5.8 m x 1.30 m) was placed into each of four 0.4 ha ponds next to the existing "grow-out" units. Also, an additional 1.0 HP aerator was added to each pond bringing the total to 5.0 HP per pond. Hybrid catfish, mean weight 31 g, were stocked into IPRS units in April 2018 and in the "stocker generator", 29 g hybrid fingerlings were stocked in July 2018 and fed a 35% and 32% CP. In year four, the last year of study (current year) an additional floating tilapia cage (36 m^3, 4.26 m x 7.16 m x 1.20 m) was placed into 2 of the 4 ponds (B2 and B4) with no aeration. There was a total of 5 HP of aeration in each pond. Hybrid catfish fingerlings for the growout units (B1 and B4) came from the third-year's project "stocker generator" and weighted 322 g. Ponds B2 and B3 were stocked with 28 g fingerlings. The stocker generator unit was stocked on June 2019 with 28 g fingerlings and the tilapia (120 g) were stocked in July 2019. Catfish were fed a 35% CP feed and tilapia were not fed and allowed to grow through ingestion of phytoplankton only.

Water quality parameters have been in acceptable ranges for all trials. The total production from 2016 to 2018 reached the amount of kg/ha produced in conventional catfish production systems. Results from years 1-3 and partial 4th year results are summarized in Table 1. It is expected that 2019 production will be greater than prior years. Full four -year trial results and enterprise budgets will be presented at the WAS meeting.

Table 1. Total and partial production of channel and hybrid catfish in In Pond Raceway System, IPRS, over the four- year project, 2016-2019.

	Pond			
	B1	B2	B3	B4
First Year - – Hybrid (kg/ha) - Growout	**15,971**	**16,502**	**13,774**	**13,666**
Second Year – Channel (kg/ha) - Growout	**11,120**	**8,515**	**9,188**	**9,151**
Third Year – Hybrid (kg/ha)				
- Growout	11,281	12,841	13,193	10,963
- Stocker	3,540	4,276	4,388	4,332
- Total	**14,821**	**17,117**	**15,295**	**15,295**
*Fourth Year - Hybrid + tilapia (kg/ha)				
- Growout	11,763	3,037	2,761	10,448
- Stocker	2,124	2,434	2,331	1,905
- Tilapia	-	1,550	-	2,210
- Total	**13,887**	**7,022**	**5,092**	**14,563**

*Partial results (thru August 2019) with 90 days remaining in this trial

2019 IN-POND RACEWAY SYSTEM PRODUCTION TRIALS GROWING STOCKER AND FOODSIZE HYBRID CATFISH PLUS TILAPIA

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Continuous pond water circulation and destratification, increased control of the raceway production environment and efficient feed conversion ratio (FCR) allows In-Pond Raceway Systems (IPRS) to produce higher fish yields (greater than 15,000 kg/ha) than in conventionally managed catfish ponds (8,000 kg/ha). The goal of this study was to evaluate the fish production and growth performance of hybrid catfish (channel catfish, *Ictalurus punctatus*, ♀ x blue catfish, *I. furcatus*, ♂) raised in IPRS and Tilapia (*Oreochromis nilotica*) grown in cages. Large floating IPRS units, called "growout" units, were placed into two 0.4 ha ponds (B1, B2) and were 64 m^3 (4.9 m x 10.7 m x 1.2 m deep) and smaller growout units were placed into two 0.4 ha ponds (B3, B4) and were 45 m^3 (3.1 m x 12.2 m x 1.22 m). These units grew fingerlings and stockers to foodsize fish. In addition, "stocker generator" IPRS units (14 m^3 - 1.8 m x 5.8 m x 1.30 m) were place into each pond next to the growout units and were used to grow fingerlings to stocker size. Once growout units were harvested, the produced stockers would be harvested and placed into the vacated, adjacent growout unit for growth to foodsize fish. Additionally, a tilapia cage was placed into 2 of the 4 ponds (B2 and B4), were 36 m^3 (4.26 m x 7.16 m x 1.20 m) and were used to reduce blue-green algae populations. Each pond had a total of 5 HP of aeration: a 1.5 HP blower for the growout unit, a 1.0 HP blower for the stocker generator unit, a 1.5 HP and 1.0 HP blower for the two in-pond water mover-destratification units.

Hybrid catfish fingerlings for the growout and stocker generator units were stocked in June 2019 and tilapia were stocked in July 2019. Catfish were fed a 35% CP commercial diet twice daily; tilapia were not fed and allowed to graze the phytoplankton flow generated by the growout and stocker IPRS units. Initial tilapia biomass stocked was 506.1 kg in B2 (mean wt 126.5 g) and 460.3 kg in B4 (mean wt 115.07 g). Current biomass estimates for B2 and B4 are 884.4 kg and 620.6 kg respectively. Each l tilapia has gained an average of 94.6 g (B2) and 41.4 g (B4). Catfish sampling at day 61 (Table 1), showed an estimated biomass (growout wt + stocker wt + tilapia wt) of 13,887 kg/ha for B1; 7,022 kg/ha for B2; 5,092 kg/ha for B3 and 14,563 kg/ha for B4. Ponds B1 and B4 have produced more than the average yield of traditional pond production systems already with 90 days to go in the study. Full trial results and enterprise budgets will be presented at the WAS conference.

Table 1 – Partial data from hybrid catfish production in IPRS (at 61 day of the trial) in 0.4 ha ponds, 2019

	Grow-out IPRS				Fingerling-to-Stocker IPRS			
	Ponds (0.4 ha)							
	B1	B2	B3	B4	B1	B2	B3	B4
IPRS volume (m3)	64	64	45	45	14	14	14	14
Number of fish stocked	8,222	8,317	7,759	10,203	9,706	9,797	9,845	9,549
Avg weight of fish at stock (g)	360.4	34.1	33.2	283.9	28.8	28.8	28.8	28.8
Stocking biomass (kg)	2,963	284	258	2,896	280	282	284	275
Current weight (g)	573.6	146.1	144.1	413.0	88.0	100.0	97.0	81.0
Estimated current biomass (kg)	4,705	1,215	1,104	4,179	850	974	932	762
Estimated FCR (sampling date)	1.78	1.67	1.63	1.70	1.60	1.42	1.56	1.77
Specific growth rate (g/fish/day)	3.49	1.84	1.82	2.12	0.97	1.16	1.12	0.85
Estimated standing crop (kg/ha)	11,763	3,037	2,761	10,448	2,124	2,434	2,331	1,905

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*Partial results (thru August 2019) with 90 days remaining in this trial

ANALYSIS OF THE ECONOMICS OF FISH MARKETING IN EKITI STATE, NIGERIA

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Efforts at ensuring sustainable fish production has not produced expected outcome because of inefficient marketing system, thus slowing the performance or efficiency of the fishing industry. Hence, there is an urgent need to assess fish marketing system by analyzing the fish marketing structure, conduct and performance in Ekiti State, South-western Nigeria. A multi-stage sampling procedure was employed to select respondents for the study. A sample size of 12 fish farmers/producers, 6 fish wholesalers and 22 fish retailers were proportionately and randomly selected from each of the Local Government Areas (LGAs) of the ADP zones (three zones) in the study area. Data obtained were analyzed using descriptive statistics, Gini-coefficient, Lorenz curve, gross margin, net margin analyses, marketing margin, marketing efficiency, operational efficiency, multiple regression and Likert rating scale. The market structure analysis revealed the Gini-coefficient of 0.7100 indicating an Oligopolistic market in Ekiti State. The market conduct results revealed that greater proportion (61.0%) of the fish marketers determined their selling price based on the marketing cost of fish and the profit desired. Also, the degree of market transparency posited that 51.4% of the marketers use weighing scale as their unit of measurement. The market performance analysis revealed that fish marketing business in Ekiti State is a lucrative one. Based on the findings, it is essential that fish marketers in Ekiti State improve their market characteristics (market structure), behaviour (market conduct) and also ensure a reduction in their marketing cost (market performance) to become a Perfect market by strictly pursuing policies and strategies that can lower the costs of marketing in order to increase the market efficiency.

EFFECT Of COMMERCIALLY AVAILABLE PREBIOTIC AND PROBIOTIC ON THE GROWTH, INNATE IMMUNE RESPONSE AND OXIDATVE ENZYME ACTIVITY OF RAINBOW TROUT *Oncorhynchus mykiss*

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Using prebiotic and probiotic as functional ingredients in the aquafeed is a new approach to prevent disease and improve fish production. Thus an 8-week fish feeding trial was conducted to evaluate the effect of commercially available prebiotic Safmannan© and probiotic Actisaf© and mixture of these two (synbiotic) ingredients on growth, lysozyme and oxidative enzyme activities of rainbow trout, *Oncorhynchus mykiss*. A total of two hundred trout were distributed equally into 4 different groups, each with 5 replicates. A commercial diet was used as a control. Prebiotic, probiotic and synbiotic diets were prepared by supplementing the commercial diet with 3 g/kg of the Actisaf © 3 g/K of SafMannan© , and 3 g/Kg Actisaf© plus 3 g/Kg SafMannan© . Fish initial mean weight was 52.4 ± 0.28 gram. Fish were harvested at the middle (week 4) and end (week 8) of the trial. Food conversion ratio, weight gain, survival, conditioning factor, hepatosomatic index, and viscerosomatic index of fish were calculated. There were no significant differences($p>0.05$) in the growth, biometrics, and survival of fish fed experimental diets 4 and 8 weeks after the feeding trial. A decreasing trend (around 20 %) in FCR of prebiotic, probiotic and symbiotic diets were observed. Lysozyme activity in plasma of fish fed the control diet was significantly higher than that of fish fed the probiotic at week 8 but there were no significant differences ($p>0.05$) observed in lysozyme activity between control prebiotic and symbiotic diets at week 4 and week 8. Plasma glutathione peroxidase activity was significantly higher($p<0.05$) in the prebiotic and symbiotic diet of fish at week 8. Superoxide dismutase activity of plasma was significantly ($p<0.05$) higher than control in prebiotic, probiotic and synbiotic at week 8. The present study demonstrates that commercial prebiotic and probiotic at the concentration used in this study do not affect the growth of fish, however significant increase in oxidative enzymes activity of plasma of fish fed pre/pro and synbiotic diets as well as decrease in the FCR suggests that these functional ingredients have a great potential to improve fish health, hence the sustainable aquaculture production of fish.

DIETARY EVALUATION OF BETA-GLUCAN ON GROWTH AND HEMATO-BIOCHEMICAL RESPONSES OF *Clarias gariepinus* FINGERLINGS

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The ban on the use of antibiotics and synthetic drugs has stimulated research efforts on alternative therapeutic approach to aquatic animal health management. Among the alternative product tested so far, Beta-glucan has become prominent and widely used in aquaculture. Although, several studies have been conducted on the use of Beta-glucan in fish such as Rainbow trout (Ji et al., 2017), Silver catfish (Janine Di Domenico et al., 2017), Tropical gar (Nieves-Rodriguez et al., 2018), however, scanty information is available on African catfish, *Clarias gariepinus*. Thus, the current study was designed to examine the effect of β-glucan on growth, nutrient utilization, and hemato-biochemical responses of *Clarias gariepinus* fingerlings.

Beta-glucan (BG) prepared from brewer's yeast (*Saccharomyces cerevisiae*) was incorporated into the diet at different level viz., 0 g/kg (control), 0.25g/kg (BG 0.25), 0.5g/kg (BG 0.5), 1g/kg (BG 1) and 2g/kg (BG 2). Fifteen fish averaging 4.60±0.01g were distinctly distributed into each tank, in triplicate and fed for 60 days under aerated condition. The fish were fed daily with their respective diets at 9:00 h and 17:00 h. Upon the completion of the feeding trial, growth performance and nutrient utilization indices, hematology, and serum biochemical parameters were analyzed following standard laboratory procedures.

The highest weight gain (WG), weight gain percentage (WG %), daily growth coefficient (DGC), specific growth rate (SGR) and thermal growth coefficient (TGC) were observed in fish fed BG 0.25g/kg which is similar to the control but differ significantly from other fed groups (P<0.05). However, the nutrient utilization indices such as feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER), and lipid productive value (LPV) were unaffected (P>0.05). No significant variation was noticed in the whole-body crude protein content whereas significantly higher crude lipid content was recorded in fish fed BG 1%. The administration of β-glucan had no effect (p>0.05) on the hematology parameters among the various groups; however, the lymphocyte and neutrophil values were significantly influenced by the dietary BG inclusion. The serum total protein, albumin, globulin, and A/G ratio values were not significantly different (P>0.05), while cholesterol, total bilirubin, total antioxidant capacity and glucose concentration differed significantly (P<0.05) among the various experimental groups. The results of the current findings showed that the addition of β-glucan at a level not higher than 0.25g/Kg positively enhanced the growth, lymphocyte level, and reduces cholesterol, total bilirubin, and glucose concentration of *Clarias gariepinus*.

Table 1: Growth performance of *Clarias gariepinus* fingerlings fed β-glucan

Variables	Control	BG 0.025%	BG 0.05%	BG 0.1%	BG 0.2%
WG	$4.39±0.26^{ab}$	$5.61±0.66^{b}$	$3.92±0.26^{a}$	$3.78±0.54^{a}$	$3.11±0.30^{a}$
WG%	$95.01±.52^{ab}$	$121.37±13.87^{b}$	$85.37±5.63^{a}$	$81.67±11.46^{a}$	$68.64±7.04^{a}$
SGR	$1.11±0.05^{ab}$	$1.32±0.11^{b}$	$1.03±0.05^{a}$	$0.99±0.10^{a}$	$0.87±0.07^{a}$
TGC	$3.28±0.19^{ab}$	$4.20±0.49^{b}$	$2.94±0.20^{a}$	$2.83±0.40^{a}$	$2.46±0.24^{a}$
DGC	$2.44±0.14^{ab}$	$3.12±0.37^{b}$	$2.18±0.15^{a}$	$2.10±0.30^{a}$	$1.73±0.17^{a}$

Data presented as Mean±SE, n=3. Values in the same row with different superscript differ significantly (P<0.05). BG; Beta glucan.

INSECT (BLACK SOLDIER FLY) LARVAL MEAL AND OIL AS A SOURCE OF FEED INGREDIENT FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Currently, soybean meal is most commonly used feed ingredient in the aquafeed industry. High replacement of fishmeal with soybean meal in trout diets cause negative impacts on performance. Recent findings indicate that insect meal contains bioactive peptides, which might have role in boosting the immune function and reducing intestinal inflammation in fish. Therefore, the aim of this study was to: (i) evaluate the additive effect of insect protein in plant-based diets; (ii) examine the insect oil as a substitute for fish oil and vegetable oil in diets for rainbow trout. Experiment I: Four diets were formulated to contain a fishmeal (FM) based diet (Control), and three plant-based diet (21% soybean meal) with and without insect meal (IM) (0%, 8% and 16% supplementation level). Experiment II: Four FM based diets wherein fish oil (FO) was completely replaced with either vegetable oil (VO) or insect oil (IO); and one additional insect oil-based diet supplemented with bile acid (1.5% Ox-Bile) (IOB). Rainbow trout (525, average weight: 32 g) were stocked at 25 fish per tank (145-L) in triplicate. Each tank was supplied with 8 L min^{-1} of constant temperature (15°C), gravity-fed spring water. Fish were hand fed to apparent satiation twice daily for 10 weeks.

The results showed that in experiment I, growth performance and nutrient utilization parameters were not significantly different among the groups. However, numerically higher growth performance was observed for IM (8%) and control (FM) fed groups than other groups. Fatty acids profile of fish fillet was significantly affected by supplementation of IM. The total polyunsaturated fatty acid (PUFA) of the muscle was influenced by the diet with a significantly higher deposition observed in insect protein supplemented groups.

For the oil study (experiment II), the lowest growth performance and highest feed conversion ratio was observed in the bile acid supplemented diet group (IOB) compared to other groups (Fig 2). Fatty acids profiles of the muscle showed that fish fed insect oil-based diet had the highest total saturated FA (SAT) and docosahexaenoic acid (DHA) deposition than other oil sources (Fig 2). Immune function and fatty acids metabolism related genes and oxidative stress enzymes will be presented. In summary, the insect protein might act as an additive for plant-based diet for sustainable carnivore fish production. Furthermore, the insect oil could be used as an alternative source of oil without any negative impact on the fish performance and fillet quality.

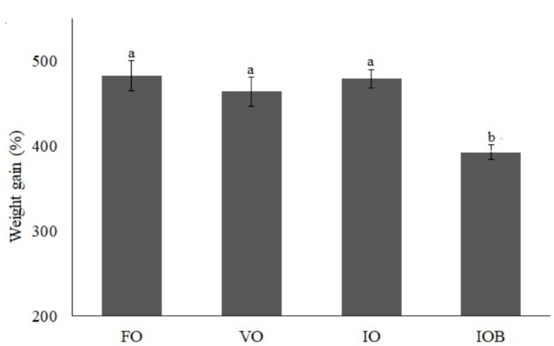

Fig 1: Growth performance (Expt. 2)

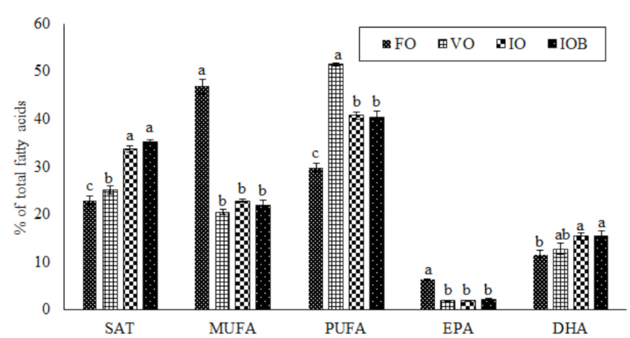

Fig 2: Fatty acids profile of fillet (Expt. 2)

IDEAL RELATIONSHIP AMONG DIETARY ESSENTIAL AMINO ACIDS FOR PACU *Piaractus mesopotamicus* IN THE FINISHING GROWTH PHASE BY THE DELETION METHOD

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Deletion method is a technique used for determining the amino acid requirements of fish, which aims to calculate the optimal relationship among essential amino acids (EAAs) in a single experiment. The main advantage of using this method is to avoid the negative effects of factors such as difference in the rearing environments, fish stocks, diets and trial durations, which may affect the performance results.

The present study aimed to determine the ideal relationship among essential amino acids for pacus in the finishing growth phase by the deletion method.

A total of 165 fish with an average initial body weight of 1,109.83±14.26g were distributed in 33 tanks of 1,000L capacity, each being connected to a water recirculation system. The experiment lasted 60 days, consisted 11 treatments with three replicates each, distributed in a completely randomized design. A control diet (CD) was formulated meeting 100% nutritional requirements for the fattening phase of the species under study. The other treatments i.e., EAA limiting diets (LD) were kept isonitrogenous and isoenergetic with a 45% deficiency of each of the 10 EAAs, respectively. The relationship among EAAs was determined by the relationship between body nitrogen retention (N) and the amount of amino acid being deleted from the test diet.

To quantify body N retention, a group of 10 fish was sampled out at the beginning of the experiment and 4 fish from each experimental unit/replicate at the end. Body N content was determined by the Kjedhal method (AOAC, 2000). The values found were used to measure the body N deposition for each treatment during the experimental period using the following equation:

$$N\ deposition = \frac{(wf * Nf - wi * Ni)}{\frac{1}{2}\left(\left(\frac{wf}{1000}\right)^{0.75} + \left(\frac{wi}{1000}\right)^{0.75}\right) * \Delta t}$$

Where, wf and wi are the final and initial weights (g); Δt is the number of days of the experiment; and Nf and Ni are the average final and initial body N content.

Through the relationship of N deposition levels which were determined for each specified treatment, the ideal EAA ratio was calculated by the following equation:

$$Indicated\ level = EAA_{CD} * \left(2 - DEL - \left(\frac{ND_{EAA}}{ND_{CD}}\right)\right)$$

Where, EAA_{DC} is the concentration of EAA in CD (g / kg, MS); DEL is the deletion of LAA in LD; DN_{EAA} is the N deposition (mg / BW0.75 / kg / day) for LD; and ND_{CD} is the N deposition (mg / BW / day) for CD.

The optimal balance among EAAs was obtained when the estimated level of each EAA was divided by the estimated lysine level (lysine = 100%).

The data obtained for zootechnical performance and N retention were submitted to One-Way ANOVA and mean contrast analysis by the Dunnett test.

The ideal profile of EAAs obtained for pacu in the finishing growth phase in relation to lysine (100%) was; arginine 41.18%, phenylalanine 31.18%, histidine 14.87%, isoleucine 42.07%, leucine 38,97%, methionine 26.48%, threonine 54.94%, tryptophan 8.58% and valine 45.55%.

Acknowledgments: CNPq (Process No. 130873/2019), FAPESP (Process No. 06343-3/2019, and 25761-4/2013).

EVALUATION OF MANNAN RICH FRACTION IN ATLANTIC SALMON *Salmo salar* FOR CONTROL OF SEA LICE *Lepeophtheirus salmonis*

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The mannan oligosaccharide (MOS) component of the yeast cell wall has been shown to improve fish performance when applied through the feed. A water-soluble fraction of MOS (MRF) has been developed to improve the health of fish through modulation of the innate immune response. MRF was evaluated in Atlantic salmon to reduce the impact of sea lice. A total of 300 Atlantic salmon (50 grams) received one of four injections in the peritoneal cavity: 1) saline, 2) commercial vaccine, 3) commercial vaccine plus MRF, 4) MRF. The fish were followed up on a group level and the groups of fish were ink labelled prior to the experiment. After feeding a commercial diet for 9 weeks, sampling of intestine from 10 fish per group was performed and the remaining fish were maintained on the commercial diet. The fish were starved for 24 hours and euthanized by an overdose of benzocaine. Real time PCR was used to screen the expression level of six marker genes. A total of three of the six genes were down-regulated compared to the saline injection. CDKN1B (p=0.0036) and CD200 (P=0.0015) were both significantly down-regulated and STIP1 (p=0.0625) was down-regulated with a trend. The other three gene markers were not significantly changed. The remaining fish were then exposed to a sea lice challenge and the number of mature sea lice per fish counted. The challenge was a bath challenge model, and fish were challenged with a known number of lice copepodites. Sea lice were counted 15 days after the challenge. Fish treated with MRF had a higher percentage of fish with fewer than 20 sea lice per fish, 37%, compared to fish that were not injected with MRF, 29% (Figure 1). The results of this study suggest that MRF may be a possible tool for reducing the impact of sea lice on Atlantic salmon.

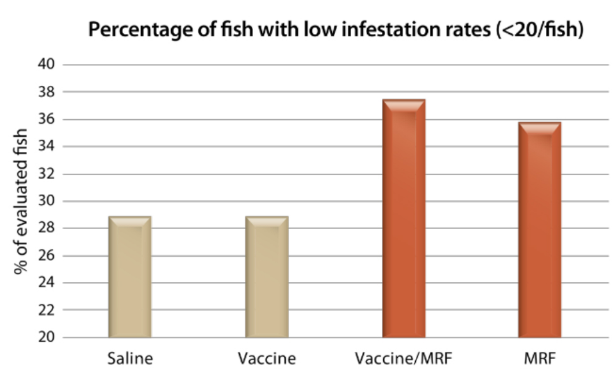

Figure 1. Percent of fish with less than 20 sea lice per fish following sea lice challenge.

EVALUATION OF A SOLUBLE MANNAN RICH FRACTION IN THE FERTILIZATION OF RAINBOW TROUT *Oncorhynchus mykiss* EGGS

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The mannan oligosaccharide (MOS) component of the yeast cell wall has been shown to improve fish performance when applied through the feed. Increasing challenges in the aquaculture industry provide an opportunity to evaluate novel applications of MOS compounds to improve performance while reducing the impact of disease. A water-soluble fraction of MOS (MRF) has been developed to improve the health of fish through a modulation of the innate immune response. Multiple studies were performed to evaluate the impact of MRF when applied during the fertilization of Rainbow trout eggs. The initial study evaluated the eyed egg percentage and hatching percentage followed by an 8-week feeding trial. A total of 300 Rainbow trout eggs in 100 ml of D532 (5.52 % NaCl; 3.74 % Glycine; 2.42 % Tris; pH 8.8) were exposed to two levels of MRF (0.10 mg/ml and 0.50 mg/ml) with a no exposure control during fertilization. The exposure of eggs to MRF during fertilization increased the percent of eyed eggs from 90 percent (no exposure) to 95 percent for the 0.10 mg treatment and 94 percent for the 0.50 mg treatment. The percent hatch increased from 83 percent (no exposure) to 93 percent for the 0.50 mg treatment and 86 percent for the 0.10 mg treatment group . Average weight gain was numerically increased following exposure to MRF but the differences were not significant (Table 1). In a second study, following exposure and maturation to the eyed egg state the changes in gene expression patterns were determined using a salmonid gene chip (Affymetrix). The design consisted of one MRF level (50 mg) and a control with no MRF exposure. Exposure of MRF to Rainbow trout eggs altered the expression of 430 transcripts in the eyed eggs. The exposure of Rainbow trout eggs to MRF resulted in the change in expression patterns of genes that are important in embryo development, represented by increased expression of genes important in calcium metabolism and bone development, eye lens development and muscle cell growth. Exposure of Rainbow trout eggs to MRF during fertilization changed the expression pattern of eggs as soon as 21 days after exposure as well as 8 weeks after hatching,

Table 1. Weight data from 8 week feeding following exposure of Rainbow trout eggs to MRF.

Treatment	Avg Initial Weight (grams)	Avg Final Weight (grams)	Avg Weight Gain (grams)	FCR
0 mg MRF8	0.411	13.8	13.4	0.687
10 mg MRF8	0.409	14.3	13.9	0.675
50 mg MRF8	0.388	14.4	14.0	0.666

RESEARCH AND DEVELOPMENT OF STANDARD OPERATING PROCEDURES TO CONTROL SAPROLEGNIASIS IN PRE AND POST SMOLT ATLANTIC SALMON *Salmo Salar*

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Aquaculture production in the United States has historically been constrained by competition for limited water resources and strict regulations on pollution discharge. As a viable and sustainable means of expanding domestic aquaculture production, land-based closed containment operations utilizing water recirculation aquaculture system (RAS) technologies offer numerous benefits, including: enhanced biosecurity, a high degree of control over the fish rearing environment, technologies to effectively capture wastes in order to reduce environmental impact, and flexibility in site selection for proximity to markets and/or low power rates. There are at least a dozen commercial ventures around the world producing food-size salmon in such facilities, and there is increasing land-based production in the planning stage in the Great Lakes region. At this critical juncture in the growing land-based salmon industry, scientific research and development to support this sector will facilitate domestic agricultural economic contribution, job growth, and food security.

The ability to effectively control saprolegniasis at vulnerable fish life-stages would be a critical advancement for the Atlantic salmon industry, particularly for facilities utilizing freshwater and furthermore for those that include plant production, for which plant health would also need to be considered when applying treatment (e.g., salt, a typical therapeutant, could not be used to control saprolegniasis in an aquaponics facility). We propose to examine a regimen of high and low dosages of both hydrogen peroxide and peracetic acid as Atlantic salmon are reared at an early, low immunocompetence life-stage (5g-20g), and secondly following photoperiod manipulation to induce smoltification (a highly stressful physiological process that nonetheless, when induced, provides growth performance benefits for salmon raised entirely in freshwater). We will conduct these investigations in facilities with both hard and soft water, to determine any differences in treatment efficacy under these conditions

At the time of the abstract submission deadline, data collection and processing for these research trials was still underway. Complete results will be available at the conference.

INFLUENCE OF TRANSITIONING TO DRY DIETS ON GROWTH AND POTENTIAL IMPLICATIONS ON THE RATE OF DEFORMITIES IN BIGHEAD CARP *Hypophthalmichthys nobilis*

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Two experiments were conducted to evaluate how bighead carp juveniles transition to dry diets after larval rearing with live feeds and potential impacts of diet on growth, survival, and deformities.

The first experiment followed a larval density experiment (12.5, 25, 50 fish L^{-1}) where two live feed regimes were examined and 30 fish from each initial density and feed regime, in triplicate, were transitioned to a commercial dry diet for 11 weeks. Samples were taken to measure growth, feed coefficient ratio, and deformities. Deformities were classified in two categories, slight (cataracts in or missing one eye, and minor spinal, jaw, and operculum deformities) and severe (cataracts in or missing both eyes, easily noticeable dorsal uplift of the caudal peduncle or curvature of the spinal column and severe jaw deformities). No significant effect of initial stocking density or initial feed on survival (97 ± 2%, mean ± SD), growth (4.9 ± 1.3 g) the rate of deformities was identified. Proportion of non-deformed (0.62 ± 0.13), slightly-deformed (0.27 ± 0.10), and severely-deformed fish (0.11 ± 0.08) were found. Samples of both non-deformed and deformed fish, from similar history, were taken to measure whole body mineral content. While there were some general trends observed, such as lower total ash and phosphorus content in deformed than non-deformed fish, no significant differences were found in mineral contents. The rate of deformities was rather high in the experiment, but these levels are not previously unseen in the culture of other carp species.

The second experiment occurred the following year and was conducted to examine whether the initial commercial dry diet impacted the rate of deformities. Following a similar live feeding period of 20d, the rate of observed deformities was 7 ± 2%. Fish were stocked (n = 38) to 10L tanks and three diets were tested, in triplicate. The same commercial diet as the first experiment, as well as 2 formulated diets with either 50% of the protein source being from soybean meal or spirulina powder. The experiment is currently on going, but will follow a similar design to the first experiment, with growth, feed coefficient ratio, deformities observed and whole-body mineral analysis of the fish from each diet group being examined. Fish will be grown to a similar size before being sampled for mineral analysis due to large differences apparent in growth rates between the commercial and formulated diets (**Table 1**)

Table 1 Weight, specific growth rate (SGR), survival, and feed conversion ratio (FCR) are presented for 14 days of feeding (mean ± SD). Data was Box Cox transformed and analyzed using a one-way ANOVA. A post hoc Tukey's HSD was conducted to generate a connecting letters report.

	Weight (mg)		SGR (%)	Survival (%)	FCR
Diet	**Initial**	**14d**	**14d**	**14d**	**14d**
Otohime	283 ± 8	912 ± 38^{a}	8.4 ± 0.1^{a}	99.1 ± 1.5^{a}	0.99 ± 0.06^{a}
Soybean meal	277 ± 5	500 ± 9^{c}	4.2 ± 0.1^{c}	69.3 ± 11.9^{b}	3.51 ± 0.63^{c}
Spirulina	277 ± 3	537 ± 17^{b}	4.7 ± 0.2^{b}	99.1 ± 1.5^{a}	2.53 ± 0.14^{b}
P-value	0.4128	<0.0001	<0.0001	0.0013	<0.0001

SUITABILITY OF KALE WINTERBOR F1 HYBRID *Brassica oleracea* IN BRACKISH WATER AQUAPONICS APPLICATIONS

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Brackish water recirculating aquaculture systems (RAS), especially in inland locations, have unique challenges in water reuse and disposal of effluent due to high salt content. Although there are marine and halophyte plant species that can be used in brackish water aquaponics systems, marketability of these species may be challenging. Brackish water aquaculture is established in Kentucky and the surrounding region, presenting a need for sustainable effluent management. Kale belongs to the Amaranthacaea family, members of which show a general tolerance to sodic soils. Kale has become a very popular leafy green in recent years and performs well in freshwater aquaponics systems. This study examined the salt tolerance of kale and its viability for production in a brackish water aquaponics system.

Three trials were conducted using 18 L tanks, each aerated with a 5 cm ceramic air stone and located under LED grow lights providing 12 hours of light per day. In each study, four plants were stocked in each bucket and floated using 2.5 cm polystyrene foam; each treatment contained three, randomly assigned replicate tanks. The first 21- day study was conducted at five salinities (0, 5, 10, 15 and 20 ppt.) to determine salt tolerance and effects on plant performance. A second 28-day trial analyzed two acclimation times (A1 and A2) based on plant age (28 and 35 day old seedlings), two salinities (10 and 20 ppt.) and a treatment assessing the plant's response to excess nitrate (80 mg/L $NaNO_3$). Treatments included A1-10-N, A1-10, A1-20-N, A1-20, A2-10-N, A2-10, A2-20-N and A2-20. The third 21-day trial compared unused, clean water with reused shrimp water, and the effect of shrimp (*Litopenaeus vannamei*) on plant performance. Treatments included clean water with plants and fertilizer (CW-P-F), clean water with shrimp and plants (CW-S-P), clean water with fertilizer and no plants (CW-NP-F), shrimp water with plants (SW-P), shrimp water with shrimp and plants (CW-S-P) and shrimp water with no plants (SW-NP). At the conclusion of each trial, plants were harvested and a series of growth parameters measured. Ammonia, nitrite and phosphorus were measured weekly, and initial and final water samples were analyzed for a suite of dissolved nutrients and elements.

Although growth was reduced with increased salinity, kale had 100% survival in all trials and salinities and produced marketable foliage. Acclimation to brackish water using older plants resulted in better plant performance overall. Average chlorophyll content index (CCI) was highest at 10 and 15 ppt. with significant differences between both salinity and acclimation time. Average dry weights of kale biomass were consistent across salinities except for the youngest plants in the acclimation trial that showed significantly lower weight with increased salinity. Results from the third trial are pending. Future studies will focus on a kale grow out with multiple harvests in a brackish water aquaponics system with shrimp, in addition to testing other kale varieties and evaluating consumer preferences for brackish water-produced kale.

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NAVIGATING OXYGEN SOURCING FOR AQUACULTURE

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The average size fish farms are in the midst of an explosive scale increase having not long ago considered 1,500 tons per year (TPY) very large. Now it is quite common to see plans of 10,000 tpy fish production and more per farm. When farms were small (~<300 tpy), so was the volume of oxygen required to support fish growth. There was one dominant source of oxygen supply – liquid oxygen (LOX) – and an occasional oxygen PSA onsite generator.

With much larger fish farm sizes, the volume of oxygen demand has proportionately grown as well. No longer is trucked-in LOX alone the go-to source to achieve the best economics and reliability. Now there are a plethora of oxygen source technologies and suppliers to choose from – LOX, PSA, VPSA, and cryogenic generators. No one source is a panacea for all farms. However, each source has its place in achieving the best cost and reliable oxygen supply. And each has its own technical and commercial advantages that also impact the design of the fish farm itself. When designing the farm, it is imperative to understand the oxygen source so that they are compatible with each other plus deliver the ideal economic farm business case.

This presentation intends to provide the fish farm owners and designers the technical, reliability, commercial and economic differences among each technology and how to navigate the custom selection process to determine the best fit for the specific farm application, thus ensuring sustainable aquaculture operation.

SUITABILITY OF SEA PURSLANE *Sesuvium portulacastrum* IN BRACKISH WATER AQUAPONICS

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Effluents from marine and brackish water aquaclture systems can be difficult to manage, especially for inland operations. Although such effluient is often rich with dissolved nutrients, the high concentration of salt can prevent its use as a fertilizer for terrestrial plants. Halophytes, or salt tolerant plants, have been grown in effluents from marine systems, however many of these plants have little value or do not produce edible vegitation. Sea Purslane, an edible, halophytic species, is native to coastal areas around the world. Although this plant will grow in saline conditions, the limits of salt tolerance and interactions between the plant and animals cultured in the same system are unknown. We conducted a series of experiments to examine the salt tolerance of sea purslane and interactions between the plant, cultured animals, and water quality dynamics in aquaponics systems.

The first study examined the salt tolerance of Sea Purslane. Fifteen 18L tanks were assigned in triplicate to 5 treatments: 0 ppt, 5 ppt, 10 ppt, 15 ppt, 20 ppt. Four plants were suspended in a 2.5cm thick raft floating on the surface of each tank and a 5cm ceramic air diffuser was placed at the bottom of each tank to prevent stagnation. LED grow lights provided 12 hours of illumination per day. A commercial marine salt mix, Crystal Sea® Reef Crystals© was used to reach the target salinities and each tank was fertilized with 50ml of a commercial hydroponics fertilizer. Water quality parameters (temperature, DO, pH, salinity, total ammonia nitrogen, nitrite, , and phosphate) were measured weekly. Harvest took place after 28 days, at which time plant growth parameters and nitrate were measured. The second study looked at the use of these plants in reused shrimp culture water and the interaction of plants and shrimp. The experiment used the same systems described above but with 18 tanks and 6 treatments with 3 replicates each. All treatments were filled with 15 ppt brackish water; three treatments used new, freshly salted clear-water (CW) and three treatments used water from a biofloc shrimp production system (BF). The treatments were CW with plants and fertilizer (CW-P), CW with fertilizer and no plants (CW-NP), CW with plants and shrimp (CW-S), BF with plants (BF-P), BF without plants (BF-NP), and BF with plants and shrimp (BF-S). Each tank in the treatments with shrimp had four white shrimp (*Litopenaeus vannamei*) added.Water quality parameters and harvest data were collected as descibed above, along with growth metrics for the shrimp at harvest.

Results from the first experiment showed a peak in plant performance at 15 ppt, with the lowest growth at 0 ppt. Edible biomass was highest in 15 ppt, along with root weight and root length. Final results from the second study are still pending, however preliminary results indicate increased plant performance in CW tanks with fertilizer and BF tanks with shrimp. Survival was 100% across all treatments and all plants exhibited new growth. Plants in tanks with shrimp had reduced root mass, likely due to the shrimp feeding on them. Plants in BF systems did accumlate organic matter on their roots, however this did not appear to severly impact production. The results of these studies indicate that *Sesuvium* is a suitable plant species for brackish water aquaponics.

EVALUATION OF COMPLEX PARTICLE FEEDING BY SABLEFISH *Anoplopoma fimbria* AND STEELHEAD TROUT *Oncorhynchus mykiss*

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Disease continues to be a problem in finfish aquaculture. Current disease management strategies such as vaccination often involve immersion and injection delivery methods. Injections are effective but often costly and stressful on fish whereas immersions are generally less effective. Current options for an oral vaccine delivery route are limited. Oral delivery methods often utilize commercial-type feed particles that are top-coated, which is necessary to obtain high and even ingestion rates in fish. Vaccines cannot be added to commercial type feeds as the high temperature of production destroys bacterial-based vaccines. In this study, we investigate novel complex particles which utilize the entire particle volume for vaccine delivery and are produced with a cold production method. However, in order for these particles to be effective, the particles must be ingested and partially digested (once in the hindgut) by the target organism. For this study we investigated the rate of ingestion and level of digestion of complex particles fed to sablefish (*Anoplopoma fimbria*) and steelhead trout (*Oncorhynchus mykiss*).

We investigated the uptake of these particles by each species of finfish. We used a control complex particle that contained liposomes encapsulating a saline solution added to a solution of 2% alginate. For the experimental treatment, the complex particles contained liposomes encapsulating an amino acid mix of alanine, betaine, and glycine added to a solution of 2% alginate. A significantly higher portion of sablefish and steelhead fed on the amino acid mix particles when compared to the saline treatment. Sablefish that were offered the amino acid mix treatment had significantly more particles in their gut when compared to the saline treatment, while the steelhead did not have a statistically significant difference. We investigated the digestion of complex particles and incorporated a third experimental treatment that included liposomes encapsulating the amino acid mix added to a solution of 6% gelatin from cold water fish skin and 0.5% alginate. Fish were collected at 1, 2, 4, and 8 hours to track placement of particles in the gut and investigate level of digestion. Particles containing the 2% alginate mix with no gelatin faced minimal digestion (Image 1) while particles that contained 0.5% alginate and 6% gelatin were highly digested (Image 2).

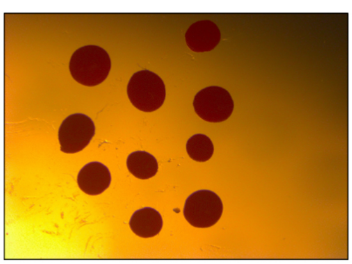

Image 1. Pigmented complex particles containing 2% alginate and liposomes encapsulating an amino acid mix. Particles were dissected from hind-gut of sablefish after 8 hours

Image 2. Pigmented complex particles containing .5% alginate, 6% gelatin, and liposomes encapsulating an amino acid mix. Particles were dissected from hind-gut of sablefish after 8 hours

ACES: A FREE ONLINE SHORT-COURSE FOR SEAWEED AND MICROALGAE AQUACULTURE

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The Algae Foundation's Algae Technology Educational Consortium (ATEC) formed the Algae Cultivation Extension Short-course (ACES) to serve as a workforce preparedness program for those interested in growing both micro- and macro-algae.

The seaweed course is a free online compendium of videos chosen and newly created to give a thorough initiation into the culture of various commercial seaweeds, including kelps, for those interested in getting started in algal-based aquaculture. The course includes: a large number of videos produced by several New England Sea Grant programs; international content; guided PowerPoint presentations. Additionally, newly created videos include industry pioneers; history of wild harvesting and culturing macro-algae; seaweed products; longline setting; harvesting methods; drying techniques; conversations about peoples' experiences in seaweed culture; and the permitting process.

The microalgae for aquaculture course is an online compendium of videos and online lectures chosen to give a thorough introduction into the culturing of various commercial microalgae. The course includes: a large number of videos produced by national and international programs, and guided PowerPoint presentations; including selections from the Santa Fe Community College, Introduction to Algae Cultivation online course. Offerings include cultivation and harvesting technologies, microscopy, algae species collections, nutrient media recipes, algae to fuel, carbon sequestration, food products and interviews with industry experts. There are additional chapters for the online algae culture collections, interviews and Ted Talks. There are several longer webinars and documents about microalgae culture that can be downloaded. There is now an additional section on algal culture for shellfish hatcheries.

These free courses require a simple registration and analytics about the student's experience with the course recorded for program evaluation. The short-courses can be found on the web at http://www.algaefoundationatec.org/acesintro.html.

CONSUMER WILLINGNESS TO PAY FOR ATTRIBUTES OF FISH FILLETS IN BRAZIL

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Tilapia and tambaqui are the two most important farmed fish species in the Brazilian market. This study provides insight into consumer demand for the product attributes of these species. Seafood supply chains, from fish farmers to supermarkets selling direct to consumers, must understand consumer demand for product attributes to ensure production and availability of desired products. There are no relevant economic studies in Brazil of the impact of variables such as species, processed form (fresh/frozen), and fish knowledge on consumers' fish selections. This study analyzes the impact of factors like product price, consumers' demographics, and product attributes such as species and processing on consumers' willingness to pay for tilapia and tambaqui in the Brazilian market.

Random utility theory is used to model consumer demand for the fish attributes of the species in fillet form. A random parameters logit (RPL) also known as mixed logit model is used to for the analysis. The data were collected through a choice experiment on fish consumers administered in person at seafood counters in supermarkets in each of the five Brazilian Regions.

The mean WTP estimates are presented in Table 1. Consumers are, on average, willing to pay more for tilapia and fresh fillets than tambaqui or frozen fish. For tilapia, the lowest WTP was found in Northeast Region where consumers are willing to pay a premium of US$ 1.64 to purchase one kilo of tilapia instead of tambaqui on average. On the other hand, Southeast and Midwest regions have the highest WTP for tilapia of almost US$ 4. Overall, the results indicate a preference for tilapia especially in regions where tambaqui does not have a mature market. Freshness appears to be the most important attribute for consumers in Brazil. In the Southeast, consumers are willing to pay a premium for fresh fillet above the average price of the choice experiment that is US$ 7.50. Even in the Northeast where the WTP is lowest for freshness, the premium value of US$ 5.50 per kilo represents a high preference for this attribute.

Table 1 – Mean WTP estimates for tilapia and tambaqui fillet for five Brazilian Regions

	Regions				
	South	**Southeast**	**Midwest**	**Northeast**	**North**
Tilapia WTP (US$)	2.78	3.99	3.99	1.64	2.53
Fresh WTP (US$)	6.65	8.29	7.06	5.51	7.26

A SPATIAL EQUILIBRIUM ANALYSIS FOR FISH SUPPLY AND DEMAND IN BRAZIL

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Analyzing the chain relationships in aquaculture is important to understand the synergies within the supply chain, how demand and supply agents interact, and what is the effect of specific changes on the whole sector. This study assesses the supply chain of the two main fish farming species in Brazil, tilapia and tambaqui, using a spatial equilibrium model. This approach allows evaluation of the impact of factors like governmental incentives (subsides on fish feed prices), international oil price shocks (changes in the cost of transportation), increase in the consumers' income and decrease in the retailers gross margin, on final product consumption.

The model scope includes farmers, the processing sector, the transportation sector, retailers and consumers. The final fish to be consumed in the model can be fresh or frozen and gutted or fillet, resulting in four possible final forms for each species. For each sector of the economy, the country is divided into regions according to the concentration of consumers and producers. The model works with five demand regions, represented by one large city of each region, and eight supply regions (five for tilapia and three for tambaqui). Each supply region produces either tilapia or tambaqui, which reflects the Brazilian fish supply chain.

Table 1 displays the percentage variation of demand and final price due to factor changes in the spatial equilibrium model. A change in consumers' income does not affect the final price because the supply curve in the model is horizontal being represented by the total cost. An increase in consumers' income of 10% will increase the total tilapia and tambaqui farmed in Brazil by 15.2%, showing that fish is a normal good in the country. Retailers' gross margin is an important factor in the chain. A 10% reduction will decrease prices by 5.2% and demand quantity by 41% in the country. On the other hand, the impacts of changes in transportation cost are much lower. A 5% change will impact only 0.1% in the final price and 1.1% on the demand. The impact of a reduction in fish feed cost on demand is also large.

Table 1 – Percentage Variation of Demand and Final Price Due to Parameters Change

Parameter changed:	**Income (+10%)**	**Retailer gross margin (-10%)**		**Transportation cost (-5%)**		**Fish feed cost (-8%)**	
% variation in:	Demand	Demand	Final price	Demand	Final price	Demand	Final price
National	15.2	41.0	-5.2	-1.1	0.1	16.5	-1.9
Tilapia	16.4	37.9	-5.1	-0.3	0.1	19.0	-2.0
Tambaqui	11.6	50.3	-5.5	-3.5	0.2	8.8	-1.7

ECONOMIC EVALUATION OF TILAPIA FINGERLINGS AND LETTUCE PRODUCTION IN AN AQUAPONICS SYSTEM IN BRAZIL

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The literature on Brazilian aquaponics is incipient. While some experiments have been done, there are only few reported relevant economic results. There is economic and environmental potential for aquaponics activities in Brazil given the sustainable characteristics of the production and the water shortage problem in some regions. Therefore, this study intends to fill the gap in the literature by evaluating the economic returns to aquaponics with different levels for key control variables in the production system.

The input variables considered in this study are fish stocking density (fish/m3) and quantity of fish feed (% difference relative to the factory recommendation). The output attributes at harvest time (lettuce and fish) are color (on a classification scale of three color shades between yellow and green) and weight (grams) of lettuce and weight (grams) of the fish (tilapia). The final output for fish to be analyzed is juveniles (big fingerlings) that have a good market potential and can be harvested within the 30-day production. Output levels were measured every day starting on the 20th day of experiment.

The data needed was collected from an experiment conducted at UNESP (Sao Paulo State University) in Brazil. The facilities utilized included 16 aquaponics units with three rows of plants each, where different levels of the same variable were measured at the same time. Four different levels of density and four levels of fish feed were measured in a 30-day experiment. An analysis using linear programming was done to identify which combinations of fish density, feed level and harvest day are economically efficient. A cash flow was also calculated for selected efficient systems to show the economic feasibility of the most efficient combination. Figure 1 summarizes the experiment scheme.

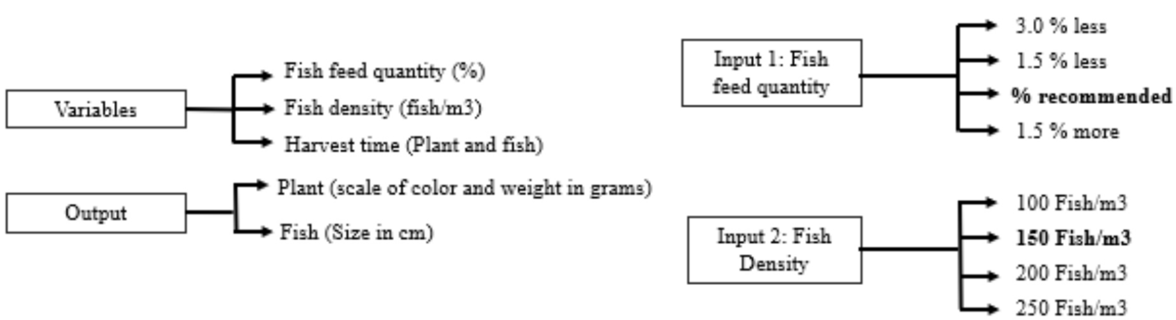

Figure 1 – Data collection on the experiment

WHOLE OCEANS: LAND-BASED AQUACULTURE AND INDUSTRIAL REVITALIZATION

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Whole Oceans, LLC is a Maine-based company constructing a land-based recirculating aquaculture facility in Bucksport, Maine. In May of 2019 we purchased a parcel of the former Verso paper mill on the Penobscot River. We plan to complete our facility in multiple phases over the next ten years, beginning with 5,000 metric tonnes annual production of Atlantic salmon and increasing to an estimated 20,000 metric tonnes at full buildout.

Selecting an industrial Brownfield site for a land-based aquaculture facility requires a long diligence period and rigorous evaluation of existing infrastructure. Land-based aquaculture facilities require significant water and power infrastructure, both of which are available at this site, which operated as a paper mill with several owners from 1934 to 2014. This site also has access to a nearby deepwater port and is adjacent to a declining railroad spur.

We hope that our facility can be a model for others in New England and beyond. Large scale land-based recirculating aquaculture has the potential to help revitalize factory towns with distressed infrastructure and provide jobs to help depressed economies thrive again. We also hope our project will help spur additional economic development in the area through relevant supply and service companies, as well as opportunities for workforce development including both vocational and academic training programs.

WHOLE OCEANS™
MAINE SUSTAINABLE SALMON

FINDING YOUR FINS! TRAINING OPPORTUNITIES IN AQUATIC ANIMAL MEDICINE

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Aquatic veterinary medicine can be incorporated successfully into both companion and livestock production practices. Veterinarians may be called upon for VFDs or health exams for aquaculture production clients as well as questions from clients with pet fish. DVM students and graduates of institutions lacking formal aquatic training may be unsure of the opportunities that exist to further their knowledge and experience in the field of aquatic animal medicine.

Others may be looking to expand on the training they have already received. Attendees will be guided through the formal and informal training experiences of the author as well as learn about additional opportunities available. This dynamic talk includes descriptions of the trainings, an honest assessment of each opportunity, photographs, videos and time for questions participants may have about the trainings. This presentation will also provide practitioners with resources for consulting or connecting clients with experienced aquatic veterinarians

VETERINARY FELLOWSHIP IN AQUACULTURE MEDICINE: A NEW APPROACH FOR TRAINING PRACTICING VETERINARIANS TO SERVE AQUACULTURE PRODUCERS

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In 2017 the University of Florida received funding from USDA-NIFA to provide advanced training in aquaculture medicine to 8 practicing rural veterinarians selected from a national pool of applicants. Participating veterinarians, Fellows, were selected from 6 states (California, Florida, Idaho, Texas, Utah, and Washington) and areas of expertise ranged from traditional dairy, to rural and companion animal practice. When the program was initiated, two Fellows were engaged in some level of aquaculture practice. Two years of training is being provided and includes 12 credit hours of on-line formal graduate education; additional learning materials including 14 hours of lecture material designed to supplement information provided in formal course work; and a one-week hands-on practicum to provide training in diagnostic methodologies as well as on-the-farm interactions with producers. Currently the program is providing support for the Fellows in development of professional relationships with aquaculture businesses in their local communities.

All Fellows have completed the one-week practicum and are on track to complete required course work by August 2020. All fellows are actively engaged in providing professional services to aquaculture businesses in their communities.

Acquiring an appropriate level of understanding of water quality parameters and life support systems, particularly the nuance of recirculating systems and associated technology, was considered a barrier by most Fellows prior to participating in this training program. The experience of working on farms and speaking directly with producers about production issues and successes was also a skill that Fellows felt they needed help with. After the first year of the program Fellows reported an improved understanding of animal physiology and production systems. We believe this approach of targeted training has demonstrated its effectiveness and will improve veterinary support for some aquaculture businesses in rural parts of the U.S.

THE MOORING-LINE TENSION REPSONSE FROM IN-SITU OCEAN INSTRUMENATION OF AN EXPOSED SITE AQUACULTURE FARM OF SUGAR KELP *Saccharina latissima*

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As part of the Department of Energy, ARPA-E program to conduct MacroAlgae Research Inspiring Novel Energy Resources (the MARINER projects), a field program has been established to examine the performance of a sugar kelp (*Saccharina latissima)* farm at an exposed site in Saco Bay, Maine in the U.S. The objective of the field program, operated as a partnership between the United States Naval Academy and the University of New England, is to obtain datasets to characterize mooring tension responses of densely grown sugar-kelp aquaculture lines in waves and currents. A 122 m line was seeded and deployed in late autumn 2018 and left to grow in a fully exposed open ocean site during the harsh Maine winter. By the end of April 2019, the kelp had grown to 1-2 m lengths at densities between 6-12 kg/m. At this time, newly developed load cells were deployed on each mooring leg of the kelp line to measure system tension responses to environmental conditions. Environmental conditions, including tides, waves and currents, were measured with two Acoustic Wave and Current (AWAC) sensors each deployed near the load cells. Ocean engineering data show clear relationships between environmental conditions, morphological characteristics of the kelp, and the tension responses in the mooring lines. Datasets to validate a computational model (called Hydro-FE) are being used to accelerate the engineering, testing, permitting, and operation of new macroalgae systems for exposed environments.

EGG AND DIETARY FATTY ACID CONCENTRATIONS AFFECT N-3 FATTY ACID METABOLISM IN LARVAE OF RED DRUM *Sciaenops ocellatus*

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Mounting evidence has shown that variations in egg and dietary lipid composition can differentially affect lipid metabolism in marine fish larvae. Previous work on red drum (*Sciaenops ocellatus*) has shown that the docosahexaenoic acid (DHA) content of the egg is positively correlated with fatty acid composition of whole larvae weeks after hatching when larvae are fed a diet containing sufficient DHA. The current study examined the influence of endogenous (maternally derived) and exogenous nutrition of red drum larvae on subsequent whole-body accumulation of n-3 highly unsaturated fatty acids (HUFAs).

Two groups of red drum broodstock were fed different diets to produce eggs containing high or low levels of DHA. Larvae from these groups were fed live prey that were enriched to provide high levels of DHA or low levels of DHA with high levels of a-linolenic acid (ALA). These larval diets were presented during the rotifer-feeding stage and during the *Artemia* feeding stage. Larvae were sampled at 7, 10, 17, and 21 days post-hatching (dph) to assess n-3 HUFA content.

When levels of DHA in the larval diet were low, larvae from eggs containing low levels of DHA had higher levels of ALA and some other fatty acids in the n-3 biosynthetic pathway than larvae from eggs containing high levels of DHA. In addition, larvae that were fed low levels of DHA in rotifers (fed 3 to 12 dph) had higher tissue levels of docosapentaenoic acid at 10, 17, and 21 dph, irrespective of egg DHA content.

These results suggest that embryonic early larval DHA restriction upregulates the n-3 biosynthesis pathway, resulting in a stronger compensatory response (n-3 biosynthesis) to a subsequent dietary challenge (low intake of DHA) compared to embryos that receive higher amounts of DHA.

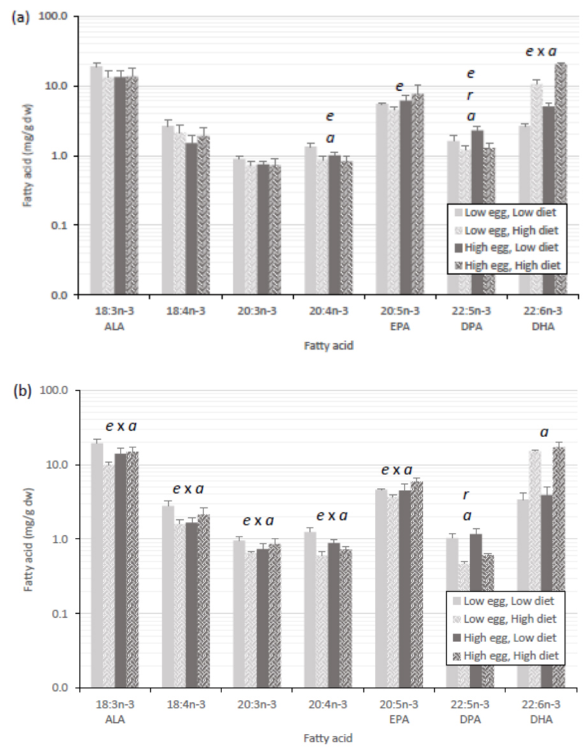

Figure 1. n-3 HUFA content (± S.E.) of (a) 17 and (b) 21 dph red drum larvae by egg DHA group and Artemia *enrichment (diet). Significant main effects of egg DHA level (e), rotifer diet (r),* Artemia *diet (a), and interactions (denoted by main effects separated by x) are indicated for each fatty acid. Vertical scale is logarithmic.*

CUTTING EDGE SUBMERGED FEEDING TECHNOLOGY IN OPEN OCEAN AQUACULTURE

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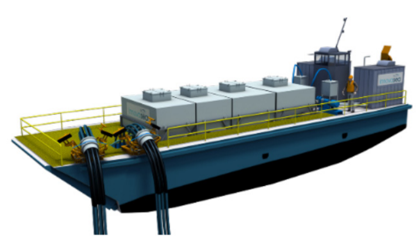
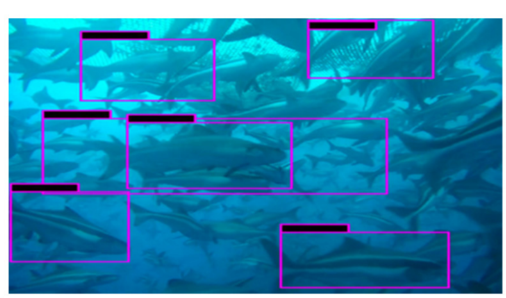
Innovasea Systems Inc. delivers a wide array of equipment and services to the aquaculture industry. Offerings range from land-based hatchery/nursery technology, open ocean farming platforms, as well as offshore wireless instrumentation with data collection, and analysis. Innovasea supports its clients from concept to harvest. With a staff of more than 200, some with 20+ years' experience, Innovasea has pioneered submerged aquaculture feeding; from single pens to entire pen grid systems.

Traditional air blown systems will not efficiently deliver feed to pens located 10-15 meters below the surface. For submerged feeding, pellets must be mixed in water then pumped efficiently to the selected pen and broadly dispersed in the grow-out volume at depth. As the pens and fish are underwater, not visible to the farmer, a reliable and cost-effective camera system is needed. This and its supporting software are necessary to produce a competitive FCR (Feed Conversion Ratio).

Innovasea has developed the underwater feeding system to address these challenges and capitalize on the advantages of submerged feeding. Select advantages are, keeping stock below sea lice and harmful algal blooms, which typically inhabit the first few meters near the surface and fewer lost days of feeding due to inclement weather. The submerged feed pipe network is kept away from the destructive energy from vessels and buoys moving at the surface. The underwater feeding System delivers feed via laminar water / feed mixture to a pen up to 400 meters away. Once feed has arrived at the pen, the Helical Disperser evenly scatters the pellets which provides equal opportunity for feeding throughout the entire biomass population. A sophisticated monitoring system assists the feed manager to optimize the FCR. Innovasea's cost effective submerged cameras transmit hi-resolution real-time video to both the operator on the vessel and the remote-control center. The underwater vision system helps feed operators identify and quantify feed loss from other particles such as fecal matter. Coupled with a fish appetite satiation index, the underwater vision system provides a powerful tool to help optimize FCR. Finally, Innovasea's Biomass Estimation platform collects and reports near real-time fish weight biomass after a few hours of data collection each week. An accurate assessment of fish mass enables the operator to make informed decisions regarding scheduled feed amounts.





PRODUCTIVE POTENTIAL OF THE CLAM *Venerupis corrugata* UNDER OCEAN ACIDIFICATION AND WARMING SCENARIOS

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The pullet carpet shell *Venerupis corrugata* (Gmelin, 1791) is a clam species distributed in the East Atlantic –from Norway to North Africa– and in the Mediterranean Sea. It has been traditionally harvested at the Southwestern and Mediterranean Europe, mainly in Portugal, Spain, France and Italy where overfishing, indiscriminate digging and pathogen-induced mortality determined stocks' reduction, forcing aquaculture production. The high market demand for this species, that supports its high market value (global average price over $20USD per kg), is mostly driven by fragile stocks' recovery under high consumption and increased mortalities due to disease outbreaks and extreme climatic events, predictably more frequent under modern to future climate conditions. The present study aims to assess the performance of the culture of this clam from early life, throughout ontogeny, metamorphosis, settlement and up to 1-month post settlement, under projected ocean acidification (OA) and warming (W) scenarios, in an attempt to foresee its productive potential in the expected future climate.

Broodstock was collected in Ria de Aveiro (NW Portugal) and induced to spawn in the laboratory by thermal stimulation. Fertilized eggs were incubated to hatch in 1-µm filtered, UV-sterilized artificial saltwater under control conditions: unmanipulated pH (~8.2) and 18 ºC. The D-larvae retained in a 60µm-sieve 48 hours post fertilization (48h pf) were exposed to nine OA-W scenarios (Figure 1) resultant from a factorial design of three pH levels at three different temperatures. Beyond the unmanipulated pH control, two acidified treatments were produced by CO_2 bubbling, while temperatures were kept constant by immersion of experimental containers in continuously controlled water baths.

Mortality, development stage and shell growth were assessed every 3 to 4 days until complete settlement (i.e., 100% settlement verified in all experimental treatments) recorded after 28 days of exposure (T28). Mortality and shell growth of settled juveniles were reassessed at T60.

Apart from an initial increased survival under acidity, potentially due to changes in the microbiome, there was an apparent lack of effect of the treatments on mortality. Moreover, development and growth were notably accelerated by warming, despite a relatively delayed settlement and growth under acidity, effect attenuated by the concomitant rise in temperature. These results reveal the resilience of this species to future OA-W and its productive potential under the tested scenarios.

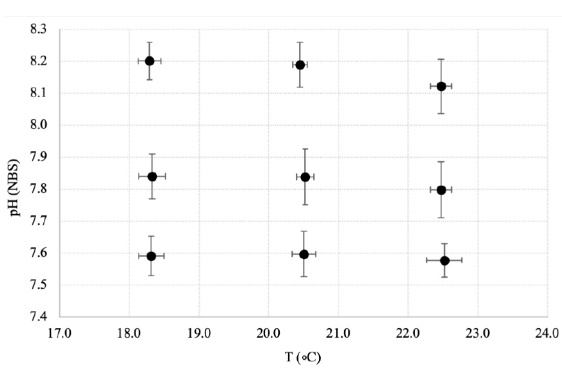

FIGURE 1. Mean temperature (T, expressed in ºC) and pH (NBS scale) calculated from probe measurements performed twice a day, and respective standard deviation.

ON-FARM EVALUATION OF AN ALTERNATIVE SALT MIXTURE FOR SALINITY ACCLIMATION OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Pacific white shrimp, *Litopenaeus vannamei* exhibit a remarkable ability to tolerate a wide range of salinities. However, proper acclimation to an appropriate environment is critical to ensure good survival. In the case of low salinity culture, post larvae are often transferred into systems for which the salinity has been adjusted with reconstituted sea salt (RSS), which are formulated for salt-water aquariums. However, the price is not within a sustainable range considering the volume of salt necessary for the acclimation process. Therefore, during the current study, a Low-cost salt mixture (LCSM) constituting sodium, potassium, calcium, and magnesium concentrations of 298, 9, 17, and 39 mg/L respectively in 1-g/L solution was prepared, which is comparable closely with the major cations in 1-g/L dilute seawater. Following several successful laboratory studies, the current study was conducted to evaluate the efficacy of LCSM to replace RSS under actual farm conditions. Experimental trials were carried out in two on-levee tank systems installed adjacent to a shrimp production ponds on a commercial farm in west Alabama. Each tank system consisted of 12, 800-L tanks supplied continuously with low salinity pond water via a regulated water pump. Upon arrival from the hatchery (at 32 g/L salinity), 100 shrimp post larvae (0.009±0.02g) were stocked into each tank with different ionic solutions (LCSM 0, 50, 75 and 100 %) at 30 g/L salinity. Salinity acclimation was done within 2-3 days by controlling the inflow to the tank to reduce the tank salinity from 30 g/L to 6 or 1.5 g/L. Following the acclimation, one tank system-maintained flow-through with the adjacent pond (1.5 g/L) while the other maintained static at 6 g/L by ceasing water circulation. Shrimps were fed six times during the day ad-libitum for 21 days. According to the statistical analysis, no significant differences were observed in survival and growth between different ionic solutions in both experimental systems. Outcomes of the current study revealed the potential of LCSM as an excellent source for acclimation of shrimp post larvae in practical farm conditions to bring down the cost of production in low salinity shrimp farming.

Table 1: Response of Pacific White shrimp (0.009 ± 0.02 g) acclimated to low salinity pond conditions using different combinations of LCSM solutions and reared for 21 days under flow-through (1.5 g/L) and static conditions (6 g/L). Values represent the mean of three replicates.

Treatment	Flow through system		Static system	
	Final weight (g)	Survival (%)	Final weight (g)	Survival (%)
1: 100 RSS	0.89	90	0.73	98
2: 50 RSS/50 LCSM	0.88	94	0.70	97
3: 25 RSS/75 LCSM	0.89	89	0.80	100
4: 100 LCSM	0.94	90	0.86	96
PSE	0.11	3.57	0.08	2.60
p-value	0.90	0.30	0.18	0.34

EFFECT OF MAGNESIUM TO CALCIUM RATIO IN LOW SALINITY WATER ON GROWTH OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Ionic composition of water is a vital factor for shrimp growth and survival and can be more crucial than the salinity itself, since deficiencies in certain ions such as sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) lead to negative impacts on growth and survival of shrimp. Remarkable variability in ionic compositions of low salinity ground waters around the world emphasize the need to elucidate optimum ionic concentrations and ratios for shrimp production. Magnesium has been identified as one of the key cations for the survival of Pacific white shrimp reared under low salinity conditions. However, in west Alabama water used for shrimp production, the availability of Mg is low relative to Ca which is the opposite of full strength or diluted sea water. Therefore, farmers are required to add an additional Mg source to remediate Mg:Ca ionic ratios (hereafter Mg:Ca ratio) in pond water which is costly. Even after Mg is added to shrimp ponds in west Alabama the ionic ratios do not closely mirror the Mg:Ca ratio of seawater (Mg:Ca ratios of 0.5 - 1.0 or lower are quite common). A study was carried out to determine the effect of different Mg:Ca ratios in low salinity (3 g/L) water on growth and survival of Pacific white shrimp. A Low-cost salt mixture (LCSM) was used (comprised of Na, K, Mg, Ca 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:Ca ratios (2.7:1, 2:1, 1.3:1, 0.7:1, 0.3:1, 0.2:1 and 0.1:1). Reconstituted sea salt (RSS) was used as a control treatment with an Mg:Ca ratio of 3.2, which is similar to sea water. Different Mg:Ca ratios were achieved by gradually decreasing the Mg level of LCSM, while keeping the Ca level constant. A growth trial with Pacific white shrimp (initial weight = 0.11 ± 0.01g and stocking density = 20 shrimp/tank) was carried out in 150 L plastic tanks, each equipped with a miniature fluidized bed bio-filter. Shrimp were fed four times per day using a standard ration over a 42-day growth trial. Results indicate a positive relationship between growth performance of shrimp and Mg:Ca ratios of low salinity water while no significant effect was observed on shrimp survival (Figure 1).

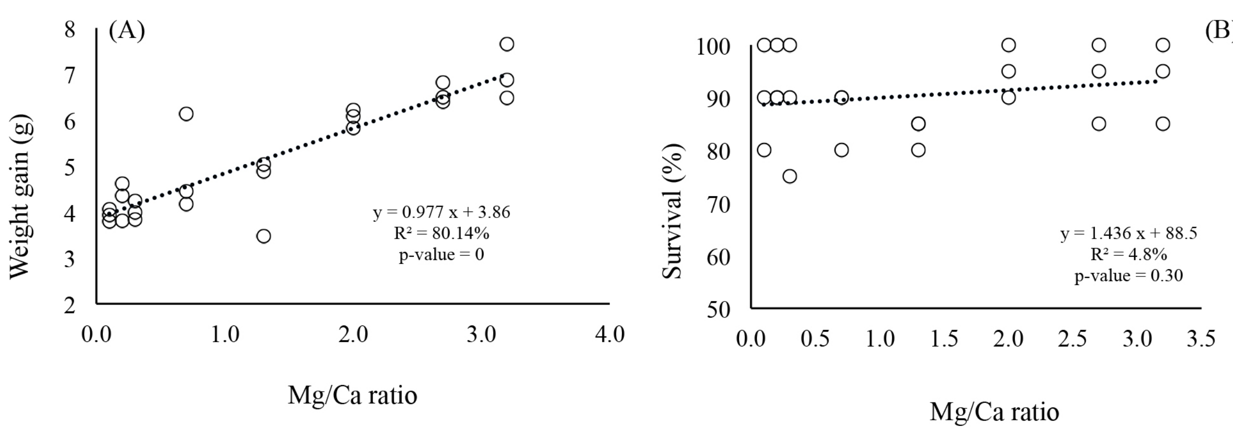

Figure 1: Effect of Mg: Ca ratio in low salinity water (3 g/L) on the growth (A) and survival (B) of juvenile Pacific White shrimp (0.11 ± 0.01 g) reared for 42 days. Values represent the mean of three replicates.

CONSERVATION AQUACULTURE FOR ENDANGERED KLAMATH BASIN SUCKERS *Deltistes luxatus* AND *Chasmistes brevirostris*

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Lost River suckers (LRS, *Deltistes luxatus*) and shortnose suckers (SNS, *Chasmistes brevirostris*) native to the upper Klamath Basin were determined to be endangered in 1988. Loss of habitat, degradation of water quality including increased nutrient load contributing to increased algal growth, increased suspended sediment and increased water temperature are considered some of the reasons for their decline along with overharvest and entrainment in water management structures. The remaining populations continue to show poor survivorship and low recruitment. In addition to habitat restoration conservation aquaculture has become a very important element for maintaining biodiversity and prevent extinction by supporting refugia populations.

Preliminary work has begun to establish a conservation aquaculture protocol for the suckers. Larval suckers are collected as they move out of their stream spawning sites into the lakes. These fish are started on feed in aquaria then stocked outside into ponds where they are raised for up to 2 years to a size (200 mm+) that will allow for increase survival in their native lakes. One of the critical needs for this culture is a knowledge of the nutrient requirements of the suckers from first feeding to grow-out size to be able to produce fish that are fit and have high survivability. This presentation will provide a current summary of the diet evaluation research for these fish.

Lost River suckers *Credit: J. Barron*

GEODUCK AQUACULTURE IN NEAH BAY AND THE ISOTOPE ANALYSES OF CARBONATE SHELLS

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The Pacific geoduck, *Panopea abrupta*, is an important clam species especially cultured in the southern Puget Sound of Washington State. Since 2007 the Makah Fisheries Management (MFM) started a pilot project on intertidal geoduck aquaculture in the Neah Bay beaches. At the same time, MFM has established a monitoring program for the geoduck aquaculture beds and weekly collected environmental parameters (e.g., temperature, salinity, pH, dissolved oxygen concentration, and pressure) and seawater samples. All these activities provide an opportunity for chemical analyses on the carbonate shells. In this study we report stable carbon and oxygen isotope analyses (□^{13}C and □^{18}O) of geoduck shells from Neah Bay and a comparison with the shells from the southern Puget Sound. Preliminary results showed that the □^{13}C values of Neah Bay geoduck shells ranged from -0.2 to +1.8‰ VPDB (Vienna Peedee belemnite), whereas □^{18}O values of the same shell samples ranged from -0.2 to +1.8‰. Over the geoduck clam's full life span (about 8 years), the □^{13}C profile showed seasonal variations with a distinct difference in □^{13}C patterns after age-1. The □^{18}O profile did not show seasonality but a trend of □^{18}O decrease starting from age-1. The MFM monitoring data indicated that seawater parameters (especially temperature, salinity, and pH) reflect a natural variation and have not changed significantly over the monitoring time. No significant changes were obtained from seawater analyses in □^{13}C from DIC (dissolved inorganic carbon), and the □^{18}O values of seawater largely correlated with seasonal changes of temperature. Overall, the isotopic comparison showed differences between the Neah Bay geoduck shells and samples from the southern Puget Sound, where distinct effects of ocean acidification were observed. In particular, the seasonal variations over the geoduck clam's life-span in Neah Bay did not show a steady decrease of □^{13}C as isotopic signatures of ocean acidification. Thus, we concluded that the carbonate shells of Pacific geoduck may be a good proxy for climate change and marine environmental studies, but the effects of ocean acidification along the Washington coast need more investigations.

GEODUCK AQUACULTURE IN NEAH BAY AND THE ISOTOPE ANALYSES OF CARBONATE SHELLS

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The Pacific geoduck, *Panopea abrupta*, is an important clam species especially cultured in the southern Puget Sound of Washington State. Since 2007 the Makah Fisheries Management (MFM) started a pilot project on intertidal geoduck aquaculture in the Neah Bay beaches. At the same time, MFM has established a monitoring program for the geoduck aquaculture beds and weekly collected environmental parameters (e.g., temperature, salinity, pH, dissolved oxygen concentration, and pressure) and seawater samples. All these activities provide an opportunity for chemical analyses on the carbonate shells. In this study we report stable carbon and oxygen isotope analyses ($\delta^{13}C$ and $\delta^{18}O$) of geoduck shells from Neah Bay and a comparison with the shells from the southern Puget Sound. Preliminary results showed that the $\delta^{13}C$ values of Neah Bay geoduck shells ranged from -0.2 to +1.8‰ VPDB (Vienna Peedee belemnite), whereas $\delta^{18}O$ values of the same shell samples ranged from -0.2 to +1.8‰. Over the geoduck clam's full life span (about 8 years), the $\delta^{13}C$ profile showed seasonal variations with a distinct difference in $\delta^{13}C$ patterns after age-1. The $\delta^{18}O$ profile did not show seasonality but a trend of $\delta^{18}O$ decrease starting from age-1. The MFM monitoring data indicated that seawater parameters (especially temperature, salinity, and pH) reflect a natural variation and have not changed significantly over the monitoring time. No significant changes were obtained from seawater analyses in $\delta^{13}C$ from DIC (dissolved inorganic carbon), and the $\delta^{18}O$ values of seawater largely correlated with seasonal changes of temperature. Overall, the isotopic comparison showed differences between the Neah Bay geoduck shells and samples from the southern Puget Sound, where distinct effects of ocean acidification were observed. In particular, the seasonal variations over the geoduck clam's life-span in Neah Bay did not show a steady decrease of $\delta^{13}C$ as isotopic signatures of ocean acidification. Thus, we concluded that the carbonate shells of Pacific geoduck may be a good proxy for climate change and marine environmental studies, but the effects of ocean acidification along the Washington coast need more investigations.

ALGAL MEALS FROM *Arthrospira platensis* AND *Schizochytrium limacinum* AS COMPLETE REPLACEMENT OF FISH MEAL, FISH OIL AND SOY PROTEIN CONCENTRATE IN FEEDS FOR TILAPIA

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Algal meals from *Arthrospira platensis* (Spirulina) and *Schizochytrium limacinum* were used to completely replace fish meal (FM), fish oil (FO) and soy protein concentrate (SPC) in feeds for juvenile hybrid tilapia (*Orechromis niloticus* x *O. mossambicus*). Five isonitrogenous and isoenergetic feeds were formulated to replace fish and soy ingredients at 0%, 25%, 50%, 75% and 100% by algal meals (ALG). Feeds were supplemented with lysine and methionine at increasing levels of ALG inclusion to meet the essential amino acids requirements of fish. Taurine was also supplemented in all feeds. A feeding trial was performed in fish with an initial weight of 0.9 ± 0.1 g. Each feed treatment was tested in triplicate 90 L tanks in a water recirculation system at a density of fish 15 per tank. Feeds were manually fed to fish twice per day at 8 am and 2 pm for nine weeks. Water quality parameters during the trial were: temperature: 28.3 ± 0.2°C, salinity 0.1 ± 0.0 ppt, dissolved oxygen 6.5 ± 0.1 mg/L, pH 6.7 ± 0.3 and total ammonia nitrogen 0.0 ± 0.0 mg/L. At the end of trial data on fish weight gain, feed utilization and nutrient retention among treatments were evaluated with ANOVA and regression analyses.

Fish feed intake was significantly higher ($P<0.05$) with increasing algal meals content in feeds, the highest found in treatments ALG 50, ALG 75 and ALG 100. The FM, FO, SPC-based feed (ALG 0) produced significantly lower growth ($P<0.05$) than all the feeds containing ALG, including ALG 100. Fish growth was the highest in feeding treatments ALG 75 and ALG 50. A quadratic regression analysis to study the relationship between fish weight gain and increasing replacement of FM, FO and SPC by algal meals indicated that maximum fish weight gain can be achieved at 70% replacement level. No significant differences ($P>0.05$) were found in FCR or feed efficiency among diets. However, nitrogen retention in fish was significantly higher ($P<0.05$) with ALG 50 and ALG 75, followed by ALG 100. No significant differences were found in final body proximate composition, with the exception of ash content. It's concluded that a feed made with 100% *A. platensis* and *S. limacinum* as the sole sources of protein and lipid produce higher growth and feed utilization in hybrid tilapia juveniles than conventional fish feed ingredients, confirming the quality and suitability of these algal in feeds for omnivorous farmed fish.

EFFECTS OF TAURINE SUPPLEMENTATION IN DIET WITH PARTIAL REPLACEMENT OF FISHMEAL BY SOYBEAN MEAL ON JUVENILE OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Higher prices and scarcity of fishmeal creates new paths to formulate feeds to replace fishmeal with cheap plant proteins. A study was conducted to investigate the effect of taurine supplementation in order to compensate negative effects replacement of fishmeal (FM) with Soybean meal (SBM). A FM based diet was considered as the control and six other diets were formulated to replace FM at 20, 30 and 40% without taurine supplementation (SM20, SM30 and SM40) and with taurine supplementation at a level of 1% (SM20-T, SM30-T and SM40-T) respectively.

Three replicated groups of juvenile olive flounders were fed one of the experimental diets for 10 weeks and their growth performance, feed utilization, innate immunity, intestinal morphology and liver IGF-1 gene expression levels were examined.

The SM20-T group showed higher growth and feed utilization compared with all other groups. However, with the increasing levels of FM replacement, taurine did not completely compensate for the growth reduction compared with the control.

Total Immunoglobulin (TIg) levels and lysozyme activity of plasma and serum increased with the supplementation, which could be an evidence of the health of the fish.

Plasma Alanine transaminase (ALT) and Aspartate transaminase (AST) levels were decreased compared with the control group in taurine supplemented groups compared to un-supplemented groups. Therefore, taurine might have completed compensated the liver damage.

There were clear IGF-1 relative expressions in supplemented groups, however, only SM20-T had a higher level than the control.

These results reveal that neither growth nor health of the fish were altered with FM replacement by 20% SBM with taurine supplementation. However, when the level of replacement increased, taurine couldn't compensate completely for the observed adverse health effects.

There were clear IGF-1 relative expressions in supplemented groups, however, only SM20-T had a higher level than the control.

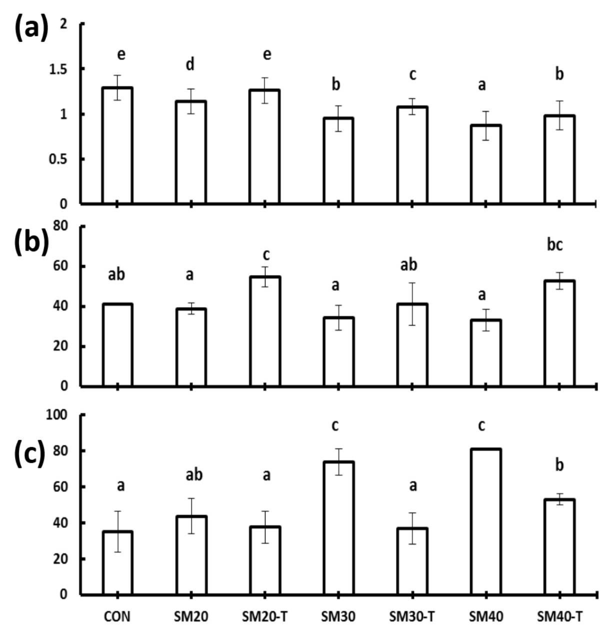

Figure 1: (a) Specific Growth Rate%, (b) TIg (mg mL^{-1}), (c) AST (U/L)

PURPLE URCHIN BARRENS: AN OPPORTUNITY FOR AQUACULTURE AND FISHERIES TO WORK TOGETHER TO SOLVE AN ENVIRONMENTAL ISSUE

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Sea urchin barrens and can stretch over 1000's of kilometers and last decades at a time. They are characterized by a predominance of urchins and coralline algae where kelp forests once existed. In contrast to barrens kelp forests provide habitat supporting thousands of vertebrates, invertebrates and plant species. Because kelp forests are keystone hosts their presence is vital to sustaining commercial and recreational industries including fishing and tourism. However, these kelp forests can collapse and shift to alternate stable states whereby urchin barrens persist. Over the last 4 decades, transitions between kelp beds and sea urchin barrens have been widely reported along temperate coastlines globally. During a kelp forest phase, urchin predation is the primary mechanism keeping sea urchin populations in check. However, due to various factors including climate change, predator densities can be reduced leading to shifts toward urchin barrens. Development of urchin fisheries has been implicated several times in recent history as a driver to return urchin barrens to kelp forests. However, this driver most recently has not worked in California where a large barren is currently persisting. California presently has an urchin fishery but it has been uneconomical for the fishery to operate given urchins in the barrens had little gonad development or undesirable human consumption traits necessary for commercialization. Aquaculture in the US has potential to restore kelp forests by collaborating with fisheries to harvest wild urchins from barrens and fatten them in an aquaculture setting prior to sale. This is of particular interest in California given U.S fisheries and environmental groups have long opposed aquaculture development due to concern of competition and environmental damage respectively. A collaboration between aquaculture and fisheries to positively impact the environment via urchin barren restoration would help to develop aquaculture in the US and California. Although urchin ranching operations exist there are still technical limitations to the activity, primarily being availability of macroalgae diets given seasonality and the propensity of urchin barrens to deplete kelp forests. Development of sustainable alternative diets for urchins is necessary for future commercial urchin aquaculture. In this student lead study a preliminary replicated diet trial was performed for ranching purple sea urchins (*Strongylocentrotus purpuratus*) collected from California barrens using 4 diet treatments including giant kelp (*Macrocystis pyrifera*), ogo (*Gracilaria pacifica*), formulated commercial diet (Urchinomics) and an unfed control. During the 10 week study duration gonadal somatic index (GSI) was measured in a subset of urchins from each replicate tank every 2 weeks. Baseline GSI at the beginning of the trial was <0.5%. A GSI of 10% was reached most rapidly in the formulated diet treatment at 6 weeks, followed by ogo and kelp at 8 and 17 weeks respectively. This study was a preliminary examination of the feasibility of urchin ranching in California, showing biological potential for alternative diets to develop urchin gonads with a view to restore kelp forests and develop a nascent aquaculture industry in California.

AQUACULTURE PRODUCTION AND FOOD SECURITY

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The United Nation's Sustainable Development Goals recognize food security and nutrition as priorities for the global development agenda. Until recently, the contribution of fisheries and aquaculture to global food security and health outcomes has been overlooked. Fish and seafood are a key source of protein and, more importantly, are rich in bioavailable micronutrients, such as zinc, calcium and essential fatty acids that are commonly deficit in plant-based diets. While global consumption of fish and seafood is increasing, consumption varies across and within regions due to geographic, economic and cultural factors. More importantly, while it is widely acknowledged that increases in per capita consumption of seafood have been made possible through increased supply of farmed seafood, there are concerns that farmed seafood, which is predominately produced in economically underdeveloped countries, is exported to wealthier nations and may undermine local food security in the poorest regions of the world.

In this paper, we use country-level aquaculture production and apparent seafood consumption data from the United Nation's Food and Agriculture Organization to examine the effect of aquaculture development on consumption of seafood in economically underdeveloped countries. We find that countries who have participated in the blue revolution are those that needed it the most (i.e., they had lower per capita consumption prior to aquaculture development than countries that do not develop aquaculture). Additionally, the development of aquaculture has, on average, positively affected per capita seafood consumption in developing countries where production has occurred, and the effect has increased over time. The analysis is highly informative to understanding the domestic effects of aquaculture production on food security in developing countries.

TEMPORAL DYNAMIC OF DNA METHYLATION PATTERNS IN RESPONSE TO HATCHERY-REARING IN STEELHEAD (*Oncorhynchus mykiss*)

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There is considerable evidence that steelhead reared in hatcheries differ from wild fish in phenotypic traits related to fitness. Genetic selection is often implicated as the underlying cause of these phenotypes, however, environmental conditions experienced during early life can also have long-term effects on fitness related traits. This form of developmental plasticity may be mediated by epigenetic mechanisms such as DNA methylation. We tested whether hatchery-rearing conditions can influence patterns of DNA methylation in steelhead with known genetic backgrounds, and assessed the stability of these changes over time.

In this study, eyed-embryos from 22 *O. mykiss* families were split across traditional hatchery tanks or a simulated stream-rearing environment for 8 months followed by a second year in a common hatchery tank environment. DNA methylation patterns were examined in the liver of juveniles at two time points: after 8 months of rearing in either a tank or stream environment and after a subsequent year of rearing in a common tank environment. Further, DNA methylation was analyzed in the sperm of mature males to assess the potential of environmentally-induced changes to be passed to offspring.

We found that hepatic DNA methylation changes in response to hatchery versus stream-rearing in yearling fish were substantial, but few persisted after a second year in a tank environment. However, the early rearing environment appeared to affect how fish responded to developmental and environmental signals that shaped the liver methylome, as novel DNA methylation differences were identified after a year of common rearing. Furthermore, we found profound differences in DNA methylation due to age, irrespective of rearing treatment. Although few rearing-treatment effects were observed in the sperm methylome, strong family effects were observed. These data suggest limited potential for intergenerational changes, but highlight the importance of understanding the effects of kinship among studied individuals in order to properly interpret DNA methylation data in natural populations.

A LOW-COST AUTOMATED ROTIFER *Brachionus spp*. COUNTING METHOD USING BACKGROUND SUBTRACTION AND CONNECTED COMPONENTS ANALYSIS

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Rotifers *Brachionus spp*. are crucial starting diet for fish aquaculture. In commercial scale rotifer production, sampling and counting are essential for examining the rotifer population. The counting of rotifer samples is usually manually executed using microscopy by aquaculturists and is extremely time consuming and inefficient. Automation of rotifer counting would improve the production efficiency and release aquaculturists from tedious work. Current automated cell counting systems are typically very expensive and not designed for commercial scale rotifer culture. The present study developed a low cost computer vision system that can detect and count rotifers by analyzing microscopic images of rotifer samples using fine-tuned background subtraction (BS) and connected components labeling (CCL) algorithms. This method can accurately (error rate 5.16%) count rotifer samples (0.1 ml) from commercial scale production.

The image processing and analysis program[1] was implemented using Python 3.7.3 and OpenCV 4.1.0. The testing procedure for this method is: 1) Take a 30s microscopic video clip of the 0.1ml rotifer sample. 2) Process the video frames with Gaussian Mixture Model based BS to generate foreground binary images. 3) Process the binary images with Scan plus Array-based Union-Find CCL to detect binary large object (blob). 4) Threshold the blobs by size to identify and count rotifers.

The equipment for data collection includes: iPhone7, microscope cell phone adapter (iDu LabCam, 10x), Leica GZ6 (1.1x). There are a couple of parameters such as the frame extraction stride, background model learning rate, blob size threshold, etc. that were fine-tuned by the authors. The counting method was tested on 42 rotifer culture samples taken from University of Miami Experimental Hatchery (UMEH) with densities that ranged from 50 ind. ml^{-1} to 450 ind. ml^{-1}. For each sample, the algorithm took and counted around 40 frames from each video clip and used the mean as the final count after removing the outliers. The method is also able to provide real-time counts for each frame of the video.

Figure 1 shows the performance of this method by comparing the counts using this method and manual counts of the Lugol-fixed samples. The performance index is the average absolute error rate (%) using the manual count as the ground truth. The R2 for fitting the data on y=x is 0.9934. The total cost of this method is only around $200 USD for the adapter assuming that most commercial hatcheries have the other tools such laptop computers and microscopes.

The program can be obtained from the author by request via email.

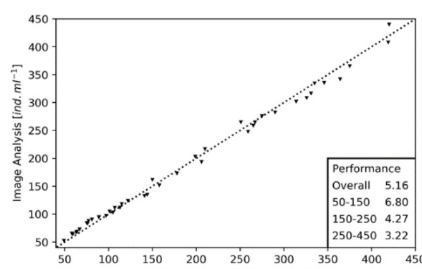

Figure 1 Method Performance

IMPROVERS PROGRAM FOR ECUADORIAN SHRIMP INDUSTRY TO PROMOTE A BETTER USE OF PRODUCTION RESOURCES AND A PATHWAY TO SUSTAINABLE PRACTICES

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Ecuadorian shrimp producers are moving towards more responsible production via several commitments and pathways. One of the fundamental components of the SSP improver program includes measuring the efficiency of water, land, energy and wild fish to produce shrimp. If we want farmed seafood to be an important part of the solution for feeding people, efficiency in natural resource inputs is critical. Local agencies, authorities and researchers have not been able to determine these metrics and if so, they haven´t made results accessible to interested parties.

WWF, SSP, IDH and ASC are collaborating to undertake a comprehensive farm level survey to measure a baseline of resource use in the country. Big groups currently operate under appropriate parameters and most of them are already eco-certified or pursuing an eco-certification, but smaller and mid-size producers, are also an important link in ecuadorian aquaculture pathway to sustainability and may perform an adequate role in an efficient resource-use.

For this reason, the Improvers Program is aiming to engage actors that are a part of this group of farms, which seeks better output regarding environmental impact and an efficient and profitable production system but can lack guidance on how to implement this as well as competitive pricing and/ or access to key markets.

With this information, we as WWF, expect to have a good picture of the real situation in ecuadorian shrimp culture that may contribute to the conservation of natural resources such as water, land, and energy and will contribute to optimizing the performance within the industry.

Surveys and sampling in field have been carried out for several months since July 2019, and preliminary results of different farming sites, both in continent and insular areas, will be shared in the presentation.

Figure 1 Sampling Weekly Growth in farm (Guayas-Province)

DEVELOPMENT OF AN OYSTER SHELL RECYCLING PROGRAM PILOT PROJECT IN PORTLAND, MAINE FOR COASTAL ACIDIFICATION REMEDIATION

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There is a clear opportunity for the development of an oyster recycling program in Maine as the aquaculture industry is expanding rapidly, and the importance of environmental sustainability and circular economies grows. Every state on the eastern seaboard has an oyster shell recycling program in place except Maine. Portland, ME has become a foodie destination for tourist with a focus on local seafood. Currently all the waste from oysters in Portland is either composted or thrown in the trash. All current oyster shell recycling programs on the east coast use the shell to create new habitat for wild oysters. With the waters of Maine being too cold for a natural oyster spawn a different purpose must be developed including the use in buffering ocean acidification.

A two-year pilot project funded by the EPA's Climate Ready Estuaries program has been developed to investigate the potential for an oyster shell collection program in the state of Maine using a small-scale demonstration in Portland, ME. The goals of the project are to develop a collection plan with restaurants in Portland, investigate potential uses for the shell, run an experiment testing the use of oyster shell as a buffer for ocean acidification, and create an opportunity for local youth engagement. The project is being conducted by the Maine Coastal Program in collaboration with the Casco Bay Estuary Partnership and began in the Fall of 2018.

After conducting stakeholder meetings with government agencies, restaurants, waste disposal companies, and interested community members an oyster shell collection program began in June of 2019. From the start of collection until September 2019 there has been over 30 cubic yards of shell diverted from composting and landfills. The shell needs to cure for 12 months in order to remove the possibility of the transfer of pathogens. During this time a variety of uses for the shell will be investigated.

Since the collection of shell began there has been a great deal of interest from a variety of stakeholder groups including the aquaculture industry, environmental non-profits, and local community members. There have been preliminary studies to test the effects of using crushed shell as a buffering agent in shellfish hatcheries. Also, living shoreline trails are being conducted to develop the best designs for northern areas such as Maine. The results of these tests have not been published yet, but the importance of a robust oyster shell collection program is clear. With successful determination of the uses of oyster shell in Maine an oyster shell collection program can contribute greatly to a sustainable future for shellfish consumption.

CONSUMERS' WILLINGNESS-TO-PAY FOR NEWLY DEVELOPED U.S. FARM-RAISED CONVENIENT CATFISH PRODUCTS: A CONSUMER BASE SURVEY STUDY

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Increased understanding of the factors that influence the U.S. consumers' willingness-to-pay (WTP) are critical as it may likely to help restoring the economic profitability of catfish business. A survey was conducted at four different states/regional-base programs during 2018-2019. An ordered probit model was developed to examine the variables influencing consumers' WTP. Current results suggest that the five newly developed U.S. farm-raised convenient catfish products such as panko breaded standard catfish strips, panko breaded standard catfish fillet, panko breaded delacata catfish fillet, sriracha marinated delacata catfish and sesame-ginger marinated delacata catfish fillet, could be widely accepted by the U.S. consumers. This is evident from the consumers' higher WTP for all these five products. The study also indicated that 41%, 42%, and 47% participants would like to pay the average price of \$3.89/108 g, \$3.29/108 g and \$2.69/80 g to the panko braded catfish delacata fillet, standard fillet and standard strips products, respectively; while 70% and 69% participants' WTP were \$3.19/108g for sriracha and sesame-ginger marinated delacata catfish fillet, respectively. The potential variables found to affect the WTP to these five products include product appearance, eagerness to buy, preference ranking, preferred form of eating, catfish consumption pattern and certain socio-economic parameters such as gender, education, race, age, and annual family income (Fig.1). Besides, participants belong to certain annual family income group (\$15,000-\$30,000, \$30,001=\$60,000 and \$ 60,001-\$100,000) are willing-to-pay the higher price (>\$2.69) to these catfish products. Entrepreneurs can potentially target these consumer segments (based on income group) in order to increase their revenue by selling these five convenient catfish products.

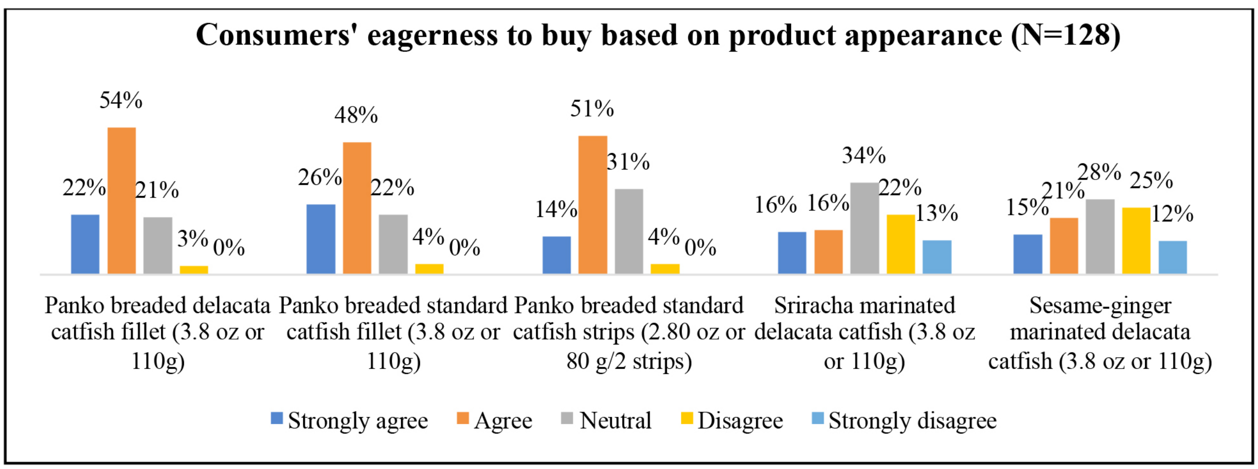

Fig.1: Consumers' eagerness to buy based on product appearance to these five newly developed U.S. farm-raised convenient catfish products

REVIEW OF AQUAPONICS SYSTEM: SEARCHING FOR A TECHNICALLY FEASIBLE AND ECONOMICALLY PROFITABLE AQUAPONICS SYSTEM

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The adoption rate of aquaponics has been, to some large extent, increasing. The potential reason for such an outcome is the interlinking procedures of aquaculture and hydroponic systems that resulted to a promising source for sustainable food production (Goddek et al. 2015). This system can also ensure the protein security to the ever-growing urban population (Alexandratos and Bruinsma 2012). Review study shows that aquaponics system is likely to be a good option for adoption (Rakocy 2012), but the overall technical and economic feasibility of this system has not been yet fully realized. Current study suggests that the management of the aquaponics system is quite complex as it deals with three different concepts of fish, plant, and microorganism together. Failure to maintain the unique water quality parameters, particularly the P^{H} stabilization and nutrient balance may likely to jeopardize the whole system, when the production is the main concern. In addition, few other technical points should be addressed before transforming this system from a small scale to a commercial level (Sverdrup et al. 1981); (1) improved nutrient solubilization and recovery for a better use of the nutrient input and reducing extra-mineral addition, e.g., phosphorus recycling; (2) adapted pest management; (3) reduce water consumption to a high degree by limiting the need for water exchange; (4) use of alternative energy sources for hot/cold and arid areas.

Review study also showed that deep-water culture system and nutrient film technique could be the good option to adopt at a commercial level. Lettuce, herbs, and specialty greens (spinach, chives, basil, and watercress) are suggested as they have a low to medium nutritional requirements and are well adapted to aquaponics systems (Diver 2006). Vegetables yielding plants (tomatoes, bell peppers, and cucumbers) have a higher nutritional demand and perform better in a heavily stocked, well established aquaponics system (Diver 2006). Among warm and cold-water species, tilapia, trout, perch, Arctic char, and bass are well adapted to recirculating aquaculture system (RAS) (Diver 2006). Tilapia is highly favored in commercial aquaponics system due to their high adaptive nature and tolerating capacity in fluctuating water conditions (Diver 2006). In terms of economics point of views, vegetables and other plants derived from hydroponic system is likely to be more profitable than fish produced from RAS (Bailey and Ferrarezi 2017)conserves water, reduces waste discharged into the environment, and recovers nutrients from fish production into valuable vegetable crops. A standard protocol has been developed for the production of tilapia yielding 5 MT per annum. The production of many vegetable crops has also been studied but, because of specific growth patterns and differences of marketable product, no single protocol can be promoted. Each crop yields different value per unit area and this must be considered when selecting varieties to produce to provide the highest returns to the farmer. Variables influencing the value of a crop are density (plants/m2. Hence, additional costs and risks associated with this complex system should be analyzed before investing the money in adopting the aquaponics technique.

U.S. DEPARTMENT OF ENERGY INVESTMENT IN ALGAE CULTIVATION AND BENEFITS AND OPPORTUNITIES FOR THE AQUACULTURE INDUSTRY

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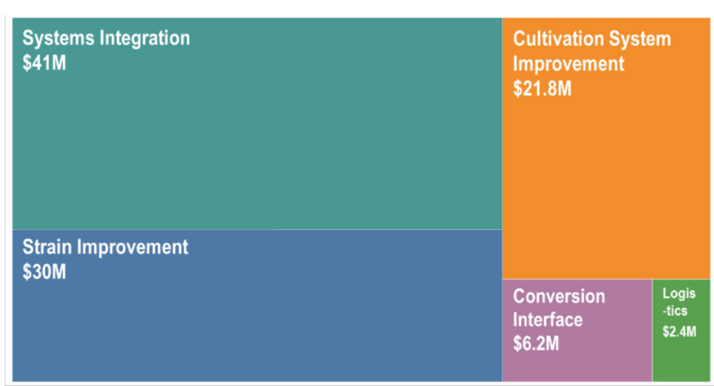
The U.S. Department of Energy (DOE) funds research, development, and demonstration to help develop sustainable and cost-competitive biofuels, bioproducts, and biopower. A key office within the U.S. DOE which serves this role is the Bioenergy Technologies Office (BETO). BETO is focused on forming cost-share partnerships with key stakeholders to develop, demonstrate, and deploy technologies for advanced biofuels production from lignocellulosic and algal biomass.

The Advanced Algal Systems (AAS) program of BETO is carrying out a long-term applied research and development (R&D) strategy to increase the yields and lower the costs of algal biofuels by working with partners to develop new technologies, to integrate technologies at commercially-relevant scales, and conduct crosscutting analyses to understand the potential and challenges of an algal biofuel industry that is capable of annually producing billions of gallons of renewable diesel, gasoline, and jet fuels.

AAS R&D covers the entire pipeline from feedstock to fuel. Activities support improvement in algal strains, cultivation systems, logistics and preprocessing technologies (harvesting, dewatering, and concentration), conversion of biomass, and systems integrations. Advancements in the production and conversion of algae feedstocks, achieved through BETO efforts, can be leveraged to support related industries including aquaculture. Furthermore, the development of aquaculture feed from the nonfuel components of algae, could be a strategy for increasing the value of biomass to enable biofuels. In fact, AAS has invested in several projects that support the co-production of aquaculture feed from algal biomass.

The purpose of the paper is to present AAS program structure, strategy, and investments to conference participants, as well as share how participants can apply for AAS funding opportunities.



Algae R&D investments by the Bioenergy Technologies Office, Advanced Algae Systems program for fiscal years 2017 to 2019. Total funding $101.9 million.

OFFSHORE MACROALGAE FARM DESIGN – EXPERIENCE FROM ALASKA, NEW ENGLAND, FLORIDA, AND PUERTO RICO

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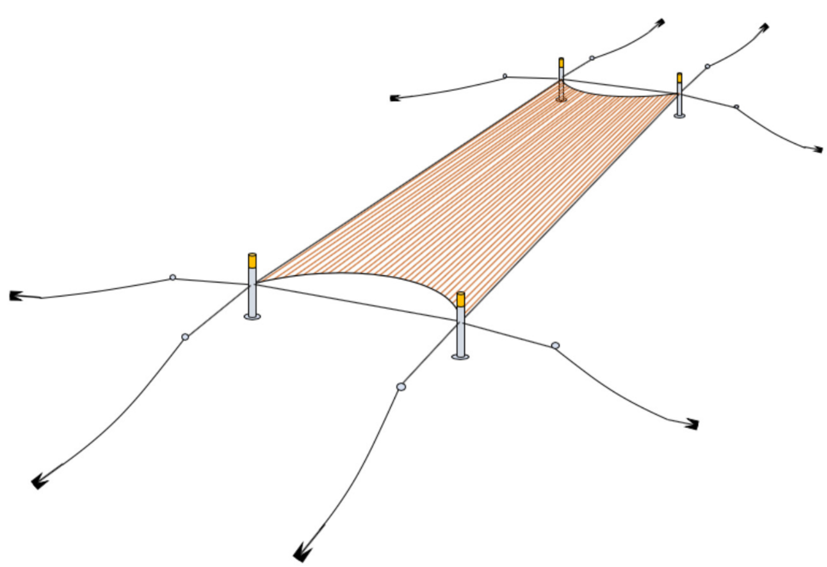
While seaweed farming is a $6B global industry producing over 15 million tons, it remains largely a labor-intensive, near-shore industry and less than 0.1% of this economic activity happens in the US in spite of our nation's huge EEZ. To prosper domestically, farm technologies suitable for offshore locations are needed, particularly systems that lend themselves to mechanized planting and harvesting and that reduce the risks imposed on marine mammals or other protected species.

We have developed farm systems addressing these needs for sugar kelp (*Saccharina latissima*) farming in Alaska and New England, and for a tropical red alga (*Eucheuma isiforme*) in Puerto Rico and Florida. In all cases our systems are modular and support multiple parallel growlines within an overarching structure that provides dimensional stability and is readily expandable. Notable in all these longline systems is a high ratio of growline length per unit farm area and the maintenance of pre-tension in the growlines. This pre-tension enables closely spaced lines, and offers opportunities for efficient multi-line harvesting. Our installation processes will be described along with our experience to date servicing these structures around the U.S.



SUSTAINABLE SEAWEED AQUACULTURE: WILL CALIFORNIA PLAY A ROLE IN THE GLOBAL PUSH?

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The global demand for farmed seaweed has increased dramatically, with recent interest in developing small- to large-scale farming in novel locations to provide biomass to feed humans and livestock, create biofuels, agricultural additives, and cosmetics, and to combat climate change. Although the academic infrastructure for studying seaweed biology is well established in California, commercial seaweed farming in the state is limited. Here, I discuss the current state of commercial seaweed farming in California and highlight some of the primary constraints on the seaweed aquaculture industry, especially as developed for human consumption. Increased interest among restaurants and food services in California for sustainably-produced seafood suggests that the potential market for farmed-seaweeds in the state is broad, as seaweeds offer an alternative source of protein and essential nutrients, while providing a diversity of flavors and textures. Although offshore seaweed farming is possible in California, many species desired by chefs are unable to be propagated in offshore systems and permitting can be time-consuming and costly. Consequently, the industry is currently limited to land-based tumble-culture farms that offer a sustainable and cost effective method for producing seaweeds for local markets. As permits become available for offshore farming in California state- and federal-waters, the production of lower cost seaweed biomass for animal feeds, biofuels, and agricultural products may become feasible. I will also discuss some case-studies for integrating seaweed farming with existing shellfish cultivation systems in California to combat local eutrophication and ocean acidification issue.

SHELLFISH GROWERS TACKLE MARINE DEBRIS AND EXPLORE RECYCLING OPTIONS IN SOUTH PUGET SOUND

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The Pacific Coast Shellfish Growers Association (PCSGA) represents growers in Alaska, Washington, Oregon, California, and Hawaii. PCSGA works on behalf of its members on a broad spectrum of issues, including environmental protection, shellfish safety, regulations, technology and marketing. This presentation will discuss PCSGA's current initiative of tackling marine debris and how we are working to reduce plastics entering the waterways by encouraging best farming practices, supporting shoreline clean ups, and finding recycling options. PCSGA and its members have been participating in marine debris removal efforts collectively since 2001. Twice a year, shellfish growers join forces with local agencies and non-profit organizations to remove marine debris of all kinds from the South Puget Sound. The debris is collected by boat, then transported to a land-based sorting station where it is sorted, counted, and its location documented.

Shellfish growers acknowledge their role in taking care of the shorelines where they farm and understand that keeping our beaches clean is a group effort. One of PCSGA's primary goals is to work with our members to minimize the amount of gear escaping from their farms during storm events and supporting cleanup efforts to remove the gear that does enter our waterways. It is important to note that over the last 18 years, the shellfish industry has expanded, and yet the composition of debris collected at our cleanups remains fairly consistent. Non-aquaculture related debris collected at our cleanups continues to make up roughly 80%. Most of the aquaculture gear that is recovered is returned to the company it belongs to or is offered to our members to take and reuse.

As the shellfish industry continues to expand, it is crucial that there are standards and practices put in place to ensure the stewardship of our tidelands continues. PCSGA is now forging partnerships with plastics recyclers to tackle the next issue – how to recycle plastics that have been exposed to the marine environment, either from use on the farm or collected via beach cleanups.

THE NORTH CAROLINA SHELLFISH LEASE PROGRAM

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The North Carolina Division of Marine Fisheries (NCDMF) is responsible for the stewardship and sustainability of the state's marine and estuarine resources for the benefit and health for all North Carolinians. In doing so, NCDMF manages shellfish resource development, habitat enhancement, benthic mapping, shellfish leasing, and water quality for shellfish growing areas. The NCDMF administers the Shellfish Lease Program through the Habitat and Enhancement Section for the purposes of commercial shellfish aquaculture within the State of North Carolina.

In North Carolina, the leasing of public trust bottom for the use of shellfish aquaculture has been around for over 150 years. Shellfish leases are divided into two types; *Bottom* and *Water Column*. Traditionally, bottom leases were utilized to grow oysters on cultch, or to bed clams under netting. The ability to lease the water column for shellfish aquaculture was enacted in 1989 with the first shellfish water column lease issued in 1991. However, it has been only recently that this type of leasing has expanded. The shellfish aquaculture industry has expanded tremendously over the last seven years in North Carolina with a 5,200 percent increase in shellfish lease applications since 2011.

Standards and requirements for shellfish leases in North Carolina are mandated through general statutes. The North Carolina Marine Fisheries Commission (NCMFC) has authority to develop rules for shellfish aquaculture to further ensure the statutorily required standards are being met, while considering other uses of the public trust. Federal permitting for shellfish aquaculture is also required by the US Army Corps of Engineers through the Nationwide Permit #48. Each shellfish lease application must be vetted through processes including state/federal review to ensure compliance with state and federal laws and NCMFC rules. Key management components include: application receipt and review, biological investigation, public comment and hearing, and the final recommendation.

Coinciding with the expansion of the shellfish aquaculture industry and its use of water column leases, there has been a substantial increase in the number of user conflict issues which has led to an increase in litigation and takes tremendous staff time and resources. Achieving and sustaining a successful shellfish aquaculture industry in North Carolina will depend on, among other things, resolution of user conflicts.

SHELLFISH HATCHERY CRASHES ALONG THE ATLANTIC COAST OF THE UNITED STATES

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Oyster aquaculture in the United States is highly dependent on hatchery-produced oyster seed to fuel production. The role of hatcheries has also expanded over time to support fisheries augmentation and restoration efforts. Given the importance of hatcheries for commercial aquaculture and increasingly for oyster restoration projects, considerable effort has been placed on increasing the output and production efficiencies of these facilities through improved husbandry practices and technological development.

Despite these resulting improvements, many hatcheries regularly experience substantial and unexplained die-offs or prolonged periods of slow larval growth (termed crashes) without clear signs pointing to causative factors. The frequency (e.g. decadally, annually, etc.), scale (i.e. amount of larvae lost), and the duration of crashes varies greatly among hatcheries. At a minimum, short-lived hatchery crashes waste valuable resources (i.e. labor, utilities, broodstock, etc.), but prolonged droughts in production among larger facilities are likely to limit regional shellfish production through seed shortages. Although crashes are well recognized within the industry, effective diagnostic and response strategies are ineffective. Indeed, hatchery staff are often forced to focus on one potential cause and simply dispose of the 'bad' larvae and water, and hope 'good' water will be available upon restarting the production process. Research is needed to improve production reliability by predicting, diagnosing, and mitigating sources of hatchery crashes.

Here we review recent crashes at research hatcheries along the Atlantic Coast and note facilities experiencing fewer crash events. Attention is given to a recent, prolonged crash at the Horn Point Laboratory Oyster Hatchery (**Fig. 1**), the largest hatchery supplier of oyster larvae and seed on the Atlantic Coast. We describe the complexity of identifying sources and discuss how crashes affect hatchery profitability, growers, and shellfish management and policy. Finally, we offer new approaches and strategies for identifying and avoiding crashes to maintain shellfish production.

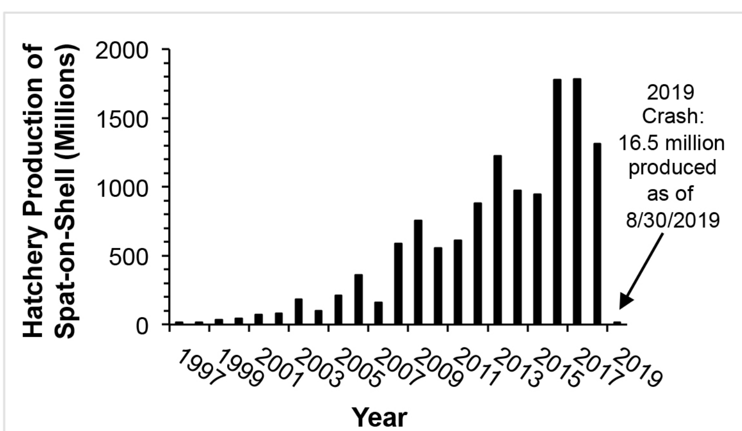

Figure 1. *Millions of oysters spat produced at Horn Point Laboratory oyster hatchery over time.*

TESTING FOR DOMESTICATION SELECTION IN BIVALVES: PHYSIOLOGICAL RESPONSE TO EXPOSURE DURING LARVAL DEVELOPMENT

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Domestication selection (shifts in traits due to adaptation to the hatchery environment) is an important consideration when using hatchery propagation for oyster stock enhancement or supplementation during restoration efforts. The hatchery environment is drastically different from the natural environment. Conditions are controlled, predation is removed, crowding may influence behavior and food is provided *ad libitum*. One concern is that many generations of hatchery propagation (selective breeding) can lead to inadvertent domestication selection that may impact long term fitness in the wild.

To test domestication effects on the Eastern oyster (*Crassostrea virginica*), hatchery produced larvae from local wild (Choptank River, Maryland, U.S.) and a selectively bred aquaculture line (Lola) were challenged under starvation stress. Specifically, we tracked the physiology (growth, feeding, and respiration) of 5-day old larvae that were subjected to a short (3-day) starvation period followed by a recovery period during which *ad libitum* diet was resumed.

Growth and survival were similar between wild and selectively bred lines. Starvation induced metabolic depression that inhibited growth, as expected in a low food environment, followed by compensatory metabolic activity before resuming growth and development. Although most physiological metrics were similar between wild and the selectively bred line during starvation, ingestion and assimilation of leucine (a proxy for micronutrients) were significantly reduced in Lola relative to wild larvae at end of the 3-days starvation. Gene expression analysis revealed distinct differences among all treatments (Fig.1). For all pairwise comparisons, proteins related to metabolism made up >30% of the significantly differentially expressed transcripts supporting the varying feeding response of larvae under starvation stress and further distinguishing the aquaculture line from the wild type larvae despite similar phenotypic responses.

Our work shows that while the physiological response to starvation stress is similar between larvae produced from wild and selectively bred broodstock, differences in gene expression between lines, in both starved treatments and fed controls, suggest domestication selection has led to molecular changes within larval life-stage.

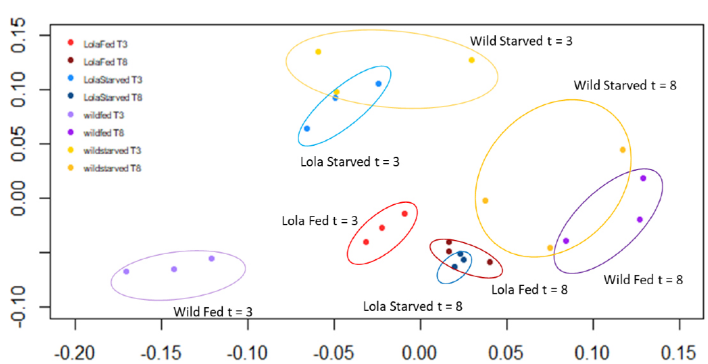

Fig. 1: Multidimensional scaling plot showing how replicates pool based on similarities and differences in differential gene expression between lines and treatments

REPLACEMENT OF FISH OIL WITH CONVENTIONAL AND HIGH OLEIC ACID SOYBEAN OIL IN THE STARTER DIETS OF RAINBOW TROUT *Oncorhynchus mykiss*

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The replacement of fish meal and fish oil with plant-based ingredients in fish feeds is a promising solution to sustainability and profitability concerns associated with Salmonid aquaculture. While conventional soybean oil has been extensively studied as a lipid source in in Salmonid feeds, no studies to date have evaluated the high oleic acid soybean oil (HOSBO) that is rising in popularity with the U.S. soy industry. This investigation examined the culture performance, gastrointestinal histology, and lipid composition of rainbow trout fry fed experimental starter diets with conventional and high oleic acid soybean oil sources.

Rainbow trout fry (165 mg) were randomly distributed to eighteen 20 L flow-through tanks with initial stocking of 60 fish/tank. Fish were fed six experimental diets for eight weeks on a restricted feeding regime (5-6% biomass/day), and tanks were weighed and sampled every two weeks. Six experimental diets were formulated to have 41% protein and 20% lipids. Two diets were formulated with fish meal protein and cod liver oil or conventional soybean oil (FM+CLO and FM+SBO, respectively). The other four diets had 1:1 fish meal and soybean meal protein and either cod liver oil (SBM+CLO), conventional soybean oil (SBM+SBO), HOSBO (SBM+HOSBO), or HOSBO supplemented with n-3 polyunsaturated fatty acids (SBO+HOSBO+PUFA). Soybean oils were provided by Archer Daniels Midland™ and SmithBucklin™.

The mean weight and feed conversion ratio (FCR) of fish were significantly different ($\alpha=0.05$) between diet groups throughout the first four weeks of feeding ($p<0.0001$; Figure), but the FCR was not significant at six and eight weeks ($p>0.1$). The fishmeal diet groups had significantly faster growth and lower FCR than all soybean meal diet groups throughout the trial. While the two HOSBO diet groups had a higher FCR at four weeks of feeding, this trend was not apparent as fish grew larger. These results suggest that rainbow trout have reduced growth efficiency on high oleic acid soybean oil in starter diets, but that this may be an appropriate lipid source for grow-out stages.

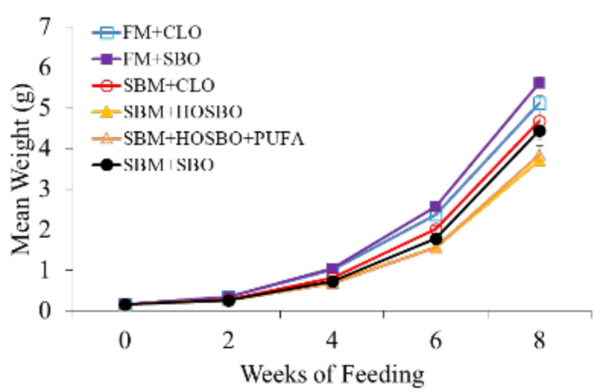

Figure. Growth of rainbow trout during the first eight weeks of feeding on experimental diets with either fish meal (FM) or soybean meal (SBM) as the primary protein source and cod liver oil (CLO), conventional soybean oil (SBO), high oleic acid soybean oil (HOSBO), or high oleic soybean oil with supplemented n-3 polyunsaturated fatty acids (HOSBO+PUFA) as the lipid source.

THE NITROGEN BIOREMEDIATION POTENTIAL OF *Saccharina latissima* CULTIVATION AND HARVEST IN THE GULF OF MAINE

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Kelp uses dissolved inorganic nitrogen (DIN) from temperate marine environments to grow. When used as a management tool, assimilation and extraction of anthropogenic DIN by strategically-sited kelp farms is known as nitrogen *bioremediation* (Fig. 1). Here, we provide the first estimates of N bioremediation by *Saccharina latissima* in the western Gulf of Maine (GOM). Wet biomass produced, total tissue N and C throughout the growing season, origin of tissue N (anthropogenic or marine), and nitrate reductase activity was measured in kelp farmed at four sites from 2016 - 2019. Ambient DIN measured at the farms was compared to nitrate reductase and total tissue N in the kelp tissue to determine quantity and rate of nitrogen assimilation by the kelp. Origin of the assimilated nitrogen was estimated by measuring nitrogen isotope values ($\delta^{15}N$) in the kelp tissue.

The mean kelp biomass measured at the end of the growing season (late May) was 9.84 (± 2.53) - 14.84 kg (WW) m^{-1}. Mean total tissue N was 1.04% - 3.82 % DW (± 0.22). These results suggest that harvesting 1 ha of *S. latissima* from the region in late May removes 18.5 – 55.6 kg N ha^{-1} with 6 m spacing between longlines. Nitrogen isotope ratios in the sampled tissue do not show a clear pattern of anthropogenic nitrogen use. High variance was observed in nitrate reductase activity of individual laminae. We conclude that kelp aquaculture may complement other N mitigation strategies, but it is not a panacea for nutrient management in the region. Modifications to harvesting schedules, alternative species or co-culture, or more informed siting may increase total potential bioremediation by kelp farms in the GOM.

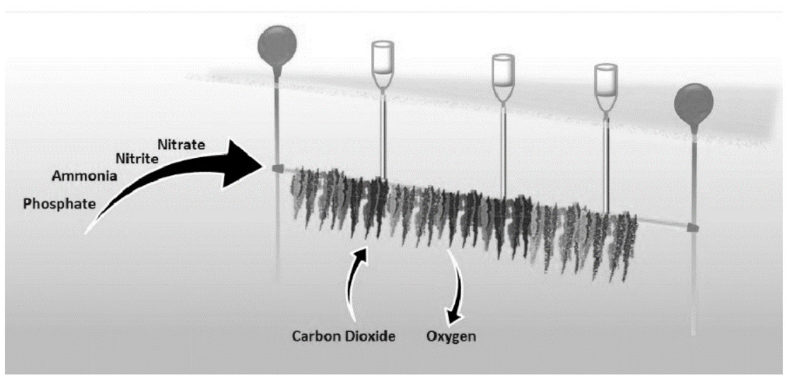

Figure 1. A typical kelp longline in the Gulf of Maine.

PRODUCTION OF CHANNEL CATFISH USING ONE-YEAR-OLD OR UNUSED WATER IN A BIOFLOC TECHNOLOGY PRODUCTION SYSTEM

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Re-using water from an established mixotrophic biofloc technology production system over multiple production cycles would be beneficial because there would be no lag in the onset of biotransformation of excreted feed nitrogen. We conducted the present study in an outdoor biofloc technology production system stocked with channel catfish (*Ictalurus punctatus*) reared in one-year-old waters with low or high total suspended solids that was used previously for two consecutive biofloc experiments or in unused (new) water. Objectives of the study were to evaluate the impacts of the different waters on fish production characteristics and mineral status, common microbial off-flavors, and water quality dynamics. Tanks (18.6 m^2, 15.7 m^3) were stocked with fingerlings (47.5 ± 0.8 g/fish) at 13.5 fish/m^2 (16 fish/m^3) and grown for 181 days. Total suspended solids were maintained at 300 to 400 mg/L in the unused and low total suspended solids used water treatments and allowed to accumulate in the high total suspended solids used water treatment. Water type did not affect significantly any channel catfish production characteristic. Gross fish yield averaged 10.2 kg/m^3, fish averaged 642 g/fish, FCR averaged 1.36, and survival averaged 99.6%. Nitrate accumulation rate was affected by total suspended solids concentration with a significant reduction observed at the highest discharge of solids from the system, suggesting wash-out of nitrifiers. Treatment effects on water quality dynamics, macro- and trace-mineral status of water, feed, and fish, 2-methylisoborneol and geosmin off-flavors and associated phytoplankton populations also are discussed. Results of this study suggest that one-year-old biofloc water can be used without adverse impact for a second year of channel catfish production in the biofloc system.

EFFECT OF SETTLING CHAMBER FLOW RATE ON WATER QUALITY DYNAMICS IN A HYBRID STRIPED BASS BIOFLOC TECHNOLOGY PRODUCTION SYSTEM

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A mixotrophic biofloc technology production system is characterized by high stocking and feeding rates. Excretion of metabolized feed stimulates primary and secondary productivity and leads to increased total suspended solids and nitrate-nitrogen concentrations. Published literature suggests that the culture species is impacted negatively at high total suspended solids concentration. Thus, a side-stream settling chamber is activated to maintain a desired total suspended solids concentration. The impact on water quality dynamics of three settling chamber flow rates (retention times) were tested in a hybrid striped bass (*Morone chrysops* x *M. saxatilis*) biofloc technology production system located outdoors. Each of nine 2.4-m diameter tanks (4.7 m^2, 3.6 m^3) was equipped with a 130-L conical bottom settling chamber into which tank water flowed via air lift pump at 1.5, 3, and 7.2 L/min. Hybrid striped bass (104.9 g/fish) were stocked into tanks at 8.5 fish/m^2 (1.16 kg/m^3) and grown for 125 days. Water quality and production data will be presented and discussed.

A NOVEL SOCIAL-ECOLOGICAL CLAM GARDEN SITE SELECTION PROCESS

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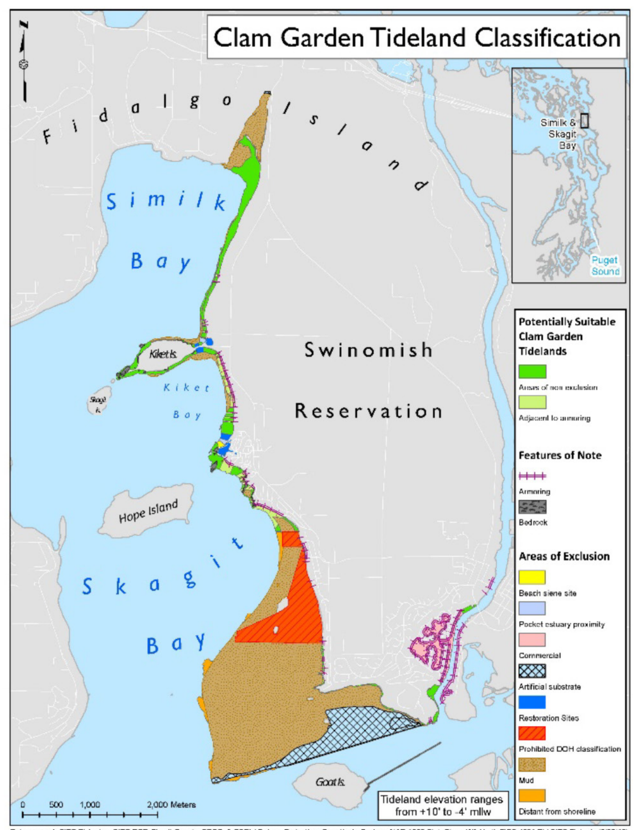
Clam gardens are intertidal features modified by Northwest Coastal Indigenous people to enhance clam habitat for optimal shellfish production. The Swinomish Indian Tribal Community (SITC) recently initiated a clam garden project to address the suite of ecological and cultural concerns documented in SITC's *Climate Adaptation Action Plan*. This effort will promote the integration of traditional ecological knowledge in contemporary resource management and climate change adaptation strategies as well as encourage local food security, support tribal treaty rights, and provide ecological and cultural benefits to the community. Because biophysical conditions and community engagement are both important to the success of a clam garden, SITC's Fisheries Department and Community Environmental Health Program have co-designed an ecological and socio-cultural site selection process. Since relic clam gardens have not been identified in Washington state waters, SITC staff are collaborating with knowledge holders and researchers in British Columbia to better understand this ancient practice and advise the Tribe's clam garden efforts.

Thus far, information gathered from B.C. experts has helped build a SITC-specific spatial exclusion model in ArcGIS to map viable clam garden locations on the Reservation (Fig. 1). Biophysical intertidal data collected from 15 candidate sites identified in the map were then used to design a multi-criteria decision analysis (MCDA) model. The MCDA provided a ranking of the candidate sites which will ultimately be presented to SITC community members for prioritization and final site selection.

Coinciding with the site selection process, Swinomish community members have been visiting B.C. clam gardens and participating in restoration events. Visiting these clam gardens has provided invaluable opportunities for transboundary and intergenerational knowledge exchange. As understanding and enthusiasm about clam gardens spreads throughout SITC, local support for the clam garden project grows. By addressing both the socio-cultural and ecological aspects of clam gardens, SITC staff aim to promote the long-term success of this adaptation strategy.



Data sourced: SITC Fisheries, SITC DEP, Skagit County, SRSC, & ESRI / Datum, Projection, Coordinate System: NAD 1983 StatePlane WA North FIPS 4601 Ft / SITC Fisheries (2/27/19)

Figure 1. Map of viable clam garden locations on the Swinomish Reservation near La Conner, WA. Results are based on a social-ecological spatial exclusion model.

SENSORY AND ANALYTICAL DETERMINATION OF OFF-FLAVOR COMPOUNDS IN WATER AND FARM RAISED CATFISH

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Off-flavors in aquaculture are a global and persistent problem, which decreases product quality and increases production costs. Remedial treatments include salting, smoking, marinades and purging prior harvest. In this report we used a sensory panel to show that consumers can perceive the earthy, musty, muddy aromas produced by the metabolites 2-methylisoborenol (2MIB) and geosmin in the low parts per trillion range in water. Due to the presence of other aroma producing compounds found in aquaculture products such as salmon, trout, or catfish, the off-flavor aromas are less noticeable and require higher levels of concentration for sensory detection. Using a forced triangle test (3-AFC), 3 out of 7 panelists were able to perceive a difference in a sample of catfish spiked with geosmin at a concentration of 100 parts per trillion, where 5 of 7 panelists were able to perceive a difference in a sample spiked at a concentration of 400 parts per trillion. The odor was not necessarily classified as objectionable or identifiable, but it could be detected.

Whereas off-flavor detection by sensory analysis is rapid and inexpensive, analytical detection can provide quantitation and improved sensitivity in most cases. Instrumental analysis is valuable for determining the efficacy of whether a remedial treatment such as purging has been effective. A pass/fail method for the screening of samples has been developed to increase sample throughput at the expense of sensitivity. The pass/fail method uses only 1 gram of tissue, which is placed in a 10 ml sample vial, with 5 ml of H2O and 3 g of NaCl. The vial is then sonicated for 30 minutes and analyzed using solid phase microextraction gas chromatography mass spectrometry (SPME/GC/MS). Using the pass/fail method, 2MIB and geosmin in catfish at concentrations on a par with sensory thresholds can be detected. A decrease in sensitivity relative to the microwave distillation/SPME/GC/S method results from a decrease in sample size (20:1). Additionally some of the analyte remains in the sample primarily as a result of the presence of lipid which in turn inhibits the quantification of the off-flavor analytes. With limits of detection below sensory thresholds for both analytes, this technique is useful for screening large numbers of samples for off-flavor.

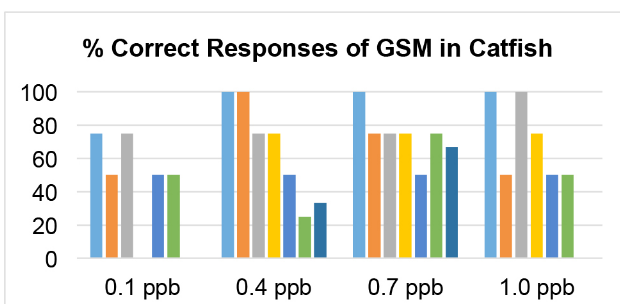

Figure 1: Correct responses of seven panelists at different concentrations of geosmin in catfish.

PLAN FOR ADDRESSING EARLY BOTTLENECKS IN *Zebrasoma flavenscens* LARVAL SURVIVAL

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In recent years, marine ornamental fish aquaculture has been on the rise. An increase in the total number of species successfully bred in captivity, as well as the number of species being commercially produced has subsequently increased the quantity and species diversity of marine ornamental fish available to consumers. The Acanthuridae family consisting of tangs, surgeonfishes, and unicornfishes are very popular in the aquarium trade. Yet, only five species of acanthurids have been captive bred and only one species has recently become commonly available to consumers, the yellow tang *Zebrasoma favenscens*.

In 2015, the yellow tang became the first acanthurid to be successfully bred in captivity at the Oceanic Institute (OI) in Hawaii. Now in 2019, four short years later, the life cycle of this species has been completed and commercialization has become a reality. Despite immense success, research focusing on addressing early bottlenecks in the yellow tang larval rearing process is still greatly needed as survival to settlement currently averages only 1%. The most severe bottleneck, coinciding with high larval mortality, occurs around seven days post hatch (DPH) with larval survival dropping to about 25%. This high mortality period early in yellow tang larval development will be investigated by focusing on improvement of lighting conditions and prey densities.

Lighting conditions have been shown to have strong effects on early larval feeding and survival in many marine fish species, and therefore are worthy of further investigation to determine its effect on early yellow tang survival. Historically, full spectrum fluorescent lighting with abrupt "on" and "off" settings was used at OI when culturing yellow tang. Lighting trials will determine the effects of light source (LED vs. fluorescent), spectrum, and intensity on yellow tang larval feeding incidence and survival. Prey density is another incredibly important factor in early larval feeding success and survival. The effect of prey density on early yellow tang larval survival to seven DPH will be investigated. Prey type will be the calanoid copepod *Parvocalanus crassirostris*, which is cultured at OI. Prey density treatments will be 5 and 8 prey items per mL. Replicates for all early larval rearing trials will consist of 200L round, black, fiberglass tanks on a flow through system with overhead lighting.

By focusing on reducing mortality to this first bottleneck, yellow tang larval survival to settlement is expected to improve and subsequent commercial production efficiency of this iconic marine aquarium trade species. The goal is to improve larval survival to seven DPH from 25% to 40% through these investigations.

MICROBIAL COMMUNITIES AND FOOD SAFETY IN RECIRCULATING AQUAPONIC SYSTEMS

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Aquaponic food production combines two well-studied models - recirculating aquaculture systems (RAS) and hydroponics - into a complex multitrophic production system for fish, fruits, and vegetables. Of the three major commodity classes produced in aquaponic systems, fruits and vegetables are implicated in about 11.7% and 34.2% of foodborne illnesses in the US, while fish is only responsible for 2.7%. For this reason, ensuring the safety of the fruit or vegetable crop is a research priority. While provisional good agricultural practices (GAPs) for recirculating (coupled) aquaponic producers have been published by one industry group, universal evidence-based standards for produce safety in aquaponics have not been developed.

The Food Safety Modernization Act's Produce Safety rule regulates microbial water quality in agricultural production, but final agricultural water rules have yet to be published by the Food and Drug Administration. Aquaponic culture water will likely be regulated as either manured water - precluding recirculating aquaponic crop production entirely - or at minimum as surface water, requiring 20 tests for generic *E. coli* annually as an indicator of microbial water quality. Additionally, one of the provisional GAPs for aquaponics would require growers to design systems that prevent contact between the shared culture water and the edible portion of the crop. However, disinfection of culture water prior to distribution to the plant system could have negative impacts on its microbiome. The inherent pathogen-suppressive effects of the indigenous microbial communities should be preserved if possible. Separation of culture water from the edible portion of the crop also precludes the production of root crops. To evaluate the potential food safety hazards introduced by contact between crop and culture water and inform responsible policy decisions, a thorough investigation of microbial water quality is needed.

Researchers at the University of Hawai'i published the first study of food safety in 11 commercial aquaponics operations in 2012. This survey found very low levels of generic *E. coli* in culture water—far below the maximum concentration allowed under FSMA—and no *E. coli* or *Salmonella* in fish or plants sampled from the systems. Two more recent studies using molecular techniques to characterize bacterial communities have provided some preliminary data. However, both studies were a single, non-replicated snapshot in time. While these previous efforts have focused on bacterial communities, no published work has characterized the rest of the aquatic biome in an aquaponic system, including archaea and eukaryotic organisms. Whole genome sequencing (WGS) offers an opportunity to comprehensively characterize all organisms in the system, which could lead to useful discoveries about microbial ecology and microbe-plant-fish interactions in aquaponics. The present study will employ both WGS and traditional microbiological techniques to investigate aquatic microbial communities in farm-scale replicated research systems as they relate to food safety and human pathogens. Effects of aquaponic unit processes on microbial abundance, community composition, and pathogen presence in culture water will be measured over time. Results to date will be presented.

CHARACTERIZING NUTRIENT PRODUCTION FROM RAINBOW TROUT AND TILAPIA GROWOUT IN RECIRCULATING AQUACULTURE SYSTEMS FOR POTENTIAL USE IN HYDROPONIC CROP PRODUCTION

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Aquaculture produces 44% of the 167 million tons of seafood consumed annually worldwide. Capture fisheries continue to decline at a steady rate, while the aquaculture industry has grown nearly 6% annually in the last decade. However, the costs associated with the treatment of nutrient-rich effluent produced from aquaculture production systems is typically internalized, thereby increasing the break-even price of aquaculture products. Integrating aquaculture with other agricultural cropping systems offers an opportunity to mitigate this costly strain on production by combining fish farming with plant production similar to other animal agricultural production systems. Past waste-solids research on recirculating aquaculture systems (RAS) suggests that the nutrient load in the captured solids meets and may exceed the demands of terrestrial plants. Using the waste nutrients from aquaculture may reduce or eliminate the need for exogenous synthetic nutrient supplementation to achieve adequate plant growth, however a complete characterization of plant-required nutrient production must be conducted to understand what treatment is required to make the nutrients plant-available for hydroponic crop production. The integration of aquaculture and hydroponic production systems also reduces water consumption when compared to separate aquaculture and hydroponic system water use.

Aquaculture waste nutrient research characterization studies were conducted at the University of New Hampshire in the Anadromous Fish and Invertebrate Research (AFAIR) lab located on the Durham, NH campus. Three replicate 5 m^3 recirculating aquaculture systems (RAS) utilizing drum screen filtration and nitrifying moving bed bioreactors (MBBRs) were used to evaluate nutrient production and fractionation in the effluent stream (dissolved and particulate) from tilapia and rainbow trout growout in terms of plant-required macro- and micro-nutrients. Results will be presented demonstrating effluent stream nutrient profiles and the fractionation of plant-required nutrients in the dissolved and particulate forms, respectively. Nutrient production rates will be quantified and normalized to provide estimates for larger feeding operations, and allow for scalable integrated aquaponic system designs.

OPTIMIZING MICROBIAL DIGESTION AND NUTRIENT SOLUBILIZATION OF RAINBOW TROUT AND TILAPIA SLUDGE FOR USE AS A NATURAL FERTILIZER IN INTEGRATED AQUACULTURE FARMING SYSTEMS

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The worldwide aquaculture industry has shown consistent growth over the past decade. The United States ranks only 15th in aquaculture production and is the largest importer of farmed seafood. Improving aquaculture production in the United States is required in order to develop a more productive economically and environmentally sustainable industry. Aquaculture is currently the only form of animal agriculture that internalizes waste treatment without offsetting costs through integration with other agricultural crop production systems. The development of a waste treatment and reuse approach will allow for the monetization of sludge treatment and offset the operating costs associated with recirculating aquaculture systems (RAS).

Due to the high liquid content of RAS sludge, waste nutrient reuse is ideally suited for soilless crop production in hydroponic and integrated aquaponic systems. Facilitating the most effective use of treated RAS waste nutrients in aquaponic systems, and the fastest adoption into existing hydroponic cropping systems, requires achieving two goals: 1.) effective reduction of organic carbon, and 2.) maximized waste nutrient mineralization. To achieve these goals, RAS sludge digestion requires the implementation of both aerobic and anaerobic microbial processes. Organic carbon may be detrimental to plant growth, facilitates disease outbreak in hydroponic systems, and causes excessive biofilm accumulation and distribution system fouling. Maximizing nutrient mineralization requires an overall reduction in microbial biomass. However, mineralized waste sludge is high in ammonia, thus requiring subsequent oxidation to nitrate prior to use as fertilizer. As the first step to developing an optimized treatment process for wastes with high liquid content to be used as hydroponic fertilizer, the aerobic and anaerobic digestion processes must be better characterized to understand the effects on plant nutrient availability.

The University of New Hampshire agricultural engineering research program, directed by Todd Guerdat, is researching RAS waste treatment in two facilities on the Durham, NH campus: Anadromous Fish and Invertebrate Research (AFAIR) lab and the Kingman Farm Aquaponic Greenhouses. The AFAIR lab houses three replicate, pilot-scale RAS with drum screen filters and moving bed bioreactors (MBBRs) used in the growout of rainbow trout or tilapia. The UNH Kingman Farm Aquaponic Greenhouses are three replicated farm-scale (133 m^2 each) research facilities as part of the New Hampshire Agricultural Experiment Stations (NHAES). Research presented will include the presence of plant-required nutrients found in RAS waste, lab-scale characterizations of aerobic and anaerobic digestion of the mineralization of solid waste from both rainbow trout and tilapia, and insight into the optimization of these processes for implementation at industrial scale.

ASSESSING THE ACUTE AND CHRONIC TOXIC EFFECTS OF POTASSIUM ON RAINBOW TROUT (*Oncorhynchus Mykiss*) AND EVALUATING THE MITIGATING EFFECTS OF SODIUM ON ACUTE POTASSIUM TOXICITY

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Global aquaculture production has increased greatly over the last 40 years and it represents the fastest growing food production sector. However, further expansion of this practice is greatly limited by the current approach to waste management. Waste management poses a significant problem for aquaculture since it internalizes all the costs. The removal of suspended and settleable solids from a RAS waste stream is an intensive and costly process in terms of capital investment, maintenance, operation, and storage and discharge of removed solids as per EPA standards. Discharged RAS waste is considerably more concentrated than effluent streams from other production methods due to low water usage and can serve as a major point source pollutant. A capture-and-reuse model must be developed to mitigate environmental impact and offset costs of waste treatment and disposal. Currently, the most suitable capture-and-reuse method for RAS couples with hydroponic crop production.

Integrating RAS with hydroponic cropping systems requires a systems approach, considering the needs of each component prior to integration. One important consideration is nutrient balance. RAS effluent sodium concentrations often far exceed the phytotoxic threshold of 50 mg/L requiring remediation prior to use as hydroponic fertilizer. To resolve this issue, many practitioners have replaced sodium salts used for pH balancing in RAS with potassium derivatives. Potassium is an essential nutrient for plants that is available at insufficient levels in RAS effluent. However, complete replacement of sodium with potassium salts is considerably more expensive and potentially poses health concerns with fish production, both lethal and sublethal. Sublethal effects include stunted growth and gill irritation. Practitioners have also noted rapid mortalities as a result of high potassium levels in a number of species including rainbow trout (*Oncorrhynchus mykiss*) and hybrid striped bass (*Morone chrysops* x *M. saxatilis*). It has also been suggested that sodium can mitigate some of these toxic effects when maintained at an electrochemical balance. Unfortunately, the toxic and sublethal thresholds for potassium have not been established in the literature for any commercially important fish species to date, nor have the mitigating effects of sodium.

Research is being conducted currently at a pilot-scale intensive RAS research facility at the University of New Hampshire in Durham, NH in the Anadromous Fish and Invertebrate Research (AFAIR) lab to assess the acute and chronic effects of potassium on rainbow trout (*Oncorrhynchus mykiss*) and the ability of sodium to offset acute toxic effects of potassium. Findings to date will be presented.

OPTIMIZING AQUAPONIC STRAWBERRY PRODUCTION IN THE NORTHEAST FOR YEAR-ROUND PRODUCTION

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In the Northeastern US, locally produced strawberries are only available during the summer months. The majority of fresh market strawberries come from field-production in California, Florida, and Mexico. To meet consumer demand for locally produced food, controlled environment agricultural (CEA) systems can enable year-round strawberry production. The use of CEA systems can reduce the impact of extreme weather on crops, reduce pest and disease pressures, and automate environmental parameters to target specific crop needs. There is a need to identify optimal cultivation techniques specific to day-neutral cultivars to justify economic viability of CEA strawberries in the U.S. To do so, we first (1) evaluated cultivars and substrates best suited for greenhouse production as preliminary research for the following two experiments. The three cultivars Albion, San Andreas, and Seascape were grown in a peat-based and peat-coconut coir blend, and yields were collected over 8 weeks. Results showed that there was no significant effect of the interaction between cultivar and substrate on yield, however there was a trend in the data revealing Seascape producing the highest yields regardless of substrate. Furthermore, Seascape produced significantly greater yields than Albion and San Andreas at week 3 of production. Secondly (2), we compared yields and overall quality of strawberries grown using a synthetic nutrient solution, a naturally-derived solution, and a naturally-derived solution supplemented with phosphoric acid. The naturally-derived solutions were sourced from a recirculating aquaponics system. Using aquaculture effluent as a fertilizer for plants has shown to be extremely successful for leafy greens, but more research is needed to evaluate the feasibility of using aquaculture effluent for strawberry production. Results from this experiment revealed a trend where both aquaponic treatments had higher total yields and greater number of fruit than the hydroponic treatment. Non-augmented aquaponic plants irrigated with low levels of phosphorous produced higher yields than hydroponic plants irrigated with higher levels of phosphorous, however plant biomass was significantly reduced. Thirdly (3), we quantified the supplemental lighting needs, based on daily light integral (DLI), to optimize production during the ‘off seasons’ when rates of photosynthesis are greatly reduced. Plants were grown under 14, 20, and 26 mol photons^{-1} m^{-2} day^{-1} of light with an 18 hour photoperiod to determine the effect of DLI on plant performance of day-neutral strawberries. Results from this study revealed that plants grown under a DLI of 26 produced significantly higher yields, a greater number of fruit, and higher sugar content than when grown under a DLI of 14. These results illuminate certain growing techniques that should be implemented in large scale greenhouse operations to optimize production of day-neutral strawberries grown in greenhouses.

CORAL RESTORATION IN HAWAII: LAND-BASED CORAL NURSERIES FOR SLOW-GROWING MASSIVE CORALS

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Throughout the world, most coral nurseries focus on in-water production of fast-growing branching species to relatively small sizes for outplanting; alternatively, the State of Hawaii has implemented an innovative program which focuses on land-based propagation of slow-growing Hawaiian coral species into large-sized colonies for outplanting. By collecting small (10 – 15 cm) coral colonies or fragments from within public harbors and using microfragmentation and advanced aquarium husbandry techniques, the State's land-based Coral Restoration Nursery can create large-sized (42 cm and 1+ m) massive colonies in a fraction of the time (1 year) it would take to occur naturally in Hawaii (20+ years, 100+ years respectively).

The resulting large colony modules are then placed onto degraded natural Hawaiian coral reefs in an effort to restore these reefs back towards their earlier ecologically-complex state. The outplanted colonies are evaluated using the State's Coral Ecological Services and Functions Tool and the resulting offset can be used by developers and Responsible Parties to pay for coral and habitat loss incurred elsewhere in Hawaii. The result is a dynamic program to put out large, live, fully-covered coral colonies; paid for without large expenditures of public monies, and without the extremely long natural recovery rates (one year instead of decades) for large corals normally seen in Hawaii. The program is now expanding to focus on even larger corals, extremely rare coral species, and large upright branching species, to re-introduce them back into the wild using similar mechanisms.

HYDROLYSED SALMON MEAL AS A REPLACEMENT FOR SALMON MEAL IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Given that the nutrient profile of salmon meal (SM) and hydrolyzed salmon meal (HSM) are similar this provides a good model to evaluate the use of silage products in feeds. A series of growth, feed stability and consumption trials were conducted to evaluate the efficacy of salmon by-product meal (SM and HSM) in practical diets for Pacific white shrimp, *Litopenaeus vannamei*. This included a meal produced under traditional processing (SM) and one produced by a hydrolysis process (HSM). The basal diet contained12% SM, which was incrementally replaced (0. 25, 50, 75, 100%) on an isonitrogenous basis to produce five test diets used in two growth trials. A sixth diet was included which evaluated gelatin supplementation (Trial 1) or pH neutralization (Trial 2). In trials 1 and 2, juvenile shrimp (initial weight 0.17 g and 0.24g) were hand-sorted to uniform size and randomly stocked into 75-L aquaria or 800 L circular tanks which are a component of a 2.5 m^3 or 21 m^3 recirculation system at 10 shrimp per aquarium or 30 shrimp per tank, respectively. In trial 1, each diet was produced using two processing conditions (laboratory extruded and formed with meat grinder) and offered to shrimp over 42-day in a clear water RAS system. The results showed that 5.85% HSM can be used to replace salmon meal without an effect on the growth performance of the shrimp. Diets with the highest level of HSM had reduced stability and a lower pH as compared to the basal diet. Leaching of aromatic amino acids (AA) was evaluated across the diets. There is clear increase in AA leaching as the level of HSM increases in the diet. The addition of gelatin reduced leaching but there was limited effect of processing on leaching. Trial 2 was conducted over 56-day in an outdoor green water system. Results indicated that up to 6.08% HSM can be used to replace 50% salmon meal without effect the growth performance of the shrimp. There was no effect of pH adjustment on shrimp performance. Based on the observed results, HSM is a suitable protein source for shrimp feeds but it is recommended to limit the inclusion of HSM to 6% of the diet.

Table 1. Response of juvenile shrimp (0.24g mean initial weight) to the various test diets over a 56-day growth trial under green water conditions. Each value represents the mean of four replicates.

Diet	HSM level	Mean weight (g)	Survival (%)	Weight gain (g)	Weight gain (%)	FCR
1	0	9.79[b]	100.8[b]	9.55[b]	3961[b]	0.99[a]
2	3.04	9.46[ab]	99.2[ab]	9.22[ab]	3895[ab]	1.05[abc]
3	6.08	9.48[ab]	100.0[ab]	9.26[ab]	4154[ab]	1.04[ab]
4	9.13	8.39[a]	99.2[ab]	8.16[a]	3548[a]	1.18[cd]
5	12.17	8.90[ab]	94.2[a]	8.66[ab]	3636[ab]	1.17[bcd]
6 (pH adjusted)	12.17	8.40[a]	95.8[ab]	8.16[a]	3382[ab]	1.22[d]
PSE[1]		0.260	1.457	0.258	130.18	0.335
P-value		0.0037	0.0307	0.0035	0.0116	0.0003
Independent T-test (D5 and D6)						
P-value		0.127	0.594	0.125	0.299	0.409

[1.] PSE: Pooled standard error.

VIRULENCE OF *Photobacterium damselae* subsp. *damselae* IN AUSTRALIAN YELLOWTAIL KINGFISH (*Seriola lalandi*)

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Yellowtail kingfish (YTK), *Seriola lalandi*, is a marine, pelagic, piscivorus species with a circumglobal distribution. The high market value and its adaptability to sea cage culture make YTK a viable candidate for commercial production in Australia. However, one of the major impediments to the growth of Australian YTK industry is infectious diseases. *Photobacterium damselae* subsp. *damselae* (Pdd) is a gram-negative, halophilic bacterium which has been isolated from a wide variety of fish species around the globe. In recent years, the bacterium has been reported for the first time in wild and cultured fish species, including YTK, throughout Australia. The major virulence factors of Pdd are plasmid (pPHDD1)-encoded phospholipase-D damselysin and pore-forming toxin phobalysin P. However, strains of Pdd without plasmid have also demonstrated pathogenicity due to the presence of chromosome I-encoded virulence factors. The present study was conducted to test the pathogenicity of plasmid-positive and plasmid-negative Australian isolates of Pdd towards YTK.

An infection trial was conducted in a flow-through system with healthy, unvaccinated cultured YTK, weighing an average 152.0±17.7 g. Three isolates of Pdd (one plasmid-positive, AS-16-0963#3; and two plasmid-negative, AS-15-3942#7 and AS-16-0963#1) were selected for experimental infection. Throughout the experiment, water temperature was maintained at 20-22 °C and fish were fed twice per day to satiation with commercial feed. Dissolved oxygen was monitored daily and kept within 80-100%. Fish were randomly allocated to 20 (300 L) tanks, with 10 fish per tank. There were six experimental treatments, with intraperitoneal injection of one of the three test isolates of Pdd at two different concentrations, 10^4 CFU/fish and 10^7 CFU/fish, and three replicate tanks per treatment. Control fish in two replicate tanks were sham-injected. Fish mortality was recorded daily for 10 days post-challenge. Blood samples from infected fish after 4 days of infection were collected to measure hematology indices.

Mortality rates for the plasmid-positive isolate, AS-16-0963#3, were 47% and 100% at 10^4 CFU/fish and 10^7 CFU/fish respectively, compared to13-20% and 3-13% respectively for the two plasmid-negative isolates. No mortality was observed in the control fish. There was little difference in hematology parameters among isolates. These results suggest that, while presence of the pPHDD1 plasmid is not essential for pathogenicity, the plasmid does increase the virulence of *Photobacterium damselae* subsp. *damselae* to *Seriola lalandi* in Australia.

Ulva, PERIPHYTON, AND THEIR COMBINATIONS FOR IMPROVING TOTAL NITROGEN REMOVAL IN MARICULTURE EFFLUENT

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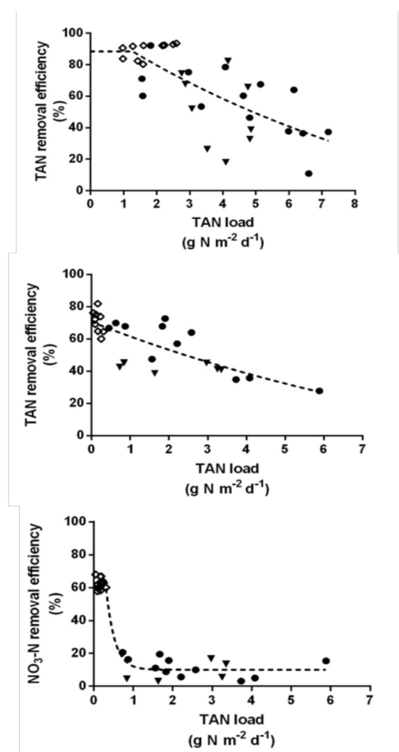
The environmental footprint of mariculture is a major obstacle towards the expansion of this agro-industry. Biofilters made of Ulva and periphyton differ in their effectiveness in removing ammonia and nitrate from mariculture effluents. In order to overcome the relatively minor uptake of nitrate by Ulva, a periphyton- based biofilter was integrated as a polishing unit in a combined Ulva-periphyton biofilter. The practicality of a combination of these two biofilters in improving the overall removal of dissolved N was evaluated by exposing the paired Ulva-periphyton biofilter to various areal loads of ammonia (as total ammonium nitrogen, TAN) and nitrate (as NO_3-N), the primary nitrogen forms in fishpond effluents. The upstream macroalgae biofilter was fed with fishpond effluents at different areal loads of ammonia and nitrate, while the downstream periphyton biofilter was fed with the remained nutrients in effluent of the upstream *Ulva*.

While the Ulva preferred uptake of TAN over NO3-N, the periphyton showed no preference between them, demonstrating flexible shifts between TAN and NO3-N uptake.

Ulva removed ammonia at a rate of 0.7 – 5.4 g TAN m^{-2} d^{-1}, in correlation with the TAN areal load, with Vmax of 5.1 and Km of 4.4 g TAN m^{-2} d^{-1}. Downstream periphyton was exposed to a lower TAN, but nitrate-rich effluent, and revealed similar capacities for the removal of both N forms, at removal rates of up to 1.7 and 1.8 g N m^{-2} d^{-1}, respectively. Compared to nitrate, areal load of TAN had a greater impact on the removal dynamics of both N forms by periphyton.

Overall, the paired biofilter resulted in a nearly total depletion of ammonia (97%) and efficient nitrate removal (67%), when areal loads in fishpond effluents were below 2 and 4 g N m^{-2} d^{-1} of TAN and NO_3-N, respectively.



Performances of the different units in the integrated *Ulva-periphyton* biofilter in N removal. TAN removal efficiency by *Ulva* (top) or periphyton (middle), and periphyton NO3-N removal efficiency (bottom) with respect to TAN areal load in mariculture effluent. For each nutrient, Plateau followed by one-phase decay curves were fitted to the data set of removal efficiency as a function of nutrient areal load.

FEED UTILIZATION BY LARGEMOUTH BASS *Micropterus salmoides* AND BLUEGILL *Lepomis macrochirus* AT THREE TEMPERATURES SUITABLE FOR PRODUCTION OF LEAFY GREENS IN AQUAPONIC SYSTEMS

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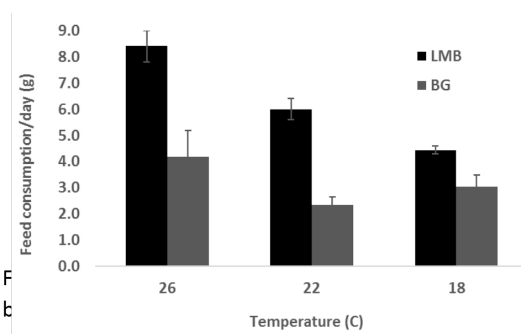
Aquaponic (AP) farmers in temperate regions must utilize a greenhouse or insulated buildings to produce year-round. In cold weather, heating water is not economically viable for tilapia production (28°-30°C) in AP, particularly when optimal temperatures for growth of leafy greens is 16°-24°C. Native fish species may provide producers with an alternative. While temperature ranges for optimal growth are known for many native Kentucky fish, little research has been conducted on feed utilization at lower temperatures. This study evaluates Largemouth bass (LMB; *Micropterus salmoides)* and bluegill (BG; *Lepomis macrochirus*) feed utilization at three temperatures suitable for production of leafy greens in AP.

A 10-week feeding trial was conducted in three separate RAS containing six 0.42m^3 fish tanks. Each system was kept at one of three temperatures (26°, 22°, and 18°C) suitable for growth of leafy greens in AP. LMB and BG were stocked into separate tanks at 15 fish/tank, with an initial stocking density of 107 and 91.7 g/m^3, respectively. Fish were fed 40% protein floating feed to satiation 1x/d. Total feed consumption (g) was recorded. TAN, NO_2, NO_3 and alkalinity were tested 1x/wk. Temperature, pH, and DO were recorded daily. TAN production/day was estimated based on feed consumption per tank using the formula TAN = feed input (g) x % protein of feed x 0.092/time.

LMB consumed significantly ($P \leq 0.05$) more feed than BG at all temperatures evaluated (Fig 1). However, LMB consumed significantly ($P \leq 0.05$) less feed as temperatures decreased. Feed consumption by BG was not significantly ($P>0.05$) impacted by temperature. Projected TAN was highest for LMB at 26°C (Fig 2), higher than BG, but still half of what is projected for tilapia at 26°C fed a diet containing 32% protein. In order to match levels of TAN produced by tilapia at 26°C, 1.9m^3 and 4m^3 of fish production would be required for LMB and BG, respectively.



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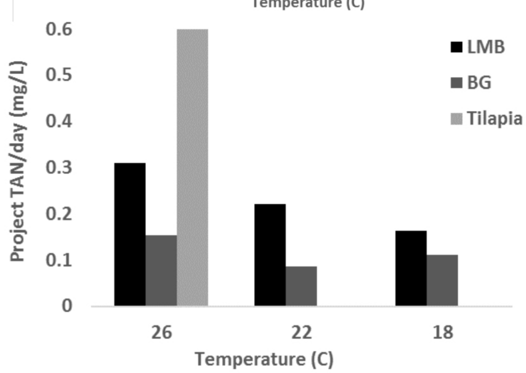

Figure 2. Projected TAN production per day for Largemouth bass (LMB), bluegill (BG), and tilapia.

CAPTIVE REARING OF THE ENDANGERED ORANGEBLACK HAWAIIAN DAMSELFLY *Megalagrion xanthomelas* AND REINTRODUCTION ATTEMPTS ON O‘AHU

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The genus *Megalagrion* (Odonata: Coenagrionidae) includes about 25 damselfly species, all endemic to Hawai‘i. The endangered orangeblack Hawaiian damselfly (*Megalagrion xanthomelas*) was formerly one of the most widespread Hawaiian damselflies, breeding in lowland wetlands across the state. The species suffered rapid declines following the introduction of mosquito fish (*Gambusia* spp.) in 1905, and is now restricted to scattered populations on several islands. It was thought to be extinct on O‘ahu until 1994 when a small population was discovered in a short stretch of stream at Tripler Army Medical Center (TAMC).

Since the rediscovery of *M. xanthomelas* on O‘ahu, multiple translocations have been attempted through direct transport of adults, naiads (aquatic larvae), and eggs from TAMC to other sites, but none have succeeded. In Nov 2018, we began a captive rearing and reintroduction program for *M. xanthomelas*. Our strategy is a two-month process of collecting eggs from TAMC, hatching and rearing naiads to the final instar, then releasing them into fish-free habitat. The predaceous naiads must be reared in individual containers and fed daily with live brine shrimp and freshwater zooplankton. Survival in captivity is high (>90%), and with current resources and space, we can produce about 300 naiads in each rearing cycle.

As of Oct 2019, we have released over 1,000 captive-reared naiads at two sites: artificial ponds at the University of Hawai‘i Lyon Arboretum and a natural stream at Wai‘anae Kai Forest Reserve. At Lyon Arboretum, adult damselflies were consistently observed mating and laying eggs for about a month after releases, but reproduction was confined to taro patches that were heavily infested with mosquito fish. No reproduction was observed in the fish-free ponds where naiads had been released, and the site is therefore not considered viable.

At Wai‘anae Kai, preliminary results are more promising. We continue to observe damselflies laying eggs in the fish-free stream over 40 days after the last naiad release. Although monitoring is ongoing, and it remains to be seen whether damselflies will persist at Wai‘anae Kai over the long term, our captive rearing strategy has been more successful than previous efforts relying on direct translocation of wild-caught individuals. The limiting factor is not whether large numbers of individuals can be reared, but whether we can identify suitable reintroduction sites that provide predator-free habitat for naiads as well as attractive breeding sites for adult damselflies.

DEVELOPMENT OF VIRTUAL AQUACULTURE TOURS FOR EXTENSION AND EDUCATIONAL PURPOSES

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It is challenging for many students, potential farmers and others to visit aquaculture sites, due at least to 1) their distance from urban centers or large roads; and 2) biosecurity concerns. This project describes and shares preliminary results of the development of virtual tours of aquaculture facilities. Specifically, a team from NCSU including extension personnel, students, distance education specialists and film and technical communications specialists worked together to share practical information on farms in virtual form. Story lines included two ways to look at most farms: from a physical/equipment/engineering perspective; and from a biological/life history/culture point of view. Videos as well as infographics were developed for each of these.

Videos included views of physical equipment, workers monitoring, feeding, grading and harvesting; and interviews of extension specialists, farmers and others providing details of critical areas, challenges and solutions. Infographics included basic information on the flow of water through various unit operations, for example pumps, filters and sterilization equipment in recirculating aquaculture operations. Other infographics depicted the life cycle and necessary culture steps during the life cycle from fry arriving on an airplane to shipping of live fish to markets.

Additional work with editing software, 360 degree and two dimensional images with interactive graphics imported via ThingLink added to the pedagogical value. For example, 360 degree images of a quarantine facility (which might be off limits due to biosecurity concerns) allow users to turn, zoom in on specific pieces of equipment or areas of the room, and access additional descriptive information (text, graphics or technical information) as desired. Preliminary graphics and videos were presented to students in undergraduate and graduate aquacultural engineering courses in 2019, and further documentation will be used to enhance courses and extension websites in 2020.

ENGINEERING SENIOR DESIGN COURSE FOR DEVELOPMENT OF A NOVEL AUTOMATED LIVE FEED SYSTEM FOR LARVAL FINFISH

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This project focused on developing an automated feeder for live Artemia, by way of an integrated engineering Senior Design course. Team members came from Electrical, Computer, Mechanical, Biological and Agricultural Engineering as well as Biology and Nutrition. Early life stage development is a critical time for culture of finfish. For many species, live feed such as Artemia or copepods may be required during this period, and, due to the high metabolism, feedings every few hours are required. As a result, this time period is a critical and highly labor intensive period. In this study, a live feed pumping system was developed to pump multiple different types of Artemia to customize feed for different treatment types.

Among critical aspects of the study were use of live feed (addressed in another paper); larval development of internal organs; and overall growth and health of animals fed different prebiotic and probiotic formulations. This presentation will focus primarily on the educational and pedagogical challenges and opportunities at the intersection of engineering and biology with specific focus on live feeds in larval aquaculture. Ongoing development of the system, as well as educational objectives and outcomes will be presented.

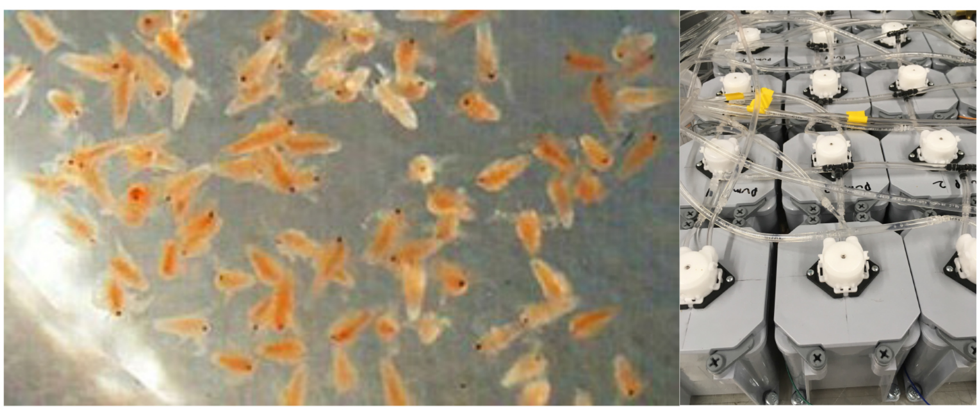

Figure 1. Artemaia typically range in size from 100-500 microns and can be kept viable under refrigeration for several days. Testing showed they can be pumped via peristaltic pumps and metered via automated feedback to optimize feeding to larval finfish. This system also measured density and flow rates and had monitoring and alarms.

ENGINEERING HYBRID UNMANNED AERIAL/SURFACE VEHICLE SYSTEMS FOR AQUACULTURAL AND AQUATIC APPLICATIONS

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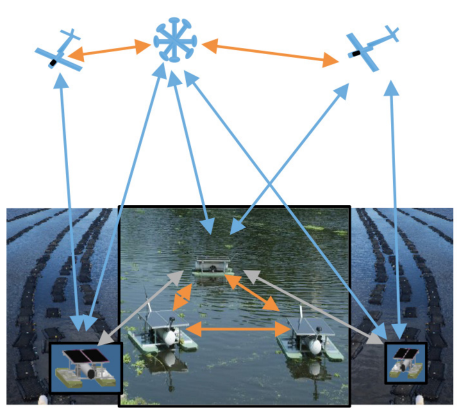
As marine aquaculture moves further offshore, many operations traditionally handled by humans become more problematic. Feeding, cleaning, assessing fish stocks, health related interventions, harvesting and other activities are more difficult both due to distance from human habitation; and safety concerns associated with high energy n the open ocean. This project looks toward this era, and is focused on developing a hybrid system that includes smaller and larger surface vehicles, as well as rotor and fixed wing vehicles for application to aquacultural and related aquatic enterprises.

Specifically, this project will test these in simulated and nearshore applications (specifically bivalve culture operations), with an eye to future far offshore and more highly automated systems. Focus areas include software for both "digital twin" modeling in different situations; open source code including enhanced learning and use of automated systems; and testing of specific monitoring, mapping and aquaculture management techniques. In particular, sensors to assess location, time, dissolved oxygen, pH, temperature and other relevant parameters have been or are in development. A system to manage floating cages (e.g. to image and manipulate cages) will reduce a difficult and potentially dangerous operation; while providing humans with improved information to help make better decisions. These have been deployed in nearshore water bodies and will be tested with collaborators in near shore oyster leases to assess effectiveness, speed, accuracy and improve understanding of limits to autonomy and human-robot interfaces.

Previous work focused on ponds and other onshore applications, and/or single vehicle deployments nearshore. This expands on both of these, testing multiple different vehicles in the nearshore/offshore environment; and studying interactions between vehicles; and with humans. The coastal environment is dynamic and subject to high energy events, but is also an extremely productive zone. These systems should enhance sustainability, improve monitoring and productivity, and may be able to provide improved information on coastal water quality, biological and ecological conditions, thus allowing improved decision making.

This paper documents applications of individual and hybrid systems to date in aquatic and aquaculture applications; and outlines needs for future research.



HISTOLOGICAL AND HAEMATOLOGICAL PARAMETERS OF *Clarias gariepinus* (Burchell, 1822) SUB ADULT INOCULATED WITH *Staphylococcus AUREUS* AND TREATED WITH NEEM LEAF (*Azadirachta indica*) EXTRACT

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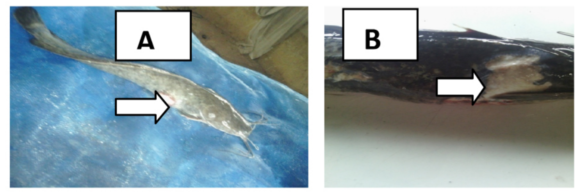
This study assessed the efficacies of Neem leaf extracts treated on fish inoculated with *Staphylococcus aureus* and their effects on the histological and haematological indices of sub-adult *Clarias gariepinus* (Burchell, 1822).

Molecular characterization of bacteria was carried out. Eighty African mud catfish were infected and treated with extracts. Neem leaf (*Azadirachta indica)* was extracted using ethanol, phytochemical screening carried out after being concentrated. Catfish were inoculated with *Staphylococcus aureus* and thereafter treated with Neem leaf extract at concentrations of 2.5 mg/g, 5.0 mg/g, 7.5 mg/g, 10 mg/g and aquacery antibiotic 10 mg/g. Completely randomized design was used. Data were analysed using one way analysis of variance Fish samples were analysed for haematological and histological profiles. Quantitative phytochemicals revealed that alkaloids had 1.34±0.02, flavonoids 0.01±0.00, tannin 5.63±0.02, saponin 0.85±0.19 and phenol 1.47±0.01mg/g. The anti-nutrients in Neem leaf were Phytic acid 2.65±0.05, Oxalate 1.44±0.83, tannin 5.63±0.02mg/g while cyanide was not detected. The haematological profile of fish post inoculation showed that White Blood Cells (WBC) increased significantly ($p<0.05$) to 11.0±0.05 compared with control of 9.50±0.05. After treatment with plant extracts, the blood parameters including WBC, Haemoglobin (HGB), Red Blood Cells (RBC), Haematocrit (HCT), Mean Corpuscular Volume (MCV) and Mean plateletcrit Volume (MPV) increased significantly ($p<0.05$) with treatment (T3) at 7.5mg/g with mean values of 15.8± 0.5, 10.7±0.05, 2.65± 0.05,37.7± 0.05, 142.5± 0.05, and MPV of 10.60± 0.05 fento litre at ($p<0.05$). White blood cell differentials revealed that significant difference exist among the values of heterophils and lymphocytes after treatment with the extracts at Treatment T3 with values of 28% and 72% respectively. The liver and the gills were normal after treatment with the extracts especially at treatment T3 of 7.5 mg/g. This study concluded that 7.5 mg/g of Neem leaf extracts improved the haematological and histological profile of inoculated fish.



Source: Awe, F.A. 2019

Plate 1: **A**, abscess on body close to the pelvic fin seven days after inoculation with *Staphylococcus aureus.* **B**, haemorrhagic lesion on body of fish within seven days after inoculation with *Staphylococcus aureus*

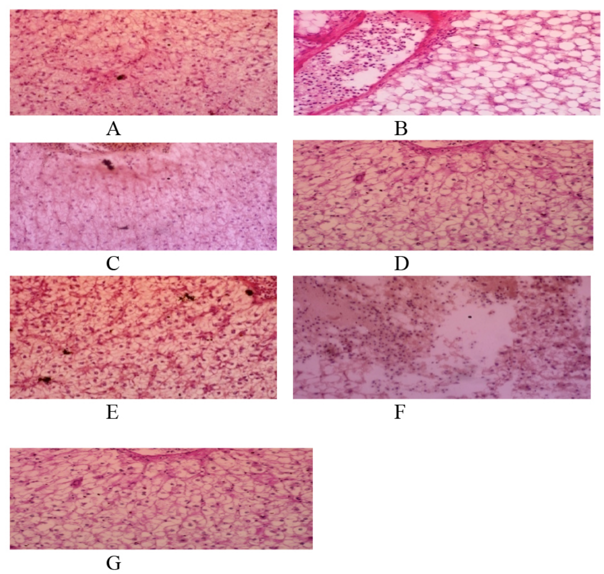


Plate 2: Histological section of liver cells to different concentration of Neem leaf extracts on catfish inoculated with *Staphylococcus aureus.* **A**, Normal liver from catfish used as control experiment. **B**, Diffuse vacuolar degeneration of the liver cells post inoculation with *Staphylococcus aureus.* **C,** Mild vacuolar degeneration of liver cells seven days after treatment with Neem leaf extracts at 2.5mg/ml concentration against catfish inoculated with *Staphylococcus aureus.* **D,** Normal to mild vacuolar degeneration of liver cells seven days after treatment with Neem leaf extracts at 5.0mg/ml concentration. **E**, Normal liver cells seven days after treatment with Neem leaf extracts at 7.5 mg/ml concentration. **F**, Diffuse vacuolar degeneration of liver cells seven days after treatment with Neem leaf extracts at 10 mg/ml concentration. **G**, Normal to mild vacuolar degeneration of liver cells seven days after treatment with aquaceryl antibiotic 10 mg/ml concentration against catfish inoculated with *Staphylococcus aureus;* Haematoxylin &Eosin, x 400.

COMPARATIVE NUTRITIONAL COMPOSITION (PROXIMATE, MINERAL AND AMINO ACID) OF *Chrysichthys nigrodigitatus* AND *Clarias gariepinus* CONSUMED IN OJO AREA OF LAGOS STATE, NIGERIA

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The proximate, mineral and amino acid contents of two commercially consumed species of catfish in Ojo area of Lagos, Nigeria was carried out to ascertain their nutritional benefits.

A total of one hundred and fifty samples each of Silver Catfish (*C. nigrodigitatus*) and the African Mud Catfish *(C. gariepinus)* weighed between 500-800g, were procured from a fish landing site in Ojo for analysis at the Biochemistry Teaching Laboratory section of the College of Medicine University of Lagos, Idi-Araba, Lagos, Nigeria. Proximate and mineral compositions were determined using the Association Official Analytical Chemists (AOAC), Atomic Absorption Spectrophotometer (Shimadzu AA 650), while amino acid was determined by Gas chromatography. Data were analysed with t-test and the significant means were compared using Graph pad prism version 8.0. Crude protein was found to have the highest value of 44.93±0.04 and 38.61±0.19 and ash had the lowest value of 3.96±0.02 and 3.77±0.02 for *C. nigrodigitatus* and *C. gariepinus* respectively. There was a significant difference ($p<0.05$) between the iron content of *C. gariepinus* (2.18±0.04) and *C. nigrodigitatus* (1.14±0.16). Sodium (80.07±0.55) and magnesium (74.55±1.00) contents were highest in *C. gariepinus*. Proline (2.06±0.05 and 2.72±0.07), threonine (7.31±0.34 and 3.95±0.03), glutamate (15.28±0.05 and 13.28±0.06) and tryptophan (1.66±0.05 and 0.04±0.01) were found to be significantly different ($p<0.05$) in *C. nigrodigitatus* and *C. gariepinus* respectively. The high fat contents 9.38±0.03 and 10.42±0.04 detected in *C. nigrodigitatus* and *C. gariepinus* could indicate good sources of fish oil while the high protein could indicate the fish are required for normal body functions. The abundant nutrients found in both species make them recommendable for health-conscious people and those with certain medical conditions.

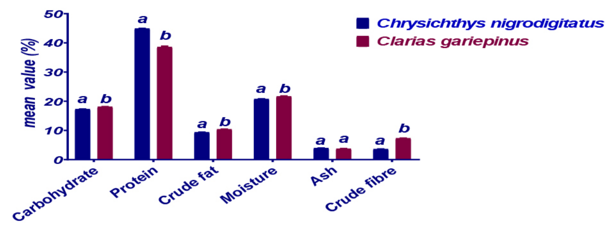

Fig. 1: Proximate composition of *Chrysichthys nigrodigitatus* and *Clarias gariepinus*, values are means ± SEM of duplicate determinations

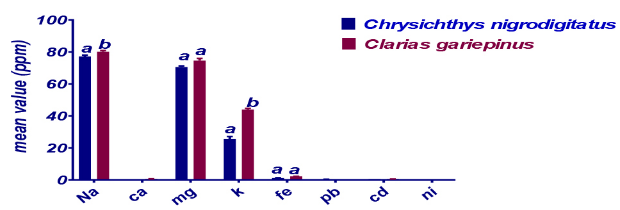

Fig. 2: Mineral composition of *Chrysichthys nigrodigitatus* and *Clarias gariepinus*, values are means ± SEM of duplicate determinations.

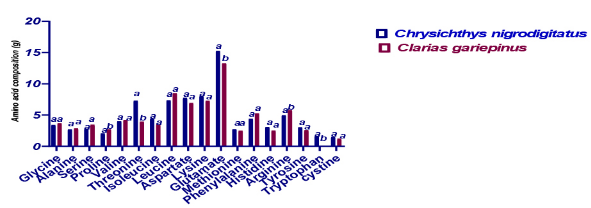

Fig. 3: Amino acid composition of *Chrysichthys nigrodigitatus* and *Clarias gariepinus* (in grams per 100 grams of protein analysed)

ACCOUNTABILITY FOR AQUACULTURE OPERATIONS IN A RESOURCE SCARCE WORLD: THE ROLE OF ELECTRONIC TRACEABILITY

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The impacts to the environment from shrimp farming happen overwhelmingly at the production farm and the extent of these impacts are largely dependent on the farming practices employed by the individuals managing the farms. Therefore, without traceability back to the farm there is no knowledge of, or route for intervention when farm management becomes unsustainable or uses illegal labor practices. It is the case today that, if any information is shared from the farm, it is done so by passing handwritten forms to buyers which makes it difficult to aggregate and analyze information in a meaningful way and also difficult for auditors to validate the origins of products. The issue is not the lack of available technology, the technology needed to collect this information has been around for decades and barriers to internet access are rapidly deteriorating through the proliferation of smart phones, so why hasn't there been large scale change in the way traceability information is recorded and shared throughout supply chains? This presentation will discuss the barriers beyond technology and the way forward to introducing technology and digitization in this industry which has historically been a data desert.

LESSONS IN MARICULTURE: EDUCATION AND CAREER RESOURCES

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In U.S. fishing communities where commercial fishing landings have declined in recent years, the next generation is considering whether mariculture can provide ample seafood supply and viable livings. Marine aquaculture education and career resources for young people are critical to developing a skilled workforce for this growing industry, and raising awareness about job opportunities.

A suite of mariculture lesson plans, careers video, and mobile aquaculture laboratory were developed to make North Carolina coastal high school students aware of this career path. Educators can now access 10 free lesson plans which are being used in science, technical and career classes. These lesson plans cover a variety of subjects, launching student explorations of aquaculture species and biology, production methods, and even business planning. Each lesson plan builds off knowledge students will gain as they progress through the lessons, but teachers also can pick and choose to suit their curriculum. Each lesson is aligned with N.C. standards and include videos, worksheets, presentations, and discussion questions. The careers video features a variety of mariculture professions – seafood producers, distributors, chefs, and scientists. The mobile aquaculture laboratory includes a recirculating aquaculture system, animal touch tank, and gear displays, as well as monitors for visual presentations.

Mariculture education and career resources can be found here: https://go.ncsu.edu/MaricultureLessons

MARICULTURE TOURISM: CULTIVATING CONSUMER DEMAND AND COASTAL COMMUNITY SUPPLY

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Shellfish mariculture has experienced tremendous growth in the United States over the past decade, but the nascent industry still has much potential to be realized with regard to revenue streams and public awareness. If the shellfish mariculture industry can leverage connections specifically with the food tourism economy, it will be better poised for sustained growth. Food tourism and agritourism provide a suite of benefits to entrepreneurs and the communities they work within, including increased consumer awareness of and demand for locally produced food, preservation of cultural practices, and diversified revenue streams.

A profile of potential shellfish mariculture tourists is in development so that coastal communities can capitalize on the growing interest in food tourism and agritourism. Through a survey of visitors to the Mid-Atlantic and Southeast coastal regions, tourists' preferences for and barriers to participation in shellfish mariculture experiences are being explored, in addition to other demand indicators (e.g., demographics). Using North Carolina as a case study, workshops are being conducted to compare coastal communities' current shellfish mariculture tourism product supply (identified through community-based asset mapping) with potential shellfish mariculture tourists' demand. This comparison allows us to identify where demand for shellfish mariculture tourism can be met and opportunities for business and tourism resource development. As North Carolina's coastal tourism infrastructure and marketing strategies are similar to that of other Mid-Atlantic and Southeast states, this assessment will generate findings that can be extrapolated to other states in these regions. Ultimately, we will use these data to create recommendations for the shellfish mariculture industry and coastal tourism leaders to support sustainable development of shellfish mariculture tourism in the Mid-Atlantic and Southeast coastal regions. These findings will also generate resources to help mariculture tourism entrepreneurs capitalize on the establishment of sustainable business practices and new revenue streams.

Preliminary results from this research will be shared, as well as recent efforts to build the North Carolina Oyster Trail whose mission is to provide oyster tourism experiences that help to sustain and grow North Carolina oyster demand and supply, resulting in economic, environmental, and social benefits to the state's seafood industry and coastal communities.

NATIONAL VETERINARY ACCREDITATION PROGRAM MODULE 14: EVALUATION OF AQUATIC ANIMALS FOR DETECTION OF REPORTABLE DISEASES AND PATHOGENS

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The goal of the NVAP program is to ensure that private veterinary practitioners who provide regulatory services to U.S. livestock, poultry and aquaculture industries, are adequately trained and well acquainted with regulatory requirements through USDA-APHIS accreditation. In addition to other accreditation requirements, accredited veterinarians are required to successfully complete a required number of 30 training Modules (http://tinyurl.com/NVAP-Modules), four of which currently cover aquatic animal health regulatory issues.

Although initiated as a 1896 agreement between the U.S. and Canada to combat equine disease outbreaks, in 1921 the U.S. Department of Agriculture (USDA) formalized the National Veterinary Accreditation Program (NVAP) so private practitioners could assist Federal veterinarians in controlling animal diseases. In 1992 regulations allow standardized procedures and requirements, and uniform administration to be managed nationally by APHIS, but with authorization of veterinarians licensed to practice on a State-by-State basis.

In 2001/2002 an "Animal Health Safeguarding Review by the National Association of State Departments of Agriculture (NASDA) to further redesign and upgrade the NVAP and suggested that "the accreditation program be the core for emergency preparedness and the response plan." Recommended revisions were published in 2002 ("New Directions for the National Veterinary Accreditation Program," J. Amer. Vet. Med. Assoc., 22(10): 1470-1472), with revised regulations implemented in 2009.

With accredited veterinarians being the first line of defense against catastrophic disease outbreaks, U.S. has successfully controlled outbreaks of several foreign animal diseases (FADs), including contagious equine metritis, equine piroplasmosis, epizootics of exotic Newcastle disease and West Nile virus, cases of screwworm and monkey pox, and pandemics of the influenza virus – and several aquatic animal diseases.

Module 14 guides users to the lists of aquatic animal diseases that are reportable to Federal, State or international governments; explains the role of the accredited veterinarian in reporting aquatic animal diseases in the United States; describes the procedures required when conducting veterinary inspections of aquatic animals; and discusses common signs of illness and disease in aquatic animal species. The module also discusses the importance of collecting appropriate samples for diagnostic testing, where to find appropriate diagnostic requirements, and overviews diagnostic sample packaging and shipping procedures.

APHIS RESPONSE TO EMERGING AQUATIC ANIMAL DISEASE DETECTIONS LIKE IHHNV

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Over the last year USDA APHIS Veterinary Services has been notified of several detections of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), an OIE listed crustacean pathogen. APHIS has been able to respond to these events but has identified challenges that will influence future responses. In the face of these types of detections, APHIS' goals are to minimize the impacts to animal health, both on and off farm, as well as to the trade status of the entire U.S. To achieve these goals farms must be able to demonstrate appropriate and adequate pathogen surveillance and risk management practices. Challenges to achieving these goals include limited resources, lack of import controls and often times incongruent state regulatory authority for aquaculture. However, these recent detections have allowed APHIS to deploy resources at the local level and establish partnerships that have worked to facilitate the containment of important aquatic pathogens and, ultimately, restore trade.

DISCOVERY OF *Ostrea stentina* (PAYRAUDEAU, 1982) ON O`AHU, HAWAI`I

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Cryptic morphology within and among oyster species causes taxonomic confusion and may complicate aquaculture and management efforts. In Hawai'i, resurgence in traditional fishpond aquaculture and an associated interest to farm both native and non-native oysters has spurred recent interests to identify unknown species. Similarly, native oyster culture has been piloted recently to determine whether these species can assist with water quality improvement in polluted areas. These commercial and environmental efforts are complicated by the wide phenotypic variation among oysters. The Pacific Aquaculture and Coastal Resources Center (PACRC) at the University of Hawaii Hilo has been culturing *Dendostrea sandvichensis* since 2010. A collaboration with the Waiwai Ola Waterkeepers Hawaiian Islands led to an effort to increase production and required collection of broodstock from the wild. *D. sandvichensis* has not been observed to exceed 3 cm (DVM) in the wild although hatchery-produced specimens may grow to at least 6 cm (DVM). The small size has led to further confusion in identifying specimens given that at least four extant native species have been documented and potentially as many as four non-native oyster species. It had been suspected for some time that additional species may exist in Hawai`i. Thus, some of the oysters that had phenotypic characteristics suggesting they might not be *D. sandvichensis* were utilized for DNA barcoding.

We sequenced two partial gene fragments, the mitochondrial cytochrome c oxidase subunit I (COI) gene and 16S ribosomal RNA (16S) gene, in 18 unidentified and 31 known oyster specimens. Molecular data identified 72% of unknown samples as *Ostrea stentina* (Payraudeau, 1826), a globally distributed species that has been previously recorded along North Atlantic, South American, Mediterranean, North African, Japan, and New Zealand coasts. The remaining unknown samples were the native *Dendostrea sandvichensis* (G. B. Sowerby II, 1871), and nonnative *Crassostrea gigas* (Thunberg, 1793). The latter is a commercial species that was introduced to Hawai'i from multiple sources during the 20th century. This study provides the first official record for *O. stentina* in the state. These findings have significant ramifications including the possibility of culturing *O. stentina*, regulatory complication and the paucity of additional information about the distribution of this species in Hawai`i.

BENCHMARK GENETICS SPF *P. vannamei* BREEDING PROGRAM DEVELOPING SPR POPULATION USING GENOMIC SELETION

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During recent years Benchmark Genetics has developed a solid presence in aquaculture genetics through acquisitions of Akvaforsk Genetics (AFGC), a leading provider of advanced breeding program design and technical services to global aquaculture, and the breeding companies SalmoBreed and Stofnfiskur (Atlantic salmon) and Spring Genetics (tilapia). With the recent incorporation of Benchmark Genetics Colombia in 2016, Benchmark expanded its breeding business to marine shrimp (*Penaeus vannamei*). Benchmark Genetics Colombia continues and expands the pioneering shrimp breeding program initiated by CENIACUA in Colombia in 1997, a program designed and supervised by Akvaforsk Genetics. Today this is the worlds most scientifically documented genetic improvement program for shrimp.

In early 2000 the shrimp farming industry in Asia was faced with instable results, mainly due to the introduction of diseases in the farm environment from the use of wild *P. monodon* broodstock. The production of *P. vannamei*, a non-native species in Asia went from 2,310 metric tons in 2000 to over 2,000,000 metric tons today. That jump was based on the Asian shrimp farmers' ability to take advantage of genetically improved, Specific Pathogenic Free (SPF) *P. vannamei* broodstock. That strategy worked but have not prevented a series of new disease epidemics. The shrimp farm sector will continue to be repeatedly exposed to new epidemics while depending on broodstock poorly adapted to local conditions. To break this vicious cycle there is the need of an alternative, integrated disease management strategy, based on: minimizing the risk of introduction of new diseases to the region; deployment of populations resistant to endemic diseases; and sanitary practices that minimize the likelihood of epidemics and delay infection.

Benchmark Genetics has successfully introduced genomic tools, used routinely in the salmon industry, into its shrimp breeding program. Combining the SPF, SPR approach Benchmark Genetics has successfully developed *P.vannamei* populations with high levels of resistance to major diseases affecting the shrimp industry today such as: WSSV; AHPND; NHP; TSV; Vibriosis.

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

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Aquaponics is a rapidly developing technology that has potential to increase food production in urban and urbanizing areas. Although aquaponics is often promoted as a resource-efficient technology, nutrient supplementation is often required to optimize plant yields in aquaponic systems. An experiment was conducted at Auburn University to determine the effects of hydroponic system type on micronutrient uptake in lettuce (*Lactuca sativa* L.) irrigated with aquaculture effluent. The experiment was a completely randomized design consisting of three treatments and four replicates. Each replicate contained 15 individual plants. Treatments were: 1) dutch bucket 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 350 to 600 mg L^{-1} during the experiment while pH and electrical conductivity remained relatively constant from 6.9 – 7.2 and from 1.2 to 1.5 mS cm^{-1}, respectively. Plant dry mass was higher in dutch bucket culture compared to deep water culture and nutrient film technique, while deep water culture was higher than nutrient film technique. SPAD index was highest for dutch bucket culture and deep water culture. There were no differences in foliar nitrogen concentrations between hydroponic system type. However, iron uptake was significantly increased in dutch bucket culture and deep water culture compared to nutrient film technique. Although all plants were market size, those grown in nutrient film technique were not as marketable as those grown in the other systems due to obvious iron deficiency symptoms visible as interveinal chlorosis. It is likely that root interaction with particulate matter, both in dutch bucket and deep water culture, led to the increase in iron uptake for those treatments. It is also likely that bacteria present in biofloc scavenged scare iron from the aquaculture system which then became available to plants during their growth cycle. Aquaculture effluent was screened to remove most particulates before being introduced into the nutrient film technique system to avoid clogging the system which may explain why plants grown in that system exhibited iron deficiency and reduced yields. Iron 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 aqauponic systems.

THE PRACTICE OF AQUATIC VETERINARY MEDICINE IN THE UNITED STATES EXCLUSIVE ECONOMIC ZONE

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The Aquatic Veterinary Medicine Committee of the American Veterinary Medical Association convened approximately 45 stakeholders representing state agriculture, fish and wildlife, and natural resources agencies; academic and research institutions; animal health laboratories; and federal agencies for a dialogue about veterinary practice in the U.S. Exclusive Economic Zone (EEZ). The objectives of the meeting were to:

1) Provide an overview of the potential challenges and opportunities facing aquatic veterinarians practicing in the EEZ;
2) Socialize and solicit feedback on the AVMA policy on "Aquatic Veterinary Practice in U.S. Waters Outside State Jurisdiction;" and,
3) Develop potential next steps for promoting, protecting, and advancing veterinarians' roles in aquaculture in the EEZ.

Challenges and Opportunities in the EEZ

The EEZ offers a significant opportunity to expand the marine food fish aquaculture sector in the United States and meet a growing domestic demand for seafood. While aquaculture has been more prevalent in state waters, very few commercial facilities have been permitted seaward of state jurisdiction under the Submerged Lands Act in the EEZ, which is under federal jurisdiction. As a result, many questions have emerged within the veterinary community about the challenges and opportunities of practicing veterinary medicine in the EEZ.

AVMA policy on Aquatic Veterinary Practice in U.S. Waters Outside State Jurisdiction

The AVMA recommends that the criteria for veterinarians to practice veterinary medicine in these waters consist of three components:

1. The veterinarian is licensed and in good standing to practice veterinary medicine in at least one state/territory/federal district within the U.S.;
2. The veterinarian holds a USDA-APHIS category II veterinary accreditation; and,
3. The veterinarian has a valid veterinarian-client-patient relationship with the facility in which he/she is practicing veterinary medicine.

Next Steps for Veterinarian's Roles in the EEZ

Participants identified the following opportunities for promoting, protecting, and advancing veterinarians' roles in aquaculture in the EEZ:

- Address gaps in regulation and oversight.
- Promote research.
- Expand education and training.
- Improve communications and outreach.

EFFECTS OF SALINITY AT ELEVATED TEMPERATURE ON MAINTENANCE REQUIREMENTS AND EFFICIENCY OF PROTEIN AND ENERGY UTILIZATION OF RED TILAPIA (*Oreochromis* sp.)

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Climate change has greatly altered environmental conditions of aquatic ecosystems in many regions all over the world in the last decades. Temperature and salinity are the main ecological factors influence to the aquatic environment. This study was conducted to assess the protein and energy requirements for maintenance and protein - energy utilization of red tilapia (*Oreochromis* sp.) in conditions of different salinity at elevated temperature. In the first experiment, red tilapia (13.0±0.12g) were fed at five different feeding rates: 0%, 25%, 50%, 75% and 100% satiation for 28 days to evaluate the efficiency of protein and energy utilization and protein and energy requirement for maintenance at different salinities at elevated temperature (0ppt–normal temperature; 6ppt–34ºC; and 12ppt–34ºC).

The results showed that energy and protein requirements for maintenance at salinity of 0ppt – normal temperature; 6ppt–34ºC; and 12ppt–34ºC were 24.94 KJ/kg$^{0.80}$/day–0.43 g/kg$^{0.80}$/day, 24.94 KJ/kg$^{0.80}$/day–0.44 g/kg$^{0.80}$/day, and 11.51 KJ/kg$^{0.80}$/day–0.16 g/kg$^{0.80}$/day, respectively. The efficiency of protein and energy utilization of red tilapia was evaluated.

Acknowledgement
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OLFACTORY IMPRINTING OF HATCHERY FALL CHINOOK *Oncorhynchus tshawytscha* IN THE ELK RIVER

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Ensuring that hatchery fish come home is the focus of the research at the Elk River Hatchery. As each tributary of water has a unique signature of amino acids, even within the same watershed, it is believed that adult Fall Chinook navigate back to their natal streams by recalling these unique amino acid signatures. Historically, at Elk River Hatchery, incubation occurred in well water to help minimize the negative impacts of sediment, temperature fluctuations, pathogens, etc. However, did this incubation strategy also minimize the ability for the Fall Chinook to imprint on the unique amino acid signature that they would encounter as they return to the hatchery as adults? In an effort to reduce straying of returning of adult hatchery Fall Chinook, the Elk River Hatchery is testing the effects of hatching performance, growth, and homing of adults from eggs incubated in River Water vs. Well Water. Additionally, Elk River Hatchery is conducting studies that test whether or not adding a unique signature of amino acids to the water during the incubation and rearing periods, if the returning adults will exhibit a better homing response to this unique amino acid signature.

STUDIES ON DEVELOPMENT OF PREVENTION METHODS AGAINST SHRIMP INFECTIONS DISEASES, AHPND

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For development of prevention methods against shrimp bacterial infections diseases, we are studying several different ways such as feeding probiotic bacteria TOA5001, immune-stimulant 5-ALA (non protein amino acid residue), Immunoglobulin Y (IgY, chicken egg immunoglobulin), phage therapy, and nano-bubble technology.

We conducted challenge test with *Vibrio parahaemolyticus* AHPND strain, transcriptome analysis of shrimp, and metagenome analysis of microbial flora in shrimp stomach and intestine for studying their mechanisms of prevention against AHPND. We also studied anti-microbial activities of TOA5001. In case of IgY, we used recombinant AHPND toxins for immunization of chickens and used dried chicken egg powder as a feed additive for studying prevention of AHPND. We studied bacteriophage and ozone nano-bubble water for bactericidal activity. I will introduce our recent research results of these research topics. In addition, I also would like to introduce characterization of shrimp hemocytes for understanding shrimp immune system.

ADVANCING MARICULTURE IN ALASKA

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Alaska has over 30,000 miles of coastline with clean, pristine, nutrient-rich waters. Over 50% of the seafood harvested in the U.S. is harvested off the coast of Alaska. The potential for mariculture to provide benefits and opportunities in coastal fishing communities in Alaska is tremendous. Mariculture also offers the potential to provide vitality and resiliency to communities which are facing future threats from declining fish stocks, ocean acidification, ocean warming and other changes. The challenge is turning the vast potential for mariculture benefits and opportunities into a reality. In 2014, NOAA provided a grant to the Alaska Fisheries Development Foundation to lead the Alaska Mariculture Initiative, which resulted in Governor Walker establishing the Alaska Mariculture Task Force (Task Force) by Administrative Order (AO) #280 and #297. The Task Force completed the Alaska Mariculture Development Plan in 2018 and the Task Force is now working with Governor Dunleavy towards the implementation of the Plan. The goal of the Plan is to "grow a $100 million mariculture industry in 20 years."

Currently there are 59 aquatic farms, 5 hatcheries, and 8 nurseries authorized to operate in Alaska. A tangible indication of the success of the Alaska Mariculture Initiative is the significant increase in applications received by the state for aquatic farms in 2017, 2018, and 2019. Approximately 50 applications for new aquatic farms have been submitted during this period.

Public investment has also increased, as new mariculture positions within NOAA and the state government are being hired in Alaska. Additionally, the Governor and Legislature are reviewing laws and regulations to improve processes and increase public-private partnerships for investing in and growing the industry.

(NOTE: In Alaska, mariculture is defined as enhancement, restoration, and aquatic farming of shellfish and seaweed. For these purposes, mariculture does not include finfish farming, which is prohibited in Alaska waters.)

INDUCEMENT OF EARLY SPAWNING IN FEMALE *Platichthys stellatus* THROUGH THE ARTIFICIAL ADJUSTMENT OF THE PHOTOPERIOD AND WATER TEMPERATURE

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Platichthys stellatus naturally spawn from March to April, when fertilized eggs are collected to be used for seed production. Seed production is completed in July-August, when the seeds are moved to fish farms. Transportation in the warm water temperatures during this period causes significant stress on the juveniles as *P. stellatus* is a cold-water species. Moreover, after one year of growing, fish mortality frequently rises due to warm water temperatures among market-sized adult fish. To resolve this problem, the present study adjusted the timing of spawning to three months earlier than the natural spawning season by adjusting water temperatures and photoperiods. This adjustment avoids the issues regarding juvenile transportation as well as harvesting in summer.

To induce the early spawning in female *P. stellatus*, we reduced the photoperiod by 10 minutes every three days from September 20, 2018 to a final photoperiod of 8 hours. Similarly, the water temperature was lowered by 1this problem, the present study of 8 reduced the photoperiod by 10 minutes every three days from S water temperatures. The first spawning occurred on December 10, 2018 in the experimental group, while on March 16, 2019 in the control group, indicating a 97-day earlier spawning in the experimental group. In terms of the floating and fertilization rate, no significant differences were observed between the experimental and control groups. There was also no significant difference in the spawning volume per 100g fish weight between the two groups. It was found that the fish in the experimental group showed the same levels of plasma sex hormone during the treatment period as at the time of spawning. This indicates that the peak of the plasma sex hormone was advanced in the experimental group.

In conclusion, the artificial adjustment of the photoperiod and water temperature effectively induced the early spawning in *P. stellatus* without no impact on the quantity and quality of the spawned eggs.

ASSESSING THE EFFECTIVENESS OF DESICCATION IN CONTROLLING BIOFOULING IN WATER COLUMN CULTURED OYSTERS *Crassostrea virginica* IN THREE CHESAPEAKE BAY LOCATIONS

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Oysters and the equipment associated with water column oyster aquaculture serve as habitat for a host of other organisms. These marine plants and animals, collectively referred to as biofouling, can detract from the growing conditions available to the oysters. Effects of these organisms range from reducing flow and food available to the oysters, to unsightly blemishes on the shell, to oyster mortality in extreme cases. Desiccation, or periodic air drying, of oysters and cages has been used to control colonization by biofouling organisms. However, the specific duration of desiccation time periods has yet to be thoroughly assessed. In this project, we research the effectiveness of different temporal desiccation regimes in controlling biofouling, and examine relevant oyster parameters associated with desiccation.

Three sites were selected for inclusion. Sites were selected with differing salinity and exposure conditions. One control and two desiccation regimes were followed at each site; control (no desiccation), 8 hours of desiccation per week, and 24 hours of desiccation per week. Oysters were deployed between July - December 2018.

In two sites, biofouling was found to be significantly higher on non-desiccated oysters but minimal differences were observed among oysters desiccated for 8 or 24 hours (Figure 1). At one site, percent coverage by a species of macroalgae increased in the desiccated oysters. Species composition varied with each site. Total percent coverage varied both geographically and temporally.

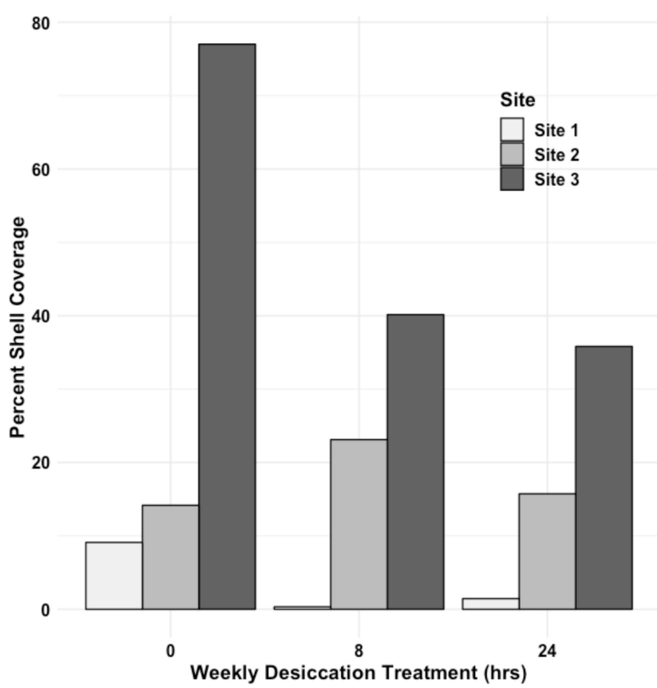

Figure 1. Percent shell coverage by biofouling organisms among oysters deployed to three sites in the Chesapeake Bay and subject to three weekly desiccation treatments (0, 8 or 24 hours of weekly desiccation).

OPERATION OF A DEMONSTRATION OYSTER FARM TO SUPPORT WATER COLUMN OYSTER PRODUCTION IN CHESAPEAKE BAY, MD, USA

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Maryland's 2009 lease law revision led to increased interest in private aquaculture using both traditional bottom leases as well as contained gear water-column leases. Interest in water-column leases utilizing containerized production equipment resulted in a need for research-based information on the variety of equipment available to provide growers with unbiased information on input costs and oyster performance. While many equipment options are available, there is a lack of empirical data useful for current and prospective aquaculture business operators to use for business decisions. The demonstration farm objectives are to provide commercially relevant data to growers, and to foster collaboration and communication among industry members and researchers to best support this industry.

The University of Maryland Center for Environmental Science's (UMCES) Horn Point Laboratory Oyster Hatchery (HPL), in cooperation with University of Maryland Extension (UME), with funding support from Maryland Sea Grant, began a demonstration oyster farm in 2016 to evaluate oyster aquaculture production equipment and management strategies.

The farm supports the industry through scientific data collection and Extension education programs. These are derived from needs assessment, organized specifically for identified audiences and evaluated to ensure that they meet the needs of the stakeholders engaged in Maryland's oyster aquaculture industry.

Since initiating the Demonstration Farm in 2016, individual consultations, public workshops and interaction with constituents at outreach events have led to new growers entering the industry and utilizing gear and management practices learned from the programs. Experienced growers have used information gleaned from this Demonstration Farm to alter their management practices or gear use in their commercial operation.

Here we present the methods used to operate this Demonstration Farm from both the applied research and Extension program perspectives, and discuss the connection between these two areas. We integrate results of known behavioral changes that have occurred, due at least partially to information learned at this Demonstration Farm, as well as results of evaluations of those engaging with Extension professionals at the Demonstration Farm projects and programs.

NOAA FISHERIES LOAN PROGRAM AQUACULTURE / MARICULTURE PROJECTS

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The NOAA Fisheries Finance Program (FFP) is a direct government loan program that receives an annual loan authority from Congress to provide long-term loans to the commercial fishing and aquaculture industries.

Eligible Projects include: Aquaculture & Mariculture Facilities • Purchase existing facility • Acquisition of facility equipment or improvements • New construction • Reconstruction; Fishing Vessels • Purchase existing vessel • Acquisition of vessel equipment • Reconstruction that doesn't materially increase harvesting capacity • Upgrades to improve collection and reporting of fisheries data, to reduce bycatch, to improve selectivity or reduce adverse impacts of fishing gear, or to improve safety; Fisheries Shoreside Facilities • Purchase existing facility • Acquisition of facility equipment or improvements • New construction • Reconstruction; Federal Harvesting Rights • Purchases involving harvesting rights in federally managed limited access systems. The program can also refinance existing debt that originated for the above purposes.

Program Benefits: Long-term, fixed rate loans with interest rates 2 percent over the U.S. Treasury's cost of funds • Loan maturities up to 25 years, but not exceeding the economic useful life of a project • Program loans may be prepaid at any time without penalty.

Additional Facts: There is no minimum or maximum NOAA FFP loan amount, however, the loan amount cannot exceed 80 percent of the eligible project cost. The only constraint is the amount of loan authority Congress authorizes for the Program on an annual basis, and the annual loan priorities. • Most financing available to the fishing industry restricts the term to meet the needs of the financial institution not the fisheries borrower. Additionally, most available financing is variable rate rather than fixed rate. • NOAA's FFP financing considers the useful life of the fishery asset in determining the term and establishes a fixed rate for the term of the loan.

A fixed rate can save money and will stabilize cash flows. The increased term allows an operation to repay the loan over the earnings life of the fishery asset. • Individuals must be U.S. citizens. • Businesses must be at least 75 percent U.S.-owned.

- Good earnings record, net worth, and liquidity behind project.
- Fully secured with borrower's assets.
- Guarantees and additional collateral may be required.
- Good credit record.
- Strong primary collateral.
- The loan applicant should have at least a 3-year history of owning or operating a fisheries project or a 3-year history owning or operating a comparable project.

NITRIFYING BACTERIA, START-UP AND MAINTENANCE OF BIO-FILTERS IN AQUAPONIC SYSTEMS - A REVIEW

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Successful rapid start-up or re-start of biological filters, defined as containers and media for the growth of nitrifying bacteria that oxidize ammonia to nitrite and nitrite to nitrate, depends on a wide variety of biological, chemical and physical factors. In many situations operators resort to adding mixtures of nitrifying bacteria, in various forms, to the system to inoculate the bio-filter and jump-start the nitrification process with little success. While much study and attention is given to the engineering aspects of the aquaponic systems and the physical aspects of the bio-filtration components the actual nitrifying bacteria and their preferences are often considered as little more than a black box. Research using a suite of molecular methods, that are culture-independent, have allowed much knowledge to be gained about how nitrifying bacteria react, positively and negatively, to the prevailing physical and chemical culture conditions. Presented here will be the major factors affecting the establishment of nitrifying bacteria during the start-up phase with or without adding an ammonia solution to feed the bacteria, how changes in basic water chemistry caused by fish in the system may degrade conditions for nitrifying bacteria and considerations for promoting nitrifying bacteria to rapidly and successfully start-up the biological filter.

HETEROTROPHIC MINERALIZATION OF ORGANIC MATERIAL IN AQUAPONIC SYSTEMS – A REVIEW

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A key goal of zero-discharge aquaponic systems is the minimization of solids discharge from the facility. This is generally accomplished using bacterial processes to degrade or mineralize organic material (uneaten fish food, fish feces, etc.). Successful mineralization depends on heterotrophic bacteria present in sufficient quantities operating either aerobically or anaerobically. Aerobic digestion depends on large amounts of aeration to maintain high oxygen levels and prevent the creation of foul odors and bulking of solids. Anaerobic mineralization is enticing as it can result in more stable water conditions (higher pH, increased alkalinity) but there is a greater chance of system failure due to the production of nitrite or hydrogen sulfide. The bacterial processes occurring in aquaponic systems are subject to many myths and misconceptions including the bacteria species responsible, how fast they may react to changing conditions and what conditions will promote which bacteria. A framework of basic knowledge regarding aquatic microorganisms in aquaponic systems will be presented along with methods for introducing and maintaining beneficial bacteria to the system. A better understanding of the microorganisms will allow operators to more efficiently manage their systems saving time, money and resources.

DEVELOPMENT OF ORAL DELIVERY SYSTEM FOR AQUACULTURE USING FLY MAGGOTS

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The global aquaculture industry is still ravaged by many diseases, causing economic losses. In recent years, vaccines for aquatic animals mostly developed as injections, which not only cause aquatic bio-impressive stress, but also cannot get vaccination on a large scale at the aquafarm. Therefore, the oral delivery system will become an indicator for greatly improving the immunity of aquatic animals. This study used white shrimp as a test species and let the fly maggot to carry vectors to develop the effectiveness of the delivery system. The antibacterial peptide contained in the maggots can effectively inhibit the disease; The recombinant protein will be accumulated in the biologically embedded manner in the maggot larvae, and improve the disease resistance by feeding the maggots, which can protect the recombinant protein not be digested by enzymes when it transported to the shrimp intestinal. In the experiment that fly maggot feeding of the vectors and the green bacterium can show the fluorescence was detected under the inverted fluorescent microscope, indicating that the system has potential application. This study will establish an effective aquatics oral delivery system and expectation to improve shrimp immunity.

USING SOCIAL MEDIA TO PROMOTE AQUACULTURE: A CASE STUDY FROM MAINE

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Aquaculture has played a small, yet increasing, role in Maine's maritime economy since the 1970s when a handful of oyster, mussel, and salmon farms cropped up along the coast. Today, Maine's mariculture industry is one of the fastest growing in the U.S., with new farmers entering the market at unprecedented rates. There are roughly 150 individual aquaculture leases in the state, with an additional 200 operations in the pre-revenue stage. The current value of the Maine aquaculture sector is estimated to be over $100 million and is expected to expand in the coming years as pre-revenue businesses enter the market.

While this growth has helped to diversify Maine's marine economy, which has become increasingly reliant on the lobster fishery over the past few decades, the recent growth in aquaculture has not been unchallenged. As with any marine industry, aquaculture must compete with existing uses on the waterfront, including commercial fishing, recreation, tourism, and riparian land ownership. The Maine Aquaculture Association (MAA) has recognized the need to reach out to Maine communities to help them learn more about aquaculture in Maine – what it is, what we grow, and who grows it. The association has devised a communication plan which addresses many of the issues Maine aquaculture faces as a burgeoning industry, including the implementation of an online industry survey and social media marketing strategy designed to target key stakeholders and maximize content impact with a focus on high-quality images and short films to deliver key to target audiences.

This case study is designed to help professionals in the aquaculture field (companies, farmers, trade associations, NGOs, etc.) think about how social media tools might be useful in helping them achieve their goals, how to gain insight from their audience or customers, and how to identify their most effective types of outreach and messaging. While the case of Maine may be unique, many of the lessons learned and strategies taken by MAA can be applied to other settings across the world where professionals are looking to communicate with a diverse audience of stakeholders and customers on a large scale.

CRABBING AND CONNECTIVITY: FISHERIES ASSESSMENT IN A TRADITIONALLY MANAGED HAWAIIAN FISHPOND

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Hawaiian fishponds, or loko iʻa, are ancient aquaculture systems that are models of sustainable aquatic resource management based on long-term experience from traditional Native Hawaiian harvest practices. An estimated 350+ fishponds provided food security for ancient Hawaiʻi, but by 1901 only 99 remained in production, and most of those were abandoned by mid-century. Reclamation efforts, beginning in the 1970s, have resulted in the rejuvenation of 38 actively managed fishponds across the State. Building on indigenous local knowledge, fishponds are being adapted to modern human population needs, because functional fishponds contribute to perpetuation of culture, improved food security and enhanced ecosystem services. In this study, we examine culturally and economically important crab fishery species to illustrate that traditional management practices are currently effective even with introduced species. This study was conducted in collaboration with Paepae o Heʻeia, a private non-provit organization that cares for the ancient Hawaiian Heʻeia fishpond (fig.1).

The state of Hawaiʻi only has size limits (6 in) for the introduced mud crab, *Scylla serrata,* but no bag limits for catch. However, with traditional management practices, limits are set and enforced in response to fluctuations in catch. During the assessment period of 2017-2019, the average number of crabs per harvest was 24 individuals, with an average keep of 7 individuals and average release of 17 individuals. Average crab size is larger during the winter months but catch is lower than during summer months. Over 95% of catch over the study period was above the size of sexual maturity of mud crab males (CW >90 mm). In order to address merging traditional and modern management practices, we investigated traditional fishing and combined it with modern collection data (assessments and abundance estimates) to propose a sustainable crab fishery model that may be tailored to fit manager preferences.

Figure 1. Heʻeia Fishpond is cared for by the non-profit organization Paepae o Heʻeia. Both are part of the Heʻeia National Estuarine Research Reserve.

THE EFFECT OF "BIOFLOC" VS. "SYNBIOTICS" ON THE GROWTH AND IMMUNE SYSTEM OF THE WHITE-LEGGED SHRIMP *Litopeneaus vannamei*

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In recent years, there has been great interest in the use of various bacterial driven systems, bioflocs, probiotics and/or prebiotics in shrimp culture. Probiotics are living microbial cells which are beneficial to the host and/or improve the quality of the environment. The synbiotic concept incorporates a nutritional supplement which is a combination of probiotics and prebiotics. This experiment was conducted to investigate the effects of culturing the white legged shrimp in biofloc type environments vs synbiotics type systems on the growth performance, immune system and water quality. Eight-week experiment was conducted at E.W. Shell Fisheries Center, Auburn University, Alabama. Static indoor polypropylene tanks were used. Each of the twenty-four tanks was stocked with 125 shrimp/m^2. Four treatments were evaluated including: biofloc (direct addition of rice bran), synbiotics with enzyme (enzyme, probiotic, fermented rice bran), synbiotics without enzyme (probiotic, fermented rice bran), and control (no supplements) with six replicates for each treatment. For the biofloc treatment, C: N ratio of the daily organic matter addition to each of the treatment tanks was approximately 15: 1. The results indicated that the final weight and weight gain of the shrimp raised in the biofloc treatment was significantly higher than the others followed by synbiotics with enzyme, synbiotics without enzyme and then the control (Table 1). However, survival, biomass (g), biomass gain (g), weight gain (%) or FCR were not significantly affected by dietary treatment ($P < 0.05$). Culturing the shrimp in Bioflocs and Synbiotics did numerically increase the number of total Haemocyte count (THC) but there was no significant difference between treatments ($P < 0.754$). Comparing these culture techniques have numerous confounding factors; however, all produced good survival and good growth and FCR indicating all are viable options.

Table 1 Growth performance and Total Haemocyte Count (THC) of the white legged shrimp cultured into the four treatments. Values represent the mean of six replicates.

Treatment	Initial wt (g)	Final wt (g)	Weight gain (g)	Biomass (g)	Biomass gain (g)	Survival (%)	Weight gain (%)	FCR	THC
Biofloc	0.39	9.36	8.97	847.83	808.93	91	2306.9	1.09	1.38E+07
Synbiotics with Enzyme	0.41	8.5	8.09	804.19	763.56	95	1996.7	1.16	1.39E+07
Synbiotics without Enzyme	0.39	8.43	8.04	806.36	767.26	96	2056.9	1.15	1.33E+07
Control	0.38	8.1	7.72	774.03	735.83	96	2022.7	1.2	1.00E+07
P-value	0.3	0.026	0.027	0.173	0.184	0.310	0.092	0.196	0.754
PSE	0.01	0.23	0.23	20.74	21.01	2.007	81.84	0.03	2.90E+06

THERMAL TOLERANCE OF THE WHITE-LEGGED SHRIMP (*Litopenaeus vannamei)* IN LOW SALINITY CULTURE SYSTEMS

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Aquaculture of white-legged shrimp (*Litopenaeus vannamei*) in low-salinity ponds is a small but growing industry in the southeastern United States. High variability in survival among ponds has been linked to variation in thermal regimes. Water temperatures in July and August can periodically reach and/or exceed 36 ºC in commercial shrimp production ponds, which are typically two meters in depth and can heat up considerably by late afternoon. In this study, we examine the physiological basis of thermal tolerance in shrimp using the concept of aerobic scope – the difference between the metabolic rate required for basic maintenance of an organism (resting metabolic rate: RMR) and the maximum metabolic rate an organism is capable of (potential metabolic activity: PMA). As aerobic scope increases, organisms can potentially utilize more energy for growth and reproduction. We hypothesize that aerobic scope can be used to predict optimal and lethal temperatures for shrimp, with optimal growth occurring at temperature(s) where aerobic scope is maximized and morality occurring at temperatures where aerobic scope approaches zero. To test the concept of aerobic scope, we exposed shrimp to temperature changes at a rate of 1°C/hr across a range of 20 - 42°C. At each temperature, we used intermittent respirometry to estimate RMR and the electron transport system (ETS) assay to estimate PMA. Aerobic scope was calculated as the difference between RMR and PMA. Critical thermal maximum (CTM) was estimated as the temperature at which shrimp could be flipped over and were unable to right themselves for at least 30 seconds. Upcoming experiments will measure growth at multiple temperatures. Data is currently being analyzed to estimate aerobic scope of *L. vannamei* and to test whether aerobic scope assays can serve as a useful tool to estimate optimal and lethal temperatures for shrimp in aquaculture systems.

OPTIMIZING CULTURE PARAMETERS OF THE CYCLOPOID COPEPOD *Oithona colcarva*

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The cyclopoid copepod *Oithona colcarva* has great aquaculture potential as a feed for marine finfish. Newly hatched nauplii are small (45 µm), have a weak escape response, and are natural prey for many fish larvae in the wild. These characteristics make *O. colcarva* an ideal candidate for feeding larvae of marine fish species with small mouth gapes. Since established production protocols for *O. colcarva* are lacking, a series of experiments were conducted to explore the impacts of culture density, photoperiod, and diet on nauplii production.

Similar experimental designs and protocols were used for all experiments and results were used to inform and modify subsequent experimental protocols. For the first study, four rearing densities (8, 12, 16 and 24 nauplii per ml) were evaluated in replicate microcosms. The mean cumulative nauplii production was significantly greater at the highest tested density with a three-fold increase compared to the lowest stocking density. In the next experiment, three photoperiod regimes (12L:12D, 16L:8D, 24L:0D) were tested and no significant differences in reproductive output were observed among treatments. Subsequent diet studies employed a 12L:12D photoperiod. The first tested four live algae diets (*Chaetoceros muelleri* only, *Tisochrysis lueta* only, *Tetraselmis chuii* only, and a 1:1:1 mix of *C. muelleri*, *T. lueta*, and *T. chuii*). The mean cumulative nauplii production was significantly greater in the mixed algae control. The *C. muelleri* only diet had low nauplii production but the *T. lutea* only and *T. chuii* only diets had close to zero nauplii production. The second diet study tested three algae mixes (1:1:1 live mix, 1:1:1 paste mix, and a commercially available “Shellfish Diet”). The experiment was stopped on day 9, when adults would have been stocked into new cups to monitor nauplii production, because growth was stunted and the copepods were only in the copepodite developmental stage. The experiment was instead harvested to take pictures and measurements and to assess survival rate from initial stocking. The live mix had the highest survival rate, with an average of 68% surviving to day 9. The paste mix had a comparable average survival rate of 67.2% but the Shellfish Diet had a significantly lower survival rate of 49.3%.

Further studies are needed to identify the ideal and maximum stocking densities, minimum light thresholds for nauplii production, and if *O. colcarva* can fully develop and produce nauplii on a diet of algae paste. Defining these culture parameters will increase the success and reliability of *Oithona colcarva* cultures and possibly even facilitate the domestication of this species and eliminate the reliance on live microalgae for nutrition. Future implications of this research include mass production of *Oithona colcarva* in aquaculture facilities and larviculture of challenging marine finfish species.

PERFORMANCE EVALUATION OF PLANT BASED SOURCE OF LONG CHAIN OMEGA 3 FATTY ACIDS FOR GROWING AQUACULTURE INDUSTRY

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Today, fast growing salmon aquaculture industry heavily depends on marine sourced long chain Omega-3 fatty acids. However, the production of fish meal and oil obtained from wild caught sources has been stable for over a decade and without projection to increase. A novel canola crop containing long chain Omega-3 (docosahexaenoic (DHA), eicosapentaenoic (EPA) and docosapentaenoic, (DPA)) fatty acids has been developed by Cargill-BASF to address an approaching fish oil supply limitation and the industry sustainability demand.

Feeding trials were conducted to evaluate the novel oil performance as long chain Omega-3 source in commercial salmon diets. Dietary treatments for studies were prepared in quadruplicates and fed to *Salmo salar* of 10g until they reached 48g in fresh water study and to *Salmo salar* of 184g until they reached 435g in salt water study. Results showed no significant difference in fish growth or food conversion ratio (FCR), and mortality between the dietary treatments at the end of the study were no different between diets in each trial, showing a value near 13% for fresh water and 7% for salt water trial. The liver histological examination revealed that fat degeneration of the liver decreased with the use of novel Canola oil diet in comparison to the other diets. EPA and DHA retention in whole fish was significantly better in the novel canola oil diet in comparison to the fish oil diet for both feeding trials.

Based on feeding trials' results, the novel canola oil (Latitude®) can be considered as a sustainable fish oil substitute in typical Atlantic salmon diets that efficiently delivers essential fatty acids to ensure performance and improving EPA and DHA retention as well as liver condition in Atlantic salmon.

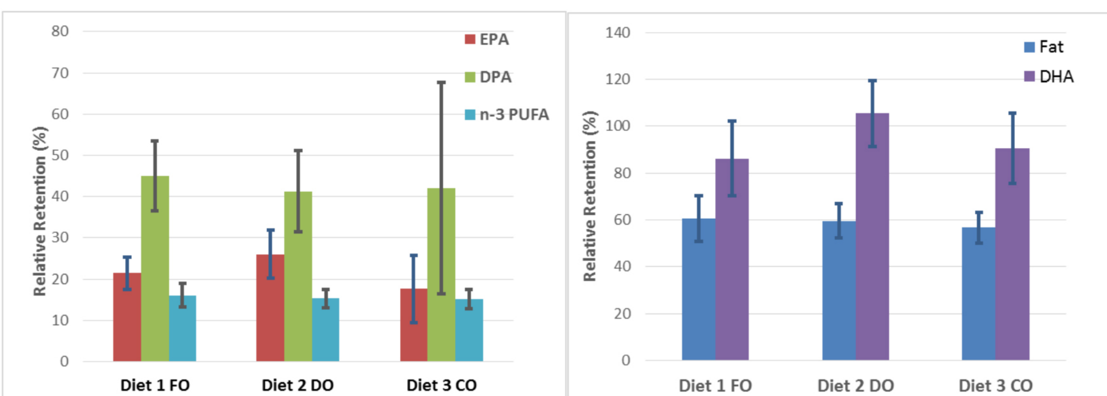

Figure 1. Relative Retention* (%) EPA, DPA, DHA, n-3 PUFA , 46 day "fresh" water trial. FO-fish oil diet; DO-Latitude®, novel canola diet; CO – commodity canola diet. *Relative FA retention = Gain factor/Intake factor * 100; Gain factor =g of FA final sample – g FA initial sample; Intake factor = total feed eaten per fish *% FA of the diet

EVALUATION OF AN EXPERIMENTAL PROBIOTIC IN A NURSERY SYSTEM TO DETERMINE THE EFFECT OF GROWTH AND SURVIVAL OF WHITE SHRIMP (*Penaeus vannamei*)

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The white shrimp aquaculture in intensive systems is a highly profitable activity in northeastern Mexico. However, in recent years, the presence of different diseases 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 and use of a microbial consortium (probiotics and BFT biofloc technology).

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 a population of 4,545,000 organisms (PL 12 with an 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. 300 mg) for their transfer into the 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 in two modes: 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 (disolved oxygen, temperature, pH, salinity, nitrite, nitrate, ammonia).

The trial results are indicated 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 temperatura (35°C) and shrimp biomass. The use and application of the experimental probiotic in feed and water worked as a management tool during the shrimp culture, however more studies and field trials in other systems as well as growout ponds are recommended.

Initial average weight (mg)	Final average weight (mg)	Population	Total biomass	Survival (%)	FCR	
3.5	555.19	4,545,000	2,382,040.50	94.39	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. Experimental probiotic performance in nursery system, shrimp zootechnical information and water quality parameters.

FISHERY DIVERSITY AND CATCHASSESSMENT SURVEY OF JEBBA LAKE, NIGERIA

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This research study assessed fish diversity and catch assessment of Jebba Lake, Nigeria for a period of five (5) months between March 2018 and August 2018. Four thousand, one hundred and eighty two (4,182) individual fish, which comprised of sixteen (16) species belonging to fourteen (14) families were recorded in the lake. The families Clariidae and Mochokidae were the most diverse with two (2) species. Cichlidae was dominant with *Oreochromis niloticus* as the most abundant species. There was low fish diversity in the lake. Diversity indices showed that upstream was the richest in diversity while downstream was the poorest. Midstream had the highest fish taxa while Downstream was lowest and there was uneven fish population at these locations. Bulk of the fish caught was from downstream and lowest at upstream. *Oreochromis niloticus,* which is a Cichlid was recorded highest at upstream and midstream and downstream. Monthly fish catch revealed highest catch at downstream in March, April and June and at midstream in May and July on the overall. It is recommended that similar study should be done for the remaining months, continuous monitoring of the fishery should be taken seriously, there is also need to monitor the activities of fishermen as well as organize workshop on management for them and the findings of this study could be used as baseline information for further research work in the lake and even other water bodies.

GROWTH PERFORMANCE OF THE AFRICAN CATFISH *Clarias gariepinus* FED VARYING LEVELS OF SOYBEAN

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Growth performance of *Clarias gariepinus* fed varying levels of soybean meal was carried out between May 2017 and July 2017. The diets were formulated at 0%, 35%, 40%, 45% and 50% soybean meal. The aim of this study is to examine the growth performance of catfish *Clarias gariepinus* to varying levels of Soybean fish feed formulation. Proximate composition of the varying levels of soybean meal showed that all the diets contain reasonable percentage of crude protein with better crude fat and carbohydrate in F3 (40%). Initial weight of *C. gariepinus* did not differ significantly ($p>0.05$) among the treatments. Diets F3 (40%) and F2 (35%) gave better body weight gain compared with F1 (0%) inclusion, which differ significantly ($p>0.05$) with only F4 (45%). Diet F1 (0%) had the best specific growth rate that differ significantly ($p<0.05$) only with diets F4. Feed conversion ratio was better in F1 and even F3 although with significant difference ($p>0.05$). Protein intake was highest in F3, which did not differ significantly ($p>0.05$) from other diets. Protein efficiency ratio was highest in F1 and can be compared with F4 and F3. Mean condition factors of fish fed diets F1 (0.71), F2 (0.72), F3 (0.73), F4 (0.69) and F5 (0.72) showed they were not in good condition in the tanks. Water quality parameters measured did not show any significant difference ($p>0.05$) among the treatments and fall within the limit for fish production. Low percentage inclusion of soybean (0%-40%) be used, further research need to be done on other processing method of soybean as well as replicated for other culturable fish species.

GROWTH PERFORMANCE OF SHARP-TOOTH *Clarias gariepenus* FED VARING LEVEL OF GROUNDNUT (*Arachis hypogeal*) CAKE

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Studies on growth performance of *Clarias gariepinus* (Burchell, 1822) fed groundnut cake meal as soybean meal replacement was carried out for 120 days (4 months) May 2018 and August 2018. The diets were formulated at 0%, 5%, 10%, 15%, 20% and 25% at 40% crude protein. Groundnut cake meal was represented as X1, X2, X3, X4, X5 and X6 respectively. The initial weight of *C. gariepinus* differ significantly ($p>0.05$) in the treatments, which indicated difference in sizes. Diets T5 (20%) gave the highest final weight and body weight gain compared other treatments and differ significantly ($p>0.05$). There was decreased in final weight gain and body weight gain as the level of groundnut cake meal increased. The specific growth rate of diet T5 can be compared with T1, which also differ significantly ($p>0.05$) with other treatments. Feed conversion ratio was highest in T3, which differ significantly ($p>0.05$) with other treatments. Feed intake was highest in T5 although did not differ significantly ($p<0.05$) with T1. Protein efficiency ratio was highest in T6 than T1, which differ significantly ($p>0.05$). The highest survival rate of fish in tank T5 and T6 compared with T1. Water quality parameters measured, which included temperature (^{o}C), pH and dissolved oxygen (mg/l) showed variations but did not differ significantly ($p<0.05$) and fall within the acceptable limits for fish survival and sustenance. It is recommended that 20% inclusion of groundnut cake meal should be used, further research should be done on replacement of soybean meal with other ingredients, there should be low inclusion of groundnut cake in the diet of the fish and there need to be carry out similar study on Tilapia, *Heterobranchus* spp. and other culturable fish species.

ANALYSIS OF THE PHYSICO – CHEMICAL PARAMETERS OF DINGI SPRING DABBAN, NIGER STATE, NIGERIA

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This study assessed the physico – chemical parameters of Dingi Spring at Dabban , Niger State, Nigeria for the duration of six (6) months between March, 2018 – August, 2018. There were fluctuations in these parameters with marked differences in turbidity, total suspended solids, nitrate and bicarbonate. Variations of these parameters at the sites, which did not differ significantly ($P<0.05$) showed that total suspended solids, dissolved oxygen and phosphate were highest at sampling station (A), pH and chemical oxygen demand at sampling station (B), total dissolved solids, nitrate and sulphate at sampling station (C), turbidity and bicarbonate at sampling station (D), while water temperature were higher at sites B, C and D. There were significant differences ($P>0.05$) in monthly parameters with temperatures highest in March, bicarbonate in April, pH, total dissolved solids and dissolved oxygen in May, total suspended solids in July, turbidity, chemical oxygen demand, nitrate, phosphate and sulphate in August. Physico – chemical parameters showed high, very low and negative correlations. Turbidity was higher than acceptable limit of World Health Organization (WHO), and Nigerian Industrial Standard (NIS), whereas temperature and Nitrate were higher than that of WHO, but within the acceptable limit of NIS. It is therefore, recommended that similar study should be done for the remaining period, water from the spring should be treated before consumption, there is the need for periodic monitoring of the physic – chemical parameters of the spring, human activities that include bathing and washing need to be discouraged and microbial analysis of the water should be carried out.

LENGTH – MASS RELATIONSHIP AND AGGRESSION OF *Astyanax mexicanus* (MEXICAN CAVEFISH) REARED IN A LABORATORY ENVIRONMENT

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Astyanax mexicanus is an emerging model organism in the field of evolutionary biology. Specifically, it is a prime candidate for numerous developmental and genetic studies including convergent/parallel evolution and metabolic adaptation. This is due to the independently evolving cave and surface morphs that remain interfertile. Unlike other common aquatic model organisms, the surface morph of the Mexican cavefish has a high rate of aggression. This study shows a potential inverse relationship between that aggression and the condition factors for certain age ranges of *Astyanax mexicanus*.

The length-mass relationship (LMR) and condition factor (K) were characterized for Surface Mexico cavefish (n=752) using a power function ($M=aL^b$) and Fulton's condition factor ($K=100*M/L^3$) respectively. When comparing Fulton's condition factor (K) of *Astyanax mexicanus*, we found at three to six months old the condition factor (2.2± 0.02) was significantly lower ($P< 0.05$) than the condition factor at two months (2.4±0.02). This difference was coupled with a spike in aggression related deaths during months three to six (n = 208).

Based on these results, a trial program has been implemented to increase the condition factor of fish post two months of age through a change in the feeding regime. With this change, we expect to see a decrease in aggression rates for fish aged from three to six months old. This is an ongoing study that will continue to be monitored during this new trial period.

DIETARY EFFECTS OF THREE SMALL ALGAL SPECIES ON GROWTH OF THE SEA URCHIN *Mesocentrotus nudus* JUVENILES

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The sea urchin *Mesocentrotus nudus* juveniles, which are densely found on barrens, would be the potential stock for sea urchin aquaculture. To develop a diet that promotes sea urchin growth, this study confirms the dietary effect of three small algal species: frozen or dried *Gloiopeltis furcata* (FG, DG), frozen or dried *Mazzaella japonica* (FM, DM) and frozen *Scytosiphon lomentaria* (FS), on the growth of *M. nudus* juveniles compared with the juveniles fed the kelp *Saccharina japonica* and the starved juveniles.

We conducted the feeding experiment during October–December in 2017. The crude protein content of *G. furcata* was 16.2%, highest among the three algal species. The food intake of FG and DG were more than those of the other algae except for kelp. The food conversion efficiencies (body weight gain × 100/ food intake) and growth rates of FG and DG were significantly higher than those of other algae (FIGURE 1). The gonad index of the urchins fed DG was significantly higher than those fed other algae. These results indicate that high crude protein content of *G. furcata* and its high food conversion efficiency and food intake (FG and DG) promoted the somatic growth of *M. nudus* juvenile. The high gonad production of DG may be due to allocation of surplus protein derived by heating in the drying process to gonad.

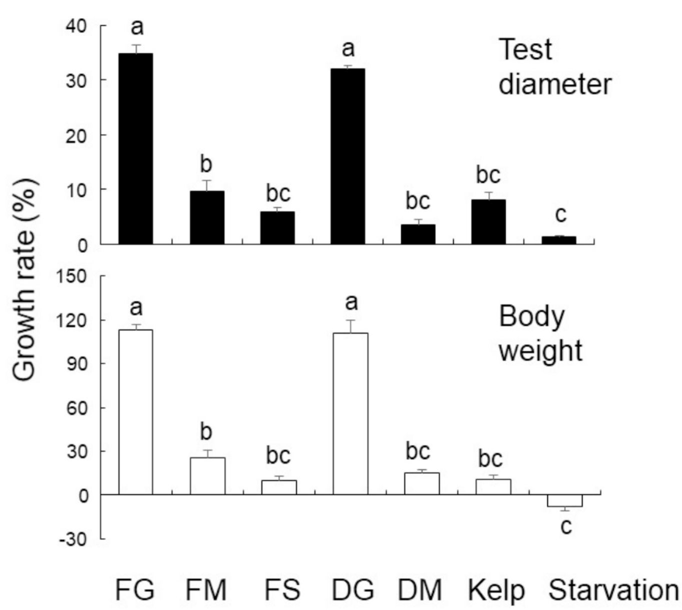

FIGURE 1. The growth rates of test diameter and body weight of *Mesocentrotus nudus*.

METABOLIC AND MOLECULAR STRESS RESPONSES OF EUROPEAN SEABASS *Dicentrarchus labrax* AT LOW AND HIGH TEMPERATURE EXTREMES

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Extreme water temperatures related to global climate change represent a significant challenge in terms of fish welfare and aquaculture productivity. Understanding the effect of more frequent and extended extreme temperature events on physiological responses, growth performance and other production-relevant parameters of major aquaculture species are essential for developing suitable mitigation methods and assuring future production. European seabass, *Dicentrarchus labrax*, a euryhaline marine aquaculture species is widely distributed throughout the Mediterranean region. This fish grows best at a temperature range of 22 ºC to 24 ºC. This fish is reportedly sensitive to temperatures below 11 ºC and over 25 ºC, which leads to impairments of growth, physiology and high mortality rates. In the Mediterranean region, maximum water temperature in ponds exceeds 33-34 ºC during late spring to August and decreases to 5-6 °C winter.

The present study evaluated the extreme ambient temperature effect on growth performance, physiological, biochemical and molecular responses of juvenile European seabass, *Dicentrarchus labrax*. Fish exposed to 8 °C and 32 °C for 30 days exhibited significantly lower final weight, weight gain, specific growth rate, survival rate, and temperature growth coefficient ($p<0.05$), compared to 16 °C and 24 °C. Hepatosomatic index (HSI), viscera somatic index (VSI), intestine somatic index (ISI) and spleen somatic index (SSI) were significantly ($p<0.05$) lower in fish at 8 ºC and 32 ºC at day 30. Plasma [Na^+] and [Cl^-] ion concentrations were significantly lower ($p<0.05$) in fish reared at 8 ºC at day 10, 20 and 30. Plasma triglycerides, lactate, cortisol were significantly ($p<0.05$) increased, while plasma glucose, protein and liver energy storage showed the inverse trend in 8 ºC and 32 ºC reared fish at day 10, 20 and 30. Heat shock proteins (hsp70) gene was significantly ($p<0.05$) upregulated in the dorsal muscle and kidney tissue of fish reared at 8 ºC and 32 ºC at day 10, 20 and 30. Whereas Interleukin 1β (IL-1β) gene exhibited a similar, but less regular expression with upregulation at day 10 across all four temperature treatments. Insulin growth factor 1 (Igf1) relative expression was decreased significantly in fish reared in 8 ºC and 32 ºC than in 16 ºC and 24 ºC at day 10, 20 and 30.

European seabass exhibits significant physiological, biochemical and gene expression alterations and marked performance reduction during extreme temperatures of 8 ºC and 32 ºC. None of the repeatedly measured parameters in the current study indicated a capacity for compensation by physiological adaptation over periods of 10, 20 or 30 days.

IDENTIFICATION OF *Vibrio* SPECIES FROM RECIRCULATING AQUACULTURE SYSTEMS AND INTERACTIONS WITH *Bacillus* spp.

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Losses during the production of marine fish larvae remain a bottleneck to the expansion of the marine aquaculture industry. In particular, the presence of *Vibrio*, a genus of bacteria associated with opportunistic disease in marine finfish and shellfish, is negatively correlated with larval fish survival in aquaculture systems. Previous studies in our lab indicate a *Bacillus* spp. probiotic significantly improves larval survival; however, the mechanism behind these benefits is unknown. The goal of this study was to characterize the interactions between the probiotic strains and *Vibrio* species present in marine recirculating aquaculture systems. In this study, *Vibrio* were isolated from a variety of fish species and life stages at the Mote Aquaculture Research Park (MAP) using thiosulfate citrate bile salts sucrose (TCBS) agar. Isolates were classified by sequencing the rpoB gene. *Vibrio* sequences were compared to the NCBI database via BLAST and reference strains that contained the highest percent sequence similarity were included in a phylogenetic tree of all found sequences. A total of 128 isolates were collected from MAP, of which 26 have been sequenced to date. Twelve species of *Vibrio* were identified, with the greatest diversity (six species) present in larval Almaco Jack. Four species were found in juvenile Common Snook, and three were found in adult Redfish. Antagonism studies were performed between two probiotic *Bacillus* strains and a subset of *Vibrio* strains. *Bacillus* inhibited growth of 6 *Vibrio* species, including 4 potential fish pathogens (*V. harveyi, V. parahaemolyticus, V. rotiferianus,* and *V. alginolyticus*). Continuing investigation of *Vibrio* communities in these systems will identify opportunists of potential concern for larval rearing and help to elucidate beneficial mechanisms behind the *Bacillus* probiotic, allowing for more targeted treatment methods to increase larval survival and promote growth of the aquaculture industry.

Table 1: Species of *Vibrio* identified in the present study.

Species Name	# Isolates	Location Isolated	Z.O.I. , mm	Other Information
V. alfacsensis	3	Juv. Common Snook	16.5	Isolated from Sengalese Sole
V. alginolyticus	1	Almaco Jack larvae	23	Symptoms include septicemia and ulcers.
V. brasiliensis	1	Almaco Jack larvae	--	Initially isolated from the Lion's Paw Scallop
V. chagasii	3	Almaco Jack larvae	--	Causes disease in sea scallops.
V. coralliitycus	1	Adult Red Drum	14	Potentially pathogenic in fish.
V. Harveyi	4	Adult Red Drum	--	Known fish pathogen causing gastric enteritis.
V. neptunius	2	Almaco Jack larvae	--	Pathogenic to oysters and mussels.
V. owensii	1	Juv. Common Snook	--	Causes disease in shrimp and lobster.
V. parahaemolyticus	1	Juv. Common Snook	27	Causes fin rot disease in Sebae Clownfish.
V. rotiferianus	4	Adult Red Drum; Almaco Jack larvae	29	Causes skin ulcers in Half-Smooth Tongue Sole.
V. tubiashii	3	Almaco Jack larvae	--	Pathogen of mollusks
V. vulnificus	5	Juv. Common Snook	15	Human pathogen- causes necrotizing fasciitis

COMPARISON OF GILL MICROBIAL DNA ISOLATION METHODS IN *Oncorhynchus mykiss* FOR APPLICATION IN MICROBIOME RESEARCH

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Microbiome research- the study of the microorganisms that live on and within a host- has been applied to aquaculture to better understand the role that bacteria play in host health. While much research has been conducted on the gut microbiota of several fish species, less priority has been given to studying microbial communities associated with other mucosal surfaces such as the skin and gills. Furthermore, due to the low abundances of microorganisms in some of these mucosal surfaces, isolating a sufficient amount of quality DNA for microbiome analyses has proven to be a challenge for the field. Failing to thoroughly isolate a representative sample of bacterial DNA can result in mischaracterizing or underestimating microbial diversity. In this study, five different isolation procedures were conducted to compare microbial DNA yield from rainbow trout gill tissue. In the first group, four gill arches were vortexed in 30mL PBS and removed. Subsequent centrifugation steps at high and low speed removed trout cells and pelleted bacterial cells respectively. Pelleted cells were washed in PBS and added the Qiagen PowerSoil Isolation Kit (PS kit). In the second and third group, four gill arches were vortexed and then removed in either 30mL of PBS or NaCl respectively. The vortexed solutions were then spun at a low speed to remove trout DNA. The supernatant was put through a 0.45μm and then a 0.22μm filter subsequently. Finally, the 0.22μm filter was added to the Qiagen PowerWater Isolation kit (PW kit). In the fourth group, a swab of the gill mucus from four gill arches was added directly to the PS kit. In the fifth group, 1cm^2 gill tissue was added directly to the PS kit. DNA yields will be measured using a NanoDrop spectrophotometer and qPCR of the 16s rRNA gene will determine the success of bacterial DNA isolation. In addition, qPCR will be conducted for rainbow trout DNA to indicate relative amounts of bacterial to host DNA abundance.

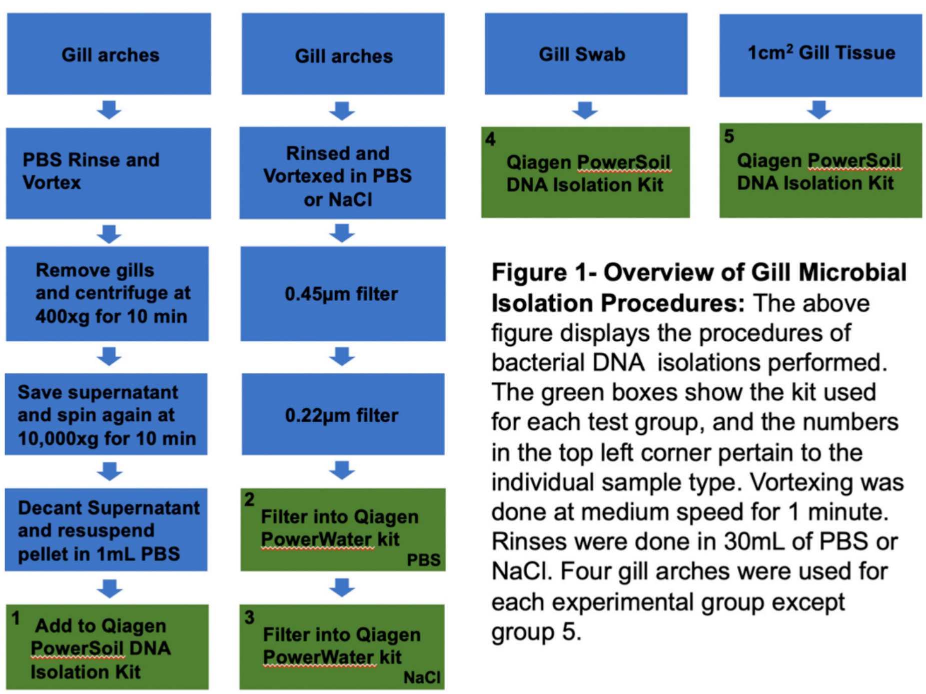

Figure 1- Overview of Gill Microbial Isolation Procedures: The above figure displays the procedures of bacterial DNA isolations performed. The green boxes show the kit used for each test group, and the numbers in the top left corner pertain to the individual sample type. Vortexing was done at medium speed for 1 minute. Rinses were done in 30mL of PBS or NaCl. Four gill arches were used for each experimental group except group 5.

EVALUATION OF THE POTENTIAL PREDATOR-PREY RELATIONSHIP OF *Procambarus clarkii* and *Litopenaeus vannamei* IN A LOW SALINITY RECIRCULATING TANK SYSTEM

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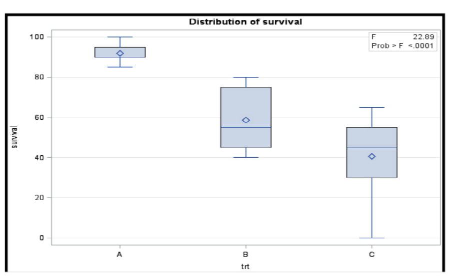
Polyculture of Pacific white shrimp (*Litopenaeus vannamei*) and red swamp crayfish (*Procambarus clarkii*) is a new production strategy being explored by commercial shrimp farmers in west Alabama. Crayfish are being produced on a pilot-scale in earthen ponds and a split-pond on one commercial shrimp farm. Since crayfish can freely cross levee systems into other ponds, hence it is important to understand the predator-prey relationship between these two species. Traditional crawfish production relies on pond primary productivity to fuel growth and reproduction of the crop, while low salinity shrimp culture involves annual stocking of post-larvae and supplemental feeding with a commercial diet. In these production systems, the growth of the crawfish population could be fueled by the primary productivity that results as a byproduct of the semi-intensive nature of shrimp culture. The main area of concern is that crawfish are known to feed on small fish and insects in addition to detritus and plant material. This feeding strategy raises the question of whether crawfish would feed on juvenile shrimp, causing a negative impact on shrimp production. In order to investigate the potential for these two species to develop a predator-prey relationship, a controlled experiment was set up using a recirculating system. The system consisted of 24 aquaria (75 L) that were continuously recirculated through a sand filter and biofilter. The salinity was maintained at approximately 2.3 g/L using reconstituted seawater. Three distinct treatments were used to evaluate the impact of crawfish presence on shrimp survival including a Treatment A - Control (20 shrimp + daily feed ration), Treatment B (20 shrimp and one crawfish both receiving a daily feed ration), and Treatment C (20 shrimp and one crawfish with only the shrimp receiving a daily feed ration). Eight replicates of each treatment were randomly assigned tanks and stocked on August 6, 2019. Shrimp and crawfish were fed a calculated ration twice daily. Following 21 days of culture the tanks were harvested and the total number of shrimp and crawfish were enumerated and group weighed to determine growth, feed conversion ratio, and survival. Shrimp survival in the control group was significantly higher (91.9%) than the other treatments (P<0.001)(Treatment B = 58.8%, and Treatment C = 40.6%). These results indicate a significant reduction in shrimp survival in the presence of crawfish. This negative effect was further increased in the treatments where feed rations intended for the crawfish were absent.



CONSTRUCTING A TRANSGENIC ZEBRAFISH WHICH EXPRESSES L-GULONO-GAMMA-LACTONE OXIDASE, IN ATTEMPT TO REVOKE VITAMIN C BIOSYNTHESIS AND BOOST THE TELEOST GROWTH

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The L-Gulono-γ-lactone oxidase (GULO) is the enzyme which catalyzes the last enzymatic reaction of vitamin C biosynthesis in animals. GULO catalyze the oxidation of L-gulonolactone into ascorbic acid. Therefore, this key enzyme plays a significant role in vitamin C biosynthesis. The animal kingdom has an ability to synthesis vitamin C in their body except for some exceptions. Some vertebrates including guinea pigs, some bat species, certain passerine birds, anthropoid primates and teleost fish have lost the ability of vitamin C biosynthesis due to the lack of functional *GULO* gene in their genome. Throughout the vertebrate evolution, GULO activity has fluctuated, and thus far, it has been a paradox in science. The animals which have lost the synthesis of vitamin C synthesis ability should consume the vitamin C by their diet, and vitamin C deficiency would lead to severe conditions such as scurvy, impaired wound healing, reduction of growth and immunity.

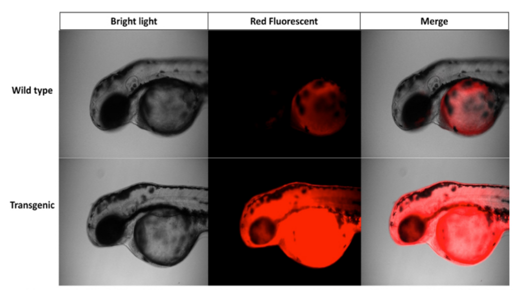

Figure 1. **The F1 wild type and Tg (b-actin:*Sgulo*:mcherry) zebrafish (48 hpf under the fluorescent microscope (Leica, DM600B).**

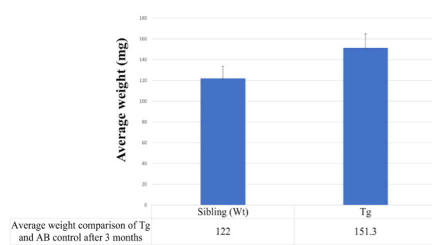

Figure 2. **Average weight comparison of Tg and Wild type control (3 months old)**

DISCLOSING AN IMPORTANT DEFENSE MECHANISM AGAINST VHSV IN FISH

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VHSV (Viral hemorrhagic septicemia virus) is a deadly fish virus, which causes devastating consequences in aquaculture. Even though interferon stimulated genes were suspected for antiviral activities against VHSV, their detailed mechanisms and pathways were not described. Korean black rockfish (*Sebastes schlegelii*) was used for this study, as it's an important species in offshore aquaculture in Korea. Interferon stimulated gene, viperin was identified in rockfish transcriptomic database. *In silico* analysis was performed to characterize the sequences and the domains that may responsible for the suggested antiviral activities. Expression was analyzed against poly I:C by qPCR. Viperin was cloned and over expressed in FHM cells. After infection, a VHSV gene transcription was analyzed. VHSV replication was determined by a cell viability assay as well as by $TCID_{50}$ method.

Bioinformatics analysis revealed availability of SAM binding motif "CNYKCGFC". *In vivo* transcription analysis revealed the substantial upregulation of viperin with the presence of double standard RNA-analogue; poly I:C. Over expression of the viperin was significantly down regulating the VHSV gene expression. And the $TCID_{50}$ values for VHSV were significantly low in the viperin overexpressed FHM cells.

According to the results, it's obvious that viperin is a radical SAM protein which can reduce viral replication and the transcription. Viperin may strongly transcribe with the presence of viral RNA in cytosol. Previous studies suggested that VHSV transcription and replication is solely carried out by the viral RNA dependent RNA polymerase (RdRp). With these results, we can suggest that viperin inhibition of RdRp leading to strong antiviral activity against VHSV.

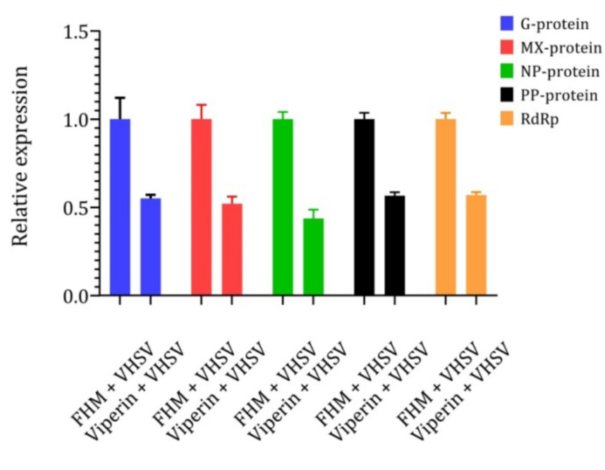

FIGURE- Expression pattern of the five viral proteins in FHM cells with the over expression of rockfish viperin

IDENTIFICATION, MOLECULAR CHARACTERISATION OF CATENIN β HOMOLOG FROM REDLIP MULLET (*Liza haematocheila*)

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Catenin β was first identified as a Ca^{2+} dependent cell adhesion molecule in the cytoskeletal structure together with catenin α and □. The function of catenin β was elaborated to the central signaling transducer in the Wnt signaling pathway. The cytoplasmic catenin β concentration is highly controlled by β-catenin disruption complex. Unless Wnt ligand enables the signaling cascade, the catenin β remain in the cytoplasm at low concentrations. However, catenin β can regulate nearly 400 genes which involve cell survival, apoptosis, growth, differentiation, and immune functions after the translocation to the nucleus. The current study was designed to investigate the transcriptional variation of catenin β of red lip mullet after the bacterial and viral pathogenic mimicry.

Catenin β sequence was identified from the constructed cDNA library of redlip mullet. Bioinformatics software and online tools were employed to characterize the identified nucleotide sequence and predicted the amino acid sequence. Healthy red lip mullets were dissected and 12 tissues including peripheral blood leucocytes, head kidney, kidney, liver,gill, and spleen were harvested to isolate mRNA and synthesize cDNA. The cDNA was used to evaluate the transcriptional difference of catenin β in different tissues in healthy redlip mullets. Healthy fish were challenged with IP injections of LPS, Poly I:C and live *Lactococcus garvieae*. The spleen was dissected at 0, 6, 24, 48 and 72 hour time points after the challenge. mRNA isolation and cDNA synthesis were performed and the qPCR was used to investigate the fold change of catenin β mRNA transcription.

The redlip mullet catenin β gene is composed of 2352 bp and predicted amino acid sequence composed with 783AA. The phylogenetic analysis cladded the sequence with other known fish catenin β homologs. However, the multiple sequence alignment shows the active domain structure of redlip mullet bears a resemblance to human catenin β. Therefore a similar function of human catenin β may observe in mullet. However, unlikely to humans the highest expression of catenin β mRNA was observed in heart muscles of mullet. The catenin β expression is drastically downregulated by different PAMP's. Previous reports documented that catenin β can affect the localization of NF-ϰB transcription factors preventing further induction of downstream genes. Therefore, the activity of NF-ϰB might be enhanced by downregulating catenin β. However, more experiments are required to reveal the functions of fish catenin β.

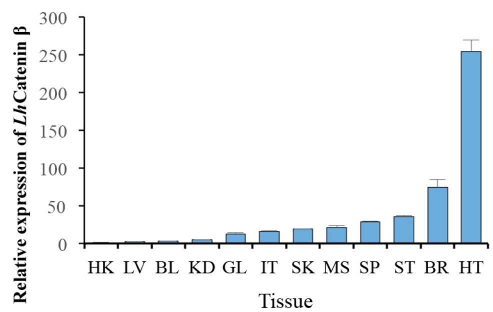

Fig.1 Tissue specific expression of the Red lip mullet Catenin β (Tissue: head kidney, liver, blood, kidney, gills, intestine, skin, muscles, spleen, stomach, brain and heart respectively)

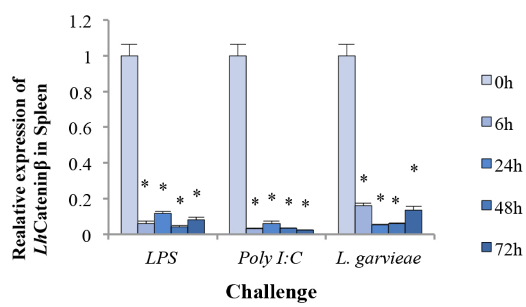

Fig.2 Relative expression of mullet Catenin β in spleen tissue at certain time points after different immune challenges

OPTIMIZING CORAL GROWTH IN EX-SITU NURSERIES: IDEAL SPECIES-SPECIFIC CONDITIONS FOR MASSIVE HAWAIIAN CORALS

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The State of Hawaii's land-based Coral Nursery quickly grows Hawaiian massive coral species to large sizes in aquarium systems then outplants these corals to damaged reefs around Hawai‘i. Hawaiian massive corals are some of the slowest growing corals in the world, with growth rates of only 1-2 cm a year. These large massive-form corals (*Porites spp.)* form the foundation of Hawaii's reefs, which are essential for protecting the coastline from wave action and also provide for diverse fisheries and recreational opportunities. However, due to these corals slow growth rates, natural recovery from anthropogenic disturbance events is extremely slow or nearly non-existent.

Although corals have been kept in aquaria for decades, not much is known about species-specific care requirements for restoration purposes, let alone the needs of unique Hawaiian species in aquarium systems. To address these needs, the Coral Nursery developed a Fast-Growth Protocol and has explored the effects of parameters such as lighting intensity, water chemistry, diet, and other factors to determine how to maximize growth of Hawaii's slowest growing corals. Small coral fragments of multiple species and genotypes were monitored in a series of experiments designed to test growth rates under different lighting intensities and feeding regimes. Species-specific growth trends were analyzed as corals were fed various cultured and commercial foods such as live zooplankton and phytoplankton, frozen processed foods, as well as amino acid solutions and powdered coral foods. Analysis of these data suggest the species-specific care and the role of mixotrophy in Hawaiian corals may be more nuanced than previously assumed. By applying these results with future research, the Nursery aims to continue refining the Fast-Growth Protocol, furthering the State's ability to restore Hawaii's reefs.

Figure 1. Experimental set up for *Porites spp.* feeding experiment.

STATUS AND SUCCESS OF CONSERVATION AQUACULTURE USED TO RESTORE KOOTENAI RIVER BURBOT

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Burbot *Lota lota maculosa* were once abundant in the Kootenai/ay River Basin; Idaho USA and British Columbia Canada, where they provided important cultural, recreational, and commercial fisheries. Beginning in the 1970's, cumulative effects of habitat loss and hydro-power operations lead to an extirpation of Burbot populations. A multi-agency cooperative program lead by the Kootenai Tribe of Idaho (KTOI), the University of Idaho Aquaculture Research Institute (UIARI), the British Columbia Ministry (BCM) and Idaho Department of Fish and Game (IDFG) has resulted in development of a program to conserve, restore and monitor a native strain of Burbot now being used to rebuild and restore populations on a basin-wide scale. From 2009 to present, the program transitioned from UIARI laboratory-scale production to larger-scale production at a new KTOI Tribal fish hatchery. The Kootenai Tribe of Idaho Native Fish Conservation Aquaculture Program now operates the hatchery to support Kootenai River Burbot conservation aquaculture and IDFG and BCM complement the program by means of in-river post-release monitoring and evaluation. Annual hatchery juvenile Burbot releases have been variable (ranging 40,000 – 750,000 beginning in 2015); however, a long-term pre-determined restoration goal of 17,500 spawning adults has been achieved. The new facility was designed to annually produce 125,000 6-month old juvenile Burbot averaging 100 mm total length and 5 – 10 g body weight. With the production target and restoration goal in mind, the program has currently rejuvenated the population beyond 17,500 spawning adults. Current population estimates of adult Burbot in the basin exceed 50,000. Based on this success, the program is now also supporting experimental releases of early life stages (e.g., eggs, larvae) into river, floodplain, side-channel, and lake habitats to further evaluate habitat constraints that remain. The information gained from these early life studies through collaboration with IDFG and BCM monitoring programs may help guide future habitat restoration in the basin. The overarching goal of the program is to restore self-sustaining, naturally recruiting populations for cultural and recreational use. In general, this presentation will highlight the progression of conservation aquaculture method developments and program research support to understand recruitment bottlenecks.

LEARNING ABOUT EUTROPHICATION: WHAT ARE STUDENTS THINKING?

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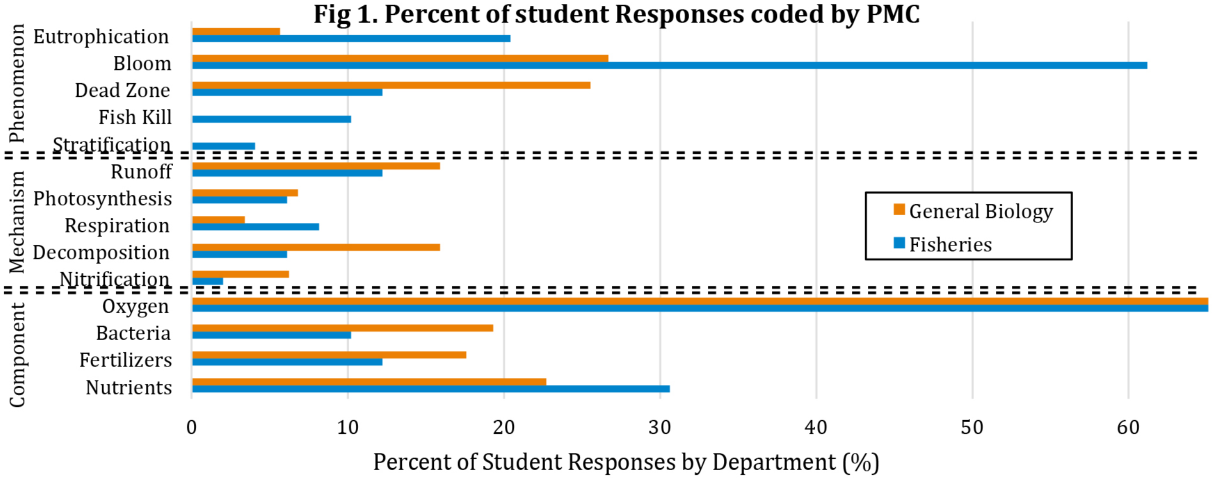
Eutrophication is an important socio-scientific issue, and an example of a complex phenomenon that many future biologists will encounter. Multiple university courses teach eutrophication in a variety of disciplines (e.g., biology, geology). However, students struggle with emergent phenomena, so educators are challenged to help students reason about the consequences of the interactions within ecosystems. This study aims to develop an open-response item (in which students use their own words to answer a question) to encourage systems thinking related to eutrophication. A lake eutrophication assessment item was given to 225 undergraduate students in four different courses (two in fisheries departments and two in general biology departments) across three universities. Student responses were coded using a systems-thinking coding scheme consisting of: 1) Phenomena, 2) Mechanisms, and 3) Components (PMC). Preliminary results (Fig 1) indicate that more fisheries than general biology students correctly stated that the phenomenon was eutrophication (20% as compared to 5%) or algal bloom (60% as compared to 25%), and fisheries students also include ideas related to fish kills and stratification. Mechanistic reasoning, as identified by Mechanism codes, was rare (<15%). Instead, students typically wrote that algae "sucked up all the oxygen" giving no mechanism for oxygen removal. Most students tended to reason in absolutes (e.g., all the oxygen) instead of relatively or proportionally. Additionally, most students (>75%) recognized oxygen as an important component in the system. Although the question provided context that the lake was next to a woodland, students added non-relevant components to the system (e.g., fertilizers, ocean). We found that students were more readily able to identify system components, but not their interactions. Data collected this fall will be used for assessment improvement. Outcomes will reveal patterns in students' reasoning about complex systems and concepts that could be addressed to students or personnel who transfer between general biology and fisheries disciplines. Findings will inform intervention recommendations for instructors (e.g., teachers, extension educators) to improve students' systems thinking and scientific literacy.

Fig 1. Percent of student Responses coded by PMC



TRACE MINERAL AMINO ACID COMPLEXES (Availa® Zn, Mn, Cu and Fe) AND Zn-L-Selenomethionine (Availa®Se) IN WHITE LEG SHRIMP (*Litopenaeus vannamei*) DIETS ENHANCE COOKED COLOR

Cinzia Boggino[1], Terry Ward[1], Silva Claudia*[1] , O. Jintasataporn[2], and S. Chumkam[3]

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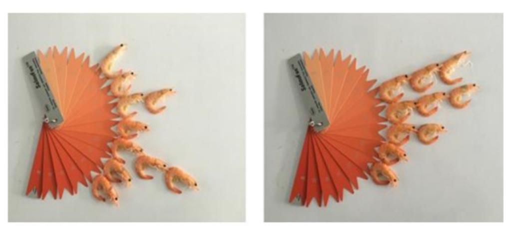
Shrimp coloration depends on carotenoid pigment content and has significant impact on market value. To enhance shrimp red color, many carotenoids like astaxanthin, canthaxanthin, zeaxanthin, lutein and other pigments are added to diets. In addition, trace minerals with anti-oxidative properties, like zinc, copper, manganese, iron and selenium may help promote expression of shrimp red color. This study was conducted to investigate effects of trace mineral amino acid complexes supplementation on the expression of red color in white leg shrimp (*Litopenaeus vannamei*). The study was a completely randomized design with 5 treatments (Table 1) and 5 replications per treatment. T1 was supplemented with 75 mg/kg astaxanthin, without canthaxanthin or trace mineral (TM) amino acid (AA) complexes. Remaining 4 diets were all supplemented with 25 mg/kg astaxanthin,25 mg/kg canthaxanthin and assigned different TM-AAcomplexes supplementation: T2 noTM-AAcomplexes supplementation, T3 supplemented with Fe and Se, T4 and T5 supplemented with Fe, Se, Zn, Mn and Cu . Diets T4 and T5 differed in their Zn, Mn and Cu levels that in diet T5 were 2 x the levels supplemented to Diet T4. The experimental period had a duration of 3 weeks. Shrimp fed T4 had better feed utilization and survival rate (P<0.05). Moreover, the color of cooked shrimp was not affected by reducing astaxanthin from 75 ppm (control diet) down to only 25ppm when combined with supplementation of both canthaxanthin and TM-AA complexes (T3-T5). Shrimp fed diets supplemented with TM-AA complexes for 3 weeks had highest redness (a* value, P<0.05) and SalmonFan score number of 28 vs 23 of the control T1 group. Shrimp fed diets containing astaxanthin and canthaxanthin in combination with the trace mineral complexes of iron, selenium, zinc, manganese and copper can improve the cooked shrimp color to consumer acceptance score within three weeks.

Table 1. Experimental diets

	Carotenoids		TM -AA Complexes[1]				
	Ast	Cant	Fe	Se	Zn	Mn	Cu
	ppm						
T1	75	-	-	-	-	-	-
T2	25	25	-	-	-	-	-
T3	25	25	100	0.3	-	-	-
T4	25	25	100	0.3	50	20	10
T5	25	25	100	0.3	100	40	20

[1] Fe,Availa® Fe; Se, Availa® Se; Zn, Availa® Zn; Mn, Availa® Mn; Cu, Availa® Cu.



T1 T2

T3 T4 T5

Figure1. Cooked shrimp color after shrimp fed 5 different experimental diets for 3 weeks

MORPHOLOGICAL VARIABILITIES OF *Saccharina latissima* FARMED IN EXPOSED VS PROTECTED SITES IN SACO BAY, MAINE

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In order to proactively asses an industry with serious economic and environmental potential the US Department of Energy (DOE0) ARPA-e program has funded research for future seaweed farming in the United States through the MacroAlgae Research Inspiring Novel Energy Resources (MARINER) program. Through this program a team lead at the University of New England (UNE) is conducting model based design validation via in-situ wave, current and resulting farm forces at two Sugar kelp (*Saccharina latissima*) farm sites in Saco Bay, Maine; one protected and one exposed.

As is typical of current siting practices for most kelp farms in the nearshore Gulf of Maine, the protected site (a 60m (200ft) single long-line kelp farm) is sited in a protected environment located in lee of an Island to decrease potential physical and biological challenges created from strong waves, currents and harsh winter storms. This approach however will limit growth of the industry and moving offshore will allow farmers to achieve greater scale with fewer stakeholder conflicts. MARINER aims to yield designs for large-scale, offshore seaweed farming in open ocean conditions.

Understanding site-specific morphological, geometric and material property differences of kelp grown in both protected and exposed sites in Saco Bay, ME will aid accurate, model driven farm design performance. Here farm and blade scale growth, morphology and material property data were collected across protected and exposed farm sites during winter 2018-2019 to assess differences between sites. Between March and May of 2019, twelve successful sampling events provided data that indicates variations in peak biomass, across the entire farm, between protected and exposed sites with the protected site showing earlier peak growth by half a week compared to the exposed site. At this growth peak blade length, stipe length and overall length of the sporophytes sampled were between 220mm-276mm longer than the sporophytes sampled from the exposed site, in each length category. A month later, the exposed site grew beyond the protected site between 817mm-851mm in total and blade length and by 34mm in stipe length. A developed understanding of morphological variabilities between protected and exposed sites could influence future modeling, farm design, and biomass and harvest projections.

Figure 1. 2019 S.latissima crop grown in exposed conditions in Saco Bay, ME showing morphological features including a short stipe and wide undulations at the intercalary meristem.

ADDITION OF LATE SEASON SUGAR KELP *Saccharina latissima* TO ALTERNATIVE PLANT BASED FEEDS FOR JUVENILE SABLEFISH *Anoplopoma fimbria*

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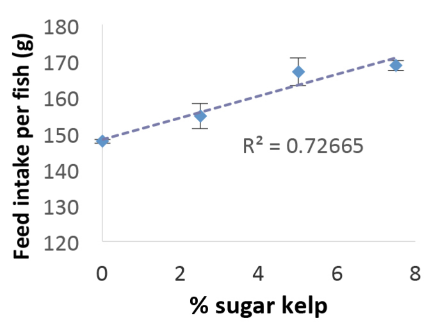
The demand for fishmeal and fish oil in aquaculture feeds, has increased dramatically in recent years. Alternative protein and oil sources are needed if further development of the aquaculture industry is to be sustained. Terrestrial plant ingredients can replace a portion of the fishmeal used in feeds for a number of species. Complete replacement, however, is seldom achieved. Macroalgae may prove a more appropriate feed ingredient than terrestrial plants products for marine fish feeds as macroalgae contains many essential nutrients from the marine environment that are limiting in terrestrial plants. There additionally appears to be health benefits associated with the use of some macroalgae species for some marine fish species.

With the emergence of kelp aquaculture in the United States, there is an opportunity to use off-product plants in marine fish feeds. Off-product kelp is generally mature plants which are approaching senescence and unsuitable for human consumption. The product is of low value and is either discarded at sea or processed into fertilizer.

In this study, we incrementally added off-product sugar kelp to alternate plant based feeds for juvenile sablefish to evaluate the effect of this ingredient on fish growth, feed intake, feed efficiency, whole body nutrient composition, and liver condition. Four isonitrogenous feeds were prepared with 0, 2.5, 5, and 7.5% late season sugar kelp. As kelp was added, soy, corn, and wheat ingredients were proportionately removed from the diets. Kelp was sourced from Hood Head Mariculture, Bainbridge Island, WA, USA in Spring 2018.

Fish grew well during the 8 week the study with 100% survival. Feed intake increased with the addition of kelp to the feeds ($P < 0.001$, Figure). Growth was similar among treatments ($P = 0.186$), but was numerically highest among 5% kelp fish. Whole body lipid content was also affected by kelp addition ($P = 0.060$), with 5% kelp fish having the highest lipid content. Feed efficiency and protein retention were inversely correlated with the addition of kelp to the feeds ($P = 0.002$ and $P = 0.001$, respectively), and we encountered physical limitations to extruding feeds containing higher than 7.5% kelp. Future research activities directed at increasing kelp content in extruded feeds and improving feed efficiencies will be discussed.



BLUE ECONOMY

Crystal Johnson

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Hatch is a business accelerator and venture fund focused on sustainable aquaculture technology ventures. Hatch bridges the gap between innovative ideas, state-of-the-art R&D and their commercialization by helping its portfolio firms to become investment and market ready and catalyzing their market entry. Hatch has a portfolio of 29 companies from all over the world as well as offices in Norway, Hawaii and Singapore.

EFFECTS OF COMMERCIALLY AVAILABLE INCREASED PROTEIN AND LIPID FEED ON THE REPRODUCTIVE PERFORMANCE OF FEMALE GOLDFISH *Carassius auratus*

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Goldfish (*Carassius auratus*) are one of the most common and popular ornamental fish used in the aquarium trade. They are also used as bait and forage in the sport-fishing industry. The majority of goldfish production in the U.S. occurs in Lonoke county, Arkansas. Commercial spawning of goldfish is done in concrete raceway systems, lined with spawning mats where females scatter their adhesive eggs, which are fertilized by males. Traditionally, producers have not used specialized brood diets for goldfish, instead, using a standard 28%-32% catfish diet throughout the year. For commercial production, goldfish brooders are spawned three to four times per summer, with three to four-week intervals between spawning sessions. In this study the effect of two commercially available high protein and lipid diets (40% protein, 10% lipid), (45% protein, 16% lipid) were evaluated as potential brood diets. In an indoor tank study, brooders were fed a control diet followed by the potential brood diets for one, two, and three weeks pre-spawn. The control group was fed only the control diet, which consisted of 32% protein and 6% lipids for four weeks. After four weeks, the fish were induced with Ovaprim® and strip-spawned. Relative fecundity was calculated for each treatment group and the control. Initial results from the study indicated that goldfish fed specialized brood diets that included high protein and lipid content appeared to exhibit better performance and higher relative fecundity than those fed the control diet. Median relative fecundities of 27,851 eggs/kg for the high protein and lipid feed and 35,507 eggs/kg for the medium protein and lipid feed were recorded for the one-week trial and two-week trials, respectively.

REDUCING *Vibrio parahaemolyticus* RISKS ASSOCIATED WITH OYSTER AQUACULTURE PRACTICES IN THE NORTHEAST UNITED STATES

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Oyster culture and harvest practices involve handling shellfish out of the water for varying time periods to reduce fouling and to harvest, cull or grade shellfish. During warm months, these practices can increase levels of pathogenic *Vibrio parahaemolyticus* populations in oysters and potentially increase the risk of illness for consumers. A more thorough understanding is needed of emerging aquaculture practices and appropriate management actions to reduce public health risks. Field-testing of *Vibrio parahaemolyticus* control measures for pre- and post-harvest husbandry practices have been conducted at harvest sites in Maine, New Hampshire, Massachusetts and Connecticut where aquaculture practices, regulations, environmental and seasonal climate conditions, and *Vibrio parahaemolyticus* populations all vary. The practices studied include exposure and re-submergence of both bottom and surface cultured oysters, relaying oysters, and temperature abuse (up to 12 h) of harvested oysters. Newly developed detection methods for regionally significant pathogenic *Vibrio parahaemolyticus* strains, i.e., ST36 and ST631, were used for evaluating *Vibrio parahaemolyticus* risk reduction.

Air exposure at elevated temperatures had inconsistent effects on the detection of pathogenic strains. Air exposed oysters at temperature equal to water temperatures caused much lower increased in *Vibrio parahaemolyticus* concentrations. Re-submergence trials in Maine, New Hamopshire and Massachusetts suggest 7 days is a consistently adequate time for re-submergence, while temperature abuse of harvested oysters for 5 hours or less did not significantly increase *Vibrio parahaemolyticus* concentrations. This study provides scientifically sound findings for each state and the region to use in managing the *Vibrio parahaemolyticus* risk in live shellfish to benefit the shellfish industry and consumer safety.

MEASURING AMOUNTS, SIZES, AND TYPES OF AQUACULTURE GEAR IN MARINE DEBRIS IN HAWAII

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Hawaii windward beaches accumulate large amounts of marine debris composed mainly of low-density polymers that float in seawater. While unidentifiable fragments from larger items are the most common type of marine debris washing ashore in Hawaii, many pieces can also be identified as once being used in fishing industries. Here we assess marine debris on Hawaiian beaches that could be tracked back to bivalve aquaculture. We collected 11,708 pieces of marine debris from 9 windward and 5 leeward Hawaiian beaches. Type, color, longest dimension (cm), and mass (g) of each individual pieces was collected. A large subset of these pieces (n = 9,758) were also analyzed using Fourier transform infrared spectroscopy (FTIR) to determine their polymer composition. All pieces have been archived and information collected for each piece has been stored in a large database. From the 11,708 pieces catalogued, 2.77% were identified as being from bivalve aquaculture (e.g. oyster or scallop spacers). This is likely an underestimate as spacers can break down into fragments and not be identified as the whole item. The sizes ranged from 1.1 - 32 cm with the majority measuring between 1.4 and 2.5 cm. Since the use of spacers in bivalve aquaculture is not active in Hawaii, these items must have traveled more than 4000 km from continental America or Asia to reach Hawaii's shores. 93.47% were identified as polyethylene (PE) while 3.78% were composed of ethylene vinyl acetate (EVA) (Figure 1), which are floating polymers and hence can be transported long distances by wind and currents. Spacers made of high-density, sinking polymers such as polyvinyl chloride were originally used in the industry and their presence on Hawaiian shorelines suggest they are still persisting and transporting slowly in the environment. Releasing spacers made of long-lasting plastic polymers, regardless of whether they float or sink, will result in habitat damage and should be avoided. There is great interest in collaborating with industry to discuss and explore alternatives (e.g. bamboo spacers) to help mitigate the impact of the global marine debris issue.

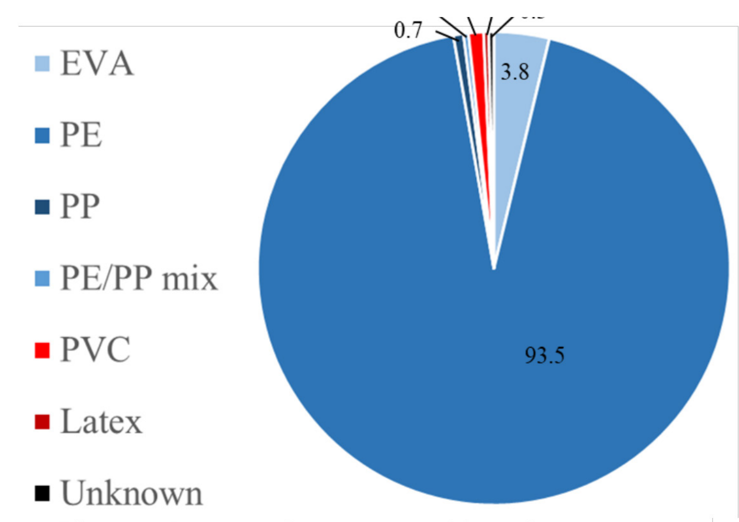

Figure 1. Percent polymer composition of oyster spacers found on Hawaiian beaches. Blue polymers (EVA, PE, PP, and PE/PP mix) represent floating plastics with red (PVC and Latex) representing sinking plastics.

INDIRECT CRITERIA TO SELECT THE RAINBOW TROUT LINES TO ENHANCE THE EFFICIENCY OF SOYBEAN MEAL UTILIZATION IN THEIR DIET

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We have developed several rainbow trout (*Oncorhynchus mykiss*) lines that show higher growth rates when fed all plant protein diet than non-selected lines of trout fed 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. We propose to test if feed intake and body weight variations during successive periods of feed deprivation (FD) and re-feeding (RF) are correlated using compensatory feeding regime with trout for genetic improvement of plant protein-based diets (PPBD) utilization using indirect selection, and to develop alternative criteria to improve feed intake of PPBD in fish.

We have used 1600 fish (av. int. wt. 30 g) from 12 families of the selected line fed plant protein (50% soy) diet (protein: 41% and lipid: 21%). Firstly, fish were tagged individually reared in common environment, fish were on *1st feeding challenge* for 4 months (one month each challenge either FD or RF), and growth performance were recorded. Thereafter, fish were separated into four groups (1331 fish) based on individual performance during FD and RF challenge studies (Fig 1A). Residual Feed Intake (RFI) 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* for 4 months) which was the same as the 1st feeding challenge. Out of 1331 fish, 143 fish exhibited a similar pattern of weight loss and gain, confirming the performance stability (Fig 1B). We have selected those individual fish (different families, Fig 1C) for spawning to challenge their offspring with PPB.

Conclusively, we have achieved the goal to select trout lines for best feed efficiency. In addition, our aim is to understand the underlying molecular mechanism on how some fish can conserve energy more efficiently compared to others and loose less weight over a FD period and gain more weight over a RF period (Fig. 1). To achieve this goal the expression pattern of the genes involved in growth, proteolysis and electron transport chain as well as the gut microbiome are being studied. At the end of genetic selection, feed (soy based) efficiency will be improved by 10-20% per generation which means feed cost will be decreased for sustainable for fish farming.

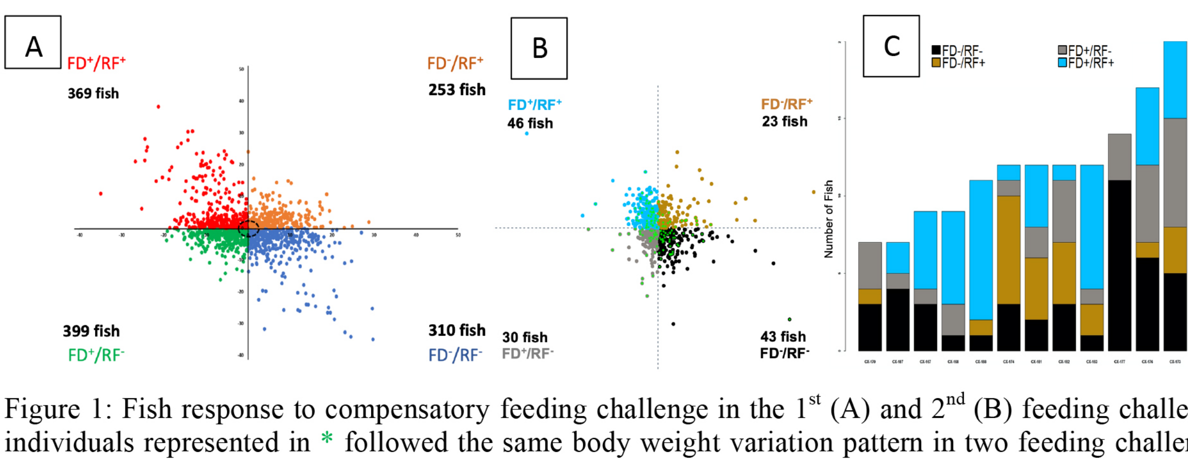

Figure 1: Fish response to compensatory feeding challenge in the 1st (A) and 2nd (B) feeding challenge, individuals represented in * followed the same body weight variation pattern in two feeding challenges and have been selected for breeding. (C) distribution of selected fish among the 12 families

GREENHOUSE GAS AND NUTRIENT BALANCE AS PART OF A LIFE CYCLE ASSESSMENT OF A COMMERCIAL SCALE AQUAPONIC SYSTEM

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Due to global populations continuing to grow at an unsustainable rate, methods of providing sufficient nutritious food and clean water must be developed all while reducing the negative environmental impacts of agricultural production systems. Aquaculture, with its high feed conversion efficiency, has great potential to meet growing global demand for animal protein. However, aquaculture systems also produce large amounts of nutrient pollutants that can potentially degrade environmental quality if improperly managed. Aquaponics presents a growing and viable solution to environmental shortcomings set forth by traditional aquaculture systems as it utilizes waste water from fish production in the production of traditional agricultural crops. Here, we look to quantify this statement by conducting a thorough life cycle assessment (LCA) of a semi-commercial scale aquaponic facility at Auburn University in Auburn, Alabama (Figure 1).

As a part of this on this ongoing assessment, extensive data has been collected in order to model and track the flow of nutrients through the system. Initial findings have traced the flow and fate of nitrogen and phosphorous helping to identify which processes throughout the system contribute most to environmental impacts. Using elemental analysis methods to provide real data from the system, a mass balanced nutrient flow model was created using the SuperPro Designer software model allowing for a virtual representation of the system. Additionally, extensive greenhouse gas sampling has taken place with initial findings identifying possible sites of high methane and nitrous oxide emissions (Table 1). We found high levels of methane emissions from the system's clarifiers and unusually levels of nitrous oxide from greenhouse crops grown in Dutch buckets. From these findings, we seek to further quantify these pollutant streams and begin to identify management methods to reduce such streams as part of a larger life cycle assessment.

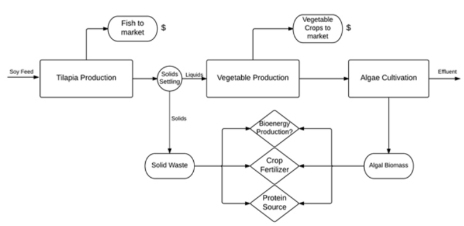

Figure 1: Schematic of process flows in the Auburn University aquaponic facility

	CO_2 (mg/day/m^2)	CH_4 (mg/day/m^2)	N_20 (mg/day/m^2)
Clarifier (Solids Settling)	5.176865951	51.68320862	0.183264548
Greenhouse Cucumber Production	21.98183737	0.005175108	0.103533669

Table 1: Average Rate of Greenhouse Gas Emissions from 5 Sampling Periods

MASS BALANCED PROCESS MODEL AS PART OF A LIFE CYCLE ASSESSMENT OF A COMMERCIAL SCALE AQUAPONIC SYSTEM

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Due to global populations continuing to grow at an unsustainable rate, methods of providing sufficient, nutritious food and clean water must be developed, while reducing the negative environmental impacts of agricultural production systems. Aquaculture, with its high feed conversion efficiency, has great potential to meet growing global demand for animal protein. However, aquaculture systems also produce large amounts of nutrient pollutants that can degrade environmental quality. Aquaponics is a growing and viable solution to environmental shortcomings set forth by traditional aquaculture systems as it utilizes waste water from fish production in the production of traditional agricultural crops. There has been a significant increase in excitement about aquaponics within the sustainable agriculture community. As commercialization of aquaponics continues to expand, rigorous quantification of sustainability using field measurements and life cycle assessment is increasingly important.

Auburn University is home to one of the nation's largest pilot scale aquaponics facilities. This facility produces tilapia that are currently sold to local markets while the aquaculture waste water is being used to produce tomato and cucumber crops that are sold to Auburn University dining services. While it is generally assumed that aquaponics is a sustainable approach to food production, we aim to quantitatively measure the system's environmental impacts utilizing a combination of field data, process modeling, and life cycle assessment. Our efforts will provide critical information about where and how environmental impact arise and provide insight into technologies and management practices that can further improve the sustainability of aquaponics.

Here we present our initial findings of the the mass balanced process model created in the SuperPro designer software which is being used to aid the ongoing life cycle assesment.

HAWAIIAN ANCESTRAL AQUACULTURE PRACTICES: LOKO IʻA [FRESHWATER FISHPONDS] AND KOʻA KAI [OCEAN GARDENS], A PANEL DISCUSSION BY NATIVE HAWAIIAN ACADEMICS:

Chaired by By Lilikalā Kameʻeleihiwa, PhD

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LOKO IʻA [Freshwater Fishponds]:

- Lilikalā Kameʻeleihiwa,* Professor, KCHS: Historical Overview of Hawaiian Islands 222 Freshwater Fishponds, 114 on Oʻahu in 1880, making 1 milion pounds of fish per year!
- Roxane Keliʻikipikaneokolohaka, Ed Specialist, Kamehameha Schools: East Hawaiʻi: Revival of Hale o Lono Fishpond,
- Kamanamaikalani Beamer, Associate Professor, KCHS: West Hawaiʻi: Kona Fishponds,
- Joylynn Paman, Director, Kimokeo Foundation: Māui: Revival of Kōʻieʻie Fishpond,
- Peleke Flores, Director, Mālama Hūleiʻia: Kauaʻi, Revival of Alakoko Fishpond,

KOʻA KAI [Ocean Gardens]

- Pelika Andrade, Extensuin Agent, UH Sea Grant Program: Koʻa Kai [Ocean Gardens]
- Noelani Puniwai, Assistant Professor, KCHS: Hawaiian Perspective of Lawaiʻa and Kanaloa [Fishing & Oceans]

Historical Overview of Hawaiian Islands 222 Freshwater Fishponds, 114 on Oʻahu in 1880. making 1 milion pounds of fish per year!

Native Hawaiians have been living in the small islands of the Hawaiian archipelago for the past 100 generations. Over time our ancestors developed unique water management systems to feed an ever increasing population. Before foreigners arrived with their foreign diseases, the population of Hawaiʻi is estimated to have been 1 million people, all being fed from the land and surrounding oceans. Today the Hawaiʻi hosts 1.4 million people with 90% of food being imported from California. Part of the Hawaiian Ancestoral strategy for increased food production was to build massive freshwater fish ponds, some as large as 523 acres, to increase the productivity of raising fish. Thus as late as 1880, Oʻahu had 114 freshwater fishponds, comprising 3,600 acres creating 1 million pounds of fish per year, just on the land! Today there are only 13 freshwater fishponds left on Oʻahu and many of them are polluted. However, many Native Hawaiians today are working to revive these ponds, creating non-profit foundations to do so!

While, Loko iʻa, or fishponds, are an amazing example of innovation, technology, and fishery production, open spaces, or Koʻa Kai, outside of the boundaries set by fences, and rock walls, were tended addressing reproduction, cultivation, and harvest. Experts understood productivity through keen observation and awareness of the natural ebb and flow of the world around them and were able to tend and harvest from open spaces successfully. Hawaiian Ancestors understood that the now termed “wild” spaces were very much tended spaces that were reciprocal in nature dependent on input for an equal or greater output. Hence, Ocean Gardens!

FISH DISEASE RISK ASSESSMENTS AND REGULATIONS

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Risk assessments have a practical application in developing a production medicine plan for fish farms. There has been limited discussion advising practitioners how to use risk assessments to build their fish medicine practice services.

We are finding new fish pathogens, but what is the significance of these findings? Fish farmers want to know whether to worry about these newly discovered organisms. How do we prioritize pathogens? What should a practitioner know about risk assessments? These are some of the questions that will be addressed in this presentation. Risk analysis involves risk identification, risk assessment, risk management, and risk communication. This presentation will present risk assessments in a practical easily replicated manner for practitioners and attempt to provide options for responding to the questions above. Risk assessment discussions can help focus the conversation with fish farmers on the most important issues on their farm.

Examples of how risk assessments have been applied in regulatory realm will be presented along with non-regulatory cases demonstrating how being well versed in risk can aid your fish farm clients. Known regulatory fish pathogens include: Viral Hemorrhagic Septicemia, Tilapia Lake Virus, Infectious Hematopoietic Necrosis among many others. Our view of risk changes with time. Several years ago Largemouth Bass Virus was viewed as a great risk to largemouth bass. Now the concern is generally less. Supposedly it has been freely spread throughout the U.S. yet it has not been associated with declines of largemouth bass. Heterosporis was also a big concern and similarly became less of a concern. We will use these and other examples to demonstrate how to assess risk. What constitutes severe fish diseases? We will discuss potential zoonotic concerns, economic impact, and environmental harm, among other considerations.

Fish farmers vary greatly in the level of concern about introducing a fish pathogen to their farms. Some are extremely cautious and other are unconvinced of the risks. What is the likelihood that a currently unknown pathogen will appear and cause severe disease? How should we put that in perspective for our fish farm clients? These issues will be discussed and the author will highlight that risk assessment discussions are a great opportunity to demonstrate the value of the fish veterinarian to fish farms.

MANAGING PATHOGEN RISKS IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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The number of new recirculating aquaculture systems (RAS) continues to grow within the United States as it has worldwide. Although several benefits of land-based RAS have been promoted, the ability to manage risk due to pathogen introduction has been one of the key reasons stated for moving from open systems e.g. sea cages to fully contained systems like RAS. While there is better control over pathogen risks in RAS, there is still a need for a robust multi-layer approach to inhibiting the introduction and establishment of pathogens within facilities. The use of quarantine facilities, biosurveillance programs, and vaccination regimens are all key to limiting the effect of aquatic pathogens whether they be regulatory, production, or political.

ON-FARM DEMONSTRATION OF A KAOLINIC CLAY FOR BACTERIAL AND ALGAL CONTROL IN AQUACULTURE

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Kaolinic clays are used in a myriad of products from Kaopectate™ to cosmetics. Recent studies have shown that certain kaolin clays bind to *Flavobacterium columnare* and *Aeromonas hydrophila* bacteria and remove them from the water column. This clay has been shown to reduce disease outbreaks in hatcheries that treat at rates of 1 g/L. Additional studies have shown that this clay will remove cyanobacteria from the water column. This past production season the cyanobacteria *Microcystis* sp. has been extremely prolific in aquaculture ponds in the southeastern US. We conducted on-farm demonstrations to determine if laboratory trials using a kaolinic clay would be successful when the technology was transferred to commercial farms. Bacterial disease in fish treated with the clay were significantly lower in hatcheries compared to non-treated fish. The use of the kaolinic clay for removal of *Microscystis* sp. from the water is being analyzed and will be presented.

INDUCTION OF STERILITY IN HYBRID CRAPPIE *Pomoxis annularis x P. nigromaculatus* **AND HYBRID CATFISH** *Ictalurus punctatus x I furcatus*

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Hybrids are produced for aquaculture and the recreational fishing industries. Black crappie (*Pomoxis nigromaculatus*) and white crappie (*Pomoxis annularis*) are popular panfish caught by anglers in the US. Hybrid crappie (*P. annularis* □ *x P. nigromaculatus* □) grow faster and weigh more than both the white crappie and the black crappie. Hybrid crappie are stocked into ponds to obtain trophy sized fish quickly. One drawback to stocking crappie or hybrid crappie is that they do reproduce and in large numbers. Recommendations for stocking white, black or hybrid crappie is to stock them in ponds greater than 25 acres. This prevents overpopulation and enables the crappie to grow to reasonable sizes. Hybrid catfish are the cross between a female channel catfish (*Ictalurus punctatus*) and a male blue catfish (*I. furcatus*). Hybrid catfish are produced commercial for the food fish market. Hybrids grow faster than the channel or blue catfish, have higher survival rates and better meat yield than channel catfish. Hybrid catfish production constitutes approximately 70% of the catfish production in the US. However, first-generation hybrid catfish can reproduce. Triploid induction is a method used to induce functional sterility in fish. Triploid induction is some species will also increase the growth rates in hybrids which would enable less time to obtain trophy fish, as with the hybrid crappie, or less time to market-size, as with the hybrid catfish. Hybrid crappie were subjected to pressure shocks to induce triploidy and stocked into ponds (4000/acre) to determine growth rates compared to hybrid crappie not subjected to a shock. For hybrid catfish, fertilized eggs were subjected to heat shock to induce triploidy and stocked into ponds (8,000/acre) for growth rate comparisons to non-heat shocked hybrid catfish. Triploid hybrid crappie and hybrid crappie were feed trained prior to stocking into ponds. All fish were fed 3% of their body weight per day and all groups were replicated four times. Growth rates, FCR, and gonad development were examined after one year. Survival rates for triploid hybrid crappie and triploid hybrid catfish were higher than those of the hybrid crappie and hybrid catfish. Reproductive function of the first-generation of offspring was significantly reduced with little viable sperm or eggs visible in the gonads. This study demonstrated that triploid induction in hybrid crappie and hybrid catfish produced more offspring and that these offspring were sterile.

RECENT PERMITTING AND TECHNOLOGICAL DEVELOPMENTS MADE TOWARD ADVANCING OPEN OCEAN FARMS IN U.S. FEDERAL WATERS

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The necessity for the United States to increase its domestic production of seafood is clearly recognized and utilizing the nation's Exclusive Economic Zone for marine farming is one alternative proposed to meet that need. Although the nation has experience with a wide range of marine farming, the majority of the existing industry lies within coastal waters thereby limiting regulatory experience to state resource managers. The potential for farming the nation's federal waters is seriously limited by the lack of a clearly defined regulatory pathway to acquire the federal and regional permissions needed to propose, permit, install and operate marine farms in federal waters.

The U.S. has rigorous regulatory processes for permitting the placement of many types of structures in the marine environment. Oil production planforms, submerged cables, LNG terminals, and sewage outfalls all require federal permits conditioned by a rigorous environmental review process. The National Environmental Protection Act and its adopted regulations provide for a process by which federal agencies review applications for placement of structures in federal waters and through this process provide conditions that set standards for placement and operation of these structures. The lack of a clearly delineated federal agency to lead the NEPA review for any proposed marine farming permit application has led to a history of application attempts being unsuccessful thereby curbing interest by investors to undertake the permit application process.

Over the past decade, NOAA agencies, oceanographic researchers and regional agencies have developed tools for a more coordinated approach to the permit process that should result in the establishment of marine farms in U.S. coastal and federal waters. Further, recent technological advances in farming systems have made the potential success of open ocean farms more likely. This presentation will summarize the regulatory and technological advances that should lead to advancing open ocean marine farming.

TRIALS AND TRIBULATIONS OF PERMITTING OPEN OCEAN FARMS IN U.S. FEDERAL WATERS: EXPERIENCES WITH STAKEHOLDER ACTIONS AND REACTIONS

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Even though it is readily accepted that the U.S. needs to significantly increase domestic aquaculture production to meet the growing demand for seafood, there is an apparent lack of action in response to this reality largely due to three primary factors: 1) a resistance by commercial fishermen to allowing competition, 2) the persistence of erroneous information promulgated to deter the public from allowing ocean farming and 3) a regulatory permitting process that is restrained by a public review that allows controversy to trump research. To resolve all of these limitations requires intensive stakeholder outreach to ensure that the correct information is properly evaluated over the clamor caused by a very vocal minority of detractors.

For more the 20 years, the Hubbs-SeaWorld Research Institute (HSWRI) has worked with regulatory agencies, investors, researchers and the public in an attempt to permit a finfish farm in U.S. federal waters. Outreach to stakeholder groups has been paramount in the approach, and many special interest groups have offered their support while others remain steadfastly opposed. As a research organization, HSWRI approaches the public's concern by providing the best scientific studies available to place into perspective the risks and benefits of marine farming. However, public opposition and the lack of a succinct regulatory pathway has led to a history of application attempts being unsuccessful thereby curbing interest by investors to undertake the permit application process, resulting in missed opportunities for the domestic expansion of sustainable seafood development.

The only way to overcome public reticence to marine farming is through education and outreach. Accordingly, over the years the Institute has worked to educate environmental groups, commercial fishers, the business community, state and local government agencies, the business community, seafood processors and distributors and resource regulatory agencies about the opportunities, risks and mitigation measures surrounding the development of marine farming. It is our hope that using HSWRI's efforts to develop the nation's first marine finfish farm in federal waters as a case study will lead to the further development of sustainable marine farming in U.S. coastal and federal waters.

RE-PURPOSING OFFSHORE ENERGY INFRASTRUCTURE FOR FOOD PRODUCTION THROUGH OPEN OCEAN AQUACULTURE

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The advancement of marine farming in the United States is mainly limited by an ill-defined and inconsistent regulatory process as well as competition for space by other uses of coastal and offshore areas. One solution might be to evaluate areas originally set aside for energy development for the potential for reassignment to marine farming. As numerous offshore energy platforms become decommissioned because the energy reserves they collected are expended, the remaining offshore infrastructure could be an invaluable, and otherwise unaffordable, resource for marine farming.

An offshore energy platform located miles from shore is an ideal environment for conducting mariculture operations. Unlike shore-based or coastal facilities, the offshore location can offer excellent water quality to promote fish and shellfish health and provide the added benefits of ocean depth and current, which eliminate environmental impacts through the diffusion of organic wastes. Further, platforms in deeper water could afford the opportunity of providing a range of temperatures by drawing water from above and below thermoclines thereby providing an energy efficient and low carbon environmental control over culture conditions. Platforms also provide infrastructure and services for research, including available deck space, utilities and daily access by supply boats.

The shore-based infrastructure developed in support of the offshore energy industry could be re-tasked or otherwise co-located to support a new farming industry. Boats that used to deliver personnel and supplies to offshore platforms could also deliver farm personnel and supplies, like feed, to farming operations and to delivering harvested product to shore-side processing and distribution centers. Further, since the space under and around existing platforms are largely restricted from other uses, like commercial fishing, the transfer of those areas to marine farming would not represent a net loss to other user groups. Also, the proximity of energy platforms to populated areas would allow for the ready delivery of locally grown product to ample seafood markets and their distribution networks.

The need to expand marine farming in the near-shore and offshore environments of the United States is well understood. Many of the inherent regulatory and infrastructure limitations could be overcome by the adoption of policies that would allow the use of decommissioned offshore energy infrastructure to marine farming.

FISH OIL AND ALTERNATIVE OIL SOURCES-BASED DIETS SUPPLEMENTED WITH OX-BILE: EFFECTS ON GROWTH, NUTRIENTS DIGESTIBILITY, BODY COMPOSITION AND INTESTINAL BILE SALT CONCENTRATION IN RAINBOW TROUT, *Oncorhynchus mykiss*

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Limited availability of fish oil has increased attention to other fat sources such as terrestrial plant oil and its byproducts. The inclusion of such ingredients may cause poor fat digestibility in fish due to a large content of saturated fatty acids. Furthermore, vegetable oils have low levels of bile salt precursors which may have a negative impact on bile metabolism. An emulsifier such as ox-bile may enhance the utilization of low digestible oil. Therefore, the present study aims to evaluate the potential of bile addition to fat metabolism when fish were fed with fish oil and alternative-based oil-sources. Nine isonitrogenous (44%, fish meal based) and iso-lipidic (20%) diets with a 3 × 3 factorial design were formulated to contain three levels of ox-bile (0, 1, 3%) and three sources of oil (fish oil, soybean oil, and fat powder). Juvenile rainbow trout (initial weight of 24.4 g) were fed to apparent satiation twice daily for nine weeks.

Results of growth performance parameters of fish were not affected ($p>0.05$) by the factors of ox-bile and/or oil source. However, fat powder-based diets fed groups resulted in lower ($p < 0.05$) fat and protein digestibility ($p < 0.05$), concurrently decreased whole-body fat and increased the protein compared to fish and soybean oil-based diets whereas supplementation of ox-bile did not affect fat digestibly. Both oil source and ox-bile addition affected the bile concentration of proximal and distal intestine ($p<0.05$). The fish oil diet led to a lower bile content in both proximal and distal intestine ($p<0.05$). Histology of liver showed that leukocyte infiltration and enlarged bile ducts in fat powder fed group whereas bile supplementation exhibited some necrotic cells. In conclusion, dietary ox-bile may not be an effective way to improve fat powder digestibility.

Table. Growth, digestibility (ADC) and wet body composition in trout fed different oil sources and ox-bile levels

Parameters	Bile	Weight gain(g)	Fat ADC%	Protein ADC%	Body Fat%	Body protein%
Fish oil	0%	69.87	97.89	87.75	12.29	15.06
	1%	71.06	98.89	86.74	12.48	15.25
	3%	72.60	97.60	85.09	12.35	15.03
Plant oil	0%	72.96	97.20	86.17	12.04	15.18
	1%	69.77	98.12	83.56	13.14	14.56
	3%	69.96	97.80	83.80	12.08	15.07
Fat powder	0%	66.66	50.04	82.95	9.61	15.97
	1%	72.01	51.67	84.12	10.11	15.62
	3%	69.25	57.27	84.69	10.21	15.68
p-values of the factors						
Oil source		0.896	0.000	0.019	0.000	0.002
Ox-bile		0.965	0.030	0.373	0.322	0.542
Oil * Bile		0.934	0.013	0.164	0.798	0.679

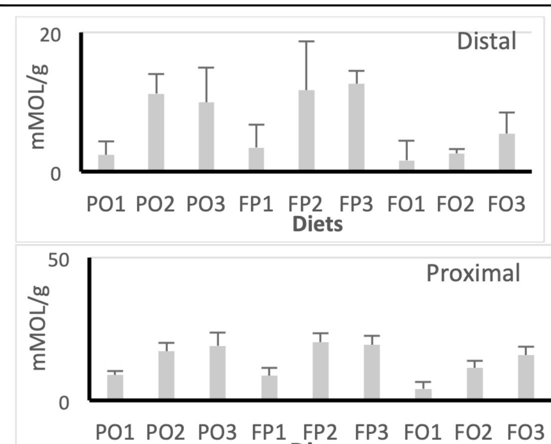

Figure. Bile salt concentration in trout intestine fed different oil sources and ox-bile levels (FO= fish oil; FP= fat powder; Po= soybean oil). The p-value for the effect of ox-bile and oil source was 0.002 and 0.000 for proximal and 0.012 and 0.003 for distal intestine. There was no significant interaction effect between the two factors

DIETARY TAURINE SUPPLEMENTATION IMPROVES GROWTH, BODY COMPOSITION AND ANTI-OXIDANT ENZYME ACTIVITIES OF GRASS CARP (*Ctenopharyngodon idella*)

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Taurine is a non-protein amino acid which is necessary for various biological processes including anti-oxidative property and detoxification. To evaluate the effect of dietary taurine on growth performance, body composition and antioxidant enzyme activities of grass carp, an experimental trail of 60 days was conducted. Four experimental diets were made containing 0, 6, 12 and 24 g/kg of taurine. The fingerlings were fed twice daily in triplicates while keeping water quality parameters constant. The supplementation of taurine significantly improved the performance of growth and antioxidant enzyme activities in fish. A significant improvement in the absolute growth, weight gain % (WG %), feed conversion ratio (FCR) and specific growth rate (SGR) was observed ($p>0.05$) while the feed intake and rate of survival showed non- significant results. The hepatosomatic index (HSI), viscerosomatic index (VSI), superoxide dismutase (SOD), catalase and glutathione peroxide (GPX) activity in the liver of grass carp fingerlings was also enhanced significantly. Among body proximate composition, the crude protein, crude fat and crude ash showed non-significant results while moisture was significantly affected. Hence, it was concluded that taurine supplementation in the diet improves the growth performance and antioxidant status of grass carp without effecting quality of fillets.

SENSORY APTNESS AND ITS INFLUENCE ON CONSUMER'S WILLINGNESS TO PAY FOR CONVENIENT CATFISH PRODUCTS

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Success of a product in the marketplace is widely determined by its superior sensory attributes and price. Standardization of sensory attributes and elicitation of consumer willingness to pay (WTP) are the key steps in the product development and marketing strategies formulation. This study evaluates the aptness of the sensory attributes and their influence on WTP for five convenient (ready-to-cook) catfish products. A total of 121 catfish consumers from Arkansas participated in the sensory evaluations of five products and elicited their WTP through Vickrey's second price experimental auctions. Using Elastic Net Regularized Regression, a machine learning approach, measured the role of the sensory characteristics on WTP for catfish products. Participants preferred Panko Breaded Delacata Fillet (PBDF) most followed by Panko Breaded Standard Strips (PBSS), Panko Breaded Standard Fillet (PBSF), Sriracha Marinade Delacata Fillet (SMDF) and Sesame-Ginger Marinade Delacata Fillet (SGMDF). Average WTP was highest for PBDF ($3.46 per packet) followed by PBSF ($2.85), PBSS ($2.84), SGMDF ($2.78), and SMDCF ($2.67). Consumers liked smell, taste and texture of all five products, but they opined that saltiness was higher for all products; crispiness was lower for PBDF, PBSS and PBSF; juiciness was lower for PBSF, SMDF and SGMDF, and oiliness was lower for SMDF. The overall liking was the most important factor affecting the consumer WTP for all products. Pruned regression tree analysis showed taste, in-mouth texture, saltiness and juiciness are significant sensory attributes affecting WTP. It may be concluded that sensory attributes determine the consumers WTP for convenient catfish products.

DISTRIBUTION OF GAMMA-AMINOBUTYRIC ACID (GABA) IN THE CENTRAL NERVOUS SYSTEM OF THE MALE MUD CRAB, *Scylla olivacea*

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Mud crab, *Scylla olivacea* is one of the most important economic value marine species in Thailand. Recently, its population is rapidly decreasing, thus the understanding of neurotransmitters/ neurohormones/ neuromodulators controlling of reproduction may help to improve the productivity in aquaculture. Many studies have reported the mechanisms of several neurotransmitters or neurohormones that involved in the reproduction of decapod crustaceans, especially serotonin, octopamine, and dopamine. They were detected throughout the central nervous system (CNS) of decapod crustaceans, which control the reproduction via regulating various neuropeptides. γ-aminobutyric acid (GABA) has implicated in the modulation of numerous physiological functions, including reproduction in many vertebrate species. However, the existence of GABA in the mud crab, *Scylla olivacea* remains unclear. We aim to investigate the distribution of GABA throughout the CNS by whole-mount immunofluorescence technique. The result revealed a ubiquitous distribution of GABA immunoreactivity (-ir) throughout the CNS. In the brain, GABA-ir was detected in the neuronal clusters 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, and fibers, including olfactory neuropil (ON), circumesophageal connective (CEC), and antenna II neuropil (AnN). In the ventral nerve cord, GABA-ir was broadly found in subesophageal ganglion (SEG), thoracic ganglion (TG), and abdominal ganglion (AG). GABA-ir was detected in the small- and medium-sized neurons in the SEG. In the TG, GABA-ir was also presented in the large-sized neurons between thoracic neuropils (TN) and fiber around the thoracic artery (TA). Moreover, GABA-ir was found in the medium- and large-sized neurons in the AG. Therefore, the existence and distribution of GABA in the male mud crab, *Scylla olivacea* may suggest that GABA may play a role in the controlling of reproduction via stimulating neuropeptides release in the CNS.

SUCCESSFUL CRYOPRESERVATION OF ZEBRAFISH EMBRYOS FOLLOWED BY HATCHING AND SPAWNING WITH LASER GOLD NANOWARMING

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The need for preservation of zebrafish embryos (*Danio rerio*) is increasingly important as a model system for both biomedical research, biodiversity and aquaculture. Although several reports claim to successfully cryopreserve fish embryos, no protocols have yet led to replication by other groups. The main challenges with zebrafish embryo cryopreservation are: 1) a multi-compartmental embryo preventing uniform diffusion of CPAs, and 2) the large size of the embryo (~1000x larger than mice oocytes), which limits both cooling and warming rates by convection. Challenge 1 can be circumvented by microinjection of cryoprotectants followed by convective (rapid) cooling. However, the massive size of the embryo has consistently led to warming failure by convection leaving Challenge 2 unaddressed.

Recently, we met both Challenges for the first time in [1]. Basically, by including GNR in Challenge 1 CPA micro-injection allows Challenge 2 to be addressed by laser irradiation of distributed GNRs to achieve rapid and uniform rewarming as shown in Fig. 1. More specifically we micro-inject cryoprotectant and GNR into zebrafish embryos and cool them at a rate of 90,000 °C/min in liquid nitrogen. We then rewarm the zebrafish rapidly ($1.4x10^{7}$°C/min) by irradiating the sample with a 1064 nm laser pulse for 1 ms due to the excitation of GNRs. This rapid warming process enables us to outrun ice formation which can damage the embryos. Like our original results in [1] we continue to show viable embryos with consistent structure at 1 hr (41%), continuing development at 3 hr (22%), and movement at 24 hr (11%). In addition, protocol refinement led to some of these rewarmed embryos hatching for the first time at 48hr (9%). By growing some of these fish to adulthood we then observed their spawn which resulted in embryo viability and growth comparable to control (> 90% survival). Other successful rewarming of coral larvae [2] and cell systems [3] suggest that this approach can be used as a platform technology for cryopreservation of fish embryos, aquatic germplasm and numerous other biological systems.

[1]) Khosla et al. ACS Nano 2017; [2] Daly et al. Sci Rpts 2018; [3] Khosla et al. Langmuir 2019.

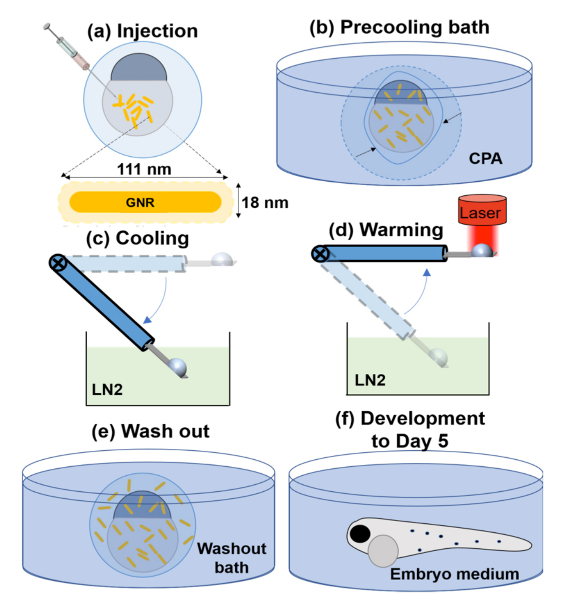

Figure 1. Step by step overview of rapid cooling and laser warming

DIETARY INFLUENCE OF TOTAL ANTIOXIDANT CAPACITY ON THE DISCOLORATION OF DARK MUSCLE IN YELLOWTAIL *Seriola quinqueradiata*

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Yellowtail *Seriora quinqueradiata* is the most widely cultivated marine fish species in Japan. One of the major problems in the end-product of yellowtail is the speed of the discoloration of the dark muscle tissue. Until now, most of the studies have been focused on the effect of supplementation of a specific or few antioxidants on the discoloration of dark muscle. However, fish meal (FM), which is the main feed ingredient, also contains antioxidant. Moreover, the antioxidant capacity of FM can fluctuate depending on many factors including the antioxidant additive level, the country of origin and the batch. Therefore, this study focused on the total antioxidant capacity (TAC) of feed, and its effect on the discoloration of yellowtail dark muscle was examined.

Two feeding trials were carried out at the Uragami Experiment Station, Aquaculture Research Institute of Kindai University, Japan. In trial 1, the control diet was designed to fulfill yellowtail vitamin E and C requirements (EC1). Three additional diets were formulated to contain 2.5, 5 and 10 times of both vitamins in diet EC1 and referred to as EC2.5, EC5 and EC10, respectively. Fifteen fish (mean weight ca. 460 g) were randomly distributed into each of eight net cages (2×2×2 m^3), set in duplicate and fed once daily until apparent satiation for 4 weeks. In trial 2, test diets D1 to D4 were prepared using four types of FM with different antioxidant capacities. The amount of vitamin E and C added was set to a value corresponding to the amount found in the commercial diets. Ten fish (mean weight ca. 1300 g) were stocked into each of eight net cages and fed once daily until apparent satiation for 4 weeks.

TAC of diets EC1, EC2.5, EC5 and EC10 in trial 1 were 5200, 5770, 6730 and 8650 μmol ascorbic acid equivalents per kilogram of feed, and those of D1, D2, D3 and D4 in trial 2 were 5230, 6450, 6900 and 9520 μmol, respectively. In trial 1, while the fillet was stored at 4°C for 96 h, the decrease in color value a* (meat redness) and a*/b* (oxidation rate of myoglobin) and the increasing of TBARS (byproduct of lipid peroxidation) of dark muscle was significantly suppressed as the TAC of the feed increased. Trial 2 also displayed similar results. The results of both trials indicate that the difference in the TAC of the feed can be attributed to various factors which can influences the discoloration of dark muscle of yellowtail. This study demonstrates the importance of analyzing dietary TAC, instead of considering the amount of a particular ingredient, prior to use in diets for yellowtail.

ESTABLISHMENT OF A DEVELOPMENT DIRECTION FOR SMART AQUACULTURE TECHNOLOGY IN THE REPUBLIC OF KOREA

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The objective of this study is to establish a direction for smart aquaculture technology development in the Republic of Korea through patent analysis and a demand survey of experts and fishermen.

The patent analysis was conducted using Wisdomain for patents in the Republic of Korea, the United States of America, Europe, Japan, and China from 2005 to 2016. This study conducted an analytic hierarchy process (AHP) survey of experts in the fields of fishery, marine, and ICT, among others. Furthermore, it carried out a demand survey of 85 fishermen in Jeonnam and Jeju.

The smart aquaculture technology market has grown moderately in the Republic of Korea until recently, and it is expected to expand further because of the expansion of national investment in the smart aquaculture field. The priority evaluation results for developing smart aquaculture technology show that land-based aquaculture has a higher priority than ocean-based aquaculture. Of the fishermen that responded, 84% said that they need to introduce smart aquaculture technology to solve problems in the supply and demand of manpower, labor cost, and maintenance expenses.

The direction of development should lie in developing biological and environment-based standard aquaculture models to spread high-tech systems and vitalize the aquaculture industry. This requires continual training of human resources in the smart aquaculture field.

SEAWEED AQUACULTURE IN KOREA – FROM HISTORIC TRENDS TO CURRENT INNOVATION

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Seaweed aquaculture production in Korea is dominated (> 99% of total production) by relatively few species: the brown kelps, *Saccharina japonica* and *Undaria pinnatifida*; and the red seaweed *Pyropia* ('gim' in Korean). The cultivation of *Pyropia* started as early as in 1623. Seaweed aquaculture industry, however, started to grow rapidly since 1960s, when *P. tenera* and *P. yezoensis* strain selections were actively occurred. Intra-species selective breeding of *Pyropia* started in 1980s and inter-species selective breeding in 1990s. For *Undaria*, strain selections started in 1970s and selective breeding since 1980s. For *Saccharina*, strain selections started in 1960s and selective breeding from 1970s. For *Pyropia* and *Saccharina*, mutant and genetic studies have also conducted recently. Seaweed aquaculture industry have grown dramatically in Korea, but there are still many challenges to overcome, including developing new cultivars considering global climate change, and developing environmentally sustainable cultivation technologies (e.g. integrated multi-trophic aquaculture, offshore cultivation, land-based cultivation, etc.), diversifying species, and new seaweed products and markets. More details about the history, current technologies and future directions of seaweed aquaculture in Korea will be presented.

COMARATIVE ANALYSIS OF THE INTESTINAL MICROBIOTA IN PACIFIC ABALONE *Haliotis discus hannai*

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To study the relationship between the internal microbial population and the grow rate of the host animal, microbial populations in the intestine of Pacific abalone *Haliotis discus hannai* of different sizes were analyzed by using next generation sequencing of 16S rDNA by Illumina MiSeq. Microbiota groups belonging to the phyla Tenericutes, Fusobacteria and Proteobacteria were found to be dominant in the intestine of Pacific abalone. Comparison of the community compositions in the large and small size groups indicated a more diverse microbial diversity in a smaller size group. Detailed analysis in the levels of class and genus of microbiota will be presented.

Microbial communities in the intestine of marine organisms reflect the physiological status affecting the growth. To study the relationship between intestinal microbial population and grow rate of abalone, Pacific abalones of 750 days post fertilization were collected and divided into small (S) and large (L) size groups.

Intestine of each abalone groups were subject to DNA purification followed by metagenomics analysis. Illumina Mi-Seq analysis led to identification of 67146.25 and 73361.13 valid reads from eight small and large individual abalones, respectively. Bacteria community structure was analyzed up to the genus level. While a 32.7% of total bacteria were classified into *Mycoplasma* _g18, only 8.45% was in small group. In addition to *Vibrio* species accounting for 14.96% and 9.93% in the small and the large groups, respectively, *Psychrilyobacter* was also detected in the small (8.15%) and large (17.3%) abalones. Relative abundance of the microbial populations in each group was compared by analyses including alpha-diversity indices. Overall, microbial composition in a smaller size abalone group showed a higher diversity than that of the large size group.

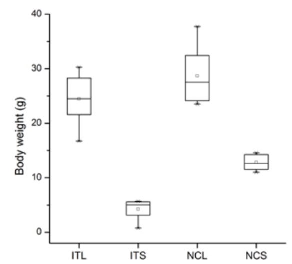

Fig. 1. Sizes of abalones subjected to metagenomic sequencing analysis.

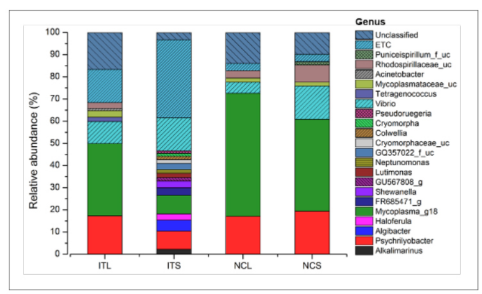

Fig. 2. Relative abundance of microbiota at the genus level detected from two different abalone groups.

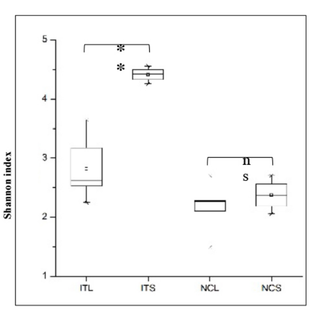

Fig. 3. Shannon diversity indices of microbial community in the intestine of the small and the large abalones.

COMPARATIVE GENOMICS OF TWO *Trachinotus* SPECIES: TOOLS TO ENHANCE THE AQUACULTURE OF FLORIDA POMPANO

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The Florida pompano (*Trachinotus carolinus*) is a finfish found in warm marine waters on the U.S. East Coast and Gulf of Mexico. This food fish demands high market value and is a prized catch for fishermen. Previous research has validated the commercial viability of Florida pompano. 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. Whole-genome sequencing (WGS) can potentially facilitate selective breeding processes by enabling discovery of genetic markers associated with favorable traits such as disease resistance, size, growth efficiency, and diversity. With a representative draft genome that we have previously assembled and annotated, our study focuses on Florida pompano as a model species for improvement using this genetic information as a tool to inform better aquaculture breeding practices. Here, we will be presenting a sequenced and assembled genome for permit (*Trachinotus falcatus*), a closely related species whose males grow nearly ten times larger than pompano, which we will use in a comparative analysis to that of Florida pompano.

The permit genome was sequenced on the Illumina HiSeq 2500 System, and the Florida pompano genome was used to perform a guided assembly of the permit genome. We will use the permit and pompano genomes to identify regions of variation that may play a role in the significant size difference between these two species. In addition, we will use a Restriction-Site Associated DNA sequencing (RADseq) method to genotype Florida pompano samples from wild and cultured populations. This will allow us to investigate population diversity and through integration with the comparative information identify candidate markers of pompano growth. Our goal is to provide a platform that will enhance the sustainable production of high quality seedstock of Florida pompano for aquaculture through the combination of WGS and RADseq technologies.

NUTRITIONAL EVALUATION OF A BYPRODUCT (FRASS) OF INSECT INDUSTRY AS AN ALTERNATIVE INGREDIENT IN NILE TILAPIA FEED

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Aquaculture is the fastest-growing food-producing sector in the world, and for this industry to continue expanding, there must be improved sustainability. One of the ways to achieve this goal is to use higher amounts of terrestrially based ingredients. Traditionally this has relied on using plant-based proteins and lipids, however, lately there is increasing interest in the use of insect meals. Recently, interest arose to evaluate a byproduct of the insect industry (EnviroFlight, LLC.) called “frass”. It is composed of spent feed and waste products from the black soldier fly (*Hermetia illucens*) larvae and contains 38% carbohydrate and 22% crude protein. Thus, it could be new ingredient for fish. The aim of this study was to evaluate the nutritional potential of frass in Nile tilapia (*Oreochromis niloticus*).

Six diets (isonitrogenous: 35% crude protein and isolipidic: 9.5% lipid) were formulated to trade the carbohydrate, lipid and protein nutrients with increasing levels of frass to replace the blend of plant ingredients that included soybean meal, corn-DDGS and wheat flour. Diets were as follows: Diet 1: Control (fish meal: 8%, corn DDGS: 30%, SBM: 33% and wheat flour: 23%); and Diets 2 – 6: 10, 20, 30, 40 and 50% frass was used to replace the blend of plant ingredients in the diets. Nile tilapia fingerlings (average weight: 2.0 g) were randomly distributed in 18 tanks (25 L, each tank contains 20 fish) in a recirculatory aquaculture system. Fish were fed 3 times per day at satiation level for nine weeks.

Fish weight gain (%) was higher in diet 6 (50% frass) than other groups (Fig.1), however, feed utilization and whole body composition of fish showed no significant differences. The gluconeogenic gene, glucose-6-phosphatase-2 (G6pca2) expression was lowest in 30% frass fed group compared to other groups, whereas no significant difference was noticed in the G6pca1. The histological evaluation indicates that the fish fed the frass-based diets showed some hepatic inflammation and vacuolization (Fig.2). Conclusively, highest growth was observed at 50% inclusion of frass in the tilapia diet, however, it does appear that the nutritive value of frass could be further improved based on the observed hepatic alteration. Nevertheless, this frass product replaced 33, 54 and 64% of the protein, lipid and carbohydrate fraction in Nile tilapia diet, respectively, and could be an additional ingredient in tilapia diets to improve aquaculture sustainability.

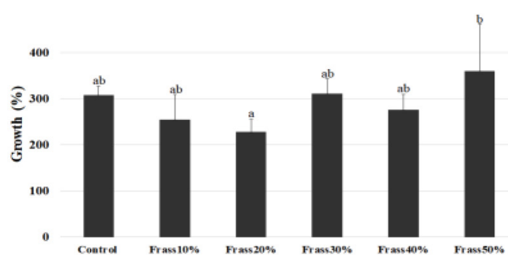

Fig 1: Growth (%) of fish fed frass-based diet. Mean ± SD, n=3. Mean values with different letters differ significantly (P<0.05).

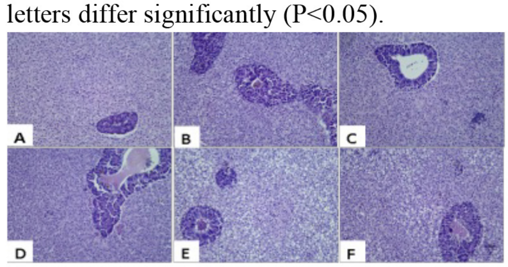

Fig 2: Liver histology of fish fed frass at 0% (A), 10% (B), 20% (C), 30% (D), 40% (E); 50% (F).

AQUATIC SPECIES AS COLLABORATIVE INVESTIGATIONAL MODELS

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Aquaculture of aquatic species has recently expanded to include applications for their use as viable experimental models. Although not commonly appreciated aquatic species have long served as fundamental tools in discovery. The wide diversity of species differentially responding to a multiplicity of life situations has provided an array of realized and more importantly unrealized investigational opportunities. Features often curtailing advancement in this arena are: 1) an under appreciation of these animals structurally, and physiologically so to identify potential contributions; 2) the animals life history and requirements of each life stage; 3) the support in an aquaculture sense to allow these species to be viable and accessible laboratory models and 4) cross-talk between aqua culturists and other scientific endeavors. Collaborative efforts provide the best opportunity for advancement. Paramount in these efforts is an understanding of the potentials and liabilities of a given model once developed. Equally important is an understanding that aquatic species are not “wet mammals”. Unconventional approaches relative to their mammalian counterparts may be necessary to deal with an aquatic animal and the aqueous environment. This talk will examine components of successful and unsuccessful approaches to collaborative investigations using aquatic and marine species.

CASTING A WIDER NET: A JURISDICTIONAL APPROACH TO AQUACULTURE IMPROVEMENT PROGRAMS

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Rapid expansion of the Indonesian aquaculture sector has outpaced the development and implementation of adequate governance and management, exposing market, environmental, and disease risks that can occur beyond individual farms. A jurisdictional approach to sectoral improvement that addresses such risks at politically, economically, and ecologically relevant scales can provide the basis for economic growth with improved environmental performance and resource efficiency and reduced disease risks. Conservation International, in partnership with Sustainable Fisheries Partnership, IDH – the Sustainable Trade Initiative, and Longline Environment is implementing a jurisdictional approach improvement program with the shrimp aquaculture sector in the Banyuwangi Regency of Indonesia to improve production and attract innovative finance vehicles. The program will test the ability of jurisdictional initiatives to transform the aquaculture sector towards increased production and profitability with reduced environmental and disease risks. Our approach is designed to address multiple commercial, political, and environmental barriers to create a scalable and investible model for improvement that can be replicated in other geographies and aquaculture sectors. This talk will highlight novel components of the program and key lessons learned during implementation.

TILAPIA AQUACULTURE IN HAWAII: LESSONS ABOUT FNO DISTRIBUTION AND SPECIES COMPOSITION FROM ISLAND STREAMS

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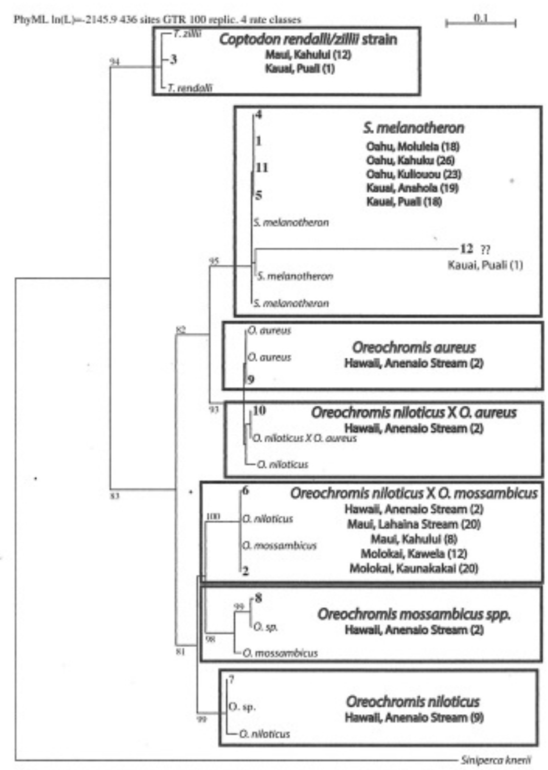
Tilapia aquaculture continues to grow worldwide, with global production of 6.5 MT in 2017. However, due to the state's isolated geography, tilapia production in Hawaii is affected by international competition with lower market costs, while local farmers are subjected to state laws that restrict importation of certain tilapia species. Additionally, due to a disease outbreak caused by the bacteria *Francisella noatuanensis* subsp. *orientalis* (Fno) around Oahu in 1994, the export of Oahu tilapia to other Hawaiian Islands is prohibited. Here we sampled tilapia from feral populations of the main Hawaiian Islands to evaluate species composition and the geographic distribution of Fno.

The research presented here includes field work on ten feral streams from five main Hawaiian Islands – Oahu, Maui, Kauai, Molokai, and Hawaii (aka Big Island) – in which tilapia were screened for species composition and Fno presence using real-time PCR. Fno was found in tilapia from Oahu, Kauai, and Maui streams. Detected species include *Oreochromis mossambicus*, *O. aureus*, and *O. niloticus* on the Big Island. The potential hybrid *O. niloticus* x *O. aureus* was also found on the Big Island, while the potential hybrid, *O. niloticus* x *O. mossambicus*, was found in Big Island, Maui and Molokai streams (Figure 1). Although Fno was found predominantly in the Black Chin Tilapia (*Sarotherodon melanotheron*) on Oahu and Kauai, Fno positive tilapia from Maui were from two potential *O. niloticus* x *O. mossambicus* hybrids and three tilapia of the *Captodon rendalli/zillii* strain. Since Fno is found in many species around the world, it is not surprising to find this bacteria outside Oahu as historically thought.

Figure 1. Phylogeny tree of Hawaii's tilapia



PRODUCTION OF SPEARMINT *Mentha spicata* WITH AQUACULTURE EFFLUENTS OF AFRICAN CATFISH *Clarias gariepinus* IN NORTHERN GERMANY

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Mint (*Mentha* spp.), a fast-growing culinary herb, was described as a plant with a high economic potential for aquaponics production. Species of the genus *Mentha* (Lamiaceae) have a high growth potential and are used for industrial and pharmaceutical products such as chewing gum, toothpastes, cosmetics or tea. Though known as a water loving plant, studies with mint under aquaponics conditions are scarce and the combined production with African catfish (*Clarias gariepinus*) is relatively new. African catfish is a common recirculation aquaculture fish in Europe, especially in the Netherlands, Austria, Hungary and Germany. In 2017, the production yield of African catfish reached 1,060 t for Germany with continuously increasing quantities, especially in Mecklenburg-Western Pomerania, Northern Germany. The present study evaluated the growth of spearmint (*Mentha spicata*) with pure effluent water from aquaculture production of African catfish (*Clarias gariepinus*) reared under extensive and intensive stocking densities without addition of fertilizer.

The experiment was carried out in the experimental state-of-the-art aquaponics facility FishGlassHouse in Northern Germany from late summer to autumn (27.08.2015- 04.11.2015) for 70 days, and African catfish with an initial weight of 275 g. The extensive aquaculture unit (EAU) was stocked with 7.6 kg/m³ and 35 fish per tank (n=9) while the intensive aquaculture unit (IAU) had a stocking density of 30.6 kg/m³ and 140 fish per tank (n=9). Fish were fed by automatic feeders with ME–4.5 44–14 Meerval (Skretting, France) with 44% crude protein, 14% crude lipid, 22.3% NFE, 10.5% ash, 1.2% crude fiber and 1.6% phosphorus. A total of 1,260 *M. spicata* were planted on ebb-and-flow tables (3.05 x 1.01 m/table) in triplicates with a control group supplied with a special low nutrient hydroponics fertilizer solution (1.12 mg/L NH_4^+–N, 9.61 mg/L NO_3^-–N, 5.54 mg/L PO_4^{3-}–P).

Spearmint (*M. spicata*) grown with effluent water from the IAU showed best growth performance (Table 1). In comparison to EAU, the mint of IAU had a 11.37% better plant height, 1.4 more shoots, 1.9 folds higher leaf area and 13.21% higher fresh biomass. Our results demonstrate the possible production of spearmint (*M. spicata*) in combination with African catfish (*C. gariepinus*) in aquaponics. Future experiments must evaluate the quality or the produce in comparison with regular production of mint in classical hydroponics or soil.

TABLE 1. Comparison of *M. spicata* plant growth parameters between low nutrient fertilizer control group (control) and effluents from extensive (EAU) and intensive (IAU) production of *C. gariepinus* ($p<0.05$).

Parameters	control	EAU	IAU
Plant height (cm)	38.4±8.2^b	45.2±11.5^a	51.0±16.9^a
Shoot number (No.)	1.6±0.7^b	1.6±0.6^b	2.3±1.2^a
Leaf area (cm²)	5.7±2.2^b	5.6±2.1^b	10.9±2.5^a
Leaf length (mm)	5.5±1.4^b	5.4±1.4^b	8.6±1.6^a
Fresh biomass (g/plant)	120.6±51.8^b	165.5±71.7^a	190.7±105.6^a
Total fresh biomass (kg)	50.7	69.5	80.1

AQUA CARE365®: AN APPROACH TO TEACHING AND ADVANCING FISH CARE AND FISH WELFARE

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Animal production agriculture including production of aquatic species is increasingly required to deal with a series of public issues. Some of these issues include operational questions and concerns, process issues, antimicrobial use, and welfare concerns to name a few. A demonstrable fish welfare program is becoming increasingly important to all segments of aquaculture production and the entire aquaculture value chain regardless of the species of fish being raised.

Aqua Care365® is an internet-based education & training resource that addresses aspects of fish care and fish welfare. Aqua Care365 initially focuses on Atlantic salmon production but does have application to the culture of other fish species as well. The components of Aqua Care365 include video training modules with individual topic lessons, quizzes, completion certificates, and customizable Standard Operating Procedures/Best Practices for each lesson. Aqua Care365 was developed in a collaborative manner with participation by salmon production companies, fish veterinarians, aquaculture industry allied organizations, subject matter experts, & Merck Animal Health. Since Aqua Care365 was also developed in conjunction with certification groups, this program can be a valuable tool to help producers meet the requirements of certification program standards.

The Aqua Care365 program is intended to be a resource to support fish farmers, fish farm employees, production facilities & sites, aquatic veterinarians, and anyone with an interest in promoting the health, well-being, and care of fish. The Aqua Care365 program may also be helpful to veterinarians interested in learning more about commercial aquaculture as well as serve as a training & educational tool for clients.

THE INCREASING CHALLENGE OF PARASITIC FRESHWATER COPEPODS, CONTROL OPTIONS, AND INTEGRATED PEST MANAGEMENT PROGRAM DEVELOPMENT

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Parasitic freshwater copepods appear to be an increasingly common issue in fish species across the United States. Parasitic freshwater copepods of the genus *Salmincola* and *Argulus* seem to be the most prevalent ectoparasites identified. Much is unknown about the biology of most parasitic freshwater copepods including details about their life cycle & stages, the primary tissues they feed on, ecology, etc. It has been determined that water temperature is a primary determinant of the time to hatching and post-hatch survival of early life stages of parasitic freshwater copepods.

In the United States there are currently no approved treatments for the control of parasitic freshwater copepods.

Attempts to control sea lice around the world has reinforced the notion that any single method of control of copepod parasites is destined to fail at some point in the future.

The Atlantic salmon farming industry around the world has developed and implemented Integrated Pest Management Programs (IPMP) to assist with the control of parasitic copepods. Components of an IPMP include operational and management decisions, biologic prevention and treatment, mechanical prevention, and mechanical treatments. Preventing infestation is fundamental to an effective IPMP and copepod control program. However, the goal of an IPMP is not the total prevention of infestations but rather to reduce infestations to levels below an established threshold based on economics, welfare, or transmission.

A group of fish health professionals, aquatic veterinarians, academicians, parasitologists, and researchers met in the summer of 2019 to discuss parasitic freshwater copepods, their biology & control, laboratory models, bio-assays, and identified knowledge gaps as well as future research project needs. Some of the outcomes of this meeting and additional knowledge necessary for the development of IPMP to assist with the control of these copepod parasites will be discussed.

PROSPECTING FOR COLD WATER PROBIOTICS

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Production of cold water (CW) fish species including trout and salmon are an important part of aquaculture. In large scale production facilities for CW fish species stressful conditions, disease and poor water quality can occur and result in significant economic loss to the industry. Often chemicals, antibiotics, and vaccination are used to improve fish health. Research efforts are looking for ecologically friendly alternatives to chemotherapeutics to improve the conditions in fisheries and to ensure that the effluent from rearing facilities are environmentally friendly.

Historically, probiotics have been used successfully in aquaculture to improve water quality, to produce inhibitory metabolites, and to competitively exclude pathogens from space, nutrients, and adhesion sites. Since, many of the *Bacillus* strains used in aquaculture products have an optimal growth temperature of 37°C they work well in warm water applications. However, salmonids grow in CW. For example, trout are reared at 15°C and Atlantic salmon grow best between 6°C and 16°C. We tested a number of our beneficial microbes and found that the supernatant from cultures grown at 30°C inhibited *Flavobacterium psychrophilum*, our warm water isolates from Ecuador and Malaysia had no activity against *psychrophilum*. Also, not every isolate of a particular species had the same activity.

Since we are looking for beneficial microbes for CW applications, it makes sense to prospect for beneficial microbes from Polar Regions and from salmonids. We have mined for beneficial microbes from temperate and polar ocean waters, cold water lakes and streams by plating the water samples and incubating the plates at 15°C. Thus far, we have isolated 9 microbes that grow well at 15°C-22°C and that have activity against *F. psychrophilum* at 15°C. Information concerning these microbes will be presented.

species	Isolate	Location	Temp °C	F. psychrophilum Inhibition
B. amyloliquefaciens				
	RA259	CT,USA, Ocean H_2O		Yes
	RA21	HI, USA, Ocean H_2O		Yes
	9H6	CT, USA,Ocean H_2O	15, 30, *35*●	Yes
	6G10	Ecuador, Ocean H_2O		No
	RA203	Malaysia, Ocean H_2O	15, *30*, 35	No
B. subtilis				
	RA26	HI, USA, oyster		Yes
	RA165	VA, USA, oyster	15, 30, *35*	Yes
	RA24	HI, USA, oyster	15, *30*, 35	No
	RA45	WA, USA, oyster		No
	RA47	WA, USA, oyster		No
B. altitudinis				
	6G11	Ecuador, Ocean H_2O	15, *30*, 35	No
B. methylotrophicus				
	RA19	HI, USA, oyster		Yes

●optimum temperature in italic and bold

THE TIMING OF FIRST FEEDING AND PREY DENSITY IMPACTS SURVIVAL IN LARVAL ZEBRAFISH-ROTIFER POLYCULTURES

Mitch Shia[1], Amy Kolb[1], Jeremy Ullmann[1], Marcia Hart[2], Marcus Crim[2], Daanya Salmanullah[1], Shane Hurley[1], Christian Lawrence[1], Michael Kent[3,4], Katrina Murray[4], and Friedhelm Hildebrandt[1]

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Rotifers (*Brachionus* spp.) are one of the most important live prey items used in aquaculture, and over the past few years, they have been increasingly utilized to feed larval zebrafish (*Danio rerio*). One of the most widely employed rearing protocols now used in this species is the "polyculture" approach in which larval fish and rotifers (*B. plicatilis* type L) are grown together in static, brackish water for the first 5 days after gas bladder inflation. We have recently observed that mortality events occur approximately 12-18 hours after fish are initially introduced to live rotifers. We attempted to understand the physiological mechanism underlying fish mortality by determining the optimal developmental stage (4, 5, 6, 7, 8, or 9 dpf) to inoculate larval zebrafish with rotifers, and if rotifer density (low vs. high) affects survival. We predicted that mortality rate would be positively correlated with later inoculation (6 dpf onwards) and high rotifer abundance. Survival was twice-daily monitored over 14 days of larval zebrafish inoculated in high (100/ml) or low (10/ml) rotifer densities between 4-9 dpf. Histopathological examination of affected fish showed distension of the intestine and severely abraded and vacuolated epithelial cells, which suggests leeching of gut bacteria to the coelomic cavity. To avoid this phenomenon, this study suggests that early inoculation (4 or 5 dpf) of larvae promotes the most optimal survival. In addition, survival is higher in polycultures with low rotifer density.

COMPARATIVE STUDIES ON MORPHOLOGICAL CHARACTERISTICS AND FILLETING YIELD OF *Clarotes laticeps* AND *Clarias gariepinus*

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Fish is a major source of protein in most Nigerian diet and Aquaculture is practiced to enhance protein supply. Samples of *Clarotes laticeps* and *Clarias gariepinus* investigated for morphometric characteristics, fillet yield and condition factor in a comparative study. Morphometric parameters and fillet yields were measured using Vernier caliper, measuring board, dissecting kit and weighing balance. *Clarotes laticeps had* significantly higher ($P < 0.05$) values of all morphometric parameters and fillet yield than *Clarias gariepinus* except the mean standard length which was higher in *Clarias gariepinus* (27.73±4.26cm) than in *Clarotes laticeps* (24.81±0.98cm). The percentage fillet yield of *Clarotes laticeps* (48.20±2.01) was significantly higher than that of *Clarias gariepinus* (45.92±2.87). The choice of *Clarotes laticeps* over *Clarias gariepinus* for consumption and aquaculture production as it gives more fillet yield per unit weight.

Table 1: Morphometric parameters and fillet yield of *Clarotes laticeps* and *Clarias gariepinus* species from Makurdi, Nigeria.

Parameter	Clarotes	Clarias	P-Value
Mean Total Weight	295.0 ± 19.3^a	162.1 ± 13.0^b	0.001
Mean Total length(cm)	31.77 ± 0.98^a	26.54 ± 0.99^b	0.001
Mean Condition factor	0.90 ± 0.04^a	0.86 ± 0.08^b	0.001
Mean Standard length(cm)	24.81 ± 0.98^b	27.73 ± 4.26^a	0.001
Mean head length(cm)	9.77 ± 0.83^a	6.45 ± 0.26^b	0.001
Mean head weight(g)	116.79 ± 7.83^a	39.28 ± 3.91^b	0.001
Mean Head width	5.57 ± 0.21^a	3.91 ± 0.13^b	0.001
Mean fin weight(g)	10.27 ± 0.65^a	3.24 ± 0.31^b	0.001
Mean vertebral weight(g)	29.16 ± 1.95^a	17.46 ± 1.64^b	0.001
Mean fillet weight(g)	140.59 ± 8.70^a	71.86 ± 7.08^b	0.001
Mean Gut weight(g)	19.14 ± 2.77^a	4.51 ± 0.44^b	0.001
Mean gut length(cm)	37.35 ± 1.47^a	19.24 ± 1.24^b	0.001
Mean hard part	38.41 ± 2.30^a	20.28 ± 1.92^b	0.001

Mean in the same row with different superscripts differ significantly (P<0.05)

Table 2: Percentage fillet yield of *C. gariepinus* and *C. laticeps* from Makurdi, Nigeria

Parameters	Clarotes	Clarias	P-Value
% head weight	29.97 + 1.55a	24.97 + 1.24b	0.001
% fin weight	3.55 + 0.14a	2.15 + 0.14b	0.001
% vertebral weight	10.40 + 0.56b	11.41 + 0.66a	0.001
% gut weight	6.81 + 1.03a	2.98 + 0.212b	0.001
% fillet weight	48.20 + 2.01a	45.92 + 2.87b	0.001
% non-edible part	51.80 + 2.01b	54.08 + 2.87a	0.001

Mean in the same row with different superscripts differ significantly (P<0.05).

IMPROVING CAPTIVE REARING METHODS FOR THE ENDANGERED ORANGEBLACK HAWAIIAN DAMSELFLY *Megalagrion xanthomelas*

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The endangered orangeblack Hawaiian damselfly (*Megalagrion xanthomelas*) was once common in lowland aquatic habitats throughout the high Hawaiian Islands, but the introduction of nonnative mosquito fish reduced this species to a few disparate populations. On Oahu, *M. xanthomelas* rests on the brink of extirpation, with one remaining population known. In 2018, the arthropod captive rearing laboratory at the Hawaii Department of Land and Natural Resources began a program to rear and release *M. xanthomelas* naiads (aquatic immatures) with the intent to establish additional populations on Oahu. Initial rearing efforts yielded a high survival rate, but naiad coloration was much lighter than that observed in the wild, and the reasons for this were unclear. We were concerned that the pale coloration may increase vulnerability to predation, and that it may be a symptom of a nutrient or mineral deficiency.

We tested the effects of diet supplementation and substrate variation on naiad developmental rates and pigmentation in captivity. To measure the effect of diet diversity, we compared naiads fed an exclusive diet of brine shrimp (*Artemia* spp.) to those fed brine shrimp and a diversity of freshwater zooplankton. To explore the roles of substrate color and composition, we compared naiads reared on six different substrates spanning a wide range of colors and textures.

We found that naiads fed a more diverse diet developed significantly faster and were slightly darker than naiads fed only brine shrimp. However, the color difference between the two groups, though statistically significant, was subtle, and supplemented naiads were still much paler than those observed in the wild. Substrate had a much greater effect on naiad coloration (Fig. 1), with naiads raised on dark backgrounds being significantly darker than those raised on light backgrounds, and more closely matching the color of wild naiads. These results have been used to modify our rearing protocols for this endangered insect. Naiads are now fed a diversity of zooplankton to promote faster growth, and their rearing cups are lined with dark gravel to produce a more natural pigmentation.

Figure 1. Naiads reared on white gravel (left) were significantly and noticeably lighter than those reared on black gravel (right).

AQUACULTURE FOOD SAFETY AND DISEASE PREVENTION PLANS

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Aquaculture is the primary component of the seafood trade which makes up the largest traded food group in the world. More than all grains, banana, cocoa, rice, and all other meats combined. Aquaculture products are also the primary source of protein for one fifth of the world's population, mainly in the developing world. This is extremely important for food security because the world's population is expected to grow from 6.8 to 9.2 billion in 2050, causing the demand for food to increase 110%. This will result in a huge demand…an opportunity… for aquaculture products. However, there are serious food safety concerns with aquacultured products. These concerns include the presence of unsafe residues, pathogens, and the potential to contribute to antimicrobial resistance. When food safety problems are found there are a range of negative impacts. These include significant costs to producers, processors, buyers, importers, and the regulatory agencies, market disruptions, an increase in the negative perception of aquaculture, lost markets, etc. This talk will describe a new way of approaching aquaculture food safety linking it to disease prevention and minimizing the risk of trade interruptions due to food safety concerns, and an approach to obtaining more customer confidence in aquaculture.

THE CRITICAL ROLES OF DHA IN FEEDING AND ITS REGULATION OF KEY GENES IN THE LARVAE OF ATLANTIC BLUEFIN TUNA *Thunnus Thynnus*

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A major factor influencing high mortality during Atlantic bluefin (ABFT) larval rearing may be poor eye development, which reduces the efficiency of prey capture leading to poor growth, survival and potential lethal tank wall collisions. The retinal membranes of the larval BFT eye are particularly rich in docosahexaenoic acid (DHA; 22:6n-3) broadly hinting at its pivotal role in photopigment function and the phototransduction cascade. In contrast, an IOLR study demonstrated a marked 59.8% decrease in whole body DHA from hatching to 2 dph first feeding larvae together with concurrent reductions in other PUFA as well as SAT and MONO fatty acids to satisfy the larva's high energy demands. Nevertheless, our group found a significant (P<0.05) dietary DHA dose-dependent effect on larval tissue DHA accumulation, ingestion rate of enriched rotifers (*Brachionus rotundiformis*) (Fig.1a) and retinal opsin abundance in 2-14 dph ABFT (Fig.1b). On the other hand, DHA is involved in the expression of a plethura of other genes modulating key physiological processes that are developmentally determined, particularly in neural tissue. In order to elucidate further the range of roles of DHA in larval BFT, transcriptome analysis was performed on extracted cDNA in a separate study that analyzed the effect of graded levels of rotifer DHA on different age (5, 10,15 dph) BFT larvae. The highest number of genes differentially expressed by DHA in 5, 10 (Fig. 2) and 15 dph larvae were those in cellular processes (225, 242 and 64 genes, respectively) that modulated neurotransmitter levels, axon and dendrite development, chemical synaptic transmission and retinal development. The results and and their relevance to the mariculture of ABFT are discussed.

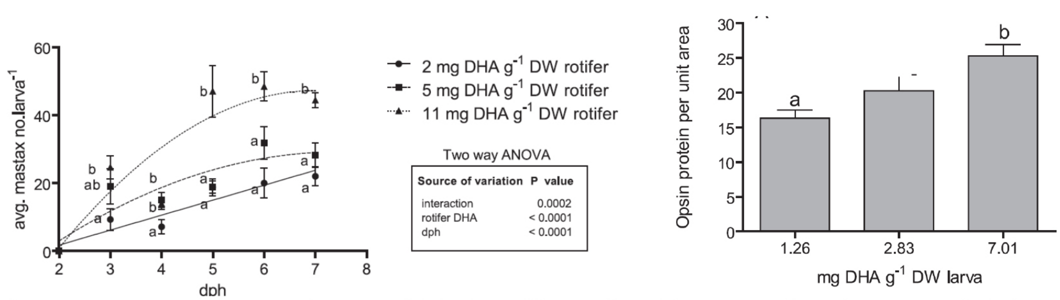

Figure 1. Regression analyses of (a) the effect of rotifer DHA level on 3–7 dph larval ingestion of rotifer mastax no. and (b) larva DHA (mg g^{-1}DW) on retinal opsin protein density (per unit area).

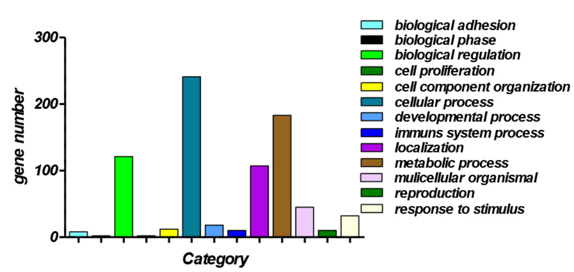

Figure 2. Physiological processes influenced by genes differentially regulated by dietary DHA in 10 dph bluefin tuna larvae.

PREDICTING AQUACULTURE FEED INGREDIENTS SOLUBILITY AND QUALITY THROUGH PROCESS CONDITIONS OF A TWIN SCREW EXTRUDER

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Ingredients used for aquaculture feed have a large impact on the quality of the feed from both a nutritional standpoint and a quality standpoint. One key aspect of feed quality is its solubility in water as it can impact both which type of marine species it is suitable for and how long it remains in the water. This can be due to both the protein and starch-based components in the feed.

During the manufacturing of feed pellets in the extrusion process, the starch and protein components can undergo a variety of chemical changes as it is subjected to the extreme pressure, shear, and heat. The chemical changes that occur can lead to a vast difference in product quality through means of solubility. While a majority of prior research has explored final product chemical solubilities and starting material solubilities, it is also important to understand the changes occurring within the extruder as the process variables change.

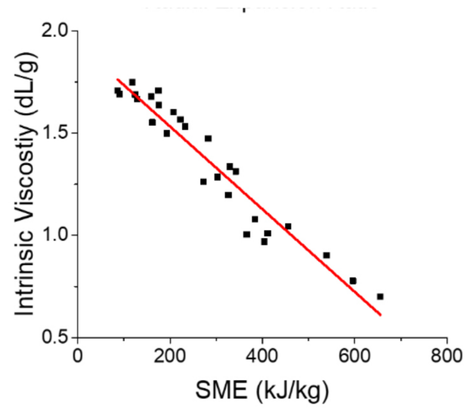

Figure 1: Correlation between intrinsic viscosity of extruded feed ingredients and Specific Mechanical Energy produced by a twin screw extruder.

In order to understand the changes for starch-based components of an aquatic feed, flour was extruded in a co-rotating twin screw extruder and brought to a dead stop under different extrusion conditions. By quickly opening the extruder, samples of starch were retrieved from the feed, transition, and cooking zones of the barrel. The ingredients were then analyzed for amount of chemical degradation as represented through intrinsic viscosity. It was found that the chemical degradation of the feed ingredients correlated strongly to the amount of mechanical energy produced in the extruder (Figure 1) and also correlated strongly with the water solubility of the final feed product (Figure 2).

By using instant measurement in the process for extruder mechanical energy, the end solubility of the feed product can be correlated and predicted accurately to design higher quality feed products that are better targeted towards different marine species.

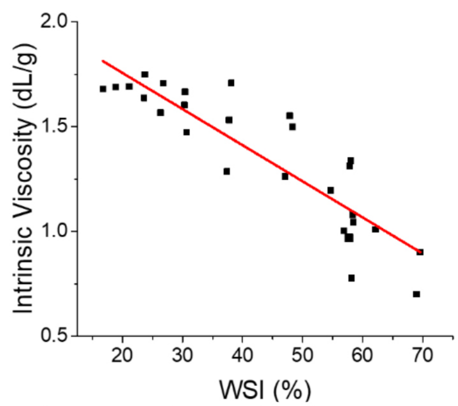

Figure 2: Correlation between intrinsic viscosity of extruded feed ingredients and Water Solubility Index of extruded feed ingredients

FORMULATION OF A. SALMONICIDA ADJUVANTED VACCINE FOR RAINBOW TROUT : IMPACT OF THE ADJUVANT OIL ORIGIN

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Injectable inactivated fish vaccines require adjuvants to enhance the immune response. Water-in-oil emulsion adjuvants are widely used in the aquaculture industry due to their cost effectiveness, stability, and long-term effect. However, oil-adjuvanted vaccines can be reactogenic and induce side effects in fish. In this study, we analyzed the impact of adjuvant oil origins in the safety and immunogenicity of *Aeromonas salmonicida* vaccines.

Two different adjuvants were tested, one with non-mineral oil (Montanide™ ISA 763A VG) and one with mineral oil (Montanide™ ISA 761 VG). Following intraperitoneal vaccination of rainbow trout, blood samples were taken at 42 and 53 days post vaccination (dpv) to assess antibody response. Adipose tissues were collected at 3, 14 and 28 dpv for RT-qPCR of genes implied in pro-inflammatory and adaptive responses. Side effects in the peritoneum were scored until 53 dpv.

Both vaccines induced high antibody titer against *A. salmonicida*. Vaccination-induced adhesion scores were both within industry-accepted limits, with lower scores from the non-mineral oil adjuvant group. Compared to the antigen-alone group, an upregulation of immune genes occurred and persisted over time. This upregulation was higher in mineral oil adjuvant group. Furthermore, a strong correlation between gene expression - modulated by the oil origin - and vaccine safety was observed.

These results showed oil origins of adjuvants impact the immunogenicity and safety of fish vaccines, and that Montanide™ ISA 763A VG and Montanide™ ISA 761 VG are efficient adjuvants for inactivated *A. salmonicida* vaccines.

CULTIVATION OF NOVEL SEAWEED SPECIES FOR CLIMATE CHANGE RESILIENT ABALONE FORAGE IN SOUTHERN CALIFORNIA

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Wild harvesting of giant kelp, *Macrocystis pyrifera*, currently supplies most of the biomass of forage for land-based cultivation of abalone. Climate change, specifically warming ocean temperature, is causing a poleward shift of kelp forest distributions worldwide. Ocean heat wave events have caused episodic die backs of giant kelp on the coast of California, destabilizing the supply of forage for abalone farms. Aquaculture of more thermally tolerant species can replace or supplement wild harvesting of kelp. The goals of our work are to identify and domesticate novel seaweed species that will provide an alternative to wild harvested giant kelp and can be cultivated on long lines in Southern California coastal waters.

Our criteria for choosing potential species were both biological and economic. Potential species must be native to Southern California, suitable for abalone forage, of known life history, with large size (minimum length 30 cm) and annual biomass yield. Resilience to marine heat waves was predicted from literature on thermal biology and/or biogeographic distribution. In addition to abalone forage, potential species must also have one or more additional markets or economic uses including human consumption, terrestrial animal feed supplement or high-value, extractable, biochemicals, for economic sustainability.

We identified 2 novel species for cultivation, the kelp, *Laminaria farlowii,* commonly called Golden Kombu (Figure 1) and the large red seaweed, *Chondracanthus exasperatus,* commonly called Turkish Towel. We are currently cultivating these two species in the laboratory, in raceways and on test lines in the field. Here, we present results for growth rates, yields and thermal tolerances of isolates of Golden Kombu and Turkish Towel in Southern California, and recommended cultivation conditions for these novel species.

Figure 1. *Laminaria farlowii,* an ideal candidate for cultivation

DIRECT-FED PROBIOTICS ENHANCES RESISTANCE TO A DEVASTATING BACTERIAL PATHOGEN (EMS-STRAIN OF *Vibrio parahaemolyticus*)

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Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) is an epizootic bacterial infection of shrimp. This disease, attributed to pathogenic *Vibrio parahaemolyticus* strains (VP-EMS), threatens shrimp aquaculture production and global food security. A valuable and alternative approach to using antibiotics for pathogen control, is the practice of incorporating direct-fed microbes (DFM) or probiotics to improve host survival and overall animal health.

Two DFM products, a single specific strain within *Bacillus subtilis*, and a blend of five strains of *Bacillus* from four different species (*subtilis*, *amyloliquifaciens*, *megaterium*, and *brevis*) were evaluated at various concentrations as feed additives (applied as a top coat on commercial feed) or as water additions for their ability to provide *Litopenaeus vannamei* shrimp protection against the EMS/AHPNS disease. Accordingly, the following assessments involved twenty-one, 20 L systems which allowed exploration of seven treatments performed in triplicate. Each trial consisted of a negative control (no VP-EMS exposure, no probiotic) and positive control (VP-EMS exposure, no probiotic), allowing for five additional probiotic treatment groups, which were fed and exposed to VP-EMS in the same manner as the positive control. Disease-challenge experiments demonstrated that while both *Bacillus* probiotic products were shown to significantly ($p<0.05$) improve shrimp survival, one specific strain provided the most consistent protection over several trials. These results also demonstrated that prophylaxis is reliant upon concentration, regardless of application. Specific probiotic strains positively influenced survival of shrimp with regard to EMS disease, serving as a promising alternative to traditional pathogen control in shrimp aquaculture.

INFLUENCE OF HOST GENETICS ON THE MICROBIOME OF TILAPIA

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An aquatic animal's microbiome, is vital to its nutrition, growth, and overall health. Exogenous factors such as diet and the rearing environment are known to affect the microbiome. However, little is known about the role that host genetics plays on the microbiome structure. To investigate the potential influence of host genetics on the community of microorganisms present in the host, skin and intestinal epithelial tissues were harvested from three proprietary family lines of Nile tilapia (*Oreochromis niloticus*) that differ by one genetic trait. Prior work demonstrated that one of the genetically-related lines has a much higher growth rate than the other two, when age and environmental conditions are controlled. It was hypothesized that the animal's microbiome was a contributing factor to the observed differences in growth. Therefore, the V4 region of the bacterial 16S rRNA gene was amplified using DNA separately extracted from the scales or midgut portions of the intestines. The resulting PCR products were gel purified and sequenced via Illumina Mi-Seq protocols. Preliminary QIIME bioinformatics analysis of the intestinal samples has revealed noticeable differences in the microbiome structure between the faster growing fish and the other family lines. The most abundant bacterial families in the more productive fish line, *Mycoplasmataceae* and *Fusobacteriaceae*, are virtually absent in the other two lines, which have higher levels of *Enterobacteriaceae*. Analysis of scale samples is on-going. Thus, fish lines differentiated only by the alteration of a single trait exhibited differences in the host-associated microbial communities.

REGULATION OF THE OMEGA-3 FATTY ACID BIOSYNTHETIC PATHWAY AND FATTY ACIDS BIOCONVERSION CAPACITY IN SELECTED RAINBOW TROUT

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Currently, vegetable oils (VO) have become the primary alternative for fish oil (FO), as they are readily available, cost effective ingredients that contain high levels of essential C18 fatty acids (FA). Through a selective breeding program we have developed rainbow trout (*Oncorhynchus mykiss*) families which are improved in their ability to desaturate and elongate α-linolenic acid (18:3n-3; ALA) to produce the n−3 long-chain polyunsaturated fatty acids [LC-PUFA, eicosapentaenoic acid (20:5n-3; EPA), docosahexaenoic acid (22:6n-3; DHA)]. Therefore, we investigated the influence of fish oil (FO) and alternative oil sources [linseed (LO), soy (SO), canola (CO), coriander (CNO) and algae (AO) are rich in ALA, linoleic acid, oleic acid, petroselinic acid and DHA FAs respectively] on growth, muscle tissue FA composition, and lipid-related hepatic gene expression in trout. Six experimental diets (D1: 12%FO, D2: 12%LO, D3: 6% LO+2%SO+2%CO+2%CNO, D4:3%SO+3%CO+6%CNO = 3:3:6), D5: 6%LO+6%AO and D6: 12%AO) were fed in triplicate to trout (initial average weight 474 g) for 9 weeks.

Dietary source of vegetable oils significantly influenced the growth performance and muscle fatty acids profile (Fig. 1) in trout.

Highest weight gain % was observed in AO fed group (D6) whereas lowest value was found in D4. Total saturated FA in muscle was affected by oil sources, diets with LO, SO, CO and CNO exhibited lower deposition than other oil sources. Among VO sources, highest EPA deposition was found in D5 (AO+LO; 1.74%) was similar to D2 (LO; 1.61%), whereas lowest value in D4 (1.26%) which was similar to D3 (1.45%). EPA was absent in diet D4 but still fish muscle contained EPA which suggests that C18:1n-12 (petroselinic acid source CNO) might have converted to EPA. Highest and lowest PUFAs was observed in D2 and D4 respectively. Total EPA and DHA content were highest in D5 which is almost three times higher than D2 fed group. Expression analysis of genes related to the desaturases, dehydrogenase, elongase, carboxylase and de-carboxylase are being analyzed in hepatic and muscle tissue. Conclusively, algae oil can be used as an alternative source of oil to enhance the growth and increased the deposition of EPA and DHA in salmonid fillet for healthy food.

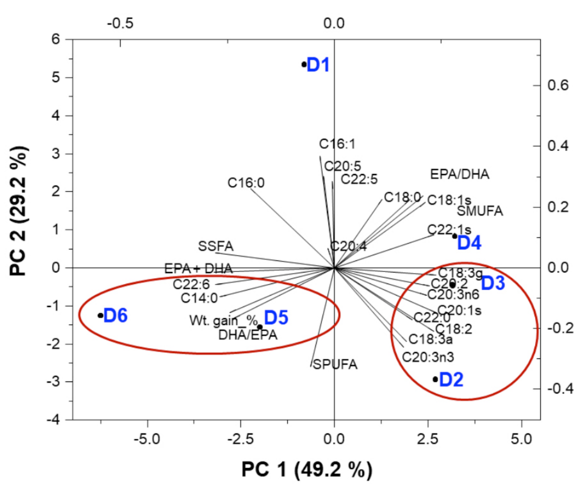

Figure 1: Principal component analysis of fish growth and muscle tissue fatty acid profiles

FISH MUSCLE HYDROLYSATE OBTAINED USING LARGEMOUTH BASS *Micropterus salmoides* DIGESTIVE ENZYMES IMPROVES LARGEMOUTH BASS PERFORMANCE IN ITS LARVAL STAGES

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An incorporation of protein hydrolysates in larval fish diets as an intact protein replacement has been shown to improve larval performance of several species suggesting that the molecular size of the protein fraction is critical to support proper development of larval fish. However, larval capacity to digest dietary components of different molecular weights changes throughout its development. Consequently, the right balance between different sizes of protein fractions in diets is critical to induce positive growth responses in larval stages. Various protein hydrolysates have been obtained using *in vitro* methods that have attempted to reproduce the physiological conditions of the digestive tract. However, the practical application of *in vitro* hydrolysis has not been routinely used by the industry due to complexity and low repeatability. The objectives of this study were: 1) to develop an optimal *in vitro* methodology for fish muscle hydrolysis using largemouth bass (LMB) endogenous digestive enzymes; and 2) to evaluate the effect of dietary inclusion of the fish muscle protein hydrolysate obtained using methodology in Objective 1 on LMB growth, survival, occurrence of skeletal deformities, and whole-body free amino acid composition.

Briefly, LMB digestive tracts were ground and used as a direct source of digestive enzymes. Each digestive tract homogenate was mixed with muscle originating from bighead carp *Hypophthalmichthys nobilis*. After temperature and pH were adjusted to the required level muscle homogenates were mixed with digestive tract supernatants (22^{o}C; initial 3-4 pH for the first one hour to mimic stomach digestion followed by 7-8 pH for two hours to mimic intestinal digestion).

At 5 dph larval LMB were randomly distributed into 50 L tanks, ~270 larvae per tank. Two diets were tested in the study: **control** - based solely on an intact fish muscle as the main protein source and **Hydro** diet - based on 50% intact fish muscle replacement with fish muscle hydrolysate. Both groups received experimental diets in a combination with Artemia nauplii to support larval LMB survival. At the end of the feeding trial LMB in the **Hydro** group had significantly larger final average weight compared to the **control** (0.20±0.01 vs. 0.15±0.02 g, p<0.05). In addition, the **Hydro** group was characterized by significantly higher weight gain compared to the **control** (6341±253 vs. 4873±647%, p<0.05). Finally, the occurrence of skeletal deformities was significantly reduced in the **Hydro** group compared to the **control** (6 vs 23%, p<0.05). These results suggest that *in vitro* hydrolysis of fish muscle using LMB endogenous digestive enzymes is a practical and cost-effective method to obtain dietary protein hydrolysate that supports LMB performance in its larval stages.

DOES DIETARY DIPEPTIDE CONFIGURATION MATTER IN FISH NUTRITION?

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Dietary amino acids (AA), critical for tissue protein synthesis, can be provided in different molecular forms: protein-bound, free amino acids (FAA), or peptides. Fish express several AA transporters in the digestive tract but only two peptide transporters, Pept-1 and Pept-2, have been identified. Although Pept-1 is able to facilitate transport of 400 di- and 8,000 tripeptides across the membrane not all AA configurations in these peptides have been reported to elicit transport currents in biological membranes. The objective of this study was to examine the effect of a wheat-gluten-based diet supplemented with lysine in different molecular forms on growth, PepT1 mRNA transcript levels, and whole body FAA composition in zebrafish *Danio rerio*.

Fish of an initial size of ~0.03 g were randomly distributed into 12 glass aquaria, 30 fish per aquarium. Fish were fed four wheat-gluten based diets supplemented with one of the following: lysine-glycine dipeptide (Lys-Gly), glycine-lysine dipeptide (Gly-Lys), free lysine (Free Lys), or no lysine (-Lys). After 30 days the mean weight and weight gain of the fish fed with the (-)Lys diet was smaller compared to the other three groups. No differences were found, however, between Lys-Gly, Free Lys, and Gly-Lys groups. The Pept1 gene expression in zebrafish intestinal tract showed that Lys-Gly induced higher expression compared to Free Lys and (-)Lys groups but no difference was detected between Lys-Gly and Gly-Lys groups. The Pept1 expression was also not different between Gly-Lys, Free Lys, and (-)Lys groups (Figure 1).

The study showed that zebrafish is able to utilize different forms of dietary Lys efficiently for growth and both Lys-Gly and Gly-Lys seem to have a similar effect on intestinal Pept1 gene expression contradicting previous studies that reported lower affinity of Pept1 towards Gly-Lys. Whole-body FAA composition will be presented during the oral presentation.

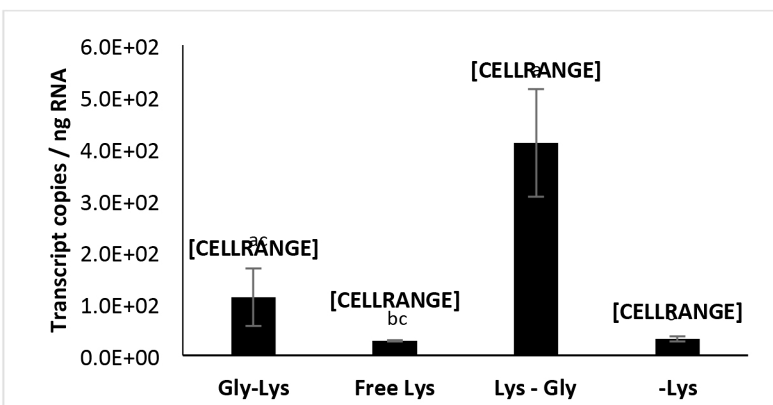

Figure 1. The intestinal Pept1 gene expression between experimental groups. Different letters indicate statistical difference at P<0.05.

EFFECTS OF NEPALI LAPSI *Choerospondias axillaris* (Roxb.) FRUITS EXTARCT ON GROWTH PERFORMANCE AND PROTEIN METABOLISM IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Lapsi (*Choerospondias axillaris*) is a large, deciduous fruit-bearing tree of Nepal. Fruit extracts of lapsi possessed high antioxidant activity. Therefore, our goal of the study was to evaluate the dietary effects of lapsi fruits extracts on growth performance and protein metabolism in rainbow trout (*Oncorhynchus mykiss*)**.** A total of 450 rainbow trout (37.24 ± 0.39 g) were randomly distributed into six groups with three replicates (25 fish per cage), fed six practical diets (isonitrogenous diet 40% protein) such as control diet without the lapsi extract (D1) and the other five diets containing incremental level of lapsi fruits extract at the rate of 100 (D2), 200 (D3), 400 (D4), 800 (D5) and 1600 (D6) mg kg^{-1}. Fish were fed twice per day at satiation level for 90 days.

Results of the study showed that supplementation of lapsi fruits extracts significantly affects ($P<0.05$) the growth performance and nutrient utilization in fish. Highest growth was observed in D4 (440 ppm) fed group whereas lowest value was found in control (D1) but feed conversion ratio (FCR) exhibited opposite trend. Growth results were concurred with protein profile content of serum (protein, albumin and globulin) in fish.

Conclusively, lapsi fruits can be used as an additive in trout diet to enhance the fish growth for sustainable aquaculture.

AQUACULTURE DRUGS: WHAT ARE THEY AND WHY DOES FDA REVIEW MATTER?

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What's an animal drug? A product's intended use determines whether it's a drug or not.
For example, when formalin is used to fix a biopsy sample from a fish, it isn't a drug because the intended use is to preserve the tissue for future study. But when formalin is used to kill external parasites on finfish, it's a drug because the intended use is to treat a disease (parasitism), which makes it a drug under federal law.

Why does FDA review animal drugs? FDA review process ensures that animal drugs meet the following standards defined by law:

- The animal drug is safe and effective for its intended use;
- Food products from treated animals are safe for people to eat (if the drug is for a food-producing species);
- The drug is properly manufactured;
- The drug is properly labeled.

FDA's review also considers:

- The drug's impact on the environment; and
- The safety of the people who will give the drug to the animal or who may come in contact with the drug.

This rigorous review process ensures that the approved animal drug is safe for animals, people, and the environment; effective; high quality; and has complete and truthful labeling.

FDA continues to monitor the drug's safety and effectiveness after it reaches the market. FDA also continues to monitor the drug's labeling, the drug's manufacturing process, and the company's marketing communications related to the drug.

Why should you care? As a fish producer or animal health professional, if you use an aquaculture drug with legal marketing status, you're assured that the drug is safe, effective, manufactured to a high quality standard, and that the label includes all information necessary for you to use the drug safely and effectively, including the risks associated with the drug.

Currently, few legally marketed drugs are available for fish compared to other animals like cats, dogs, cattle, and chickens. Drug companies are often hesitant to spend a lot of resources to develop aquaculture drugs when there is little return on their investment due to the small size of the U.S. aquaculture market. However, you can help change this. You can collaborate with government agencies and other private and public aquaculture professionals and use your facilities and expertise to help get more aquaculture drugs to legal marketing status in the U.S.

ENVIRONMENTAL EFFECTS ON BREEDING PACIFIC OYSTERS *Crassostrea gigas* ON THE WEST COAST, USA

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The Molluscan Broodstock Program (MBP) has been selecting Pacific oysters (*Crassostrea gigas*) on the West coast, USA, since 1996. The initial focus has been improvement in yields though selection for higher growth and survival of families planted at a wide range of different farm sites. Selection of these traits in oysters, and other species grown in the natural environment with little farmer input, is challenging due to high variability in the culture environment over both spatial and temporal scales. Despite these difficulties, harvest yields of MBP-derived families have been found to be 15 to 30% greater after six generations of selection, compared with those of offspring from non-selected "wild" parents.

MBP selects families that perform well across as wide range of different culture conditions, including coastal, inland (Puget Sound, Washington), intertidal and subtidal sites. Heritability values for growth, survival and yield at coastal and Puget Sound sites were moderate-to-high, ranging from 0.36 to 0.62, with higher values occurring at Puget Sound sites. Genetic correlations for harvest traits between these two locations ranged from 0.46 to 0.69 for yield and survival, respectively. Surprisingly, genetic correlations were higher for families planted at subtidal and intertidal sites, ranging from 0.64 to 0.81 for individual average harvest weight and survival, respectively.

These results indicate that Pacific oysters on the West Coast, USA, show considerable phenotypic plasticity across a wide range of farm environments; however, the limited proportion of "generalist" families in cohorts (Fig. 1) will result in long-term loss of genetic diversity unless compensating strategies are implemented by the breeding program.

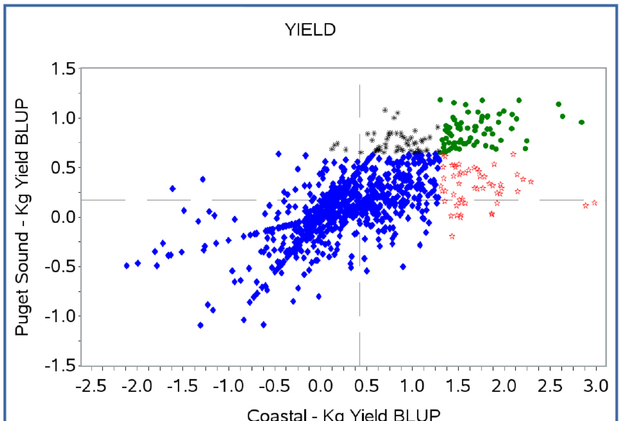

Fig 1. Comparison of BLUP values for yield (kg) of MBP families planted in Puget Sound and coastal sites: top generalists; top 12.5% coastal; top 12.5% Puget Sound; others

MANNA OCEAN FOUNDATION ORGANIC (TO THE CANADIAN STANDARDS) SEAFOOD CERTIFICATION PROGRAM

Donna Lanzetta, Manna Ocean Foundation

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Today there are more people on the planet than have ever died! We will eat more food on Earth in the next fifty years than has been consumed in all of history! Seafood production is an **IMPERATIVE** for our future, and responsibly farmed seafood is part of the solution. In order to achieve the United Nations Sustainable Development Goals (SDGs), we must double our aquaculture production.

Manna Ocean Foundation (MOF) is a 501(c)(3) non-profit corporation with an urgent mission to support sustainable aquaculture production. MOF seeks to encourage responsible farmers of seafood. The MOF Mission includes outreach and education programs to teach about Aquaculture. This mission necessarily includes the promotion of U.S. responsibly produced seafood. To that end, Manna Ocean Foundation is excited to officially announce the launch of our Organic Certification Program!

- Independent auditors will inspect and report;
- Producers compliant with the Canadian Organic Certification Standards will be eligible to apply for an annual license to use the MOF Organic label on their seafood product packages;
- Certification Program with low fees, open to all U.S. seafood farmers;

Today in the United States we are experiencing an annual trade deficit in seafood of approximately 14.5 Billion Dollars. Currently in the U.S., over 90% of the seafood consumed is imported. It is anticipated that the Manna Ocean Foundation Organic Seafood Certification Program will drive sales of U.S. farmed product, as well as provide assurance that seafood is farmed in accordance with Organic Standards of Canada.

IMPACT OF PROBIOTICS AND PREBIOTICS ON POST-LARVAL FLORIDA POMPANO PRODUCTION AND HEALTH

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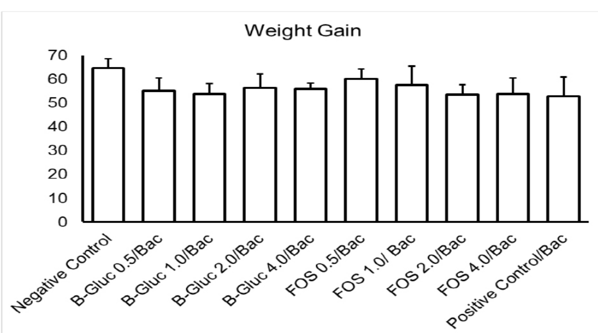
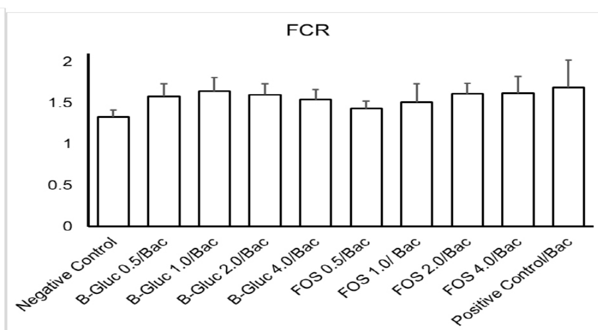
The commercial production of Florida pompano, *Trachinotus carolinus*, has been shown to be feasible based on previous reported joint research projects conducted by FAU-HBOI and the USDA-ARS. Much of the focus of those projects centered on nutrition. Probiotic and prebiotics have been shown to react synergistically, compared to the use of probiotics alone, resulting in enhanced health and production of aquatic species. The present study was conducted to determine whether the addition of various levels of prebiotics to a *Bacillus* sp. probiotic would enhance the production or health of post-larval Florida pompano over that of a basal diet or that of a basal + *Bacillus* sp. enhanced diet.

Ten diets were prepared. The basal diet served as the negative control, while a *Bacillus* sp. dietary addition served as a positive control. Two prebiotics, β-glucan and FOS were substituted for cellulose and added at 0.5, 1.0, 2.0 or 4.0 g/kg^{-1}. There were 4 replicates per treatment. Twenty post-larval Florida pompano (~ 5 g), obtained by ProAquatix, LLC (Vero Beach, FL), were added to one of 40 75-L tanks that were part of an 8,750-L RAS. Fish were reared to ~50 g (~12 weeks). Production data assessed at the end of the experiment included weight gain, specific growth rate and survival. Additional analysis conducted included proximate analysis (whole fish), enzyme analysis (intestinal tissue), and hematological (e.g. blood counts, lysozyme, ACP activity) and immune function assays (e.g. head kidney phagocytic activity, SOD activity).

Production data showed no significant difference between treatment groups based on weight gain (p≤0.120), SGR (p≤0.079), FCR (p≤0.191) and survival (Range 95-100%; p≤0.205). Although production from 5-50 g was not enhanced by addition of pro or prebiotic treatments health parameter evaluations may provide more information concerning treatment groups. These analyses are currently underway and will be reported at AA2020.





IMPACT OF DIETS AND WATER SOURCE ON PRODUCTION OF THE SEA CUCUMBER *Holothuria floridana*

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Sea cucumbers are a good candidate species for aquaculture due to their high dollar value and strong demand for human consumption and medicinal use. As detritovores they were considered as an extractive culture component for the HBOI-LB-IMTA system. Preliminary observations of the sea cucumber, *Holothuria floridana*, maintained in unfiltered system water suggested that they preferred fresh *Ulva lactuca*, over biofloc solids. Skin lesions were observed in some of the sea cucumbers, and amphipods, present in the unfiltered water, were postulated to be responsible. To better understand the impact of diet and water source on *H. floridana* production and lesion development, a 12-week 2x3 factorial experiment was designed.

Ten sea cucumbers (11.5±1.8g) were randomly added to one of twenty-seven 30-L square tanks placed into troughs, with a flow through system. Provided water sources were salt well water, filtered IMTA water, or unfiltered IMTA water. Pelleted diets were manufactured that replaced 20% of fish meal with biofloc or *Ulva* meal. Sea cucumbers were fed a 100% biofloc, 100% Ulva or 50% biofloc and 50% Ulva diet, twice per day at 5% body weight. Dissolved oxygen, temperature and salinity were taken daily and pH continuously. Ammonia, nitrite and alkalinity was measured twice per week. Mortality was assessed daily, and presence of lesions noted; individuals were weighed bi-weekly.

No significant difference was seen between treatments with regards to final growth or survival. Initially (wk 0-6), highest survival was seen in treatments receiving filtered water (93%), and lowest in treatments receiving unfiltered water (78%), but differences were not significant (P=0.0656). By week 8 survival was low in all treatments (53-77%), and following impacts bought on by Hurricane Dorian continued to decline; final survival was 27-60%. Growth initially increased in treatments receiving filtered water, regardless of diet, but decreased to initial weights by week 4, and continued to decrease in all treatments thereafter.

Amphipod presence and water chemistry may have initially played a role in terms of survival in the unfiltered IMTA treatment. Amphipods were an issue in 78% of unfiltered IMTA treatments, compared to 45% of filtered, and 18% of well water, and amphipod presence was associated with lesion development. Nitrites were higher and pH was lower in unfiltered IMTA treatments as well. However, low survival and negative growth were apparent in all treatments by week 8 indicating that other factors were responsible for poor production.

GUIDELINES AND RECOMMENDATIONS TO ENHANCE WATER BIOSECURITYAND PROMOTE FISH PRODUCTION USING ULTRAVIOLET DISINFECTION

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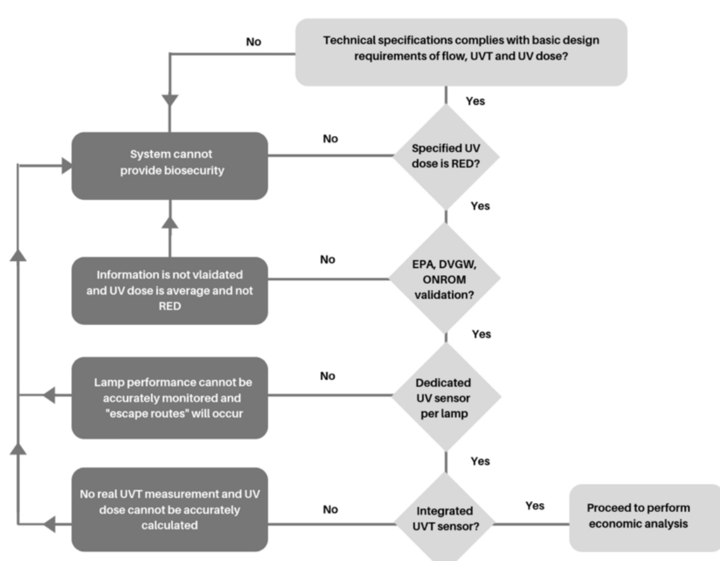
Aquaculture has been identified and welcomed as an important "remedy" to successfully address the challenges to global food security arising from climate change and intensified by the projected 2050 world population of 9.5 billion (National Research Council, 2015). However, marine and land-based aquaculture facilities are being subjected to an increase of biosecurity threats resulting from intensified production and cross pollution on the quality of their influent water. Ultraviolet (UV) disinfection is probably the safest, most effective for treatment of pathogens such as bacteria, microorganisms, and viruses.

At present, there are more than 20 different kinds of commercially available UV technologies offered into the aquaculture market, varying in performance and price. UV systems are very sensitive to water conditions, depend on proper operation of all lamps, and require routine but dedicated maintenance as control and monitoring of UV systems is difficult at best and often times, there can be no real-time indication as to the effectiveness of the UV treatment. Despite its popularity, little information is available to producers about how to qualify UV systems according to site-specific needs. Navigating the selection can be overwhelming and a systematic approach is needed. A UV system performance and cost evaluation process diagram (TABLE 1) has been developed to assist aquaculture facilities in the selection of the most appropriate UV technology to meet their application specific needs. In addition, the convoluted UV terminology needs to be simplified and clearly designated. With these tools facilities can more appropriately select a UV technology based on a defined set of parameters and requirements which will increase the chances of the UV system to provide the site with the required water biosecurity.

Following this systematic approach will aid in prevention, control, eradication of risks to life and health, and a reduction in the economic impact of diseases.

TABLE 1: UV System Performance and Cost Evaluation Process Diagram



REDUCED TANK-CLEANING FREQUENCY, BUT NOT CO-CULTURE WITH SEA CUCUMBERS, IMPROVES SURVIVAL OF LARVAL SABLEFISH *Anoplopoma fimbria*

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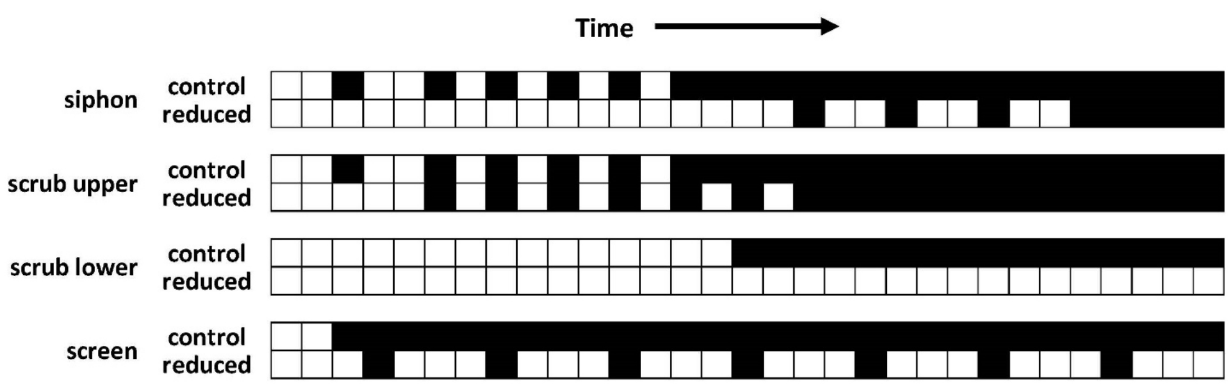
The larval stage for marine fishes is labor intensive and characterized by high mortality. These challenges reduce profitability and limit numbers of fingerlings available for further rearing. Significant labor is dedicated to tank cleaning, to remove organic matter which enters rearing tanks in the form of feeds, feces, dead larvae, and turbidity agents such as algae. However, tank cleaning protocols are often a product of tradition or intuition, rather than replicated experiments. In this study, we compared three tank cleaning protocols for larval sablefish.

Cleaning activities were typical for marine larviculture, including siphoning of tank bottoms, scrubbing of tank walls, and spraying of center standpipe screens (see figure below). The control treatment approximated our traditional sablefish protocol, while a "reduced frequency" treatment conducted most of the same cleaning activities, but at a lower frequency. A third "multi-trophic" treatment cleaned at that same reduced frequency, but also co-cultured sea cucumbers (*Apostichopus japonicas*) that we hypothesized would help remove excess organic matter and improve larval survival.

The reduced frequency treatment showed improved larval sablefish survival relative to control, but sea cucumbers drastically reduced larval survival in the multi-trophic treatment. Water quality measures did not differ among treatments, and microbial community was affected by date but not treatment, supporting the conclusion that cleaning frequency can be reduced. This reduced cleaning protocol allows for less labor without negative impacts on larval survival.



EFFECT OF DIETARY LINOLEIC ACID, EICOSAPENTAENOIC ACID AND DOCOSAHEXAENOIC ACID ON THE GROWTH AND FILLET FATTY ACID PROFILE OF OLIVE FLOUNDER *Paralichthys olivaceus*

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This study was conducted to investigate the effects of different dietary lipid sources on the growth, feed utilization, body composition and tissue fatty acid profile of olive flounder *Paralichthys olivaceus*. Five isonitrogenous and isocaloric diets were formulated by adding various lipid sources including soybean oil (SO), eicosapentaenoic acid triglyceride (EPATG) and ethyl ester (EPAEE) forms, docosahexaenoic acid triglycerides (DHATG) and a 1:1 blend of soybean oil and DHATG. Triplicate groups of fish (6.8±0.01 g) were fed one of the experimental diets to apparent satiation twice daily for 8 weeks. Fish fed the DHATG diet had the highest growth, protein efficiency ratio and feed efficiency values which were significantly higher than those fed the SO and EPAEE diets. Whole body proximate composition and somatic parameters were not influenced by the dietary treatments. Muscle of fish fed with SO diets were rich in 18:1n-9, 18:2n-6 and 18:3n-3, whereas those of fish fed with EPATG, EPAEE and DHATG diets were rich in n-3 polyunsaturated fatty acids (HUFA). These findings indicated that the inclusion of n-3HUFA oils in olive flounder feed could be beneficial for the fish while simultaneously increasing the concentration of beneficial n-3HUFA in fish fillets destined for the human consumer.

HEAT SHOCK PROTEIN 70 GENE EXPRESSION AND STRESS RESPONSE OF PACIFIC ABALONE (*Haliotis discus hannai*) TO TEMPERATURE, SALINITY AND AIR-EXPOSURE STRESS

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Hsps play an important role in the cell's response to a wide range of damaging conditions, and they are important for the recovery and survival of organisms. An important member of the Hsp family, Hsp70 is involved in chronic temperature acclimation and acute responses in aquatic animals. This study observed the expression regulation of the Hsp70 gene, and the changes in hemolymph glucose levels as a stress response in Pacific abalone (*Haliotis discus hannai*) during exposure to water temperature shock, low-salinity and air-exposure. In the initial period of high temperature stimulation, the expression of Hsp70 in Pacific abalone muscle increased up to 4 times and then decreased at high temperatures for 6 hours. In the recovery phase, it increased rapidly to 8 times its normal levels. In contrast, there was no significant difference in Hsp70 expression under hypothermic stimulation. Under low salinity stimulation, Hsp70 increased rapidly up to 8 times and then decreased as much as the control in the recovery phase. The difference from air exposure stress was small compared to the control. The hemolymph glucose was measured and gradually increased under all stress conditions and then decreased during the recovery phase. Pacific abalone is a cold water species, so it is not stressed at low water temperatures, but high water temperatures and low salinity should be considered to be a variables in the rate of Hsp70 expression.

TWO GENERATOINS OF DIVERGENT SELECTION FOR FILLET YIELD IN RAINBOW TROUT *Oncorhynchus mykiss*: DIRECT AND CORRELATED RESPONSE TO SELECTION

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Fillet yield (FY) is heritable in rainbow trout populations, and genetic improvement of FY has the potential to improve efficiency, sustainability, and profitability of production. Despite this, FY has received little attention in commercial breeding programs because it cannot be measured directly on breeding candidates, it is difficult to measure on a large number of pedigreed fish, and it exhibits less phenotypic variation compared to body weight (BW) traits. Scientists conducted 2 generations of divergent, family-based selection for FY in a closed, pedigreed population previously selected 5 generations for improved growth to develop high-yield (ARS-FY-H), randomly-mated control (ARS-FY-C), and low-yield (ARS-FY-L) lines. Approximately 100, 28, and 23 full-sib families were produced each generation for the 3 lines, respectively, and ~5 fish/family were evaluated for FY (trimmed fillet ÷ BW) at ~15 months of age (~1.8 kg). Breeding values were estimated each generation using an animal model that included fixed effects of year, harvest group, and harvest BW (linear covariate) and random effects of animal and family.

After 2 generations of selection, the ARS-FY-H line had greater ($P < 0.01$) FY (54.3 ± 0.1% vs. 52.1 ± 0.5% and 52.2 ± 0.3%) and less ($P \leq 0.02$) viscera yield (8.7 ± 0.1% vs. 10.1 ± 0.5% and 10.4 ± 0.3%) compared to the ARS-FY-C and ARS-FY-L lines, respectively, and head yield did not differ ($P = 0.22$) among genetic lines. BW at harvest tended ($P = 0.07$) to be greater in the ARS-FY-H line (1,952 ± 20 grams) compared to the ARS-FY-C (1,786 ± 88 grams) and ARS-FY-L (1,798 ± 41 grams) lines. The ARS-FY-C and -L lines did not differ ($P \geq 0.56$) for any of the traits. Whereas selection response for these traits was symmetric after 1 generation (data not shown), the asymmetric response in the 2nd generation is currently unresolved. Furthermore, a long-term feed efficiency trial was conducted using 20 second-generation families each from the ARS-FY-H and -L lines. A total of 500 fish per genetic line (25 fish/family; ~175 grams) were randomly assigned to 5 replicated tanks within line. Feed intake was recorded for each tank for ~4 months until the fish reached ~1 kg, at which time all fish were harvested and BW and head-off gutted carcass weight was recorded individually for each fish. Feed conversion was calculated for each tank for the entire trial as unit of feed consumed per unit of BW gain (F:G), or unit of head-off gutted carcass produced (F:C). The ARS-FY-H line had similar F:G (1.18 ± 0.02; $P = 0.27$), but improved F:C (1.30 ± 0.02; $P = 0.04$), compared to the ARS-FY-L line (1.20 ± 0.02 and 1.36 ± 0.02, respectively).

Collectively, these studies suggest that FY can be improved in rainbow trout populations via family-based selection, with favorable correlated responses for viscera waste yield and feed efficiency, and no adverse effect on growth performance.

GENETIC CONSEQUENCES OF POND PRODUCTION OF A NATURAL PIKEPERCH *Sander Lucioperca* POPULATION

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Our target species the pikeperch (*Sander lucioperca* L.) represents a great potential for the diversification of European aquaculture. Concerning a commercial, cultured species like pikeperch, the efficiency of production and possible breeding programs in the future are largely influenced by the genetic variability of the species. The basis of this variance is the genetic diversity of the native populations. The aim of the present study is to describe the genetic consequences of pond culture in pikeperch.

The estimation of the genetic divergence of a cultured stock originating from a native population during a 10 years period or a single generation was targeted by the comparison of the genetic variability of a wild population of pikeperch (*Sander lucioperca* L.) living in Lake Balaton, a broodstock originating from the lake and its offspring generation. A total of 15 microsatellite DNA markers were used to genotype all individuals.

The genetic variability of the wild, Balaton population and the two reared stocks are similar. According to the expectations the diversity decreased in the broodstock and in the fingerling compared to the wild population. The fingerling showed even lower diversity than the broodstock but the difference was not significant (Table 1).

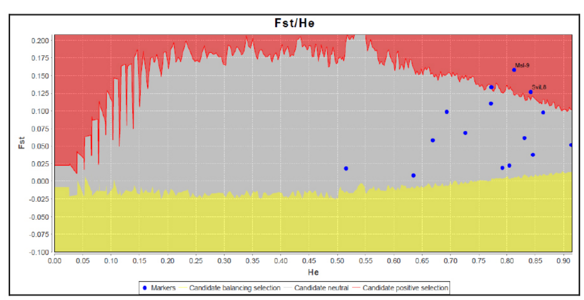
The results of the Fst outlier test (Lositan) on our dataset defined that two of the loci were outliers marked as candidate for positive (separating) selection Figure 1.

Our study demonstrates that pond culture (semi-natural conditions) could result in a similar situation as hatchery rearing. Although the genetic diversity could be maintained at a high level, the lower effective population sizes led to partially decreased variability compared to the natural populations. Despite the similar conditions and the relaxed predation pressure, alterations in the environmental parameters bring on selection even as it was detected in two of the 15 markers in the present study. The study was supported by the Government of Hungary within the project GINOP-2.3.2-15-2016-00025.

Table1.
Basic molecular genetic parameters of the populations under study.

Pop	Balaton	Broodstock	Fingerling
N	46	40	44
uH_e	0.71±0.03	0.72±0.02	0.69±0.03
H_o	0.67±0.04	0.70±0.03	0.68±0.04
N_A	10.93±1.06[a]	7.53±0.83[b]	6.00±0.36[b]
AR	10.49±3.81[a]	7.53±3.12[b]	5.95±1.37[b]
AR_P	3.74±1.94[a]	0.41±0.71[b]	0,0±0,0[b]
F_{IS}	0.053±0.03	0.016±0.03	0.001±0.04

Figure 1
Identification of outlier loci by Lositan



STATUS AND FUTURE OF AQUACULTURE IN THE GREAT LAKES BASIN: MOVING FORWARD IN A CHANGING LANDSCAPE

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Globally, aquaculture production is growing, and outpacing wild capture fisheries that have been reasonably stable the last 2 decades (Fig. 1). Despite vast freshwater resources, the aquaculture industry in the Great Lakes has faced many challenges over the past 2 decades, and aside from mollusk production (mainly marine and outside the Great Lakes basin), Great Lakes state's aquaculture sales are in the bottom 50% for all US states. Barriers to expanding production in Great Lakes states include consumer stigma, energy costs, regulatory hurdles, and lack of startup funds. More recently, efforts have been made to better understand and address barriers facing aquaculturists in the Great Lakes basin, and multiple groups are working together and individually to bolster aquaculture in the region. Here we describe the status of Great Lakes aquaculture production and efforts that are underway to identify and address factors that have hindered aquaculture production in the region.

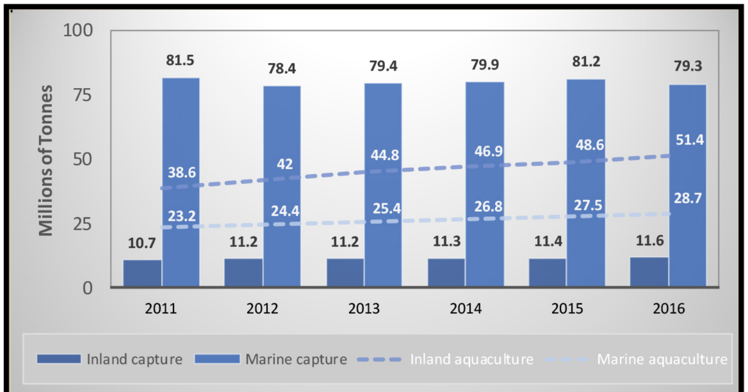

Figure 1. Global aquaculture (dotted lines) compared to capture fisheries (solid bars) with millions of tonnes in text for each production type. Data from FAO 2018.

OXIDATION OF ENERGY SUBSTRATES IN TISSUES OF WHITELEG SHRIMP

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The whiteleg shrimp (*Litopenaeus vannamei*) is the most widely cultivated penaeid shrimp in the world. The recommended dietary protein, lipid and starch levels for this animal are about 30-40%, 5-10% and 15-20%, respectively. Our previous study has reported that amino acids are the primary energy substrates in major tissues of some fish species. The present study was conducted to test the hypothesis that amino acids are also oxidized at a higher rate than carbohydrates (e.g., glucose) and fatty acids (e.g., palmitate) to provide ATP for tissues of whiteleg shrimp. The hepatopancreas, intestine, gill, and muscle were isolated from juvenile shrimp (~3.0 g) and incubated at 26 °C for 2 h in 1 ml of modified KHB buffer (pH 7.4, with 5 mM D-Glucose) containing 2 mM glutamate, 2 mM glutamine, 2 mM aspartate, 2 mM alanine, 2 mM leucine, or 2 mM palmitate with their respective [U-^{14}C]-labeled tracers. In parallel experiments, a tissue was incubated in the presence of a tracer plus a mixture of the unlabeled substrates (i.e., 5 mM glucose, 2 mM each of amino acids and fatty acids). $^{14}CO_2$ was collected to calculate the rates of substrate oxidation. In the intestine and hepatopancreas, the rate of glutamine or glutamate oxidation was greater than that of any other substrate in KHB buffer with a single energy substrate; however, the rate of oxidation of aspartate was greater than that of any other substrate in the presence of a mixture of energy substrates. In gills, the rate of oxidation of glucose, glutamate, glutamine or aspartate was greater than that of any other substrate in the presence of a single substrate or a mixture of substrates. In skeletal muscle, glutamine and aspartate were the most important energy substrate in the presence of a single substrate or a mixture of substrates, respectively. In all the tissues studied, amino acids provided the most ATP, and palmitate was a minor energy substrate. Collectively, these results indicate that amino acids were the major metabolic fuels for tissues of whiteleg shrimp, as recently reported for Largemouth bass and hybrid-striped bass.

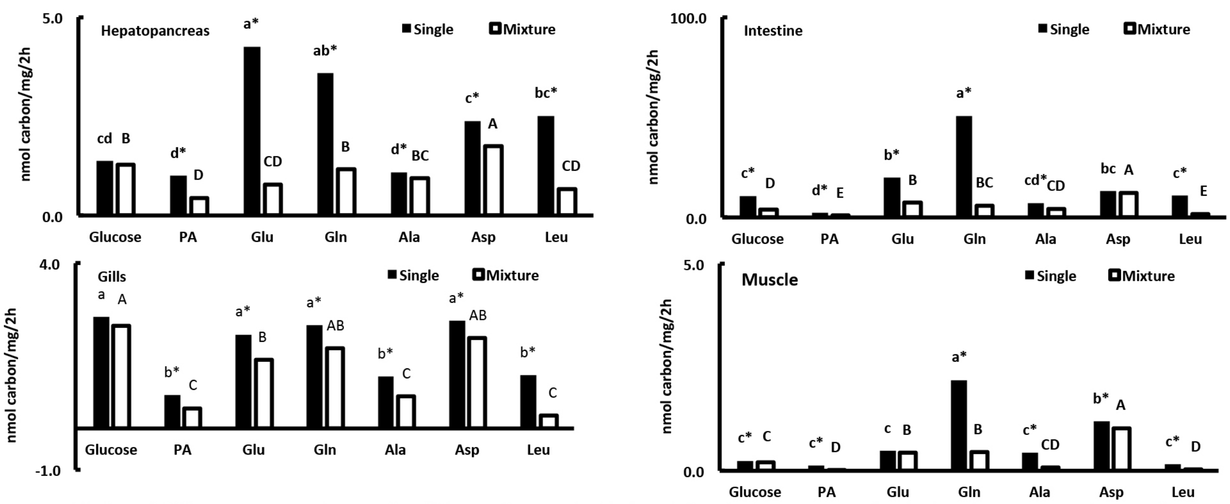

Fig 1. Oxidation of different energy substrates by different tissues of whiteleg shrimp in the presence of a single substrate or a mixture of substrates. *P < 0.05 vs the value for the mixture of substrates. Values with different letters (a, b, c) are significantly different among different single substrates(P < 0.05). Values with different superscripts (A, B, C) are significantly different among different substrates in the presence of their mixture (P < 0.05).

DIETARY SUPPLEMENTATION WITH MICROBIAL BIOMASS (NOVACQ™) IMPROVES WHITELEG SHRIMP GROWTH

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The whiteleg shrimp (*Litopenaeus vannamei*) is the most widely cultivated penaeid shrimp in the world, and is a euryhaline species that can tolerate a wide range of salinities (0.5–45 ppt). As a result, inland aquaculture of shrimp in low salinity (< 5 ppt) waters is popular in many regions worldwide. In this study, we evaluated the effect of a microbial biomass (Novaco™) on shrimp growth. Five experimental diets were formulated, including 20% fishmeal and 0, 5 or 10 % Novaco™, or 15% fishmeal and 5 or 10 % Novaco™. There were 4 thanks per treatment group with 20 juvenile whiteleg shrimp (initial body weight of ~0.5 g) per tank (50 L water, 3-5 ppt salinity). Shrimp were assigned randomly into one of the five treatment groups, and fed their respective diets to satiety twice per day. The feeding trial was conducted for 8 weeks. The results indicated that the survival of shrimp at the end of experiment did not differ among treatment groups ($P = 0.96$). Shrimp fed the 5% of Novaco™ diet exhibited improvements in growth performance (+15.8 %, $P = 0.01$) and feed efficiency (+18.6 %, $P = 0.04$) when diets contained 20% fishmeal. The weight gain of shrimp fed the 15% fishmeal diet and 5% or 10% Novaco™ did not differ (P = 0.95 or 0.80, respectively) from that for shrimp fed the 20 % fishmeal diet without Novaco™. Interestingly, the concentrations of some amino acids (including taurine and glutamine) in hemolymph were greater ($P < 0.05$) in shrimp fed the 5% Novaco™ diet, compared with the control diet. In conclusion, the appropriate supplementation with microbial biomass (Novaco™) to practical feeds enhanced the bioavailability of some dietary amino acids, growth performance, and feed efficiency in whiteleg shrimp raised under a low salinity water environment. We suggest that addition of 5 % Novaco™ to practical diets for whiteleg shrimp can reduce the inclusion level of fishmeal in the diet from 20 % to 15 % with benefits of decreasing nitrogen excretion and sustaining the environment

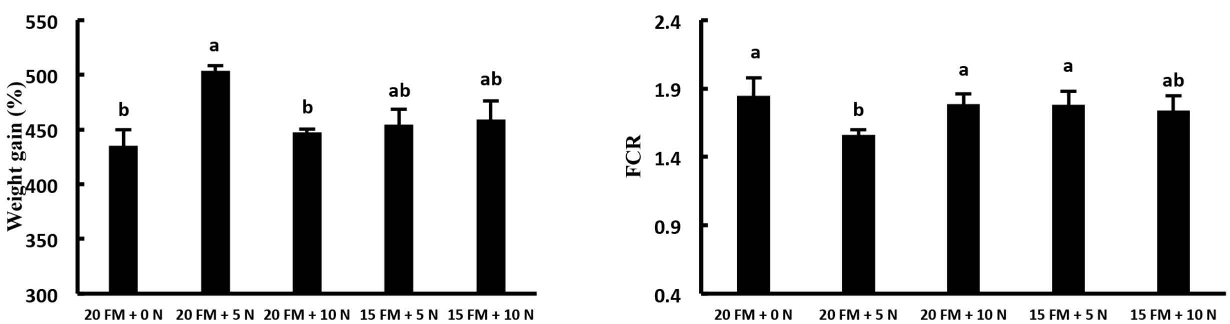

Fig 1. The growth perforemance of shrimp fed different experimental diets after 8 weeks.
20 FM + 0 N: 20% fishmeal (FM) and no microbial biomass (Novaco™); 20 FM + 5N: 20% fishmeal wand 5% microbial biomass (Novaco™); 20 FM + 10 N: 20% fishmeal and 10% microbial biomass (Novaco™); 15 FM + 5N: 15% fishmeal and 5% microbial biomass (Novaco™); 15 FM + 10 N: 15% fishmeal and 10% microbial biomass (Novaco™).
a-b: For weight gain or feed conversion ratio (FCR, feed/gain), means not sharing the same letters differ ($P < 0.05$), as analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison test.

GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* LARVAL IS SENSITIVE TO ACIDIFICATION

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Giant freshwater prawn *Macrobranchium rosenbergii* is one of the popular market demand and valuable species as freshwater crustacean delicacy especially in Asia markets. However, farming of giant freshwater prawn is experiencing inconsistent quality and quantity of post-larvae supply. Limitation of quality seed production is believes due to changing of environment quality that influencing overall food chain availability and habitat lost in nature. Although artificial seed production technology have been established for decades, but hatchery seed production still rely on wild broodstock collection. Thus, overall giant freshwater prawn seed production remain uncertain. In order to produce high quality and consistent quantity post-larvae, ensuring early life performance is a crucial key to culture freshwater giant prawn successfully. Among the environment factors, water pH is one of the factor affecting early life performance. Small degree of water pH fluctuation is known to pose significant impact to biological processes of aquatic life. Therefore, this study was planned to examine the effect of water acidification on early life performance of freshwater giant prawn with specifically focus on feeding, growth and molting performances. Experiment was designed with three different acidic water pH (neutral – 7.7±0.4; mild-acidic – 6.4±0.5 and acidic – 5.4±0.2 by using HCl titration) in triplication with stocking density at 50 larvae/L. Throughout 30 days culture period, feeding rate, survival, growth and molting stage to post-larvae were monitored. As expected, giant freshwater prawn highly sensitive to acidic pH with no larvae survive beyond 48h under acidic condition. Meanwhile, larvae exposed to mild-acidic pH significantly depressed feeding, growth and survival rates as compared to neutral pH. Larvae exposed to mild-acidic water pH experiencing longer larvae period for 30 days prior metamorphosed into post-larvae stage. Whilst under neutral water pH, larvae metamorphosed into post-larvae was recorded at day-22. Ultimately, this study proved that giant freshwater prawn *M. rosenbergii* larval is highly sensitive to acidification and farming of this species should be avoided from low water pH condition.

DEVELOPMENT OF THE WIRELESS SENSING EMBEDDED SYSTEM FOR AQUACULTURE

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This study is to develop the wireless sensing embedded system for aquaculture. Taiwan is located in a subtropical region, affected by geography and extreme climate variability. Extremely high and cold temperature occur frequently. Also, typhoons occur in summer every year. Seawater intrusions and floods in coastal areas occur large number of fish died, so aquaculture industry and fishermen suffer heavily economic losses. In addition, the remaining feeds and metabolites rich of organic and inorganic materials can affect the aquaculture environment. Nowadays people engaged in aquaculture are gradually aging, demand for manpower will face challenges. However, with the development of computer and sensing technology, the wireless sensing embedded system will be developed and established in the aquaculture environment. In this study, the central processing system and water quality monitoring platform system are established with LabVIEW software. A central processing system is set up by NI myRIO platform. The water quality monitoring platform system is set up by Arduino board and pH, temperature, dissolved oxygen and salinity sensors. A central processing system and the water quality monitoring platform system communicate via XBee wireless transmission. Data obtained from the water quality monitoring platform system are transmitted to the central processing system for storage and analysis. Data are recorded every one hour with pH, temperature, dissolved oxygen and salinity sensors. Finally, data can display in real time and fishermen can find out the water quality status of the aquaculture environment. Therefore, the wireless sensing embedded system can be successfully applied for aquaculture with high sensitive, high stability and high linearity. Fig.1 shows the system architecture of the wireless sensing embedded system for aquaculture. Layout of the wireless sensing embedded system is shown in Fig. 2.

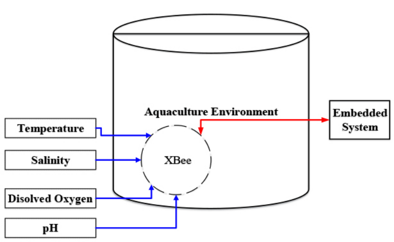

Fig.1 the system architecture of the wireless sensing embedded system for aquaculture

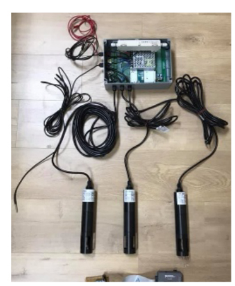

Fig.2 Layout of the wireless sensing embedded system

SELECTIVELY IMPROVING STRAINS OF SUGAR KELP *Saccharina latissima* FOR FOOD AND FUEL

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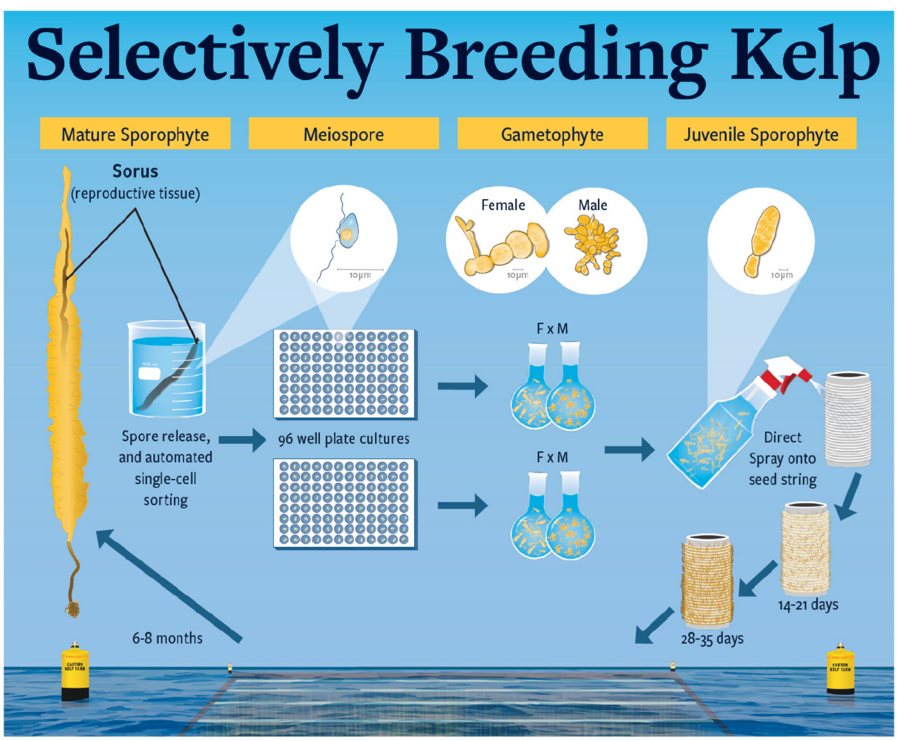
As part of ARPA-E's MARINER program, we are pursuing a selective breeding program to improve the productivity and composition of *Saccharina latissima,* which could serve as feedstock for biofuels. A MARINER goal is to develop tools and a pathway toward low-cost (< $80/DWT) feedstock that could ultimately supply 10% of US liquid transportation fuels. In our progress to date, USDA/Cornell and HudsonAlpha have employed PacBio and Illumina sequencing to create a deep-sequenced reference genome and establish a variant catalog for our founding populations and families. WHOI, UCONN and GreenWave have started a second season of field trials of hundreds of founding families. Each family consists of hundreds of unique sporophytes resulting from crosses generated from hundreds of microscopic gametophytes isolated from more than a dozen wild collections in New England. These families were planted in "common garden" farm arrays over two seasons (2018 and 2019) in New Hampshire and Connecticut. Analysis of our phenotypic and genotypic results will be presented along with our progress in identifying variants significantly associated with primary productivity and composition traits. One project goal is to develop methods to predict offspring (sporophyte) performance based upon genotype and breeding values of parents (gametophytes) for rapid cycle breeding approaches and to improve the efficiency on-farm testing. Ultimately, our goal is to select sugar kelp best suited genetically for offshore farm environments and possessing qualities of 10% increased dry matter yield per unit area per generation, and improved composition for use as a bioenergy feedstock.



CHARACTERIZATION OF LARVAL DIGESTIVE SYSTEM ONTOGENY IN *Corydoras aeneus*

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Understanding species-specific larval digestive system ontogeny is critical for formulating effective feeds and feeding protocols in aquaculture. Some species exhibit extended agastric larval stages, during which time hydrolysis of macronutrients is dependent primarily upon digestive enzymes of pancreatic and intestinal origin. Other species emerge from the egg with a fully formed and differentiated gastrointestinal system, indicating a much higher capacity for digestion and assimilation of diverse prey. The current study used histological and histochemical preparations, as well as analysis of digestive enzyme activities, to characterize the embryonic and larval digestive system ontogeny in the Bronze Cory *Corydoras aeneus*.

Samples of 15 individuals were taken at 3 days post fertilization (embryos), 1 days post hatch (dph), 2 dph, 3 dph, 5 dph and 8 dph. Five fish were assayed to determine trypsin and bile-salt dependent lipase activities, five to determine pepsin activity, while the remaining five were fixed in Trump's solution for histological processing. Histological processing consisted of fixation in epoxy, followed by staining with hematoxylin and eosin, as well as periodic acid Schiff stains to visualize the production of neutral mucopolysaccharides, which are present during acid production by gastric glands. A stomach anlage was apparent in the 3 dpf embryos, although gastric glands were not visualized. A functional stomach was apparent at 1 dph, which preceded the complete absorption of yolk and the onset of exogenous feeding. Although gastric glands were present at 1 dph, pepsin activity was not detected until 2 dph (Figure 1), when exogenous feeding began. These results corroborate preliminary research that indicated a high digestive competency of *C. aeneus* from first feeding, in which commercially available microparticulate diets outperformed live *Artemia* nauplii.

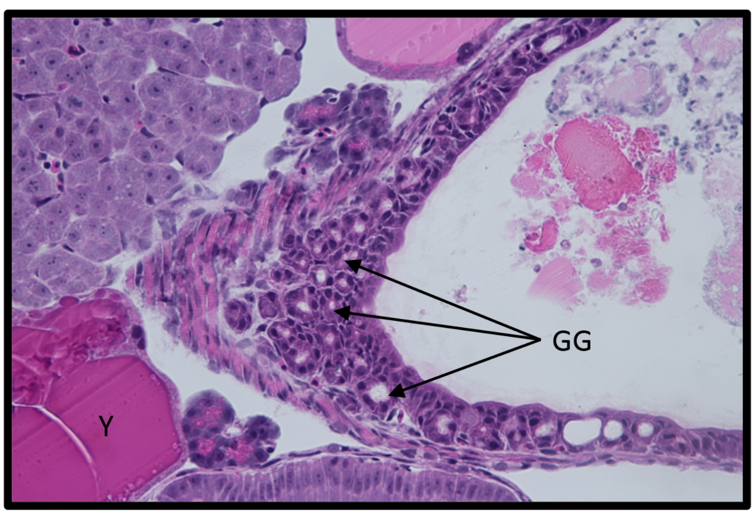

Figure 1. Photomicrograph of *C. aeneus* gastrointestinal tract at 2 dph. Gastric glands (GG), unabsorbed yolk (Y).

HISTOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF LARVAL DIGESTIVE SYSTEM ONTOGENY IN TWO CHARACID SPECIES TO INFORM WEANING PROTOCOLS

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The intensive culture of characid teleosts for ornamental trade is highly dependent on live *Artemia* nauplii through the larval stage. Live feeds exhibit disadvantages relative to prepared microparticulate diets (MDs), specifically pertaining to availability, labor, and cost. Larval Black Tetra*Gymnocorymbus ternetzi* and Neon Tetra *Paracheirodon innesi* exhibited poor survival and growth for MDs relative to *Artemia*. Following confirmation of live feed dependence at first feeding, digestive system ontogeny was characterized using histology, histochemistry, and digestive enzyme activities from the onset of exogenous feeding through the larval stage. Both species exhibited an agastric, altricial larval stage, as well as low digestive enzyme activity at the onset of exogenous feeding followed by abrupt increases in trypsin, lipase, and pepsin activity. In *P. innesi*, histological differentiation of the stomach, including gastric gland formation and production of neutral mucopolysaccharides, as well as the onset of pepsin activity, did not occur until 20 days post hatch (dph; Figure 1). For *G. ternetzi*, these developmental milestones were not reached until 22 dph. This shift from agastric to gastric digestive modes is indicative of a proliferation of digestive capacity and subsequent prey diversity.

Based on this information, experiments were conducted to evaluate different weaning times from *Artemia* to a MD. For each species, twenty replicate tanks were fed *Artemia* exclusively from hatch through the end of the trial, a MD exclusively, or were transitioned from *Artemia* to a MD at three different timepoints. For *P. innesi* fed until 32 dph, and weaning beginning at 12 dph and 17 dph, survival was similar to live *Artemia* (mean: 22.0 ± 1.7%), while weaning beginning at 22 dph resulted in lower survival (16.2 ± 1.3%); MD only resulted in the lowest survival (0.8 ± 0.3%). For *G. ternetzi* fed until 33 dph, weaning beginning at 13 dph exhibited higher survival (20.6 ± 1.8%) than live *Artemia* and weaning beginning at 18 dph and 23 dph (mean: 13.6 ± 1.5%), while the MD resulted in the lowest survival (0.8 ± 0.2%). For both species, weaning did not result in statistically higher growth than *Artemia*. These results indicate that weaning is possible prior to gastric differentiation, potentially resulting in the reduction of *Artemia* use in the larval culture of both characid species.

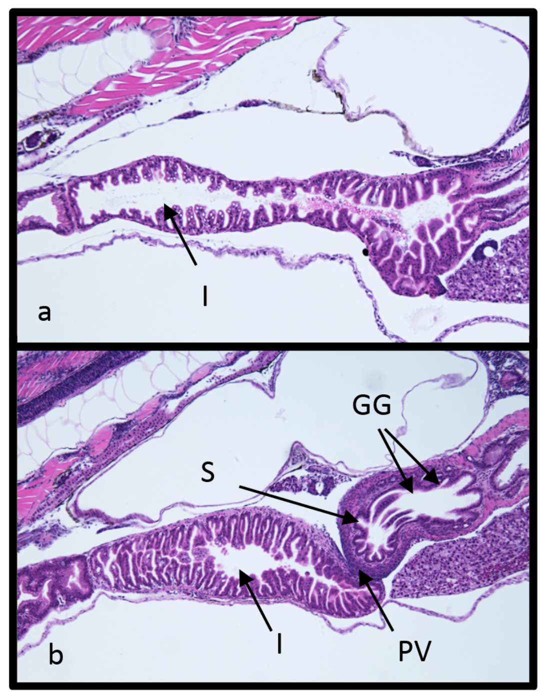

Figure 1. Photomicrographs of *P. innesi* gastrointestinal tract at 17 dph (a, 100x), 20 dph (b, 100x). Stomach (S), gastric glands (GG), pyloric valve (PV), intestine (I).

EFFECTS OF CHOLINE ON LIVER LIPID COMPOSITION, LIVER HISTOLOGY AND PLASMA BIOCHEMISTRY OF JUVENILE YELLOWTAIL KINGFISH *Seriola lalandi*

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Choline is an essential nutrient typically supplemented in aquafeed. Effects of choline-deficiency in animals include reduced growth, liver lipid accumulation and dysfunction. Yellowtail kingfish (*Seriola lalandi*; YTK) has a relatively high dietary choline requirement. A greater understanding of liver lipid composition and health condition can provide greater insight into the effect of choline on YTK health. Thus, this study aimed to identify the effects of dietary choline on liver lipid composition, liver histology and plasma biochemistry of juvenile YTK.

An eight-week feeding experiment was conducted in a recirculated aquaculture system where fish (initial weight 156.27 ± 15.27 g) were reared at 16°C and fed five isonitrogenous and isocalorific semi-purified diets containing 0.59, 1.25, 1.56, 3.11, 6.22 g choline kg^{-1} diet (Diet 1 to Diet 5) by adding graded levels of feed-grade choline chloride (CC). Diet 1 to Diet 5 contained three grams of 2-amino-2-methyl-1-propanol (AMP) kg^{-1} diet to prevent *de novo* choline synthesis. A control diet devoid of AMP (Diet 6; measured choline 3.29 g kg^{-1}) was formulated the same as for Diet 4. After the feeding experiment, apparent digestibility of choline and lipid was determined.

After eight weeks, increasing dietary choline significantly improved choline and lipid digestibility. For liver, free fatty acids were the main component of lipid in fish fed diets containing AMP instead of triacylglycerol. Increasing dietary choline did not affect liver phospholipid and sterol content; however, plasma cholesterol increased significantly. Bile duct necrosis was affected by dietary choline (P<0.05; Fig. 1a) whereas hepatocyte necrosis was affected by AMP (P<0.05; Fig. 1b) indicating that this compound may be toxic. This study indicates that improved choline and lipid digestibility might be the driver for increased neutral lipid deposition in juvenile YTK liver. It appears that dietary choline, at least in the form of CC, does not significantly alter the liver lipid composition; instead, it aids lipid digestion and protects liver health.

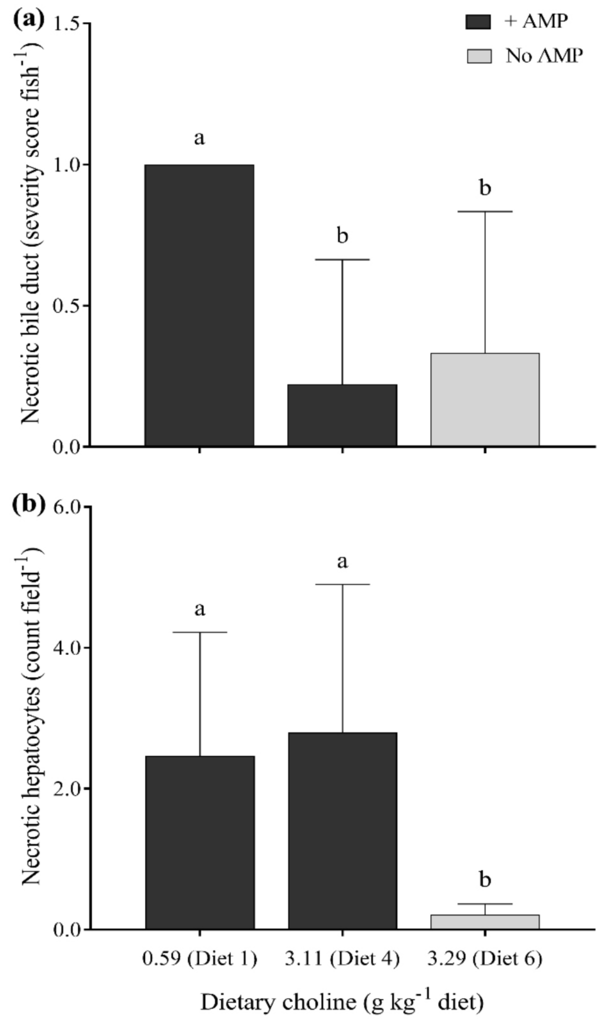

Figure 1 Histological assessment of (a) bile duct necrosis and (b) hepatocyte necrosis in YTK fed graded levels of dietary choline.

MICROFABRICATED LOW-COST COUNTING CHAMBERS FOR STANDARDIZED ESTIMATION OF SPERM CONCENTRATION

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Evaluation of sperm concentration is essential for research and procedures involving artificial fertilization, cryopreservation, and assessment of sperm quality. Microfabrication technologies have shown tremendous potential to rapidly prototype and fabricate devices to assist reproduction and fertility research, however, such utility has not yet been made available for reproduction laboratories. The goal of this work was to evaluate the feasibility of using microfabrication techniques to produce counting chambers for estimation of sperm concentration. Zebrafish (*Danio rerio*) sperm were used as a model for evaluation of functionality of the chambers. These microfabricated enumeration grid chambers (MEGC) (Figure 1) were composed of a polydimethylsiloxane (PDMS) coverslip with grid patterns (100 × 100 μm) and a PDMS base platform to create a known volume with a 10-μm height to restrict sperm cells to a single layer. The cell counts estimated by two of three early prototype MEGC devices tested were not significantly different from the control device, a commercially available Makler® chamber. The material cost for a MEGC was < $0.1 compared to ~ $100 for a standard hemocytometer and $700 for a Makler® counting chamber. This study demonstrates the feasibility of microfabrication in creating low-cost counting chambers to enhance standardization and strengthen interdisciplinary collaborations. Such device can be applied in research, hatchery management, genetic breeding programs, and commercial aquaculture applications.

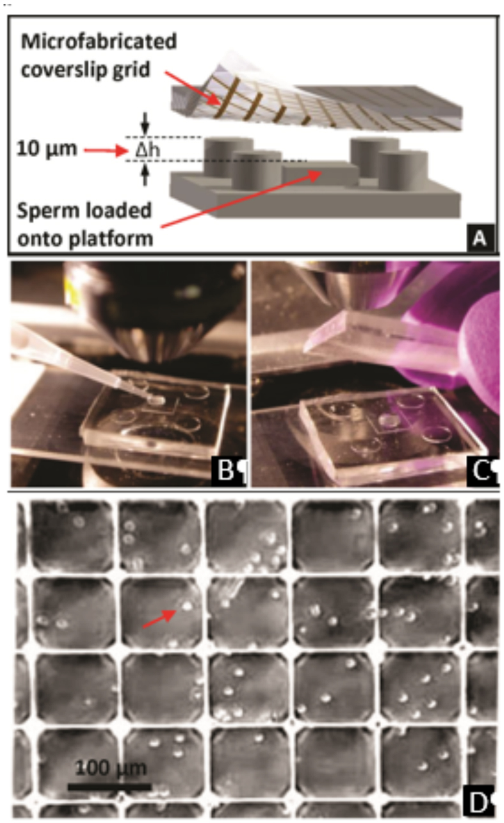

Figure 1. Fabrication and use of a Microfabricated Enumeration Grid Chamber (MEGC) for concentration estimation of zebrafish sperm. **(A)** Overview of the grid-patterned coverslip with 100 × 100 μm squares placed on top of posts to create a 10-μm tall chamber to create a known volume (1×10^{-4} μl for 100 × 100 × 10 μm) for accurate estimation of sperm concentration. **(B)** zebrafish sperm samples were pipetted onto the central platform of the base piece, **(C)** followed by placing the coverslip on top. **(D)** To estimate sperm concentration, sperm samples were observed with a dark-field microscope (200-× magnification), and the number of cells (white dots indicated by the arrow) within each square was recorded.

INNOVATIVE DEVICES FOR COMMUNITY-BASED DEVELOPMENT OF GERMPLASM REPOSITORIES

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Repositories of cryopreserved germplasm are important tools for genetic management in conservation, restoration, aquaculture, and biomedical research. However, lack of accessible devices makes it difficult for many facilities to apply cryopreservation technology with reliable reproducibility. Commercial equipment can cost tens of thousands of dollars, deterring inexperienced laboratories from seeking the utility of cryopreservation. Low-cost homemade devices are usually not standardizable, impeding process and transfer of innovative technologies into accessible tools for the user community to achieve aggregate throughput. To address this, we are employing interdisciplinary approaches that integrate cryobiology and bioengineering techniques to develop innovative cryopreservation devices that are low-cost, standardizable, and portable to assist adoption by aquatic user communities. These devices are fabricated by soft lithography (Fig. 1A-C) and three-dimensional (3-D) printing (Fig. 1D-F) with open-source files that can be shared and reused by users. The prototypes of these devices will be evaluated for closed-beta testing with the Zebrafish International Resource Center (ZIRC) and the USDA National Animal Germplasm Program (NAGP), and can be evaluated by open-beta testing with external groups. The overall goal is to provide a platform for community-based standards to ensure reproducibility and efficiency for germplasm repository development. As such, thousands of users could be provided with devices for reproducible cryopresevation to back up lines for submissions to central repository facilities, or commercialization of genetic resources.

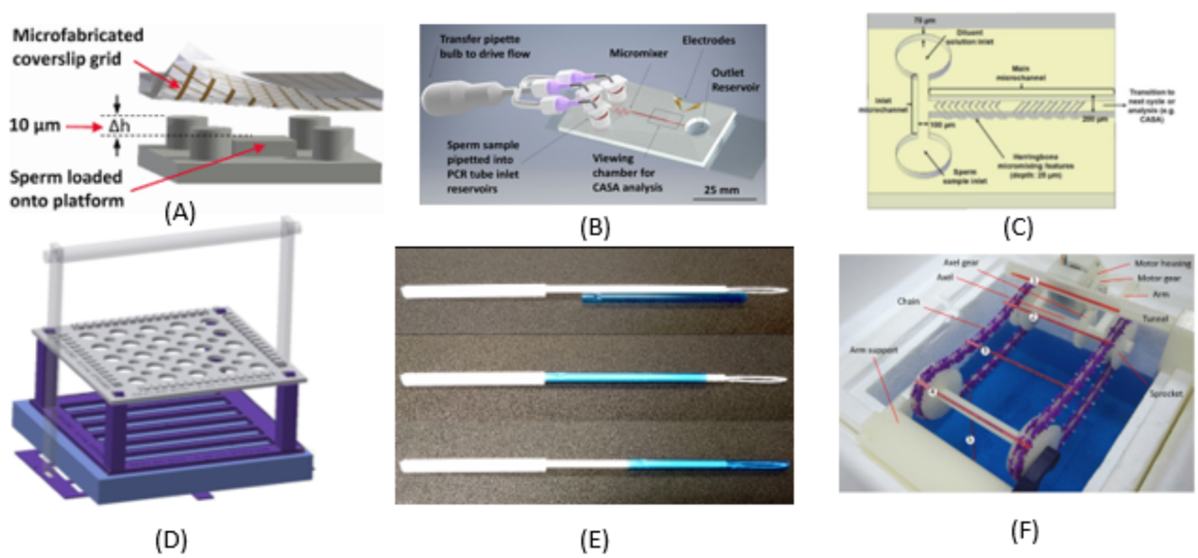

Figure 1. Examples of innovative devices to assist development of germplasm repositories. (A) A Microfabricated Enumeration Grid Chamber for estimation of sperm concentration. (B-C) Microfabricated Activation & Motility Chambers for assessment of sperm motility. (D) A Positional Cooling Platform Device for cryopreservation of batched samples. (E) Vitrification Storage & Identification Device for vitrification of individual samples. (F) A Controlled Cooling Conveyer Device for cryopreservation of individual samples.

MOLECULAR CHARACTERIZATION OF THIOREDOXIN MITOCHONDRIAL -LIKE PROTEIN FROM BIG-BELLY SEAHORSE *Hippocampus abdominalis* IN RESPONSE TO IMMUNE STIMULATION

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Oxidative stress is generally occurred due to imbalance of Reactive oxygen species molecules in cells. To maintain the homeostatic of ROS level, organisms have antioxidant defend systems including enzymatic & non- enzymatic antioxidants. Among those, Thioredoxin protein is a 12 kDa small redox protein with characteristic conserved WCGPC active site and ubiquitously expressed in all living organisms. This protein exists as either a cytosolic (TRx-1) or mitochondrial (TRx-2) isoform. TRx members are involved in wide range of biological functions for instance regulation of gene expression, control of apoptosis, protein folding and scavenging of ROS. In this present study, we examined molecular features and the biological functions of TRx-2 from *Hippocampus abdominalis* (HaTRx-2).

The cDNA & protein sequence of HaTRx-2 was analyzed molecular wise by using bioinformatics tools such as UGENE software, NCBI conserved domain database (CDD), MEGA 7, ExPASY PROSITE, SignalP 4.1 online server, ClustalW and etc. The qPCR was performed to determine the tissue specific distribution of *HaTRx-2*. Also, the mRNA expression profile of *HaTRx-2* upon immune challenged with Poly I.C, LPS, *Edwardsiella tarda* & *Streptococcus iniae* was investigated. Further, the TRx-2 was recombinantly expressed in *Escherichia coli* BL21(DE3) and the antioxidant property of recombinant TRx-2 (rHaTRx-2) was investigated by carrying out the MCO assay.

The cDNA sequence of *HaTRx-2* consists of 519 bp of open reading frame (ORF) which encoding 172 amino acids with a molecular weight of 18.8 kDa and calculated pI of 7.80. According to the phylogenetic tree analysis, *HaTRx-2* is close to its ortholog from *Fundulus heteroclitus*. The qPCR results revealed that *HaTRx-2* transcripts ubiquitously express in all examined tissues with a high expression in ovary. The mRNA expression levels in the blood showed significant up regulation with Poly I.C and *E.tarda* and significant down regulation with LPS & *S.iniae* at time post immune challenge. The metal-catalyzed oxidization assay results (Fig.1) elucidated an antioxidant property of HaTRx-2 by protecting super coiled DNA from oxidative damage. On the whole, our data indicated that *HaTRx-2* may play an important role in host immune response and regulating oxidative stress.

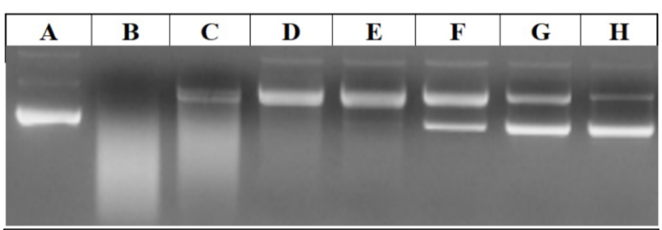

Fig. 1. Protection of super coiled DNA by rHaTRx-2 from cleavage in an MCO system: A, pUC19 without any treatment; B, pUC19 with MCO system; C, pUC19 with MCO system and MBP; D-H, pUC19 with MCO system and different concentrations of rHaTRx-2 (0.05,0.1,0.3,0.4 and 0.8 ng/μl)

DRAFT GENOME ASSEMBLY OF REDLIP MULLET (*Liza haematocheila*) USING HYBRID APPROACH

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Background: The redlip mullet, also known as *Liza haematocheila* is an economically important fish species in Korea and East Asia. Due to many disease outbreaks, there is considerable profit loss during production. Therefore, improve the understanding and knowledge of the redlip mullet genome structure will provide important data towards the increasing the production and help to elucidate their mechanisms of stress and host defense.

Findings: In this study, we constructed the redlip mullet draft genome. The total genome size was estimated to be 700 Mb and, using the Illumina HiSeq 2500 System and PacBio Sequel System sequencing data, the genome was annotated as 747 Mb. Hybrid assembly of mullet genome sequences comprised 1,453 contigs, with 3.9 Mb contig N50 value. Final gene models predicted 28,919 transcripts (21,796 genes), of which 20,310 genes showed gene ontology terms (70.23%), with 10,053 biological functions, 24,091 cellular components, and 14,180 molecular functions. The quality and completeness of the draft genome were assessed with Benchmarking Universal Single Copy Orthologs v. 2.0 and Core Eukaryotic Genes Mapping Approach v. 2.5, which resulted in 89.2% similarity to Actinopterygii (ray-finned fishes) and 248 core eukaryotic genes. Ortholog analysis resulted in 8,280 core genes, with 444 genes unique to *L. haematocheila*.

Conclusion: We built a first draft genome for *L. haematocheila* (Family: Mugilidae) using a hybrid approach. The genome can be used as a genomic reference for the discovery of genetic features and the advancement of marine science.

TRANSCRIPTIONAL MODULATION OF APOPTOSIS REGULATORY PROTEIN SIVA1 UNDER DIFFERENT IMMUNE CHALLENGES IN RED LIP MULLET *Liza haematocheila*

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Apoptosis, or programmed cell death is an obligatory event occur in the cell to keep the proper functions and cellular homeostasis of all multicellular organisms. Conversely deregulation of apoptosis could lead to a wide variety of severe pathological conditions. Siva 1 is a p53- inducible gene and the gene product is considered as an extensive inducer of apoptosis which can function both in extrinsic and intrinsic apoptotic pathways. Studies have shown that Siva 1 is overexpressed in various pathological conditions such as viral or bacterial infections and acute ischemic injury, hypoxia conditions and following DNA damage signals. Siva 1 binds with the CD27 molecule and interacts with the cell surface receptors GITR (glucocorticoid-induced tumour necrosis factor receptor) as well, to mediate the appropriate apoptotic signalling pathways where necessary.

Current study has focused on identifying a Siva 1 homolog from the Mullet cDNA library and relevant *in-silico* analysis of the protein was carried out using suitable bioinformatics software. Quantitative real-time PCR was used in determining the tissue specific mRNA expression of twelve different tissues collected from healthy red-lip mullets. Healthy fish were immune challenged individually with lipopolysaccharide (LPS), poly I:C, *Lactococcus garvieae* and Phosphate buffered saline (PBS/control) and selected tissues were collected in certain time intervals from five fish from each challenge. The collected tissues were pooled, cDNA was synthesized and applied in qPCR to determine the transcriptional modulation of the Siva 1 gene under different immune challenges.

Complete ORF of the Siva 1 of red lip mullet consisted of 867 base pairs encoding 168 amino acids with a predicted molecular weight of 18.5 kDa. The Siva superfamily domain has been conserved among the fish species. Brain tissue has the highest expression of Siva 1according to the tissue-specific expression analysis. A significant up-regulation of the gene could be observed after 24 hours of immune challenge with LPS in both spleen and blood followed by a significant down-regulation in 48 and 72 hours. This may happen because of the activation of caspase dependent mitochondrial pathway of the apoptosis of the lymphoid cells due to the pathological condition. However profound investigations are necessary to clarify the exact mechanism of apoptosis facilitated by Siva 1.

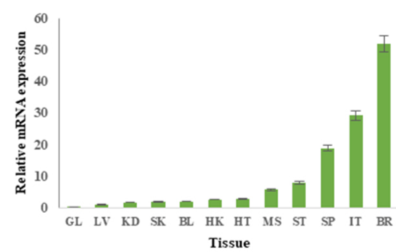

Fig.1 Tissue specific expression of the Red lip mullet Siva 1 (Tissue: gills, liver, kidney, skin blood, head kidney, heart, muscles, stomach, spleen, intestine and brain

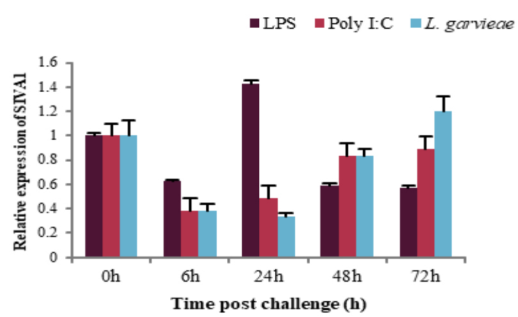

Fig.2 Relative expression of mullet Siva 1 in blood tissue at certain time points after different immune challenges

MOLECULAR CHARACTERIZATION AND FUNCTIONAL INSIGHT TO MITOCHONDRIAL GLUTATHIONE REDUCTASE HOMOLOG ISOLATED FROM RED LIP MULLET *Liza haematocheila*

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Glutathione reductase (GSHR) is a biologically important enzyme involved in the conversion of oxidized glutathione (GSSG) into its reduced form, reduced glutathione reductase (GSH) with the catalytic activity of NADPH. The precise GSH: GSSG ratio is very much important to a cell in detoxifying a variety of potentially harmful electrophilic compounds such as reactive nitrogen and oxygen species (ROS, RNS). Therefore GSHR which keeps this ratio exactly so is considered to be an imperative enzyme in the cell. Most of the aquatic organisms including fish are fortified with the above enzyme system to neutralize the oxidative stress arises due to toxic substances. In the current study, the sequence of mullet GSHR was identified from the cDNA library followed by the *in-silico* analysis of the protein using appropriate bioinformatics tools and software. cDNA samples synthesized from twelve different tissues were subjected to quantitative real-time PCR in determining the tissue-specific expression of the gene. cDNA was synthesized from selected tissues in certain time points after the immune challenge of fish with lipopolysaccharide (LPS), poly I:C, *Lactococcus garvieae* and Phosphate buffered saline (PBS/control) respectively and the recombinant protein purified from the pMAL™ Protein Fusion and Purification System was used in the GSHR activity assay.

Complete ORF of the mullet GSHR consisted of 1527 base pairs encoding 508 amino acids with a predicted molecular weight of 55.43 kDa. Multiple sequence alignment revealed the conservation of the authoritative amino acids within the fish species and the phylogenetic analysis demonstrated clustering of mullet GSHR with other fish GSHR complements with the highest evolutionary relationship. The highest expression of GSHR was observed in the gills tissue of according to the tissue distribution data. This may happened because relatively high concentration of H_2O_2 is accumulated in the gills as H_2O_2 is removed from the gills of fish. Significant up regulation of the gene could be observed in the gills tissue of the fish immunized with *L. garvieae* after 72 hours of immunization. The recombinant *Lh*GSHR activity assay demonstrated a linear graph confirming proper activity of the enzyme with a specific activity of 35.2 U/mg. Experimental results suggest the significant role of GSHR enzyme in counterbalancing the oxidative stress arises due to pathogenic attack of fish.

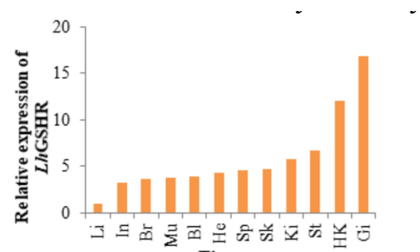

Fig.1 Tissue specific expression of the Red lip mullet GSHR (Tissue: liver, intestine, brain, Muscle, blood, heart, spleen, skin, kidney, stomach, head kidney, gills respectively)

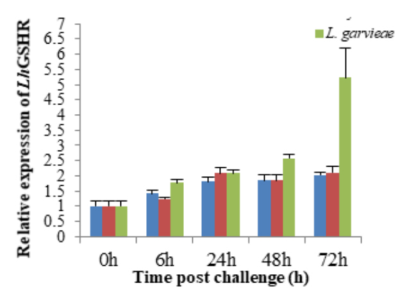

Fig.2 Relative expression of mullet GSHR in gills tissue at certain time points after different

IMPROVING YIELDS WITH DECOUPLED AQUAPONICS

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RAS biofilter effluent is an ideal supplement for hydroponically grown crops, with prior studies observing a significant (30-40%) improvement (1) in both the growth rate and total yield of lettuce when effluent was used to supplement a standard hydroponic nutrient solution (HNS). Traditionally, solid (insoluble) RAS waste recovery has not been a priority due to the relatively small quantity of solid waste produced and the limited perceived cost-benefit of additional infrastructure for solid waste recovery. Recent advancements in waste digestion may permit novel opportunities when applied to solid aquaculture waste reutilisation.

Although nutrient supplementation with RAS waste products (soluble and insoluble, Figure 1) improves hydroponic growth rates, prior experiments have shown nutrients such as N and P are not the primary drivers for improved growth rates of plants in the hydroponics compartment (increased growth rates were observed despite nutrient concentrations remaining constant between treatments). Contrary to previous claims (2), our research into the effects of RAS supplementation to standard HNS grown crops indicates that it is not the living microbial community *per say* that has the greatest effect on crop productivity, but rather stimulatory compounds produced by that community. To investigate how waste treatment systems can augment nutrient uptake and improve crop stability, we supplemented HNS with soluble waste from the biofilter. The effect of filter sterilisation (preservation of only bacterially-produced compounds without microbial proliferation), as well as community development (consolidation around a stable rhizosphere consortium) was explored. Similar experiments were carried out using digested insoluble waste.

Consistent with prior published research, soluble RAS waste supplementation to HNS resulted in increased growth rates and total leaf mass. Insoluble waste digestion showed promising results in model systems. Preliminary results indicate that a combination of soluble and insoluble waste digestion strategies can be effective at significantly improving hydroponic crop productivity compared to nutrient solutions alone. Future work will use cost-benefit models to determine the economic suitability for aquaponic operations.

References:
1. Delaide B, et al. Water. 2016.
2. Wielgosz Z, et al. Horticulturae. 2017.

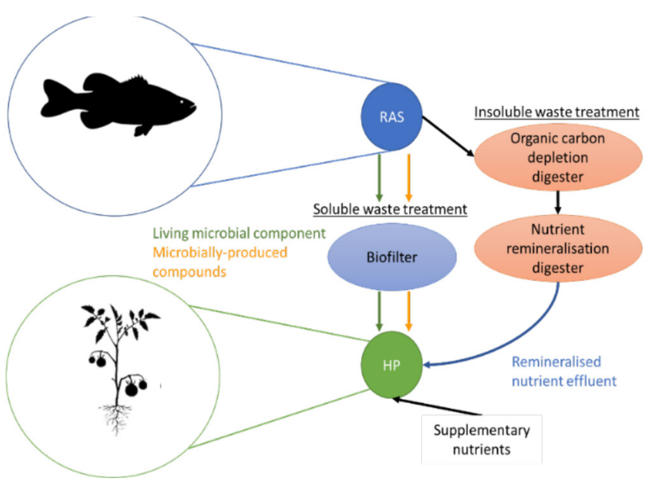

Figure 1. Overview of how aquaculture waste from an RAS may be independently treated to supplement hydroponic crops.

CHALLENGES OF USING CALCEIN TO MARK LARGEMOUTH BASS FINGERLINGS

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Distinguishing hatchery fish from wild is a fundamental part of stock enhancement programs. Batch marking with chemicals, such as oxytetracycline, is common. Calcein offers and advantage over oxytetracycline because of the non-lethal nature of mark detection. However, ultraviolet light is reported to degrade external calcein marks, so mark retention in full sunlight should be examined. We attempted to determine the retention of calcein marks on fingerling (< 1 g) Largemouth Bass *Micropterus salmoides*. This work was conducted under an agreement with the U.S. Fish and Wildlife Service (INAD #10-987). Prior work with crappies suggested osmotic induction with a 4% salt bath for 3.5 min followed by a 0.5% calcein solution for 7 min produced lasting external marks on another centrarchid. However, this procedure resulted in 100% mortality within 48 h of marking. Fish were stocked into outdoor tanks in this and every subsequent trial unless otherwise specified. A second trial was conducted with graded levels of salt (0%, 2%, and 4%) followed by a 7-min bath in a 0.5% calcein solution. Calcein solutions in this and all subsequently-described trials were always 0.5%. Mortality (48-h) was 100% in all treatment except the control, which experienced no mortality. A third trial with graded levels of salt (0%, 2%, and 4%) and a sham 7-min bath (no calcein) resulted in no mortalities after 48 h. A fourth trial with 2% salt and graded periods (3, 5, and 7 min) of 0.5% calcein bath resulted in 80% mortality after 48 h in all treatments and no mortalities in the control. We speculated that our calcein was contaminated and ordered fresh calcein. A fifth trial was conducted with 2% salt and 3.5-min bath in 5% calcein or 3.5-min in a sham bath. These fish were held in indoor tanks for 48 h and mortality in the treatment and control were both zero. A sixth trial was conducted with 2% salt and graded periods (3, 5, and 7 min) of 0.5% calcein bath or a 7-min sham. Fish were stocked directly outside. All treatments experienced 100% mortality, while the control experience none. A seventh trial was conducted with 2% salt and a 3-min 0.5% calcein bath or 3-min sham. Half the treatment and control replicates were stocked directly outside, while half the treatment and control replicates were stocked inside. All treatments and replicates experienced no mortalities after 48 h, except the treatments stocked directly outside, which experienced 100% mortality. A final trial was conducted with graded levels of salt (0%, 2%, and 4%) and a 3-min bath in 0.5% calcein. The control for this trial was 0% salt and 3-min sham calcein bath. All fish were held inside for 48 h. No mortalities occurred in any treatment except the 0% salt which experienced 10% mortality. After 48 h, fish were moved to outside tanks. Within 48 h, mortalities ranged from 60% to 80% in treatments, while the control experienced 10% mortality. A protocol for calcein marking of fingerling Largemouth Bass has not been established.

NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF NILE TILAPIA *Oreochromis niloticus* FED DIETS WITH DIFFERENT PROTEIN CONCENTRATES

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Nile Tilapia *Oreochromis niloticus* has become one of the most preferred and consumed farmed fish species globally. There are commercial diets without fish meal available for Nile tilapia, and many of these contain high inclusion levels of plant ingredients. Some of these, such as soybean meal, have favorable amino acid profiles. However, plants also contain anti-nutritional factors that can limit their use in fish diets. In addition, soy (and some other plant ingredients) are scarce in many developing countries where tilapia species are cultured, and are valued more for human food. The availability of alternative high-quality feedstuffs with a consistent nutrient profile is often limited in these regions. The addition of commercial concentrates containing high levels of digestible protein and essential micronutrients to diets could make up for the inferior quality of cheaper ingredients and improve production profitability of tilapia.

We conducted a digestibility trial and a feeding trial with the commercial products Omni 55, Elite 60, Elite 65, and Elite 70, which are proprietary blends provided by H J Baker & Bros, Tuscola. Test diets for the digestibility trial were formulated from a 35% protein commercial-type reference diet and were composed of a 70:30 mix of reference diet to the following test ingredients: Aqua-PakTM, Aqua-Pak® 60, Aqua-Pak® 65, and Aqua-Pak® 70.

Ingredient ADCs of dry matter and lipid were different for all ingredients - the Aqua-Pak® 70 had higher dry matter and lipid ADCs than other ingredients. The ADCs for protein were similar and uniformly high among all Aqua-Pak ingredients. The apparent digestibility coefficients (ADC) of dry matter and crude protein were similar for Aqua-Pak™ , Aqua-Pak® 60, Aqua-Pak® 65, and Aqua-Pak® 70 diets. A higher lipid ADC was observed for Aqua-Pak® 70 than other diets.

For the feeding trial, 5 diets (one per concentrate and a control) were formulated using the nutrient digestibility data and fed to 5 groups of 20 fish (weighing 047 g initially) per diet twice daily to satiation for 12 weeks. Mean increase in weight gain (%) was highest in the fish fed the control diet (with chicken by-product meal), but similar to the Elite 70. The fish fed the Elite 60, Elite 65, or Omni 55 had the lowest increase in weight gain. The FCR (range: 1.03-1.32) was lowest in fish fed the control diet and highest for those fed Omni 55, with others intermediate. Survival ranged from 82-91% and did not differ among diets. Alternative complement and lysozyme activity did not differ among diets. Overall, fish performance was similar on the concentrates and the control diet with no adverse effects of any of the diets. Therefore, economic analysis needs to be conducted to determine the commercial potential for inclusion of the concentrates in tilapia diets.

EFFLUENT BIOREMEDIATION BY THE POLYCHAETE WORM, *Alitta brandti,* IN A MARINE RECIRCULATING SYSTEM

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Marine aquaculture plays an important role in providing seafood for the world. One of the biggest challenges in marine recirculating aquaculture systems is the handling of wastewater. Finding organisms that have the potential to feed on these organic wastes such as sea cucumbers could be beneficial for waste bioremediation. One limitation for using this effluent is the low concentration of protein (2.3%) and fat (0.5%) with notably low omega-3 fatty acids (EPA 1.9, DHA 0.9, and linolenic 0.8 W/W% grams per 100 grams of wet effluent). The goal of this project is to provide compelling evidence that the California Sea Cucumber, *Parastichopus californicus*, can assimilate and growth by feeding enriched effluent from a Sablefish, *Anoplopoma fimbria* recirculating system.

A 25-day feeding trial was being conducted in a recirculating aquaculture system where individual polychaete worms were fed effluent with different concentrations of soybean meal. The treatment diets includde: a)72% soybean meal + 20% effluent (control), b) 46% soybean meal + 46% effluent, c) 20% soybean meal + 72% effluent, and d) 10% soybean meal + 82% effluent. The diets with soybean meal were enriched with 2% of the microalgae *Schizochytrium* sp. and 6% canola oil to supplement the fatty acids, especially the omega-3. Ammonia, nitrite, nitrate, temperature, and alkalinity are measured once per week.This is an on-going project, and the results from this study will be presented at the conference.

METAGENOMIC ANALYSIS OF GUT MICROBIOME IN FRESHWATER PERCH *Anabas testudineus*

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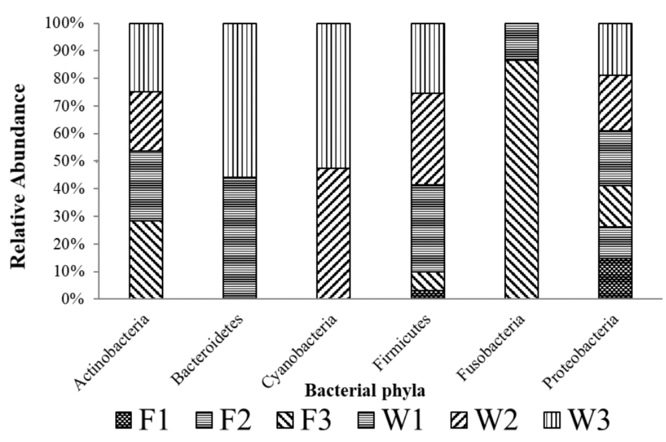
Anabas testudineus is an important aquaculture species in Southeast Asia (SEA). Like many other fish species, rapid development of aquaculture has led to disease outbreaks and a low survival rate in *A. testudineus*. Understanding the taxonomic diversity and uncovering potential probiotic stains residing in *A. testudineus* gastrointestinal (GI) microbiome is important in disease control and productivity in captivity.

In this study, metagenomics analyses using Illumina-sequencing showed that three bacterial phyla, *Proteobacteria*, *Firmicutes* and *Fusobacteria* were predominantly found in the gut microbiome of climbing perch, *Anabas testudineus*, from both farmed- and wild-type samples. The 16S rRNA gene sequence analyses revealed that opportunistic pathogens and beneficial bacteria were co-existed in the freshwater teleosts. Among the intestinal microbes, *Proteobacteria*, *Klebsiella* sp., *Cronobacter* sp., *Salmonella* sp., *Proteus* sp., *Edwardsiella* sp. and *Morganella* sp. were dominant in climbing perch's GI found in two different habitats. In contrast to most marine fishes, *Vibrio* is the dominant genus in the gut system. In general, farmed climbing perch hosted fewer microbial biodiversity and dominated primarily by *Fusobacteria* (*F. nucleatum* and *F. mortiferum*), while the wild climbing perch GI was largely harbored by *Proteobacteria* and *Firmicutes*.

Our study suggests the climbing perch GI microbiome may have influenced by geographical variations, or human factors in aquaculture. In terms of microbial similarity, the wild-type climbing perch has a more consistent bacterial diversity compared to those found on farms. Our work provides a comprehensive report on the microbial ecology in the intestines of adult wild- and farmed climbing perch. In short, the bacterial community structure is largely driven by their living environments.



FDA'S REGULATION OF INTENTIONAL GENOMIC ALTERATIONS IN AQUACULTURE

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Scientific advances such as genome editing have tremendous potential for the aquaculture industry. Intentional genomic alterations (IGAs) can be introduced to confer disease resistance, improve environmental tolerance, manipulate sex ratios, or increase growth rates for food production. FDA regulates IGAs in animals, including aquaculture, using a science- and risk-based approach. These IGAs are developed using a wide variety of techniques and for a wide range of intended uses. FDA evaluation ensures safety to animals, safety to humans, and that the alteration does what it is claimed to do by the developer. In this presentation, FDA will describe its risk-based approach to the oversight of IGAs, provide information about the risk assessment process, provide information about a pilot Veterinary Innovation Program for certain IGAs, clarify specific misconceptions about FDA's regulation of IGAs, and lastly, provide an overview of an example of an IGA approval in aquaculture, AquAdvantage Salmon. FDA also plans to communicate its intent to seek feedback from stakeholders to enhance transparency, predictability, and efficiency in the review process.

IMPORTANCE OF FEED CONSIDERATION FOR RAS

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The production of harvest size fish for human consumption is increasingly occurring on land, in intensive RAS facilities. These systems rely on optimal water quality maintained through filtration and water treatment processes and are effected by the biological loading from inputs. The largest inputs which pose a risk to the process are feed and the feces produced. Feed is a complex component when considering parameters required for each RAS operation. Feed performance, both biological and physical, is effected by the target species, the environment it will be used in, marketing requirements, ingredient restrictions, life stage and many other considerations. As feed has such an impact on land-based systems and represents the largest cost for production, determining the details of what feed will be used to accomplish the end goal should be completed at the early stages of planning. This presentation will go into depth on impacts of feed in RAS and what Skretting can offer to support the emerging sector.

POPULATION GENETICS OF APOSTICHOPUS CALIFORNICUS ALONG THE PACIFIC COAST OF NORTH AMERICA

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Commercial aquaculture production methods for the California sea cucumber (*Apostichopus californicus*) are being developed in response to high demand in overseas markets and wild stock declines. Because interbreeding of wild and farmed animals can lead to genetic risks, patterns in population structure in the wild is important to inform aquaculture practices, such as the site choice for collection of broodstock and transfer of seed across geographic areas. Here, we quantify population structure (1) at a small-scale, within Puget Sound, and (2) at a broad-scale, from Alaska to California. We used single-digest restriction site associated DNA (RAD) sequencing, the dDocent software package, and custom filtering scripts to identify 1680 single nucleotide polymorphisms (SNPs) across nine collection sites of *A. apostichopus*. Using these SNPs, we estimated population structure to be significant yet low, consistent with previous results. Additionally, we found strong evidence for isolation by distance, suggesting that dispersal is geographically limited and drives population differentiation. We detected population structure at both small and large geographic scales; even within Puget sound, the majority of pairwise population comparisons were significantly differentiated. We recommend that these estimates of population differentiation be considered in management decisions concerning movement of broodstock and seed, to limit artificial gene flow among differentiated populations.

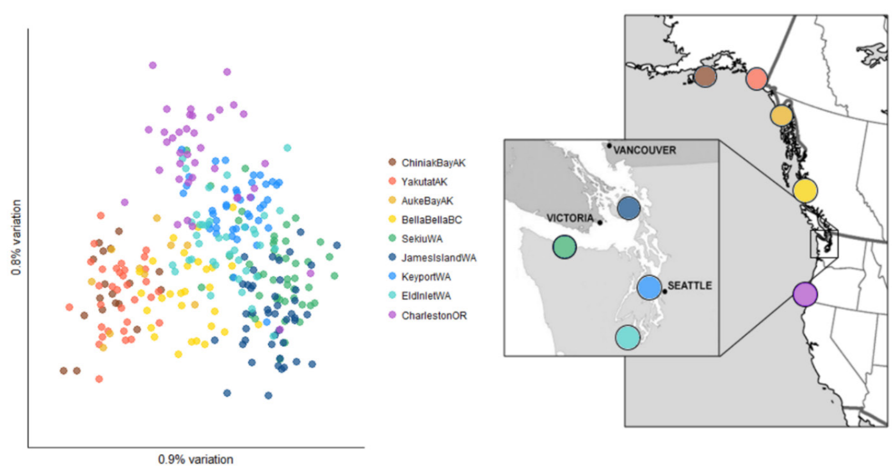

Figure 1: First two axes of a principal component analysis using 1680 SNPs shows geographic population structure consistent with isolation by distance

THE THAD COCHRAN MARINE AQUACULTURE CENTER: INFRASTRUCTURE, RESEARCH, DEMONSTRATION AND PARTNERSHIP OPPORTUNITIES

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The Thad Cochran Marine Aquaculture Center (TCMAC) is a 25 million dollar advanced research center and innovation hub within the School of Ocean Science and Engineering at the University of Southern Mississippi. The Center is centrally located in Ocean Springs, Mississippi with direct access to the northern Gulf of Mexico. The mission of the Center is to drive aquaculture innovation by alleviating the bottlenecks that constrain the production of marine species.

Facilities consist of 9300m^2 in 13 buildings including 4600m^2 of culture space for animals and live feeds and 930m^2 of experimental space designed to accommodate replicated studies in isolated and biosecure small-, medium-, and large-scale systems with single-pass climate control.

TCMAC has broodstock, hatchery, nursery and growout facilities that employ artificial seawater and recirculating aquaculture systems to produce marine species including finfish, crustaceans, molluscan shellfish, and algae. The Center focuses on advancing development and research programs dedicated to aquatic health, genetics, larviculture, reproductive physiology, nutrition/live feeds, recirculating aquaculture system design, technology, site assessment, business planning, offshore and nearshore aquaculture. TCMAC also functions as a demonstration center to provide training and technical assistance to industry.

TCMAC supports industry, academic institutions, government agencies, and non-governmental entities in the advancement of technologies for sustainable marine aquaculture on land and in coastal and marine environments. The Center offers multiple avenues for partnerships, including space and equipment leases, support laboratories, services and research contracts and is committed to establishing mutually beneficial partnerships

INVESTIGATION OF APPROACHES FOR REPRODUCTIVE STERILIZATION OF SABLEFISH *Anoplopoma fimbria*

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Escapement is an ecological concern for marine finfishes reared in net-pen systems. To mitigate this risk, we have initiated research to test several non-GMO approaches for reproductive sterilization, including high temperature exposure, triploidy, and gene silencing technology. Sablefish (or black cod, *Anoplopoma fimbria*) has been used as a marine model species for this line of research based on our understanding of its sex determination system (XX/XY) and early reproductive development.

High temperature exposure (20-22 ºC) over 19 weeks of early development inhibited sablefish ovarian development and induced female-to-male sex reversal in some individuals. However, complete germ cell loss was not observed in any individuals and ovarian development appeared to fully recover within one year post-treatment. Therefore, high temperature treatment may be useful for generating neomale (XX male) broodstock but is unlikely to be an effective approach for sterilization of sablefish.

Protocols for induction of triploidy using hydrostatic pressure or cold shock were also developed for sablefish, as well as methods for ploidy determination in whole larvae or blood samples. In brief, pressure shock of 7,000-9,000 psi applied 10 min post-fertilization for 5 min, or cold shock at -1.5 ºC for 60-120 min induced triploidy in a high percentage of fish while minimizing adverse effects on survival. Diploid (control) and putative triploid sablefish were reared until reaching a size (~200 mm in length) at which gonadal development could be assessed by histology and ploidy and genotypic sex determined from blood samples. We found that diploid females had ovaries with well-developed primary oocytes, while triploid females had ovaries that exhibited suppressed development, with mostly empty lamellae and reduced numbers of smaller primary oocytes. Diploid and triploid males had testes that appeared similar, composed of type-A spermatogonia. At one-year post weaning, the gonadosomatic index (GSI) of phenotypic females was reduced by ~10 fold in triploids compared to diploids, while there was no GSI difference between triploid and diploid males. These results indicate that triploidy was especially disruptive to female reproductive development and may be a valid approach for sterilization of monosex female lines. An important next step will be to assess the performance of triploid sablefish aquaculture.

Finally, a trial was conducted to test the efficacy of a gene silencing (morpholino oligomer, MO) procedure targeting the germ cell gene, *dead end* (*dnd*). Sablefish eggs, either pre- or post- fertilization, were immersed in a solution containing a *dnd*-MO-Vivo (at a concentration of 10 μM for 24 to 48 h) and the fish reared until gonadal development could be assessed by histology. From the pre-fertilization group, 10% of the fish had gonads completely devoid of germ cells, 15% had significantly reduced germ cell counts relative to untreated controls, and 75% had what appeared to be normal gonads. This demonstrated that it is possible to produce germ-cell free sablefish using *dnd*-MO-Vivo bath immersion. Ongoing work is focused on optimizing this method and assessing performance of these fish.

METHODS FOR TRIPLOIDY INDUCTION AND PLOIDY DETERMINATION IN A MARINE TELEOST, SABLEFISH *Anoplopoma fimbria*

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Approaches for reproductive sterilization are needed for marine aquaculture to increase sustainability and mitigate public concern associated with escapement of farmed fish and potential harm to wild stocks. One such approach is triploidization, a technique that has been widely used in freshwater fish species but not as well explored in marine species. Triploidy can be achieved by applying a "shock" to eggs shortly after fertilization to disrupt completion of meiosis and induce retention of the second polar body, which contains a complete set of chromosomes normally extruded from the early embryo. This study developed methods for triploidy induction and ploidy determination in a new marine aquaculture species, sablefish (or black cod, *Anoplopoma fimbria*).

Flow cytometry methods were assessed for ploidy determination in sablefish embryos, larvae, and erythrocytes. Microscopy techniques for ploidy determination, including nucleolar organizing region (NOR) analyses in larvae and erythrocyte major axis measurements in blood smears were also tested as lower cost alternatives. Experiments were conducted to establish initial methods for induction of triploidy using either hydrostatic pressure or cold shock. For each experiment, a minimum of four unique breeding crosses were made. Each consisted of ~100 ml of green eggs fertilized with 1 ml of sperm (diluted 1:30 with Cortland's solution) that were split equally into 50 ml vessels to either undergo a shock or no shock (control). The first experiment evaluated the optimal time after fertilization for application of pressure shock (10, 20, 30, or 40 min post-fertilization; mpf), the second evaluated the intensity of pressure shock (7,000, 9,000, or 11,000 psi), and the third evaluated the duration of cold shock (60, 120, or 180 min) at -1.5 ºC. Three criteria were used to evaluate the triploidy protocols relative to controls: embryo cell symmetry (proxy for embryo quality), percent survival to hatch, and triploidy rate.

Flow cytometry was effective for ploidy determination in embryos, larvae, and juvenile blood samples, NOR analysis was effective for larvae, and erythrocyte measurements were effective for juvenile blood samples. The timing of pressure shock (Exp. 1) had the greatest effect on the triploid criteria assessed, as all treatments, except 10 mpf, induced significantly lower cell symmetry and hatch rates compared to controls. Higher levels of pressure (9,000 and 11,000 psi; Exp. 2) significantly reduced hatch rates, whereas hatch and symmetry rates at 7,000 psi were not different than those of controls. Duration of cold shock (Exp. 3) had no effect on symmetry rates, however the hatch rate at 180 min was significantly lower than that of controls. For all treatments tested, 100% triploids were obtained based on NOR analyses (n=12 larvae/treatment/replicate). These results indicate that both pressure and cold shock can be used to induce triploidy in sablefish and that shock protocols applied at 10 mpf with 7,000 psi for 5 min or -1.5 ºC for 60-120 min maximize embryo quality and survival, while maintaining high levels of triploidy.

CUTTING DEVELOPMENT TIME: MODELING THE EFFECTS OF NOVEL FEED FORMULATIONS ON THE GROWTH OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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In aquaculture operations, feed may represent more than 50% of the total cost of operations. As the industry is expanding rapidly, the average prices for aquafeeds have increased by 20-90%, depending on region and target species. Accordingly, there is a steady demand to reduce feed production price by finding novel and alternative feed ingredients and formulations, while maintaining feed efficiency and growth performance at the same time.

Currently, it is common practice to test new ingredients and formulations in laboratory trials, mostly on juvenile fish. However, there is evidence, that results obtained in such trails cannot be transferred across different life stages, and therefore contain little information regarding rearing time and harvest size.

In the current study, we have successfully tested the possibility, to model the long-term effects of different feed formulations on cultured rainbow trout. This approach can, with little effort, be adopted to other species as well. The method also allows to predict and evaluate the goals of trait-specific breeding programs.

The experiment was performed at a commercial farm. Fish (n=900) were fed with three different extruded pellets of different experimental formulations in triplicate groups. Several models of different complexity were tested against the data. The most suitable model was evaluated by a variety of statistical tests combined in a multi-criteria analysis.

Despite the massive influence of the feed formulation on fish performance, the statistical analysis revealed the same mathematical relationships related to the growth process. This means, that different growth trajectories, triggered by different feed formulations and/or feed ingredients, can be described in the same manner and by the same function within the same species. This allows evaluation of a specific model and accordingly the prediction of the influence of specific feed formulations by a mathematical equation.

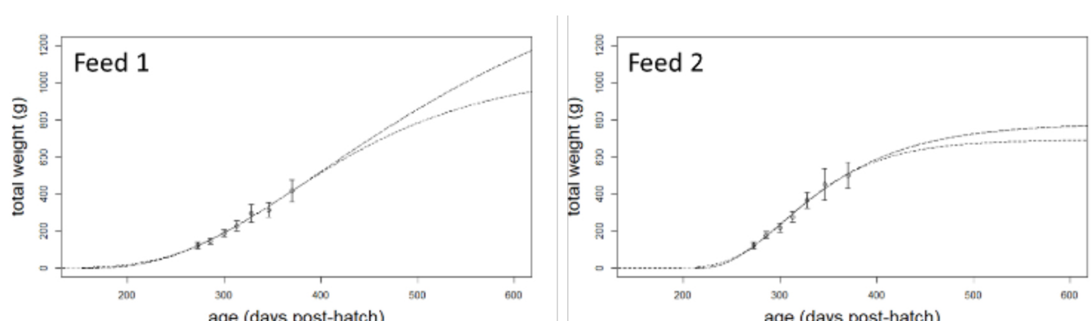

Figure 01: Simulation of long-term effects on body weight, as a result of different feed formulations displayed by the same two models. Dotted line = model 1, dashed line= model 2.

LACK OF KNOWLEDGE DOES NOT JUSTIFY MISSING ACTION: ASSESSING THE STATE OF WELFARE IN FARMED FISH

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Today already 50% of the world's food fish originate from aquaculture farms. Predictions indicate that this number will increase to approximately 65%, equivalent to 90-100 million metric tons/year, by 2030. Increasing production is often associated with increased intensification and greater environmental footprints, putting aquaculture into the center of public debates regarding sustainability and animal welfare. These debates are often controversial, emotional, and lack a solid, data-based line of argumentation.

According to German federal law, a regular corporate self-monitoring on the state of welfare is required from every fish farmer. Nevertheless, at the federal level, there is still no profound reporting structure established, and until now there is no approved set of indicators available to comprehensively implement this in an equivalent way to terrestrial livestock. We reviewed the current guidelines for the assessment of welfare in terrestrial farm animals as well as the methodology used to develop these, in order to adopt a strategy for fish welfare indicators. We collect and evaluate parameters and welfare indicators for rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) which are the most important species in the traditional, pond-based aquaculture in Germany. Despite this long tradition, little is known about the state of animal welfare of the two species, which differ fundamentally both in their biological requirements and in the husbandry systems used for rearing.

We identified several areas of activity, where welfare might be impaired or improved. Our results enable farmers and farm managers to routinely assess the status of animal welfare on their farms. This will also enable individual farms to survey the development of their husbandry conditions and to detect trends in husbandry at an early stage. Additionally, they can check the success of changes and implemented measures immediately.

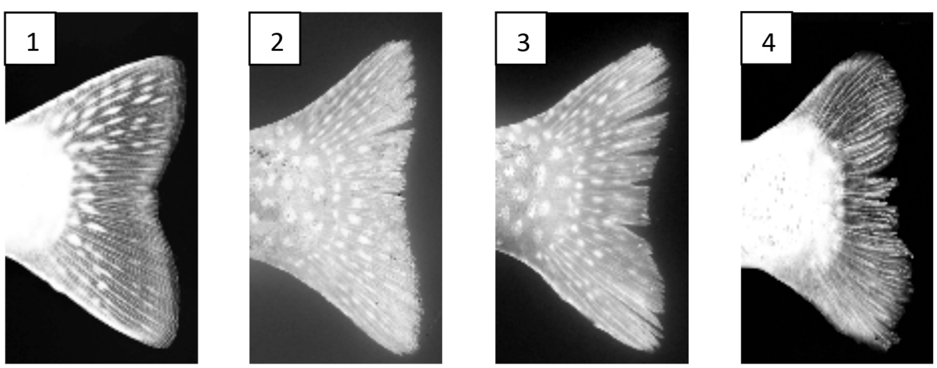

Figure 1-4: Fin status is a widely accepted welfare indicator in salmonids. However, the causes of fin erosion can be multifactorial. The figures show different levels of fin condition on the caudal fin of rainbow trout. Figure 1: 'Perfect' condition. Figure 2-4: Increasing levels of fin erosion. (© Thünen-Institute of Fisheries Ecology/Vincent Lugert, Marc Willenberg)

USE OF HYDROLYZED FEATHER MEAL IN DIETS FOR CHANNEL CATFISH (*Ictalurus punctatus)*

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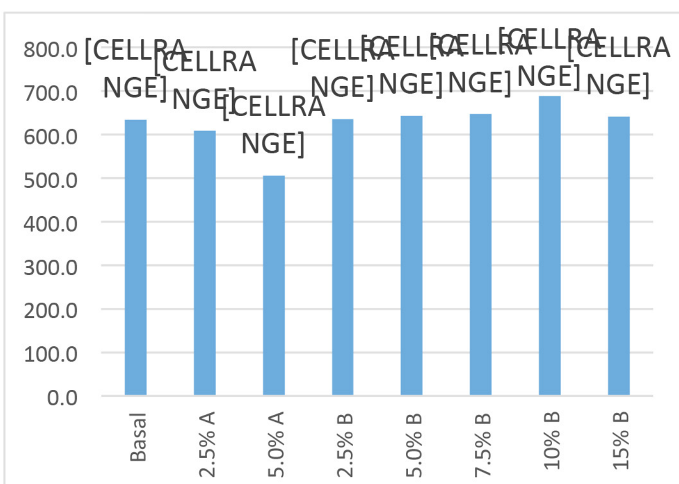
Chicken feather meal quite often is viewed as an undesirable product as most people think of ground up feathers, which have many processing and nutritional issues. However, modern feather meals are typically produced through a controlled process which improves digestibility and processing characteristics. Given the poor understanding of the quality of these meals we chose to evaluate the efficacy of hydrolyzed feather meal (HFM) in catfish feeds.

For this work we investigated the replacement of poultry-by-product meal (PBM) or soybean meal (SBM) in a practical catfish feed formulation. The basal diet contained 6% PBM and 56.5 SBM We then incrementally replaced PBM with 2.5 % and 5% HFM. Another five experimental diets were also made by replacing soybean meal with 2.5%, 5%, 7.5%, 10%, and 15% HFM. Diets were formulated to be iso-nitrogenous and essential amino acids were supplemented to maintain minimal levels that should meet the requirements. The growth trial conducted for 78 days. The catfish were stocked at 15 fish per tank using four replicates per tank. The survival of the catfish was around 94% across all treatments with not significant difference found due to diet. With the exception of one diet, the inclusion of FM did not influence the growth or FCR of the fish. Diet 3, which contained 5% FM as a complete replacement for PBM had significantly lower growth and FCR than the other treatments. As there was no effect of higher levels of FM as a replacement for soybean meal, it is likely this was due to a miss-formulation of the diet. It is likely, that the AA supplement for Lysine were left out. This will be confirmed by analysis of the diets which is ongoing. Growth, FCR, nutrient retention as well as the nutrient content of the test diets will be presented.

Figure 1. Percent weight gain of juvenile resulted 634.4 %WG (Basal), 608.2 %WG (2.5% A), 506.1 %WG (5% A), 635.8 %WG (2.5% B), 642.2 %WG (5% B), 646.5 %WG (7.5% B), 688.5 %WG (10% B), 641.6 %WG (15% B), channel catfish offered diets for which poultry by product meal (series A) or soybean meal (series B) was replace (0 to 15%) with hydrolyzed feather meal over a 78 day culture period. Diets were all formulated to be isonitrogenous and offered to four replicate groups of fish.



MULTICRITERIA PRODUCTION OPTIMIZATION IN AQUACULTURE USING POPULATION-BASED METAHEURISTICS

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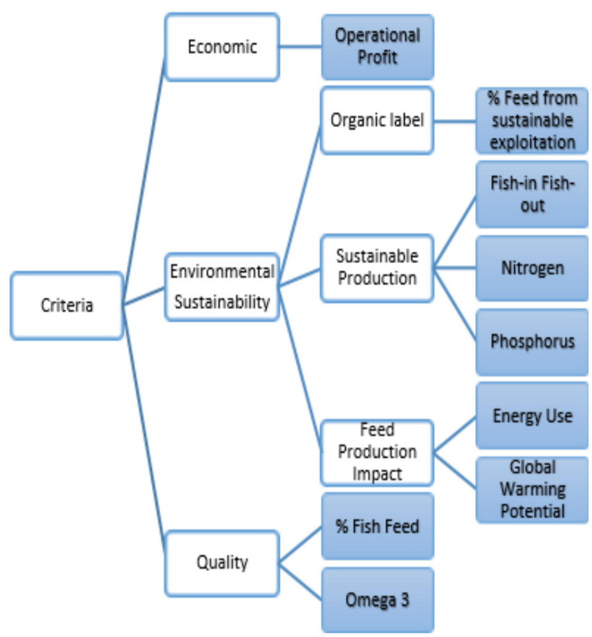
The company's economic performance is, very often, taken into account as the most important criterion for the determination of the optimal strategies in aquaculture farms. However, decision-making in aquaculture is becoming increasingly complex for producers due to the need to consider different aspects, such as product quality or the environmental sustainability, which are sometimes opposing and difficult to integrate. In addition, there is currently a wide range of possible production/market strategies, with specific values for each of these aspects and a very differentiated performance.

In this new context, market competition has increased and the complexity of managing industrial-scale production processes involving biological systems is still a growing problem in aquaculture. All this has led, in many cases, to a lack of management capacity and an increasing need for expert systems applied to decision-making processes that maximize the farm efficiency.

Like in most real-world problems, that maximization process is very complex and time consuming, so conventional optimization techniques could encounter many difficulties when attempting to address it. However, some population-based stochastic optimization techniques inspired by the social behaviour of groups of animals, such as PSO, allows to overcome this problem. Thus, it is possible to formulate an objective function and conduct a process of finding the optimal production strategy based on multiple objectives.

In this context, the present work explains the development of a novel methodology, based on the integration of multiple-criteria techniques and population-based metaheuristics, which allows decision-makers to align its production objectives to their preferences in multiple aspects and thus determine the best strategy. This directly addresses one of the key challenges in aquaculture in recent years, the ultimate goal of which is to improve efficiency in order to minimize the use of resources and maximize profits.



The results obtained show the utility of this methodology for integrating numerous objectives and prove that in most cases these techniques improve the company's results. Therefore, we may conclude that this methodology will improve the management capacity of aquaculture producers and their understanding of the performance of the main variables of the farm. Lastly, it is important to highlight the importance of the data quality so any effort aimed at increasing information recording and transparency will improve the results of implementing these methodologies.

DRIFTING FISH AGGREGATING DEVICE (dFAD) SATELLITE BUOYS WASH ASHORE ON PALMYRA ATOLL AND HAWAII: A TOOL TO SOURCE AND PREVENT NETS FROM ENTANGLING REEFS?

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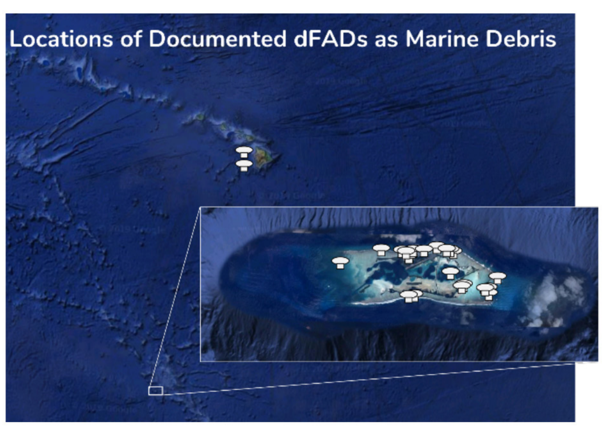
Drifting fish aggregating devices (dFADs) are commonly used in the purse seine tuna fishery to attract fish. dFADs are commonly made of bamboo rafts and floats wrapped in nets with stretches of nets hanging vertically in the water column. Fishing fleets track the location of floating dFADs using attached satellite transmitter buoys. Derelict fishing nets make up a considerable percentage of marine debris impacting the coral reefs of the Hawaiian Islands and Palmyra Atoll. Some net masses are identifiable as dFADs associated with purse seine fishing, and some of those still have attached satellite tracking buoys. Since 2014, at least 30 buoys have been recovered from marine debris in the Main Hawaiian Islands and Palmyra Atoll. Date, location, serial numbers, attached net descriptions, and photos are recorded in a database. This poster discusses how dFAD buoys can play a role in marine debris tracking, sourcing, site remediation, and prevention. Physical oceanographers could make use of previous tracks of recovered FAD buoys to predict future dFAD grounding events, if the data were made available. If abandoned buoys can be reprogrammed, the devices could be repurposed for future offshore tracking scientific studies. The unique identifiers on the buoys could be used to source the abandoned fishing gear to a particular vessel or fleet. Ideally, concerned parties could work collaboratively with the fishery to prevent abandoned dFADs from washing ashore. The fishery, satellite transmitter manufacturers, and state/federal agencies could decide together to make real-time GPS locations accessible when the dFAD drifts outside of sanctioned fishing grounds, allowing the salvage of the dFAD before it causes ecological damage in shallow coastal waters. Use of dFADs offer an opportunity to work with the fishing industry to minimize environmental harm.



EVALUATION OF A PLANT-BASED PROTEIN *Moringa oleifera* IN FORMULATED DIETS OF JUVENILE CHANNEL CATFISH

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High costs of traditional protein sources in feed limits the production of healthy proteins from farm raised fishes for both developing countries and the domestic industry. Moringa (*Moringa oleifera*) is a promising plant-based protein source due its nutritional profile, low cost, and ability to thrive in adverse growing conditions. Although previous investigations using *M. oleifera* as a source of protein in feed has been performed for several finfish species, studies have assessed its promise for channel catfish (*Ictalurus punctatus*), even though this species represents 50% of total U.S. aquaculture production in terms of metric tonnage. In the present study, growth rates and fatty acid profiles of juvenile channel catfish raised on diets with and without *M. oleifera* were compared to determine the suitability of *M. oleifera* as an alternative protein source in fish feed for this species.

A 56-day feeding trial was conducted in two dedicated recirculating systems (1438 L per) with juvenile channel catfish (mean individual weight = 3.41 g). 80 fish were randomly selected and divided into groups of 10 and stocked into eight 227-L round tanks (four replicates per system). Water was recirculated through biological and mechanical filters. Water temperature was maintained at 26-30°C and lighting was provided by overhead fluorescent lighting on a 12:12 hour light:dark cycle. Ammonia, nitrite, and dissolved oxygen were measured once per week, with temperature and pH monitored daily.

Final weight and percent weight gain were higher in fish fed the basal diet as compared to the moringa diet. Fatty acid profiles of fish fed each diet were relatively similar, indicating that lipids in the moringa diet were not a limiting factor in biomass generation of moringa fed fish. However, fatty acid levels in fish fed the moringa diet were on average lower than those fed the basal diet, with some exception.

TABLE 1. Formulation of experimental diets fed to channel catfish.

	Diet	
	Basal	Moringa
Soybean meal	47.0	32.0
M. oleifera leaf powder	0.0	15.0
Cottonseed meal	10.0	10.0
Corn meal	30.3	30.3
Wheat middlings	10.0	10.0
Dicalcium phosphate	1.0	1.0
Menhaden fish oil	1.5	1.5
Vitamin and mineral premix	included[1]	included[1]

[1]Premix added at 2.0% of total weight

TABLE 2. Average final weight, percentage weight gain, specific growth rate (SGR), and percentage survival of channel catfish fed two diets.

	Diet	
	Basal	Moringa
Starting weight (g)	32.88	35.35
Final weight (g)	71.48	30.45
Weight gain per day (g)	0.69	-0.09
Total weight gain (%)	117.60	-14.06
Survival (%)	97.50	95.00

DETERMINATION OF CLINICAL RELEVANCE OF ANTIBIOTIC RESISTANCE IN *Flavobacterium psychrophilum* STRAINS

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Bacterial coldwater disease (BCWD), caused by *Flavobacterium psychrophilum* (*Fp*), remains one of the most significant bacterial diseases of salmonids worldwide, and losses sustained in farmed salmonids are particularly substantial. Currently there are two antibiotics, Terramycin 200 (oxytetracycline dihydrate; OTC) and Aquaflor (florfenicol; FFC) approved to treat *Fp* infections in food fish by the U.S. FDA. Treatment efficacy with these two antibiotics can vary, and antimicrobial resistance (especially to OTC) has been documented in some *Fp* strains. Therefore, research investigating the relationships between antibiotic resistance in *Fp*, and *in vivo* virulence is imperative. Our lab tested two *Fp* strains (US57 and US87) showing varied *in vitro* antibiotic sensitivity against OTC and FFC. To evaluate the clinical relevance of antibiotic resistance in the *Fp* strains, experimental challenges of rainbow trout fry were conducted by intramuscular injection (IM) *Fp* US57 or *Fp* US87 strain with 10^5 CFU/fish. Five days post-challenge, fish were fed at 1% body weight (BW)/d for 10 days on the oxytetracycline-medicated feed at high rate (3.75g/100lb fish) or low rate (2.0g/100lb fish), or florfenicol-medicated feed at high rate (15 mg/kg BW/day) or low (10 mg/kg BW/day), respectively. All treatment groups except for the mock-challenged fish had mortalities with symptoms of BCWD, but the cumulative percent mortality (CPM) varied significantly among treatments. FFC treatments significantly ($p<0.01$) reduced the mortality of rainbow trout infected with US87 strain, but only had a moderate inhibition effect on US57-challenged fish. Both US57 and US87 strains showed resistance to OTC as CPM was not different from untreated controls at either high or low treatment rates. Results from this study illustrate the importance of antibiotic sensitivity testing on *Fp* strain and point to the need to utilize antibiotic treatment judiciously for control of BCWD.

CO-INFECTION OF RAINBOW TROUT (*Oncorhynchus mykiss*) WITH INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS AND *Flavobacterium psychrophilum*

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Co-infection of rainbow trout with infections haematopoietic necrosis virus (IHNV) and *Flavobacterium psychrophilum* is known to occur, and it has been speculated that a combined infection can result in dramatic losses. Both pathogens can persist in fish in an asymptomatic carrier state, but the impact of co-infection has not been well characterized or documented. In this study, it was hypothesized that fish co-infected with *F. psychrophilum* and IHNV would exhibit greater mortality than fish infected with either pathogen alone. To test this, juvenile rainbow trout were co-infected with low doses of either IHNV or *F. psychrophilum*, and at 2 days post-initial challenge, they were given a low dose of the reciprocal pathogen. This combined infection caused high mortality (76.2%-100%), while mortality from a single pathogen infection with the same respective dose was low (5%-20%). The onset of mortality was earlier in the co-infected group (3-4 days) when compared with fish infected with *F. psychrophilum* alone (6 days) or IHNV (5 days), confirming the synergistic interaction between both pathogens. Co-infection led to a significant increase in the number of *F. psychrophilum* colony-forming units and IHNV plaque-forming units within tissues. This finding confirms that when present together in co-infected fish, both pathogens are more efficiently recovered from tissues. Furthermore, pathogen genes were significantly increased in co-infected groups, which parallel the findings of increased systemic pathogen load. Extensive tissue necrosis and abundant pathogen present intracellularly and extracellularly in haematopoietic tissue. This was pronounced in co-infected fish and likely contributed to the exacerbated clinical signs and higher mortality. This study provides novel insight into host–pathogen interactions related to co-infection by aquatic bacterial and viral pathogens and supports our hypothesis. Such findings confirm that mortality in fish exposed to both pathogens is greatly elevated compared to a single pathogen infection.

COMPARATIVE TRANSCRIPTOME ANALYSIS DURING THE SEVEN DEVELOPMENT STAGES OF CHANNEL CATFISH (*Ictalurus punctatus*) AND TRA CATFISH (*Pangasianodon hypophthalmus*) REVEALS NOVEL INSIGHTS FOR THE TERRESTRIAL ADAPTION

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Channel catfish (*Ictalurus punctatus*) and tra catfish (*Pangasianodon hypophthalmus*) both belong to *Siluriformes*, one of the most taxonomically diverse orders in all vertebrates. The two species are the largest aquaculture commodities in the United States (channel catfish) and in Vietnam (tra catfish). Channel catfish does not possess an air breathing organ (ABO) and thus cannot survive in anoxic water. Tra catfish are facultative air breathers and use a modified swimbladder to breathe air when they encounter anoxic water, which is a highly advantageous survival mechanism. These two species present a unique research opportunity to study the mechanism of air breathing in teleost fish. In this study, we conducted RNA sequencing with whole fish samples for channel catfish at 2, 5, 6, 7, 8, 9, 10 dpf (days post fertilization) and tra catfish at 2, 4, 6, 8, 9, 10, 11 dpf. The timepoints were selected based on the developmental stage and a previous study rather than absolute age, and have been verified using developmental correspondences and heterochrony analysis.

In this study, we identified differentially expressed genes during development within each species. The expression profiles had considerable variation with the development of channel catfish and tra catfish (Fig. 1, 2).

Comparative transcriptome analysis revealed that a set of 193 genes were present in the tra catfish but absent in the channel catfish. Expression profile analysis and subsequent time series analyses were performed to further narrow the list of key genes. Histology experiments were also conducted to substantiate the results, which indicated that the swimbladder started to develop at 6 dpf in tra catfish. Cluster 5 (Fig. 3) was concordant with our histological experiments and contained 17 key genes potentially important for the air breathing ability in tra catfish. This study provides a model for studying evolutionary genetics during the transition from life in the ocean to terrestrial living and a novel insight for the evolution of vertebrates.

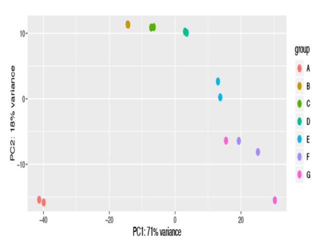

Fig.1. Principal component analysis (PCA) of Channel Catfish differential expression genes

A-G, Channel catfish 2, 5, 6, 7, 8, 9, 10 dpf

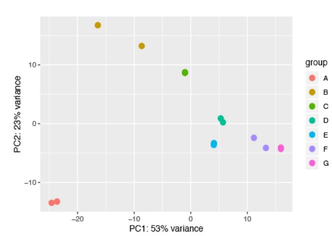

Fig.2. Principal component analysis (PCA) of Tra Catfish differential expression genes

A-G, Tra catfish 2, 4, 6, 8, 9, 10, 11 dpf

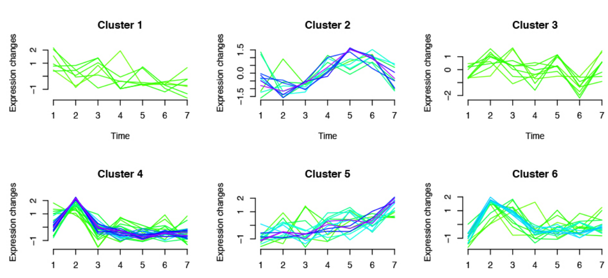

Fig. 3. Time series expression profile for tra catfish key genes.

THE EFFECT OF MICROALGAE AS A FISHMEAL AND FISH OIL REPLACEMENT ON THE INTESTINAL MICROBIOTA OF TILAPIA (*Oreochromis niloticus* X *O. mossambicus*) AQUACULTURE FROM GUT HEALTH PERSPECTIVE

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The aquaculture industry has explored alternative protein and lipid sources for aquaculture feed to reduce costs and promote sustainability. Microalgae is a desirable feed ingredient because it is grown in high volume and can be selected for high levels of lipids and protein. Additionally, microalgae have been recently explored as a prebiotic to promote the health of fish species through the modulation of the intestinal microbiota. This study investigated the use of microalgae as fishmeal and fish oil replacements and its effect on the intestinal histology and microbiota of hybrid tilapia (*Oreochromis niloticus x O. mossambicus*). Feed intake and body weight were recorded weekly and intestinal samples were collected monthly and processed for microbiota analysis using metagenomics and histological analysis of the intestinal epithelia. Tilapia growth performance was significantly higher in the 100% algae-based diet compared to the fishmeal and fish oil control (P>0.05), suggesting that microalgae can completely replace fishmeal and fish oil in the tilapia diets without negative impact. Microalgae inclusion into the tilapia diets did not significantly alter the overall intestinal microbiota. However, several species were significantly different between treatments, most notably an increase in *Parabacteroides goldsteinii* in the 100% algal inclusion. *P. goldsteinii* is a known beneficial microbe in the human GI tract, thus the inclusion of algae in the diets may be used as a strategy to modulate the gut health of Tilapia. Additionally, several novel species were reported in tilapia including the two most common species across all treatments, *Mycobacterium pinnipedii*, a closely related species to the tuberculosis-causing *M. tuberculosis*, and the non-tuberculosis causing, *M. brasiliensis*. Histological results did not show a significant difference between treatments, suggesting that algae inclusion in the diet does not negatively affect the health of tilapia.

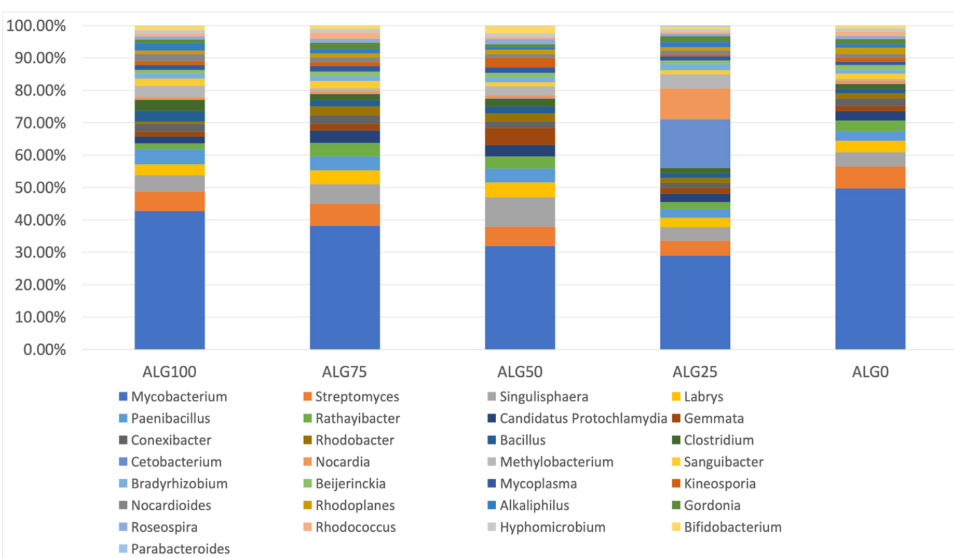

Figure 1. Top 30 Genus classification results per sample. Samples are labeled as follows:
ALG0 = control,
ALG25 = 25%
ALG50 = 50%
ALG75 = 75%
ALG100 = 100%

BENEFITS AND RISKS ASSOCIATED WITH THE INTEGRATION OF ULVA IN LAND-BASED AQUACULTURE SYSTEMS: IMPLICATIONS FOR SEA URCHIN AQUACULTURE

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Inclusion of Ulva in sea urchin and other aquafeeds has been shown to enhance feed consumption, growth, product quality and animal health. In South Africa, large-scale production of Ulva in land-based aquaculture systems has occurred successfully since 2002. However, despite the obvious benefits of growing Ulva in integrated systems for water bioremediation and recirculation as well as its reported benefits as an aquafeed component, production is still limited to a small number of farms. Biosecurity of integrated systems remains one of the main constraints preventing wider adoption of integrated technologies, since Ulva grown in effluent water is often considered to be a disease risk when fed back to animals on the farm.

To better understand potential disease risks, we studied the microbial communities associated with seawater and Ulva obtained from raceway systems supplied with effluent seawater (ESW) or natural seawater that had been fertilized (FSW). Water samples were collected at the inlet and outlet of each system. A culture (traditional plate count) and non-culture (NextGen sequencing of the 16S rRNA gene) based approach was adopted. The culture based approach utilised three selective media for the isolation and quantification of bacteria; namely tryptic toy agar (TSA), thiosulfate-citrate-bile-sucrose (TCBS) agar (Vibrio selective), and Ulvan agar (primary polysaccharide of Ulva used as the sole carbohydrate source).

In both raceway systems Ulva had a strong inhibitory effect on the total number of Vibrio species within the water column (Fig.1). The inhibitory effect appeared to be nutrient dependent, with a more substantial decrease in Vibrio species observed in ESW. NGS revealed high microbial diversity in all samples. A PCoA showed a clear separation between Ulva and seawater samples, while a linear discriminate analysis (LEfSe) showed that FSW had the largest number of genus level OTUs associated with it. Bacteria assigned to Hellea, Saprospiraceae, Gammaproteobacteria and Rhodobacteriaceae were shown to be abundant on Ulva. These organisms are often isolated from marine environments and seaweeds (Incl. Ulva), and bacterial species within some of these taxa have been shown to play important roles in the morphogenesis of Ulva. Conversely, OTUs assigned to the family Vibrionaceae had low abundance on Ulva. Our data supports the potential health and environmental benefits associated with the inclusion of Ulva in integrated systems.

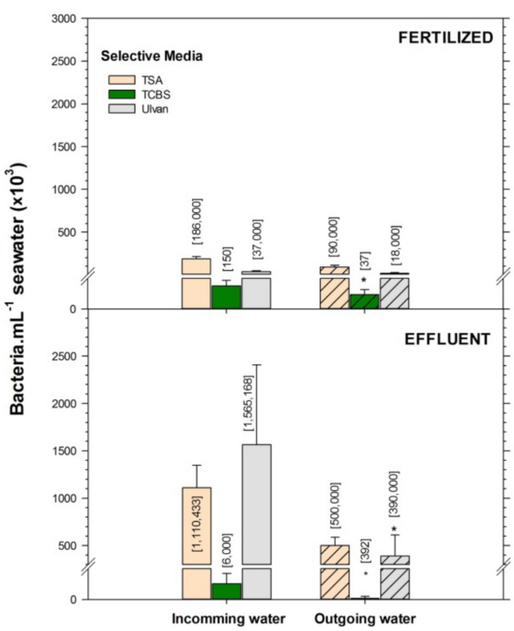

Figure 1. Culturable bacteria in effluent and fertilized seawater systems growing on TSA, TCBS and Ulvan.

USING PROBIOTIC BACTERIA TO REDUCE LARVAL MORTALITY OF *Crassostrea gigas* DUE TO THE BACTERIAL PATHOGEN *Vibrio coralliilyticus* AND IMPROVE METAMORPHOSIS

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High mortalities of Pacific oyster (*Crassostrea gigas*) larvae and spat associated with an increase in concentrations of the pathogenic bacterium *Vibrio coralliilyticus (Vcor)*, are commonly reported. This study examined the ability of probiotic bacteria, which inhibited the growth of *Vcor* cultured on agar plates, to reduce mortality of Pacific oyster larvae in the presence of *Vcor*. We have identified three high-performing probiotic strains that significantly reduced mortality of larvae exposed to lethal concentrations of *Vcor* when added 24 hours post egg fertilization. Larval mortality was minimized to background levels when all three probiotics were added in combination (Figure 1).

A thirty-day experiment showed that a single addition of the probiotic mix to larval cultures resulted in a significant increase in larval shell length 12 days post fertilization (Larvae only mean=153.0 ± SE 1.5μm, Probiotics mean=164.5 ± SE 2.6μm;(T test: $t(8) = 3.86$, P=0.0048), and greatly increased the proportion of larvae that successfully metamorphosed and grew for at least six days as spat (Figure 2). The ability of these probiotics to reduce larval oyster mortality in the presence of *Vcor* pathogens and to improve metamorphosis success independent of disease challenge could greatly improve the consistency and production of seed oysters in hatcheries

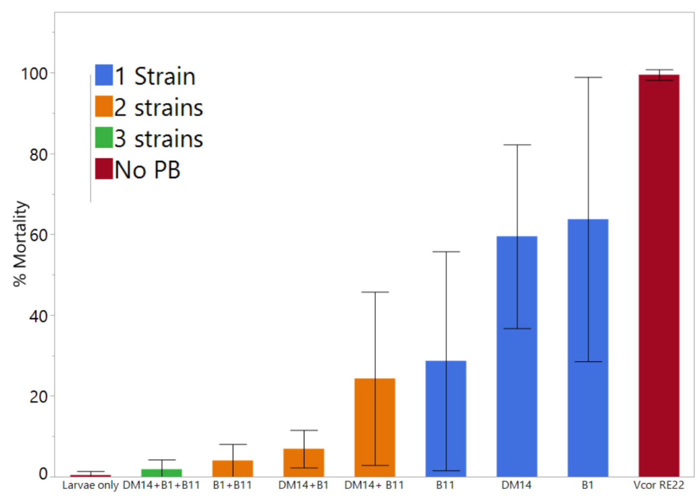

Figure 1. Mortality of *C.gigas* larvae exposed to *Vcor* RE22 in the presence and absence of probiotics. Error bars represent one standard deviation from the mean.

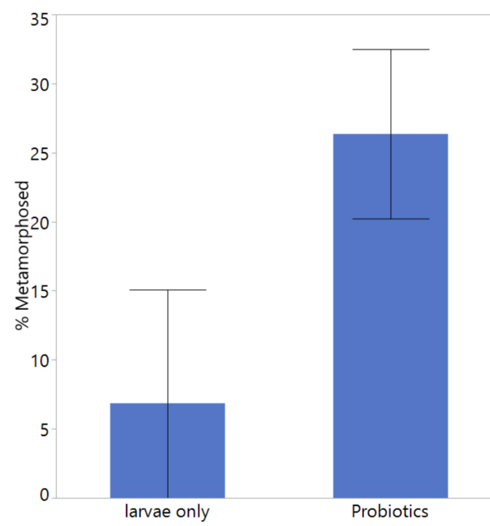

Figure 2. Percent of larvae stocked 24 hours post egg fertilization that metamorphosed and survived until day 30. Error bars represent one standard deviation from the mean.

OCEAN FARMING AND THE COASTAL COMMUNITY: A STORY MAP

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Responsible marine aquaculture can complement well-managed wild capture fisheries and responsible land-based agriculture to support a long-term strategy to create a safe, secure, sustainable, and more resilient global food system. While there is robust research and data to support the ecological, technological, and regulatory capacities of the United States to expand marine aquaculture in its waters, there is little information on how incorporating marine aquaculture into local seafood production impacts the economic, social, and cultural identities of coastal communities. Marine aquaculture has the potential to support a more stable source of income year-round by providing alternative or supplementary ocean-based livelihoods for coastal community members whose employment status is made insecure by the unpredictable impacts of climate change, urban coastal development, and policy changes on wild fisheries. Throughout the U.S., there are examples of fishermen and coastal communities that have benefited from adding marine aquaculture to their seafood production portfolios. Telling these stories can help inform understanding of how marine aquaculture contributes to the economic and social viability of coastal communities as well as identify gaps in knowledge about these interactions that can guide formal research in this area.

In collaboration with NOAA and Sea Grant, the Aquarium of the Pacific's Seafood for the Future program is curating a collection of stories that highlight the interactions between marine aquaculture and the coastal community. These stories are presented in a Story Map, a format that was chosen because it is accessible to a broad audience and with compelling images and stories that can be shown spatially. Our target audiences are legislators and stakeholders, as well as educators and the general public. We hope to expand this project and work with social scientists to help refine and develop more robust criteria to bridge stories with scientific theory to create a more complete and accurate picture of how marine aquaculture affects people and communities.

FISH CHOLINESTERASES AS BIOMARKERS OF SUBLETHAL EFFECTS OF ORGANOPHOSPHORUS AND CARBAMATES IN TISSUES OF *Cyprinus carpio*

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The present study was undertaken to assess the sub lethal toxic effects of carbofuran and profenofos on acetylcholinesterases (AChE) and butyrylcholinesterases (BuChE) in various tissues of *Labeo rohita* and was compared with control. The fingerlings of *Cyprinus carpio* (L= 92.5 ±6.3 mm, W= 33.44 ± 3.66 g) were exposed to three sub-lethal concentrations of profenofos (0.040, 0.020 and 0.013 mg/L) and carbofuran (0.199, 0.099 and 0.068 mg/L) in a three replicates for the total period of 60 days. The minimum AChE activity was recorded as 1.05 ± 0.02, 0.66 ± 0.02,1.91 ± 0.06,1.38 ± 0.24, 4.27 ± 0.03 and 1.69 ± 0.04 μmol/min/g protein in brain, gills, flesh, kidney, liver and blood, respectively with a dose of 0.040 mg / L of profenfos. The minimum BuchE activity was observed as 8.41 ± 0.06, 2.54 ± 0.31,1.91 ± 0.06, 4.41 ± 0.05,16.80 ± 0.05 and 8.69 ± 0.03 μmol/min/g protein in the brain, gills, flesh, kidney, liver and blood, respectively with a dose of 0.040 mg / L of carbofuran. The minimum AchE activity was recorded as 4.71 ± 0.05, 4.58 ± 0.04, 4.21 ± 0.14, 3.55 ± 0.12, 9.30 ±0.107 and 4.81 ± 0.04 μmol/min/g protein in brain, gills, flesh, kidney, liver and blood, respectively with exposure concentration of carbofuran (0.02 mg/L). The maximum BuChE activity was 13.75 ± 0.46, 2.56 ± 0.14, 3.91 ± 0.07, 7.40 ± 0.05,19.71 ± 0.08 and 6.82± 0.04 μmol/min/g protein in brain, gills, flesh, kidney, liver and blood, respectively with the exposure of 0.199 mg/L carbofuran. It has been concluded that profenofos was found to be the potent inhibitor of cholinesterases as compared to cabofuran.

ZEBRAFISH AS BIOACTIVITY GUIDED DRUG SCREENING ANIMAL MODEL TO IDENTIFY ANTI-ANGIOGENIC COMPOUNDS FROM SAUDI MEDICINAL PLANTS

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The phytochemicals and botanical crude extracts have proven their efficacy as natural antiangiogenic agents with minimum toxicities, there is a need to explore varieties of medicinal plants for novel antiangiogenic compounds. More than 100 herbs and medicinal plants found in Saudi Arabia were screened in this study.

Rumex vesicarius L. (Humeidh), is an annual herbal plant with proven medicinal values. The chloroform stem extract showed significant level of antiangiogenic activity in zebrafish angiogenic assay on a dose dependent manner. Thirty five (35) bioactive compounds were identified by gas chromatography mass spectrophotometry (GC-MS) analysis in the stem extract of *Rumex vesicarius*.

The anticancer activity was evaluated in MCF7, Lovo, and Caco-2, HepG2 cancer cell lines. Chloroform extract of stem exhibited strong anticancer activity in all tested cancer cells with IC_{50} values in micro molar range.

Based on these results, it is recommended that formulation prepared from *R. vesicarius* can further be tested in clinical trials in order to explore its therapeutic potential as an effective and safe natural anticancer product.

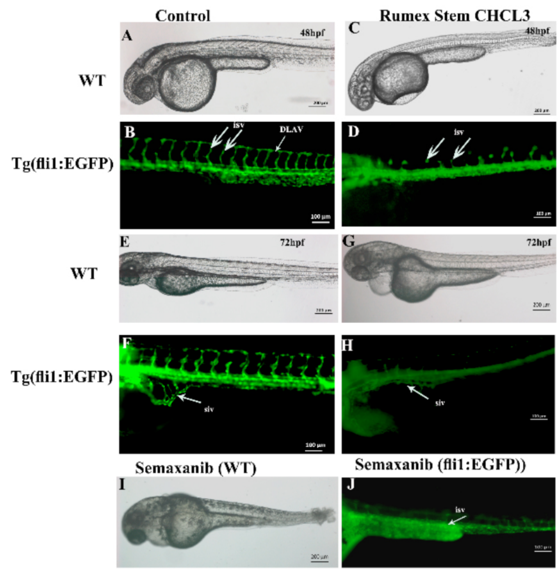

Fig1:. Rumex vesicarius inhibited the angiogenic blood vessels formation in Tg (fli1:EGFP) embryos.

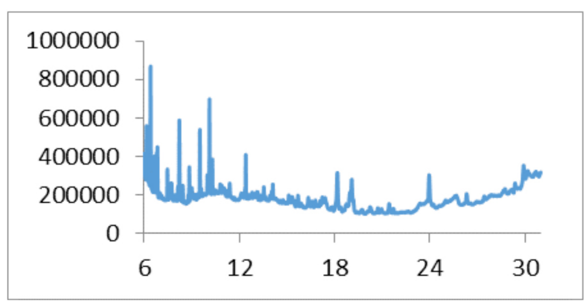

Fig2: GC-MS spectrum showing different peaks

A BIOECONOMIC MODEL OF INDOOR MARINE SHRIMP *Litopenaeus Vannamei* FARMS WITH LOW-COST SALT MIXTURES

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US domestic shrimp production is small, producing around 1,800 metric tons per year. Earthen Ponds and Recirculating Aquaculture Systems (RAS) are the two methods of domestic production. RAS systems have become increasingly popular in the Midwestern United States due to their ability to operate in colder climates indoors year-round. The problem with most RAS shrimp farms is that they are located inland, hundreds of miles from the ocean. Shrimp farmers are thus tasked with creating sea water by mixing freshwater with specialized, expensive sea salt. Recent studies on Low-Cost Salt Mixtures (LCSMs) derived from cheaper, industrial salt components have shown promise.

To analyze the impact of these cheaper salts on a commercial shrimp RAS systems, a bioeconomic model was constructed that uses biological growth parameters as well as financial and capital costs and returns in a series of financial performance measures. Three different farm scenarios were created, ranging from small to large. Commercial farms in Kentucky and Indiana provided data to validate the model's inputs. The Aquaculture Research Center at Kentucky State University has run several experiments testing the biological performance of the LCSMs and provided the costs.

Using Palisade's @Risk Excel software add-in, a series of variables were made stochastic using both perf and triangular distributions. The adoption of the LCSMs did improve financial performance in each scenario but the results were marginal. Other inputs such as growth rates, survival rates, feed costs, and post larvae costs are more sensitive in deciding the financial health of RAS shrimp farms.

MOTE AQUACULTURE RESEARCH PARK: MARINE FINFISH INFRASTRUCTURE, RESOURCES AND PARTNERSHIP OPPORTUNITIES

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Mote Marine Laboratory's Aquaculture Research Park is located on 100 hectares of land in Sarasota, Florida. Our research focuses on the development and evaluation of aquaculture technologies to produce fish and invertebrates for food and stock enhancement, as well as the development of land-based recirculating aquaculture systems. Mote has designed and evaluated the performance of recirculating aquaculture systems for breeding and rearing marine fish from egg to market size. Our facility is zero-discharge and tank-side filtration systems are linked to full-strength or brackish wastewater treatment systems to process solid waste and filter seawater for reuse. Recirculating systems are equipped with solids filtration, biofiltration, denitrification, ultraviolet light and/or ozone sterilization, and temperature control. Systems are available to support marine finfish broodstock (ranging from 25 m^3 to 44.5 m^3 in volume), replicated experimental, pilot- and commercial-scale larval, fingerling (ranging from 100 L to 8.9 m^3) and ongrowing systems (ranging from 8.9 to 71 m^3). Live food production facilities for rotifers and *Artemia* are also available. These systems have been used to culture economically important Gulf of Mexico and Caribbean species (snook, pompano, red drum, almaco jack, greater amberjack, flounder, red snapper). Stationary and hand-held instruments are used for analysis of conventional water chemistry and water quality parameters. Our science laboratories house advanced instrumentation and equipment including a UV visible spectrophotometer, freezers, incubators, and other specialized laboratory apparatuses appropriate for analysis and characterization of samples.

Current marine finfish research is focusing on common snook, red drum, almaco jack and Florida pompano. Applied research to address bottlenecks in aquaculture and system technologies has been successful due to strong collaborations with private industry, University, Foundation, and government agency partners. Examples of these collaborations include public-partnerships to improve or produce fingerlings and our long-term partnership with the Florida Fish and Wildlife Conservation Commission (FWC) to develop common snook stock enhancement technology. Our collaborations with private industry have led to research efforts focusing on optimizing broodstock and hatchery management technologies. The collaboration with FWC has led to development of improved culture technologies for reproduction and larval rearing of common snook, as well as stock enhancement research focusing on identifying critical habitats for fingerling snook. Expanded development of collaborations with research and industry partners continues to be a fundamental element of Mote's research program.

POLYCULTURE INCREASES GROWTH AND SURVIVAL OF LARVAL PACIFIC LAMPREY *Entosphenus tridentatus*

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Healthy wild river environments sustain large communities of fishes, invertebrates, and microorganisms. Laboratory environments are largely homogenous, providing little habitat complexity, few interspecies interactions, and semi-sterile conditions resulting from disinfection to control harmful bacteria or other pathogens. To test the effects of microbial inputs to our recirculating system, we examined growth and survival of lab-propagated larval Pacific lamprey (*Entosphenus tridentatus*) held in culture with speckled dace (*Rhinichthys osculus*). For the purposes of population supplementation and research, larval Pacific Lamprey have been propagated and reared at Confederated Tribes of the Umatilla Indian Reservation (CTUIR) facilities since 2012, mainly in monoculture (only lamprey). As larval lamprey are closely associated with the benthic environment in the wild, they likely have an important ecological relationship with the microbial community in substrate.

Average instantaneous growth rates for larvae reared in polyculture in 2018 were higher (7.2×10^{-3} mm/day) than those of monoculture larvae reared in 2016 and 2017 (2.1×10^{-3} mm/day; Figure 1A). Laboratory monoculture of larval lamprey in 2016 resulted in a 29.0% survival rate, adjusted to 90 days post-fertilization, while polyculture resulted in a 57.1% adjusted survival rate (Figure 1B). These results suggest that the presence of dace provided some improvement in the growth and survival of lab-propagated larval lamprey. Potential mechanisms for these results include direct consumption by lamprey of microorganisms or increased ecosystem services provided by the microbial community. Further research is needed to identify mechanisms of microbial interactions and their implications for production-scale lamprey aquaculture.

BIOECONOMICS OF *Flavobacterium columnare* VACCINE POND TRIALS FOR CHANNEL CATFISH *Ictalurus punctatus*

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The United States farm-raised catfish industry stocks channel catfish (*Ictalurus punctatus)* and hybrid catfish (*I. punctatus, ♀ x blue catfish, I. furcatus, ♂)*. In recent years, this industry has had a variety of problems, both in the ponds and in the marketplace. Increased disease pressures in the last few years have come primarily from the pathogens responsible for Columnaris (*Flavobacterium columnare*), Enteric Septicemia (ESC, *Edwardsiella ictaluri*), and virulent *Aeromonas hydrophila* (vAh). This project is conducting pond trials using comparing channel catfish vaccinated with a live-attenuated Columnaris vaccine versus an unvaccinated channel catfish control.

Goals of the project include estimating the value of the vaccine, as well as comparing growth rates and overall survival between vaccinated and unvaccinated fish. The strategy involves a pond study of channel catfish, vaccinated through bath immersion, with routine periodic sampling to track growth. Through this project we aim to create a vaccine for the US catfish industry, that when administered, will decrease mortalities due to *F. columnare*.

Experimental units include ten 0.1-acre watershed ponds. Each pond includes one 0.5 hp aerator, which runs from 6pm to 8am daily. Full water quality parameters of each pond are measured weekly and dissolved oxygen is measured twice daily. Fish are fed a 32% crude protein floating pellet once daily. Feeding rates are determined weekly for individual ponds, based on the 90/7 method. For this method, a pond is fed to satiation, and then 90% of that total amount is fed to the respective pond for the following seven days. Mortalities are counted and removed daily, while ponds are refilled as needed to maintain consistent water levels.

During monthly sampling, 30 fish are collected from each pond to record length and weight for each individual. Each fish is anesthetized with MS-222, and then measured for length and weight, before being placed in a recovery tank, and then returned to their respective pond. Additional blood samples were taken from ten fish per pond, during sampling events at months one and three. The blood samples will be processed and analyzed for antibody concentrations with an ELISA (enzyme-linked immunosorbent assay).

Harvest is planned for October of 2019, and complete data results and analyze will be presented at the 2020 Aquaculture America conference.

Figure 1. Recording the length of an individual channel catfish during a sampling event.

ASSESSMENT OF A *Flavobacterium columnare* VACCINE THROUGH POND TRIALS AND IDENTIFICATION OF VACCINE ADOPTION FACTORS IN THE US CATFISH INDUSTRY

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The catfish sector is a large contributor to the US aquaculture industry, as it processed 335 million pounds foodsize fish on 63,000 water acres of ponds in 2018 (NASS Catfish Production, April 2019). The majority of production ponds are located in rural counties of the southeastern US states of Mississippi, Alabama, Texas, and Arkansas. Fish health and disease mortality reduction are essential to this industry's profitability, success and sustainability.

Effective fish health and disease treatment begins with good farm management. Feed restriction is a common practice used to reduce disease transmission between actively feeding catfish. Use of medicated feed is popular, but it is expensive and requires a veterinary feed directive (VFD). Bath treatments, such as copper sulfate against columnaris, are often costly and require additional labor as well. Vaccines are an emerging treatment option, which could reduce medicated feed use. In addition, vaccines can be delivered through a variety of methods, including bath immersion, injection, or coated feed.

Three major diseases affecting US catfish production are enteric septicemia of catfish (*Edwardsiella ictaluri*; ESC), virulent *Aeromonas hydrophila* (vAh), and columnaris (*Flavobacterium columnare*, COL). In this project, a live-attenuated COL vaccine is being compared against a non-vaccinated control channel catfish (*Ictalurus punctatus*) treatment. If this vaccine trial is successful, it is hoped that its future use could lower the 2018 annual loss of 1.5 million pounds of catfish to COL in Alabama. Another goal of this project is to identify factors affecting COL vaccine adoption by producers in the US catfish industry.

COL vaccine efficacy will be evaluated through an on-going pond trail. Channel catfish fingerlings were stocked into ten 0.1-acre ponds, with five ponds stocked with COL vaccinated catfish via a one-hour bath immersion at stocking and the other five ponds of catfish being a control with no vaccine. Seven monthly samplings of catfish from each pond will provide data for growth rate, feed conversion ratio (FCR), and survival analyses. Blood samples taken at 30 days and 90 days post-stocking will be analyzed for IgG antibody concentration using an ELISA test. Factors affecting COL vaccine adoption will be identified by reviewing the history of the previously available COL vaccine (AQUAVAC-COL).

THE CHALLENGES AND SOLUTIONS TO LONG TERM ENGAGEMENT OF AQUACULTURE STUDENTS IN A MULTIPLE YEAR PUBLIC SECONDARY SCHOOL AQUACULTURE PROGRAM

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Students in the Academy of Natural Resources at Island Coast High School in Cape Coral, Florida are offered an academically rigorous curriculum following a course sequence of Agriscience Foundations, Aquaculture II, Aquaculture III and Aquaculture IV. These students had the opportunity to learn in the classroom over a four-year program and apply their skills to run a commercial level facility that has grown to feature two indoor vaults systems, various aquaponic systems, and multiple fiberglass finfish tanks. Students are also offered the opportunity to earn the Aquaculture Technician certification through the Florida Aquaculture Association after completing year three of the program. However, maintaining the students' interest and authentic engagement for multiple years can be challenging.

In the beginning the administration and student ownership was easy due to the unique concept of sustainability of producing products with little or no damage to the planet in which we live. This began with hydroponics which utilize minimal water compared to traditional farming techniques. First year students are charged with designing and constructing their own hydroponic system and raising a crop to fruition. Additionally, students learn and practice the fundamentals of aquaculture and raising a basic crop, tilapia. They balance pH, establish and carry out feeding regimes, monitor growth, and encourage reproduction.

Through-out the progression of the Academy, the challenges have been how to continue this ownership in the program. We have learned to incorporate whatever ecological/environmental issue that is the fore-front of the latest news-cycle. By addressing the latest issues, we not only engage our students in the issues at hand but challenge them to problem solve and devise solutions. Some of the topics we have addressed over the years include utilizing an invasive species for a positive outcome. Students removed and recycled bamboo to protect juvenile mangroves from wave action when installed in a restoration site to be utilized as shoreline protection and filtration. This project was extended when we involved a local business partner who allowed our students to install mangroves in reef balls to create a natural filtration system at the commercial dock. We were able to extend this project even further with our high school students teaching water quality to local elementary students who were afraid to go into the local gulf waters due to red tide and freshwater releases from Lake Okeechobee.

Through these projects, not only has aquaculture become a vital part of sustainability in the future, it can also be utilized for solutions to environmental issues that keep out students authentically engaged in aquaculture and becoming stewards of the environment.

BUILDING AQUACULTURE ACCEPTANCE THROUGH 4-H

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4-H began at the end of the 19th century as a way to bring new agricultural techniques to farmers through rural youth programs. Reluctance of farmers to adopt new agricultural practices was circumvented by introducing youth, who were more open to new ideas, to innovative farming methods that they would then bring back to the adults in their communities. Today 4-H is dedicated to youth development in communities nationwide. With a focus on STEM advancement, 4-H offers in and out of school programs, clubs, and camps for youth ages five to 19. With roots in agriculture and animal science, 4-H is a natural fit for aquaculture advancement programs.

Through an innovative summer internship program developed by the University of Maine's Center for Cooperative Aquaculture Research and Hancock County Cooperative Extension 4-H Youth Development Program, high school students were introduced to the role of sustainable aquaculture in the global food system. The group of seven students formed a 4-H club with the purpose of demonstrating sustainable aquaculture to the public at a local agricultural fair through a 4-H exhibit.

The students spent their summer, over an eight-week timeframe, at the Center for Cooperative Aquaculture Research (CCAR), in Franklin, Maine. While at the CCAR, students were responsible for the production of 165 yellowtail kingfish (*Seriola lalandi*). Emphasis was placed on the development of skills needed to rear fish and run and maintain a recirculating aquaculture system. In addition, participants learned about the history of aquaculture, engineering, and business.

We believe this may be the first time a farmed fish has been shown as part of a 4-H program at a rural agricultural fair. By exhibiting aquacultured products alongside traditionally farmed crops, it is our hope that one day farmed fish will be as socially accepted as farmed beef, pork, and chicken.

Funding for this project was provided by Maine EPSCoR/SEANET.

BUSINESS INCUBATION IN MAINE FOR MARINE FINFISH AQUACULTURE: 20 YEARS OF IMPROVEMENT AND LESSONS LEARNED

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The Center for Cooperative Aquaculture Research (CCAR) was established in 1999 by the University of Maine as a place where aquaculture research, development, and business incubation could be carried out at commercially relevant scale. The site had been previously developed as a privately-operated hatchery supplying salmon smolts for Maine's salmon aquaculture industry. It had access to fresh and salt water, along with six functional recirculating aquaculture systems (RAS) previously used to rear salmon and Arctic charr. The largest of these RAS consisted of two 300,000 gallon (1,100m^3) in-ground tanks, each supported with an independent RAS. Located about an hour from the University of Maine campus, the facility appeared well suited for collaborative research between university scientists and industry.

In its early years (2000 – 2008), CCAR activities were directed towards development of what were perceived to be 'alternative' or 'new' aquaculture species: Atlantic halibut, cod, and marine polychaetes. These cold-water species were chosen as viable alternatives to salmon because they were native to the Gulf of Maine, the wild stocks were depleted, they had high market value, and hatchery and culture methods were developed or under development in other regions. Parallel with these activities, aging and obsolete infrastructure was renovated or replaced and new infrastructure was developed, including a 24,000ft^2 (2,230m^2) dual-species (halibut and cod) marine hatchery and an 11,500ft^2 (1,068m^2) aquaculture business incubator building. The CCAR worked with four industry partners during this period to produce cod, halibut, and the marine polychaete *Nereis virens*. These species were reared in CCAR hatcheries from captive wild broodstock and raised to market size in existing and newly developed CCAR production units. While the companies incubated their aquaculture start-ups at CCAR they developed plans to build production facilities and worked to finance their efforts through public and private funding. Public support at the state level was essential and came through entities such as the Maine Economic Improvement Fund (MEIF) established by the legislature in 1997; the Maine Aquaculture Innovation Center (MAIC); and the Maine Technology Institute (MTI). This support is ongoing and remains critical today.

Ultimately, however, these early ventures all failed. Although failure can be attributed in part to the Great Recession of 2007-09, other underlying causes played as great, and perhaps greater, of a role, and valuable lessons were learned. These lessons include, but aren't limited to, the importance of having established sound aquaculture infrastructure as a foundation for success and having the backing of private investors with a long-term view. For a period of about three years CCAR business development slowed and attention shifted to new species such as green sea urchins, seaweed, and California yellowtail *Seriola lalandi*.

Today, in 2020 and twenty years after its founding, the Center for Cooperative Aquaculture Research is a thriving hub of activity as Maine experiences a resurgence of interest in land-based aquaculture. Three companies are using CCAR business incubation space and aquatic systems to commercialize aquaculture of marine ornamentals, American eels *Anguilla rostrata*, and California yellowtail. Green sea urchins *Strongylocentrotus droebachiensis,* marine macroalgae, and lumpsucker *Cyclopterus lumpus* are also under commercialization development at CCAR. Along with this activity, three other companies recently announced plans to build large salmon RAS facilities in Maine. These developments have led to consideration of expanding the CCAR's scope to include workforce training and development to train the anticipated need for marine hatchery and RAS expertise.

In this presentation we describe the twenty-year history of the CCAR and how it functions in greater detail. We examine issues of facility cost recovery; causes of commercialization failure and conditions for success; the critical role a broad public infrastructure plays in supporting aquaculture business development; species selection; and other factors.

FIXED FILM AND SUSPENDED GROWTH IN SMALL-SCALE INDOOR RAS PRODUCTION OF MARINE SHRIMP

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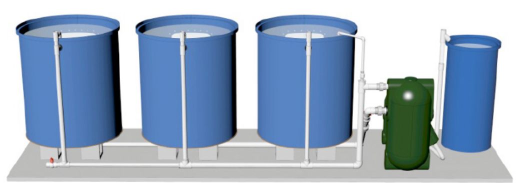
A Phase II USDA SBIR grant to develop cost effective technologies for the indoor recirculating production of the marine shrimp (*Litopenaeus vannamei)* was awarded to AST. An obstacle to obtaining commercial success was identified as low tank production densities. Phase I studies demonstrated commercially attainable production densities of 5 kg/m^3 (25 count) across both biofloc and fixed film formats. In the first Phase II study, a fixed film water treatment format was evaluated at a small 250-L scale in triplicate resulting in a mean production density of 10 kg/m^3 falling short of the 15 kg/m^3 required for cost effective tank production in small-scale operations. Loss of larger molting shrimp during periods of elevated nitrite levels (>0.6 mg-N/L) appearing to limit higher densities. The findings were consistent with the working hypothesis that that RAS densities are limited by water quality although the nitrite peaks are suspected of causing the issues here was much lower than expected. The experiments are now being repeated with more stringent nitrite control mechanisms.

Moving forward the AST RAS design will focus on the use of a fixed film PolyGeyser® floating bead filter supported by an aerated sludge basin (ASB) that will provide addition nitrification support external to the tank. Phase II studies that employed bead filter with an in tank biofloc nitrification were thwarted by the complexity of maintaining target tank suspended solids in the tank while a bead bioclarifier was constantly removing it. Balance was achievable in well trained hands, but, deemed unfeasible for small scale commercial facilities. Moving the biofloc to an external sludge basin significantly simplified the integration of the biofloc and fixed film technologies. The emerging designs will employ pneumatic sludge and water transfers between the PolyGeyser® and the ASB while water movement with the tank will be implemented by airlift. It is projected that these design improvements will lower capital cost while the density studies improve production improving economic feasibility.



THE IMPACT OF NUTRIEPIGENOMICS IN FISH IMMUNE RESPONSE

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The massive salmon farming has brought a significant increase in infectious diseases, thereby generating economic losses. The constant interaction of cultured fish with pathogens makes it necessary to search for new alternatives to reduce the mortalities generated by infectious diseases.

Epigenetics studies are all those non-genetic factors that are involved in the expression or gene silencing, without interfering with the DNA sequence. These mechanisms determine the degree of DNA packaging and therefore the ability to express a gene in the correct space and time, which it is crucial to emergence of physiological phenotypes.

DNA methyltransferase (DNMT) is an important epigenetic enzyme involucrate in DNA methylation. This study explores the effect of use natural DNMT inhibitors, as supplement in diet of salmonids.

We measure the capacity of natural supplement to diminish the DNA methylation activity in Head Kidney, Spleen and Liver of *Salmo salar* Figure 1.

We observed that DNA methyltransferase inhibitors treatments were able to modulate the mRNA expression of DNMT and interferon system genes Figure 2.

In consequence, the inhibition of DNMT enhancer the expression of interferon system genes and improve the fish response to infection with necrosis pancreatic infectious virus (IPNV). Figure 3.

Our work suggest that epigenetic mechanisms modulated trough diet, can impact the antiviral host genes expression which open a new frontier of development of epigenetics functional supplements to aquaculture feeding.

Funding Fondecyt 3170881, Fondap 15110027 and Fondef VIU16E0165.

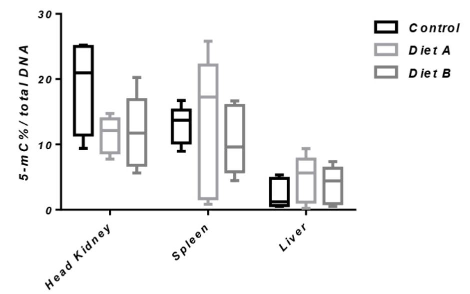

Figure 1. DNA methylation. Global Methylation of fishes feed with control, diet A and B.

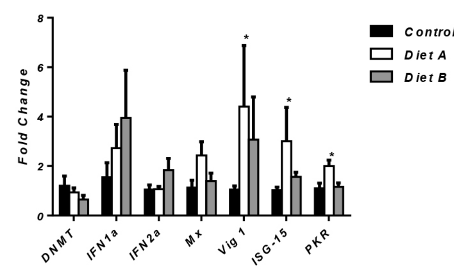

Figure 2. Relative expression. qPCR analysis of Head kidney of fishes feed with control, diet A and B.

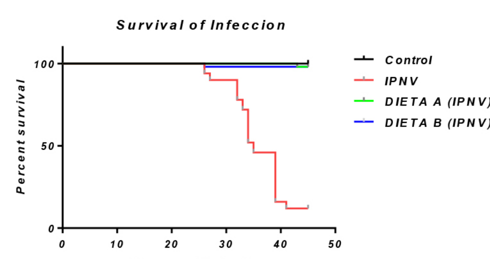

Figure 3. IPNV challenges. Survival of fishes previously feeding with control diet or diet containing natural supplements DNMT inhibitors

EFFECT OF SALINITY CHANGE AND EXPOSURE TIME ON THE EGG STAGES OF TWO ABALONE SPECIES *Haliotis discus discus* AND *Haliotis gigantea*

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Torrential rainfall is one of the main external cause that affects coastal marine species during spawning seasons. Heavy rain over a certain period may have effects on embryonic development of coastal marine organisms. The Japanese government started a marine stock enhancement programme in 1963 and abalone was one of the first targets to help restore the decrease in wild stock population. Even though the Japanese large-scale stock enhancement programme was expected to sustain the fishery production of abalone, it was claimed that stocking cultured juveniles had not contributed to the enhancement of wild abalone stocks. Therefore, the objective of this study is to find out the combined effects that salinity change, and exposure time have on disk abalone and giant abalone eggs with regards to onset hatching time, hatching success rate, percentage of abnormality and survival rate.

Disk abalone (*H. discus discus*) and giant abalone (*H. gigantea*) eggs were obtained from Mie Prefectural Hatchery in Hamajima, Japan. For each species, 6 replicates per treatment were prepared and each replicate contained 90 eggs that were transferred into six-well plastic microplates and incubated at a temperature of 20 ± 1ºC under white light conditions of 12:12 h light/dark cycle. The effects of salinity change and exposure time on both species were tested by reducing the salinity from 34, 24 and 14 psu with exposure times of 0, 1, 3 and 6 h. The experiments were carried out for a period of 5 days.

Onset hatching time significantly increased for both species as salinity dropped and exposure time increased. Both abalone species followed a decreasing trend in terms of hatching success rate as exposure time increased at low salinity levels. As for abnormality, both species showed a significant negative effect of low salinity and long exposure times. Giant abalone showed more adaptability to long exposure time at low salinity levels compared to disk abalone, hence a higher survival rate.

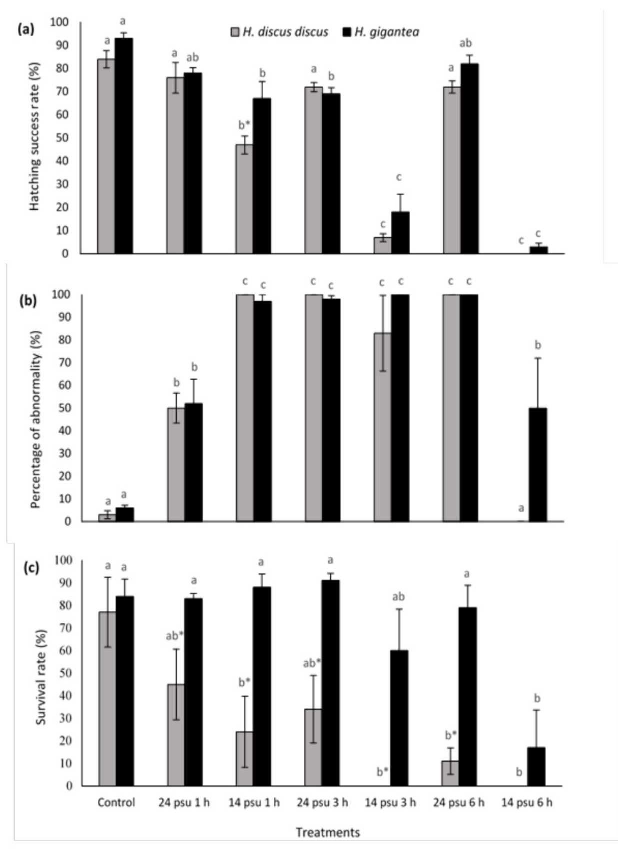

Fig. 1 Hatching success rate 24 h post-hatch (**a**), percentage of abnormality 24 h post-hatch (**b**) and survival rate 5 days post-hatch (**c**) of disk abalone *H. discus discus* and giant abalone *H. gigantea* in experiment 2. Different alphabets represent a significant difference between treatments and asterisks (*) represent a significant difference between species in the same treatment ($p < 0.05$)

STUDIES OF SPAWNING DYNAMICS AND GROWTH AND SURVIVAL DURING THE LARVAL AND EARLY-JUVENILE STAGES OF YELLOWFIN TUNA AT THE IATTC'S ACHOTINES LABORATORY IN PANAMA

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The Inter-American Tropical Tuna Commission (IATTC) has been conducting research on the early life history and biology of tropical tunas at the Achotines Laboratory in the Republic of Panama since 1986. Spawning from a population of yellowfin tuna (YFT) has taken place almost daily in the Laboratory's land-based tank since 1996. Eggs and larvae collected from the broodstock tank are used to conduct biological and physical experiments aimed at gathering ecological information on early life history stages.

The near-daily spawning of yellowfin at the Achotines Laboratory represents the only sustained spawning of yellowfin in captivity in the world. Water temperature is the most potent influence on timing and occurrence of spawning in our tank population. Broodstock fish exhibit the ability to increase their daily egg production in response to increases in daily food ration. Overall spawning dynamics of the yellowfin broodstock population will be described.

Pre-recruit research on yellowfin at the Achotines Laboratory has focused on growth and survival dynamics of larvae (the first 3 weeks), but in recent years the research focus has expanded to the early-juvenile stages (1-6 months). Growth rates of larval yellowfin (3-16 mm SL) have been estimated in the laboratory and *in situ*. Growth rates have been estimated for all transformation and early-juvenile individuals reared in land-based tanks or a sea cage; the early-juveniles have ranged from 1.6-28.0 cm in length and up to 158 days after hatch.

The studies of growth during the first 6 months have strong application to tuna aquaculture. In 2015, in collaboration with Kindai University, the first transfer worldwide of yellowfin juveniles from landbased tanks to a sea cage was successfully completed. The overall growth dynamics of larval and early-juvenile stages will be described, and juvenile rearing studies planned for 2020 will be summarized.

NATIONAL VETERINARY ACCREDITATION PROGRAM MODULE 13: AQUATIC ANIMAL HEALTH REGULATIONS AND HEALTH CERTIFICATION

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The goal of the NVAP program is to ensure that private veterinary practitioners who provide regulatory services to U.S. livestock, poultry and aquaculture industries, are adequately trained and well acquainted with regulatory requirements through USDA-APHIS accreditation. In addition to other accreditation requirements, accredited veterinarians are required to successfully complete a required number of 29 training Modules (http://tinyurl.com/NVAP-Modules), four of which currently cover aquatic animal health regulatory issues.

Although initiated as a 1896 agreement between the U.S. and Canada to combat equine disease outbreaks, in 1921 the U.S. Department of Agriculture (USDA) formalized the National Veterinary Accreditation Program (NVAP) so private practitioners could assist Federal veterinarians in controlling animal diseases. In 1992 regulations allow standardized procedures and requirements, and uniform administration to be managed nationally by APHIS, but with authorization of veterinarians licensed to practice on a State-by-State basis.

In 2001/2002 an “Animal Health Safeguarding Review by the National Association of State Departments of Agriculture (NASDA) to further redesign and upgrade the NVAP and suggested that “the accreditation program be the core for emergency preparedness and the response plan.” Recommended revisions were published in 2002 (“New Directions for the National Veterinary Accreditation Program,” J. Amer. Vet. Med. Assoc., 22(10): 1470-1472), with revised regulations implemented in 2009.

With accredited veterinarians being the first line of defense against catastrophic disease outbreaks, U.S. has successfully controlled outbreaks of several foreign animal diseases (FADs), including contagious equine metritis, equine piroplasmosis, epizootics of exotic Newcastle disease and West Nile virus, cases of screwworm and monkey pox, and pandemics of the influenza virus – and several aquatic animal diseases.

Module 13 provides information about the agencies involved in regulating aquatic animal health and trade, with an emphasis on USDA and the role of accredited veterinarians. It also addresses the proper completion of health certificates for farmed aquatic animals and provides resources for obtaining current regulations.

LAND-BASED CULTURE TRIALS IDENTIFY CANDIDATE TROPICAL MACROALGAL SPECIES AND OPTIMAL CULTURE CONDITIONS FOR MARICULTURE IN HAWAI ‘I

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When looking for potential ways to help feed and fuel our future amidst a growing population and limited natural resources, seaweed biomass production offers many opportunities. Seaweed mariculture does not use earth’s arable land, freshwater, or artificial nutrients, while absorbing excess nutrients and carbon from the ocean. It has the potential to remediate so-called “dead-zones” from eutrophication in areas of the planet where this is a problem, and counter ocean acidification at local scales.

Kampachi Farms is pioneering seaweed culture methods for offshore tropical waters, with a demonstration project on track to be deployed one nautical mile off the coast of Hawai‘i Island in 2020. Challenges include the nutrient-limited nature of tropical surface waters; offshore depths rendering fixed grid or multiple point mooring arrays too expensive; and expensive labor for harvesting. This presentation will focus on the selection of suitable native Hawaiian seaweed species to grow on the offshore demonstration project for foods, feeds, and biofuels.

After a year of land-based experimental trials, we propose three species candidates: *Caulerpa lentillifera, Gracilaria parvispora,* and *Sargassum aquifolium* for the offshore demonstration. Results will be discussed from light trials conducted to determine optimal water depth; nutrient trials to determine growth rates using nutrient-rich deep sea water; and successful sexual reproduction of *Sargassum aquifolium* that our team has performed in the hatchery.

PHYSIOLOGICAL EFFECTS OF THREE DIFFERENT DIETS ON THE REARING OF SEA URCHINS IN RECIRCULATING AQUACULTURE SYSTEM

Lindee Mason*, Asif Mortuza, Megan Rasnake, and Ahmed Mustafa

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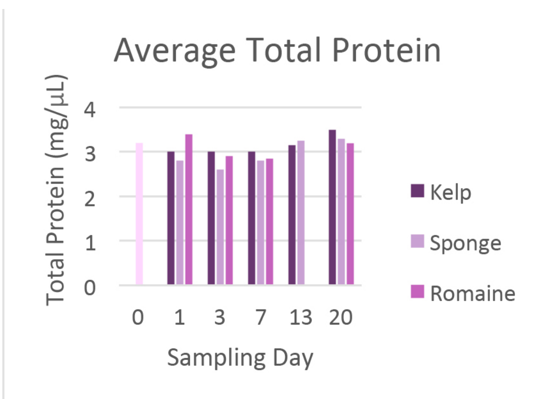
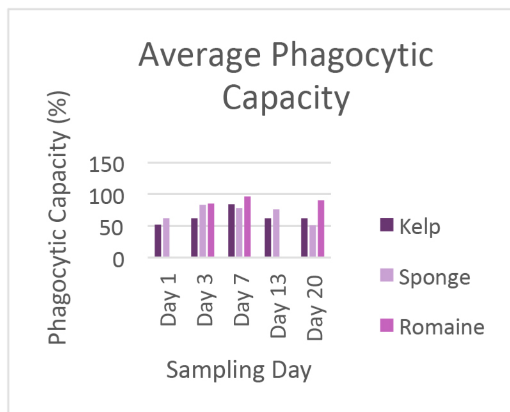
Experiments were conducted to investigate the effects of three different diets on sea urchins reared in recirculating aquaculture systems. Sea urchins were reared in three groups – sea urchins fed sponge, sea urchins fed kelp, and sea urchins fed romaine lettuce. The experiment ran for three weeks and the animals were sampled at regular intervals. Total body weight, gonad weight, gonado-somatic index, packed cell volume, coelomic fluid protein, total and differential cell counts, and coelomocyte phagocytic capacity were observed to measure the physiological and immune responses. Sea urchins were successfully reared in the lab on all three diets. Preliminary data did not show any significant differences in the physiological and immunological parameters. Further investigation needs to be done to determine which diet is best for rearing sea urchins in captivity.





Figures. Total protein concentrations of coelomic fluid and phagocytic capacity of coelomocytes of sea urchins, reared in recirculating aquaculture system and fed three different diets.

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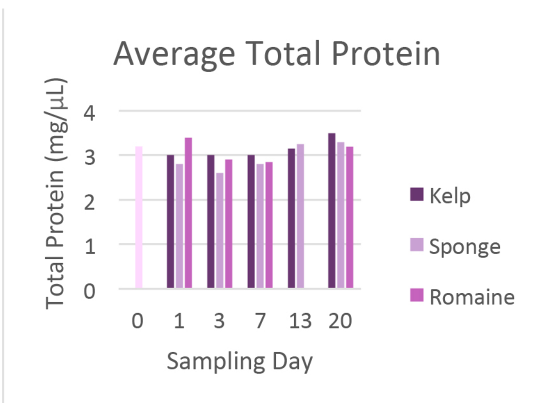

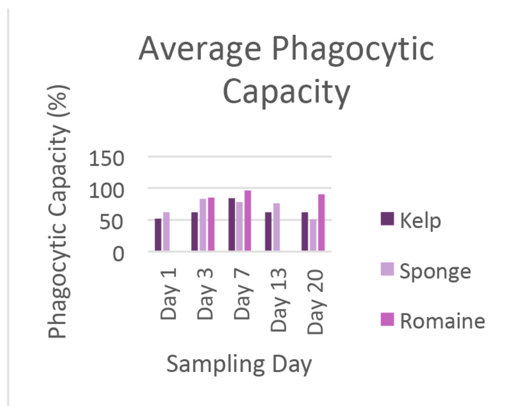

Figures. Total protein concentrations of coelomic fluid and phagocytic capacity of coelomocytes of sea urchins, reared in recirculating aquaculture system and fed three different diets.

OBSERVATIONS OF HAWAIIAN FLAME WRASSE *Cirrhilabrus jordani* REPRODUCTIVE BEHAVIOR

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The fairy wrasses in the genus *Cirrhilabrus* are popular in the marine ornamental industry due to their small size and vibrant colors. Fairy wrasses inhabit deep-water reefs, and therefore, new species are frequently discovered but are only rarely collected. The relatively limited supply of wild-caught fishes coupled with consistently high demand renders fairy wrasses ideal candidates for aquaculture, but there are numerous challenges posed by the complex reproductive biology and mating systems. For several years, the University of Hawaii at Hilo Pacific Aquaculture and Coastal Resources Center has been developing broodstock and larval rearing techniques for the endemic Hawaiian Flame Wrasse *Cirrhilabrus jordani* as part of the Coral Reef Breeding Program. Establishing and maintaining broodstock groupings for consistent egg production has proven difficult due to the propensity for females to rapidly transition to males under captive conditions. Additionally, males vary in their reproductive prowess ranging from highly active to inactive. Further complicating broodstock management, our observations indicate that there are potentially sneaker males, or individuals with female coloration that function as males. Our results have led to the development of a process of forming a group of wild-caught fish with the highest potential for maintaining a broodstock group that produces eggs nightly over protracted periods. We present our observations of this species with the intent that these techniques may be useful for the establishment of broodstock groups of other valuable and difficult to obtain *Cirrhilabrus* species.

COMBINED EFFECTS OF TEMPERATURE AND CO_2 CONCENTRATION ON THE GROWTH AND CHEMICAL COMPOSITION OF MARINE PHYTOPLANKTON *Nannochloropsis oculata* AND *Chaetoceros gracilis*

Rebeca Mata*, Jackson Gross and Tien-Chieh Hung

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Algae, the most abundant primary producers in the ocean, are organisms capable of transforming inorganic nutrients into organic matter through photosynthesis. As primary producers, they are the first trophic level in the ocean food chain and serve as a food source for consumers at higher trophic levels. To produce organic compounds, such as carbohydrates, lipids, and proteins, algae utilize dissolved CO_2. Hence, their physiology is influenced by ocean acidification and rising ocean temperatures. As temperature and CO_2 concentration change, algal cells chemically adapt to the environment producing biomass with differing chemical compositions. Temperature strongly influences algae cellular chemical composition, uptake of nutrients and CO_2, and productivity. Changes in CO_2 concentrations affect algae growth, lipid formation, productivity, and species composition. The objective of this study is to determine the independent and combined effects of various CO_2 concentration and temperature levels on the growth and chemical/nutritional composition (lipid, protein and carbohydrate content) of two marine phytoplankton species important to Northern California: *Nannochloropsis oculata* and *Chaetoceros gracilis* to further understand their impacts on primary consumers.

Mimicking projected conditions for the temperature and CO_2 levels in Northern California, where the majority of oyster farms in California are, algae will be grown in two different temperatures (10°C and 18°C) and at two different pH (8.1 and 7.8). These treatments bracket the present and projected conditions for 2100, by the Intergovernmental Panel on Climate Change (IPCC), to determine the combined effects of ocean acidification and temperature on their growth and chemical composition. Growth, lipid, protein and carbohydrate content will be analyzed for each condition. The results from this study will be used to understand the impacts of changes in primary producers, like algae, on primary consumers, like oysters, which correspond to the first and second trophic levels, respectively, in the ocean.

EVALUATION OF EARLY LARVAL WEANING OF LIVE FEED FOR BURBOT (*Lota lota*)

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Live food utilization is essential for small burbot larvae after hatching to ensure high survival and growth rates. However, the feeding plan for live food is expensive, it requires manpower and expensive equipment. The nutritional values of live foods are difficult to control. It would be economically beneficial either to use live food substitutes at first feeding or to minimize the duration of feeding burbot larvae on live foods. Therefore, the study was conducted to: 1) determine if the period of providing live feeds to burbot larvae could be reduced in order to shorten this early production phase and decrease labor and feed costs and 2) provide fish growers with applied knowledge of cost-effective strategies for weaning small burbot larvae. The study was conducted for 77days post hatch (DPH). A randomized block design with six treatments was used. 400 larvae/tank were stocked at 7days post hatch (7DPH) into triplicate 4L tanks. 10% of the total population was sampled for each treatment for survival counts, length and weight every 7-10 days. A digital camera was used to take pictures for length measurement. Length measurement was done using an ImageJ software. All data was analyzed using analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post hoc testing. The findings show that treatment 4 (weaning protocol-fed SF and GSL artemia + dry diet) yielded the highest survival rate (23%) by 77DPH, followed by treatment 3 (fish fed Commercial artemia substitute). Other treatments had low or no survivals. Growth length was higher for treatment 4(fed SF and GSL artemia + dry diet) & positive control by 77DPH, followed by treatment 3. Treatment 3 (fish fed Commercial artemia substitute) reduced on the time of feeding live feeds by 17 days (14% survival) when compared with treatment 4. Therefore, weaning small larvae directly on dry diets is challenging, however good results can be obtained when used in combination with live prey or when artificial artemia substitute is used thus reducing on production costs.

NEAR-DAILY RECONSTRUCTION OF TROPICAL INTERTIDAL SEA-SURFACE TEMPERATURE FROM LIMPET SHELLS TO INFER THEIR GROWTH RATES

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Measurements of life-history traits can reflect an organism's response to environmental factors.

In wave-dominated rocky intertidal ecosystems, measurements of key grazing invertebrates are constrained by extreme conditions. Recent research demonstrates mollusc shells to be high-resolution oceanographic climate proxies for SST as well as archival records of growth; however, no prior molluscan climate proxy has been demonstrated for the tropical rocky intertidal environment – a zone influenced by warmer waters, mixed tides, trade-wind patterns, and wave-action.

Here, we show the first near-daily, spatiotemporal climate proxy for SST in the tropical rocky intertidal environment by coupling secondary ion mass spectrometry analysis of oxygen isotopes with the sclerochronology of *Cellana sandwicensis*, an endemic Hawaiian intertidal limpet, that is a significant biocultural resource harvested for consumption. We also develop a method for reliable interpretation of seasonal growth patterns and longevity in limpets.

This study provides a robust approach to explore tropical intertidal temperature climatology and molluscan life-history.

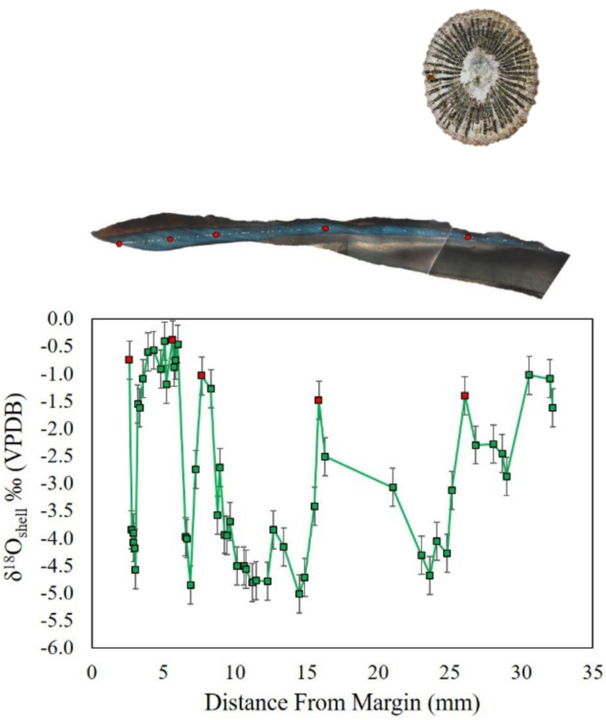

Figure 1: A shell cross-section and the associated oxygen isotope profile ($\delta^{18}O$) of a Hawaiian limpet (*C. sandwicensis*), reported in parts per mil (‰) relative to the international VPDB standard, were measured sequentially along the growth axis – starting at the shell margin. This pattern in the $\delta^{18}O$ profile of the historical shell (BPBM – green line) reflects the recorded seasonality in intertidal SST. The positive $\delta^{18}O$ measurements (red squares) were taken along and correspond with major bands (red circles) in the shell cross-section.

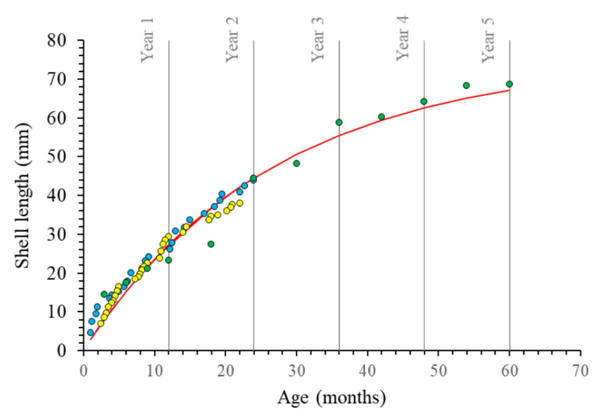

Figure 2: Observed shell length-at-age measurements of *Cellana sandwicensis* (blue circles: modern CW1 shell; yellow circles: modern CW2 shell; green circles: historical BPBM shell) and the best fit von Bertalanffy growth model for pooled measurements of all three shells (red line).

AQUAPONIC SYSTEM IMPACTS ON LETTUCE YIELD AND QUALITY

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Aquaponics is a holistic technology which integrates fish farming and hydroponic vegetables production with water continuously circulating throughout the system. The objective of our research was to determine and maintain optimal water quality parameters across a DWC aquaponics system to achieve fish and vegetable yields that exceed or are comparable to those of individual traditional hydroponic system for the same quantity of water and nutrients consumed. A completely randomized design was established with Tilapia and green leaf lettuce each with three replications for both an aquaponics and hydroponic systems. In the aquaponics system, 62 Tilapia fingerlings (7.5 to 10 cm) were stocked in each 750 L fish tank. Both systems were based on a nearly 950 L capacity plastic rectangular grow beds (1.2 m wide, 2.4 m long, and 30 cm deep). There were 4 floating rafts with 28 holes for grow cubes each totaling about 3 m^2 of grow space per bed. Both the aquaponics and hydroponics utilized Rockwool cubes as a growing media. Green Muir pelleted lettuce seed was sown into the rafts on a weekly rotation and harvested at around 35 to 40 days due to their close planting density, short growth period and frequent harvest. Lettuce growth and yield parameters plant height, photosynthesis, and chlorophyll (as a measure of N uptake using SPAD meter), biomass yield and protein content, and nutrient and heavy metal contents were measured. Results showed that lettuce grown under aquaponics had better leaf geometry and significantly higher photosynthetic rates and increased chlorophyll contents than that of the lettuce grown under hydroponic system. Of of the 13 harvests that have been done so far, aquaponics leafy biomass of lettuce totaling of 33.1 tons/ha and root biomass totaling of 6.7 tons/ha, with a shoot: root of 4.9. In contrast, hydroponics leafy biomass of lettuce totaled 21.3 tons/ha and root biomass totaled 4.6 tons/ha, with a shoot: root 4.6. In other words, aquaponics had a significantly higher (by 7%) leafy green biomass production, as compared with the hydroponic system. While both macro- (C, N, P, Ca, K, Mg, and S) and micronutrient (Fe, Mn, Zn, Cu, B, Mo, and Si) contents were higher, the heavy metals contents (Al, Ni, Cr, and pb) were lower in the leaf biomass of lettuce grown in the aquaponics, with respect to lettuce grown in conventional hydroponic system. The water quality parameters such as pH, EC, redox, ammonia, nitrate, phosphorus, total dissolved solids, BOD and COD measured on a weekly basis were much steadier and more balanced under aquaponics when compared with hydroponic system.

COOKE AQUACULTURE CANADA CLEANERFISH PROGRAM DEVELOPMENT: LAND-BASED GROWOUT AND TRANSPORTATION

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Lumpfish (*Cyclopterus lumpus*), and to a lesser extent Cunner (*Tautogolabrus adspersus*) production continues to expand in Atlantic Canada to meet the growing demand for cleanerfish as part of an integrated pest management strategy. While receiving juvenile lumpfish from Memorial University of Newfoundland Oceans Sciences Centre we continue to grow them out to cage appropriate size at 2 locations- the Belleoram facility in Newfoundland and ProNova Marine Products in Nova Scotia. This presentation will address production development at both facilities with a focus on the 2018-2019 growout cycle and development into the 2019-2020 growout. Additionally, due to the large geographic distances involved in production here the inherent unique transportation logistics and issues will be discussed as well. While continuing to expand use and development in Newfoundland future plans include expanding cleanerfish use in New Brunswick and Nova Scotia while supporting industry development in Maine.


Figure 1: Belleoram Lumpfish


Figure 2: ProNova Staff loading Lumpfish

Figure 3: Lumpfish transport trailer

OFF-SEASON SPAWNING OF WHITE BASS *Morone chrysops* AND THEIR SELECTIVE IMPROVEMENT TOWARD BUILDING A BETTER HYBRID STRIPED BASS *Morone chrysops x M. saxatilis*

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Off-season spawning of white bass (Morone chrysops) provides producers with the potential advantage of year-round availability of fingerling fish, especially when used in conjunction with cold banking. Additionally, off-season spawning permits producers with the flexibility to optimize the utilization of infrastructure investments moving toward a more continuous batch production cycle instead of the single season cycle currently used in the industry. A seasonal model involves grouping total annual production from a relatively small production window into batches of fish that are pushed for growth and others that are restricted for growth to meet market demands with neither option being optimal.

To better utilize fish rearing and live-feed facilities at Harry K. Dupree Stuttgart National Aquaculture Research Center (SNARC), white bass were conditioned for a year prior to October of 2018. Family crosses are part of the selective improvement of white bass to improve the resulting hybrid. Crosses from domestic and wild sourced white bass were made and reared in about 100 individual 35L tanks. They were fed rotifers (*Brachionus plicatilis*) and artemia (*Artemia franciscana*) until transitioning onto a prepared starter (#0) diet about 45 days post hatch. After all the crosses were conditioned to prepared diets the fish were transferred to 250L rearing tanks to quantitate growth and feed utilization on an individual cross basis on a fishmeal free selection diet for the 209 day selection time period.

From each of the family crosses, fish weight, fish length, K-condition factor, and other parameters were measured. White bass performance after the evaluation period was measured in common garden tanks of the 71 crosses and two control groups fed a commercial broodstock diet. Trends of that growth were compared to the growth of the crosses during the evaluation time period. Performance differences between the selection period growth and the post-selection period growth will be contrasted to examine if selection period trends continue based upon parental origin. Additionally, conditioning parameters for off-season spawning of white bass will be presented and discussed.

COMMUNICATIONS STRATEGIES FOR SUPPORTING STAKEHOLDERS AND BUILDING PUBLIC AWARENESS OF AQUACULTURE ISSUES, PRODUCTS AND VALUE

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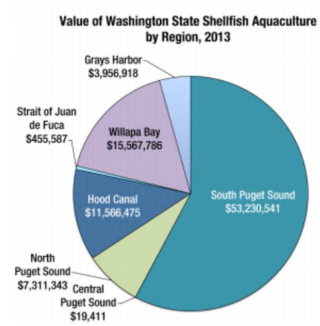
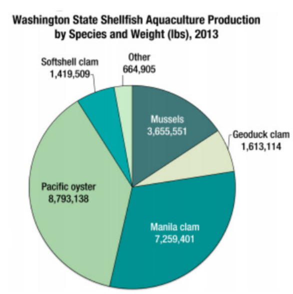
In 2011, Washington State began an ambitious effort to advance shellfish research, ensure clean water, improve the aquaculture permitting processes and address ocean acidification through the Washington Shellfish Initiative. In 2017, Washington State Department of Agriculture (WSDA) created the State Aquaculture Coordinator position to act as a liaison between growers and the many state and federal regulatory agencies that interact with industry. In addition the aquaculture coordinator is tasked with coordinating efforts of the Shellfish Initiative.

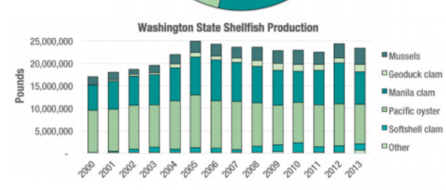
To meet these goals WSDA's communications team worked with the State Aquaculture Coordinator to develop strategies to promote the industry, work with the regulatory agencies and support the public understanding of aquaculture. The team employed social media, traditional media and stakeholder networking. This presentation explains some of the issues, hurdles and the techniques we have employed in our efforts to bring together sometimes disparate groups to make sure our state lives up to our commitment to Puget Sound and coastal communities in all aspects of shellfish production and harvest.

Shellfish are critical to the health of Washington's marine waters and the state's economy. In particular, shellfish farming is a foundation for rural Western Washington economies. Washington produces more shellfish than any other state in the country. Shellfish aquaculture adds more than 3,000 jobs and almost $300 million to the state economy. Shellfish farming has been an important component of Washington's growth for more than 160 years. It provided a foundational pillar for western Washington's rural economy and it's an important part of our state's heritage. Shellfish growers are the largest employers in at least two rural counties in Washington.

There is no question that shellfish farming is "farming" and can be supported in many of the same ways we support other aspects of the agriculture industry while at the same time protecting the public interest and the environment. We will discuss how to meet the challenge of facilitating interagency coordination to streamline regulatory processes and identify areas where rules or regulations are redundant.







Source: The Washington State Shellfish Initiative fact sheet.

EFFECT OF PHOTOPERIOD ON GROWTH, DEVELOPMENT, FEED CONSUMPTION, AND SURVIVAL OF RED SNAPPER *Lutjanus campechanus*

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Progress towards the development of reliable hatchery technology for red snapper, *Lutjanus campechanus,* has been limited by inconsistent spawning and difficulties in larval rearing. We report on recent progress to improve this technology. A trial assessing the effects of two different photoperiods (18L:6D and 24L:0D) was conducted at the University of Miami Experimental Hatchery (UMEH). A detailed protocol used for red snapper larval rearing from embryo through the completion of weaning from live to dry feeds is described.

Embryos were collected from a cohort of red snapper broodstock that are volitionally spawning regularly in copious quantities. Embryos were stocked into a cylindrical 400 liter incubation tank, treated with 100 ppm Formalin for one hour, and hatched approximately 24 hours after collection. A total of, 114,000 larvae were split evenly across (8) 400 liter larval rearing tanks at a stocking density of 35 larvae/liter. Four tanks were subjected to a photoperiod of 18 hours of full spectrum light, while the remaining tanks were subjected to 24 hours of full spectrum light through 11 days post hatch (DPH). Beginning at 12 DPH, all tanks were maintained with an 18 hour photoperiod. S-strain rotifers, *Brachionus rotundiformis*, were used as the exclusive first feeding source and were maintained at a density of 25/ml. *Artemia sp*. were introduced as a second feed source at 15 DPH and were co-fed with rotifers until 20 DPH. Both rotifers and *Artemia* were enriched with astaxanthin, a commercially available lipid supplement, algae extract, and taurine. Exchange rate was maintained at 250% per day and increased incrementally as larger feeds were introduced. Pure Oxygen and air were each lightly bubbled into each tank to maintain dissolved oxygen saturation from 6.5-9.0 mg/L. Temperature was maintained from 24.0 – 26.0 °C. Standard length, swim bladder inflation, and feed consumption were measured for each tank at 3 DPH, 6DPH, 9DPH, 12 DPH, and 16 DPH. Survival and was quantified at 12 DPH, 26 DPH, and at the completion of weaning 45 DPH. No significant differences in growth, feed consumption, or survival were detected between treatments at the conclusion of the trial. However, tanks maintained with 24 hour light showed completion of swim bladder inflation in >95% of larvae sampled by 9 DPH, while tanks maintained with an 18 hour photoperiod only showed 60% swim bladder inflation at this sample date. By 12 DPH, all tanks showed >95% swim bladder inflation. Due to this subtle, but potentially impactful difference, future larval rearing procedures should consider use of 24 hour light treatments through the completion of swim bladder inflation. Total survival across treatment groups through 12 DPH was 66%; survival was 31% at 26 DPH, and final survival at 45 DPH was 5.5%. This trial and other larval rearing trials at UMEH have demonstrated that red snapper can be raised solely on rotifers from first feeding through early larval stages. As broodstock continue to produce large quantities of high-quality eggs, a number of additional trials are planned to further optimize larval rearing protocols.

THE IMPORTANCE OF SHRIMP BREEDING IN EVOLVING THE MODERN SHRIMP INDUSTRY

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Prior to 1999 the world shrimp production was stagnant with many issues developing from known diseases and unknown syndromes. Prior to 1999, early work on domestication and shrimp maturation by the Oceanic Institute provided a basis for an industry in crisis to move from wild caught broodstock and post larvae to closed domesticated lines reproduced in closed maturation systems. The two crisis that were picked in 1999; provided the necessity for such a drastic world change in the way the industry operated; the WSSV epidemic that swept through the Americas and the Slow Growth Syndrome that made culturing *P. monodon* not profitable (Fig 1)

This talk will describe the early history and problems faced by the adoption of domesticated shrimp by the industry; and the move of Specific Pathogen Free (SPF) *P. vannamei* to Asia and how this adoption of SPF *P. vannamei* in Asia lifted the world production from one million tons to three million tons in 10 years. The domestication of shrimp also provided the platform to increase the efficiency of shrimp production that resulted in increased volumes of shrimp at lower costs to consumers. Also, it disrupted the shrimp industry and related industries with the application of genetic selection to the domesticated stocks. The progress in breeding is generating more efficient shrimp for culture and the newly developed and evolving molecular technologies will provide the basis for the next big disruption in the shrimp industry.

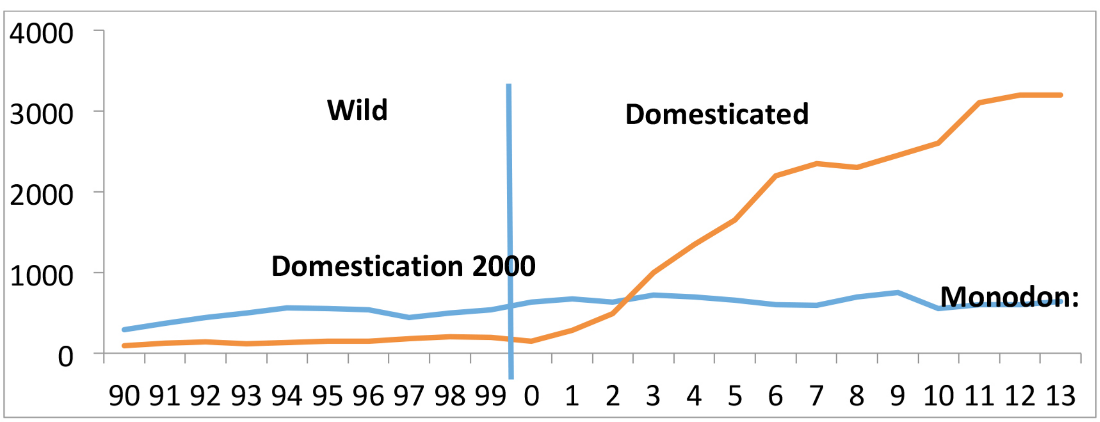

Fig. 1: 1999: The year the shrimp industry switched from wild shrimp stocks to domesticated shrimp stocks

CLAM PROCESSING COPRODUCTS AS AQUAFEED INGREDIENTS

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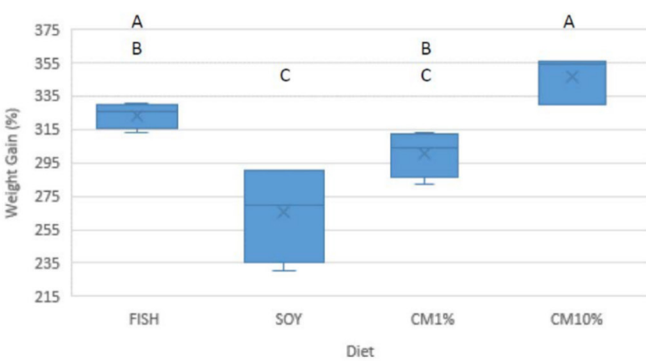
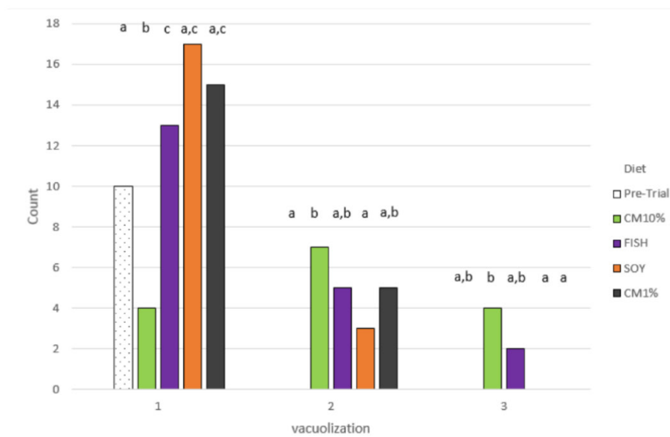
While fish meal and fish oil represent the nutritional 'gold standard' of feed ingredients in many aquaculture diets, the research community has continued to explore reduced fish meal diets and fish meal alternatives. Given that feed costs are commonly the major variable cost in aquaculture production, there is strong justification to continue to reduce the reliance on not only fish meal as a feed ingredient, but also to further reduce the cost of feed through the identification of suitable alternatives.

In an effort to find viable alternatives to fish meal, we are working with a corporate partner to evaluate the utilization of seafood processing waste streams, and their potential as fish feed ingredients. We have analyzed the nutritional profiles of the handful of unique byproduct streams, have identified which byproduct streams are promising as aquaculture feed ingredients, and derived limited quantities of a dried meal using these clam processing coproducts.

Beginning in 2017 experimental feeds were manufactured, containing either differing levels of clam processing coproducts as attractants or control diets absent of clam processing coproducts. Two trials have been completed with these original formulations being fed to populations of hybrid striped bass and barramundi. Initial results indicate that these diets are readily accepted by both species, and adverse effects attributable to clam-processing coproducts were not observed. A recently completed third trial with re-formulated diets including a diet containing clam processing coproducts at a 10x (CM10%) safety factor has shown that growth, feed conversion and feed intake did not differ between the positive control (FISH) and recommended inclusion rate (CM1%). CM10% demonstrated higher average growth and feed intake than FISH but these differences were not significant. Survival did not differ among all treatments ($p = 0.124$). All liver tissue subjected to histological examination were considered non-pathological across all four diets.





COMPARISON OF GROWTH PERFORMANCE AND SURVIVAL OF LARVAL GIANT KOKOPU *Galaxias argenteus* FED VARYING LEVELS OF INSTAR-I AND INSTAR-II *Artemia* NAUPLII

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Giant kōkopu (*Galaxias argenteus*) are a threatened, amphidromous fish species endemic to New Zealand. The larvae of this species are one of five making up the whitebait fishery, a local delicacy with a market price of NZ$150/kg and considerable aquaculture potential. However, in current production the *Artemia* feed regime (LD1) accounts for a significant proportion of production costs, inhibiting commercialization. Therefore, the purpose of this study is to optimize the live feeding regime to improve the financial feasibility of cultured giant kōkopu.

Three experimental live food treatments (Table 1), for feeding larval giant kōkopu were tested over a 28 day period starting on the first day of feeding (3DAH). Larvae were reared in round, 20 L tanks in RAS with water 35 ppt and 16-18 °C. Each tank, initially stocked with 2000 larvae, received 3 g of feed three times a day at 0830, 1230 and 1630 hours throughout the experimental period. Each feed treatment was conducted in three randomly selected replicate tanks.

After 28 days larvae in the LD2 treatment achieved the greatest growth, with length and mass being greater than that of the LD3 which in turn was greater than LD1. From day 11 onward LD1 had significantly greater mortality than the other two diets. There was no difference in total productivity between LD2 and LD3, but both were greater than LD1 (Fig.1). The extrapolated time to harvest was significantly different amongst groups with LD2 to achieve harvest weight at 74 DPH, LD3 78 DPH and LD1 80 DPH (Fig. 1). Consequently, switching to the LD2 or LD3 diet would result in 21% and 25%, respectively, savings on *Artemia* costs as well as 17% and 9% respectively on artificial dry food costs.

TABLE 1. *Live Diet (LD) regimes showing ratios of instar-I:instar-II Artemia nauplii, where LD1 is a replica of the current commercial feeding regime.*

	Week 1	Week 2	Week 3	Week 4
LD1	1:0	1:0	1:1	0:1
LD2	1:1	0:1	0:1	0:1
LD3	0:1	0:1	0:1	0:1

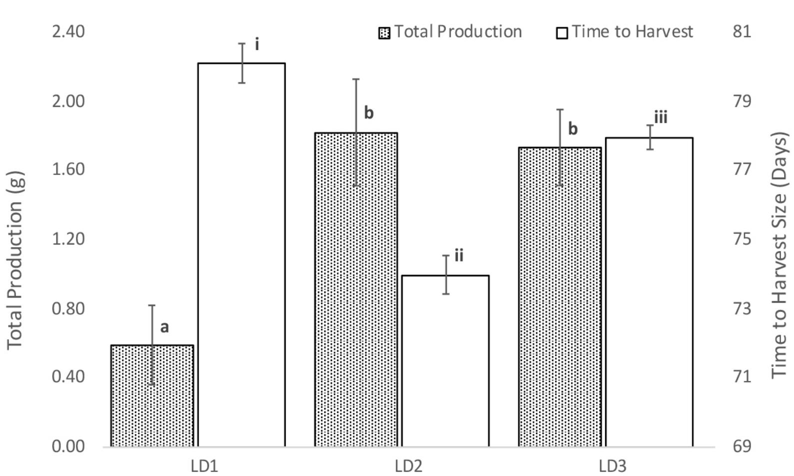

FIGURE 1. *Mean total production (primary axis) and mean time to harvest size (secondary axis) for larval giant kōkopu in three different feed treatments; LD1, LD2 and LD3 over the 28 day experimental period (mean ±SE). Means with different superscripts are significantly different (P<0.05).*

AN ECONOMIC AND ENVIRONMENTAL SUSTAINABILITY DECISION-SUPPORT TOOL FOR FISH-FREE AQUAFEED

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The U.S. aquaculture value chain—from companies developing alternative ingredients to aquafeed manufacturers and aquaculture farms—lack a scientifically verified and common platform for comparing economic and environmental performance of alternative ingredients (e.g., yeast, bacterial biomass, insect meal, microalgae) versus conventional ingredients (e.g., fish meal, fish oil corn meal, soybean meal, canola oil) for aquaculture feeds. Available tools for evaluating economic costs of conventional aquafeed ingredients lack robust methods for evaluating alternative ingredients. Existing tools also do not consider critical aquaculture performance parameters (e.g., digestibility of ingredients or growth metrics of fish fed the feed) nor do they consider environmental impacts. We are developing an open-access decision-support tool that will help drive innovation, commercialization, adoption and acceptance of more sustainable aquafeeds. Our research will address four inter-related objectives that culminate in developing a decision-support tool: (1) develop a meta-model database of life-cycle assessments (LCA) of alternative aquafeed ingredients compared with conventional ingredients, that will help decision makers compare environmental effects (e.g., marine eutrophication, global warming potential (GWP), freshwater consumption, land use change) of ingredients across LCA studies; (2) develop a meta-model database of techno-economic analysis (TEA) of alternative aquafeed ingredients compared with conventional ingredients that will help decision makers compare economic performance (e.g., least cost formulations, economics of scale) of ingredients across TEA studies; (3) develop a meta-model database of digestibility and nutritional feeding studies on alternative and conventional aquafeed ingredients that will help decision makers compare the nutritional performance metrics of alternative versus conventional ingredients in aquaculture that will help decision makers compare the nutritional performance metrics of alternative versus conventional ingredients in aquaculture feeds across studies;. and (4) develop a decision-support tool, with open-source software, that integrates the three meta-model databases and ingredient-specific process models.

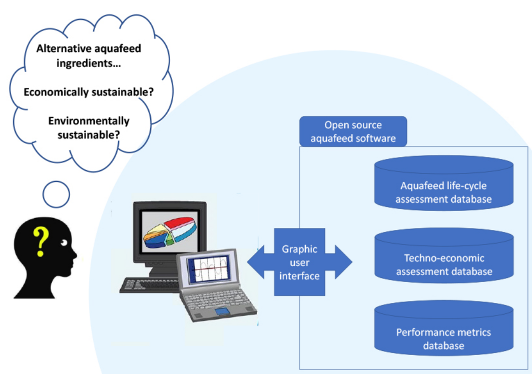

Figure 1: *Schematic of alternative aquafeed decision support tool.*

KA PŪNAEWELE MEA‘AI O NĀ LOKO I‘A: THE USE OF δ15 NITROGEN AND δ13 CARBON ISOTOPES AS AN EXTENSION OF ONE’S KILO TO UNDERSTAND TROPHIC INTERACTIONS WITHIN A LOKO I‘A

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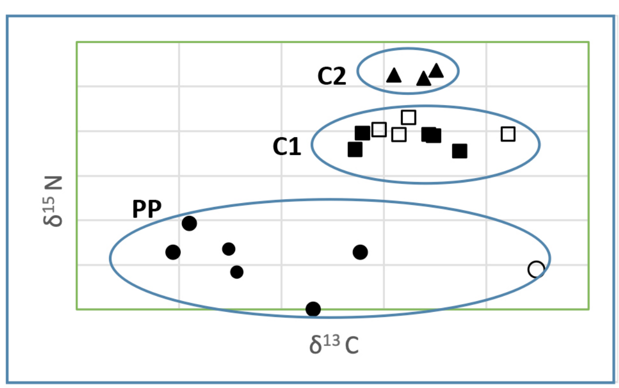
The east side of Hawai‘i Island, with its abundant rain and fertile soils, is susceptible to invasion by non-native flora and faunal species. The traditional functioning of the *loko i‘a* (traditional Hawaiian fishpond systems) of Waiāhole and Kapalaho are challenged by an abundance of non-native invasive species including California grass (*Brachiaria mutica*), Mexican mollies (*Poecilia mexicana*), Marquesan mullet (*Moolgarda engeli*), and tilapia (*Sarotherodon melanotheron*). Not only are these invasive species physically reshaping the environment through reduction in open water and increased sedimentation, but they may also be changing the water chemistry and the structure of native food webs within these *loko i‘a*.

In an attempt to quantify the energy dynamics at the *loko* i‘a, Kamehameha Schools Kumuola Marine Science Education Center (Kumuola) partnered with Ke Ana La‘ahana Public Charter School (KALPCS) to collect and process samples of aquatic and semi-aquatic native and non-native organisms for δ^{15} nitrogen and δ^{13} carbon stable isotopic analysis to further understand present-day energy sources and trophic niches at the *loko i‘a* of Waiāhole and Kapalaho.

The generalized functional groupings of primary producer (PP), primary consumer (C1) and secondary consumer (C2) were assigned based upon δ^{15}N clustering for all resident loko i‘a organisms analyzed (Fig. 1). The Mexican molly, kanda and tilapia, the three significant non-native invasive fish species at the loko, group with the native primary consumers and detritivores and have δ^{13}C signatures that overlap with important native food fish and crustaceans. Secondary consumers in the studied loko i‘a were all native, clustered tightly in δ^{15}N isotope signatures, and had broadly overlapping δ^{13}C signatures. Observational data by KALPCS students supports their grouping as the top-level consumers in these loko i‘a.

The invasion and establishment of non-native species to the Hawaiian Islands is a reality that we will continue to face going forward; understanding the nature of the resultant impact allows us to manage and combat the invasions to maintain the functionality of our culturally important places.

Figure 1. Native (closed) and non-native (open) species at the *loko i‘a* in functional groupings of primary producer (PP), primary consumer (C1), and secondary consumer (C2) based on δ^{15} N signatures.



APPLICABILITY OF MICROBIAL PHYTASE TO INCREASE PHOSPHORUS AVAILABILITY IN SOY-PROTEIN-BASED DIETS FOR LARGEMOUTH BASS *Micropterus salmoides*

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Over 50% of the phosphorus (P) in feeds containing high inclusion levels of soy-protein feedstuffs can occur as phytic acid (PA), which is not digestible by fish. Supplementation of microbial phytase (PTS) into soy-based formulations to degrade PA can increase P availability to fish and minimize P excretion to receiving waters.

An eleven-week feeding trial was carried out to evaluate the effects of supplementing PTS to soy-protein-based diets on the growth performance and mineral composition of largemouth bass (LMB). A P-deficient (~ 0.25% total non-PA-P), PTS unsupplemented basal diet was formulated to contain 42% crude protein and 12% lipid and was used as the negative control (D1; - Control). The basal formulation was then supplemented with PTS to obtain five additional diets (D2 to D6) with PTS activities ranging from 250 to 2000 units (U)/kg. Finally, a positive control diet (D7, + Control) was formulated by supplementing Ca-P monobasic to the basal formulation. Each diet was fed twice daily (8:00 and 16:00) and to apparent satiation to triplicate groups of 20 LMB (initial weight ~10.25g) stocked in 110-L aquaria operating as a recirculating aquaculture system. Statistically significant LMB responses to dietary treatments were considered at $P<0.05$.

Linear regression analysis on resulting data revealed no effects of PTS supplementation on LMB survival, feed intake, weight gain, and feed conversion efficiency; but positive trends were observed (Table). Orthogonal contrast analysis on selected treatments indicated that: i) + Control-fed groups consumed more feed, grew faster, and converted feed more efficiently than - Control- fed groups; ii) the supplementation of 2040 PTS U/kg of soy-protein-based feeds can improve LMB production performance and reduce the need for inorganic P supplementation. Results for P retention and concentration in whole fish and skeleton mineralization will be presented.

Production performance metrics (survival, feed intake [FI], weight gain [WG], and feed conversion efficiency [FCE]) of largemouth bass after 11 weeks of growth trial. ND = not detected; PSE = pooled standard error.

Diet	PTS U/kg	Survival %	FI %BW/day	WG % of initial	FCE
D1 (- Control)	95	88	2.8	243	0.68
D2	345	98	2.7	268	0.69
D3	759	78	2.7	257	0.74
D4	519	90	2.7	275	0.73
D5	948	97	2.8	268	0.67
D6	2040	90	2.9	316	0.77
D7 (+ Control)	ND	87	3.0	371	0.84
PSE		2	0.03	13	0.02
Linear Regression (Pr>F)		0.90	0.06	0.08	0.10
Contrasts (Pr>F)					
D7 vs D1		0.81	0.02	<0.01	<0.01
D7 vs D6		0.64	0.10	0.19	0.24

ALTERNATIVE FEED INGREDIENT TESTING FOR *Seriola Rivoliana*

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Reliance on fishmeal and fish oil is a significant constraint to the expansion of finfish aquaculture. Alternative feeds for marine finfish can increase the economic performance and decrease the ecological footprint of commercial mariculture. This is critically important to sustainability, scalability, and profitability of the industry both in terms of the bottom line and attracting capital investment. Commercial opportunities and sustainability concerns are arguably greatest for high-value marine finfish, such as kampachi (*Seriola rivoliana*).

In partnership with both leaders and newcomers to the alternative feeds space, Kampachi Farms has tested experimental diets for over a decade on high-value marine finfish. This presentation will highlight the resulting trial data from these partnerships, as well as the value of alternative feeds in commercial culture. A status update will be presented on current feeds in development for *Seriola*, including fishmeal free and fish oil free formulations.

DIETARY REQUIREMENTS OF ORGANIC AND INORGANIC ZINC IN A COMMERCIAL STRAIN OF DIPLOID AND TRIPLOID RAINBOW TROUT *Oncorhynchus mykiss*

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As the world population increases, finding adequate and sustainable food sources is of paramount importance, and aquaculture offers an appealing solution. Among essential nutrients, zinc is an important micro-mineral for fin fish and is required in small quantities, participating in a wide variety of biochemical processes. In aquaculture, triploids are utilized for their sterility to decrease risk of genetic pollution and for their potential for faster growth. However, to our knowledge there is no information regarding zinc requirements for triploid trout, despite support from previous studies that mineral requirements may differ. This experiment aims to determine zinc requirements in a commercial strain of genetically similar diploid and triploid rainbow trout (*Oncorhynchus mykiss*) using various levels of both organic and inorganic zinc. Eleven diets, including one basal diet (33 mg/kg, Zn_{33}) and 10 experimental diets, with incremental levels of inorganic ($ZnSO_4$) and organic (Alltech) zinc supplemented to the basal diet (Zn_{63}, Zn_{93}, Zn_{123}, Zn_{153}, Zn_{183}) were fed for 9 weeks. Treatments (22 total) were run in triplicate for a total of 66 tanks, with 11 fish per tank (66 l).

Results showed that neither ploidy (triploid vs. diploid) nor type of mineral (organic vs. inorganic) significantly influenced the growth performance of fish. However, in diploid trout, final whole-body protein (%) was significantly higher with organic Zn_{123} compared to other groups.

Fish fed organic Zn_{183} had significantly higher lipid retention than those fed Zn_{33} (control), inorganic Zn_{153}, and organic Zn_{123} and a trend of higher lipid retention than inorganic Zn_{183} (p=0.00387) (Fig. 1). Dietary zinc had no significant effects on histology of the distal intestine or cataract formation and lens histology. Only one fish developed bilateral incipient cataracts. Other parameters being analyzed are density of vertebrae and opercula, mineral analysis of whole-body and vertebrae, oxidative stress enzymes in plasma (superoxide dismutase, glutathione peroxidase) and gene expression in liver (lipoxygenase, NADPH oxidase, NF-kB, xanthine oxidase) and opercula/vertebrae (osteopontin, osteonectin, osteocalcin, calcitonin).

In conclusion, significantly higher nutrient retention was observed in fish fed organic zinc than inorganic zinc. Zinc requirements tended to be numerically higher in the inorganic diets when compared with the organic diets. Results of this study will increase the portfolio of high-quality organic zinc available to the fish feed industry.

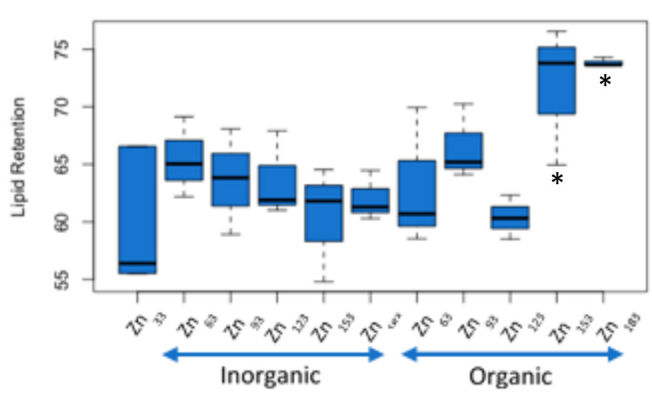

Figure 1: **Lipid retention (%) in triploid trout**

SUCCESSFUL HORMONAL INDUCTION OF SPAWNING IN BONEFISH (*Albula vulpes*), DESCRIPTION OF EARLY ONTOGENY, AND CHARACTERIZATION OF LIPID UTILIZATION

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Bonefish (*Albula vulpes* L.) are a highly prized sportfish and part of a recreational fishery. Despite their economic importance, their populations in the Florida Keys and numerous locations in the Caribbean are in decline with much of their early life history and development undescribed. Using hormonal induction of *A. vulpes*, we successfully induced spawning and describe previously unknown stages of egg and larval development and lipid characterization and utilization for the species. Egg size increased following intraperitoneal injection of Carp pituitary extract as well as at spawning. Zygote and cleavage stages lasted 1 to 2 hours. The blastula, gastrula, and segmentation stages lasted 6 to 9 hours and the pharyngula stage lasted 2 to 4 hours and preceded hatching. Upon hatching at 26 hpf, larvae were morphologically primitive and had no mouth or teeth and the eye was undeveloped. Average total lipids in eggs, before fertilization, accounted for 171.2 μg mg^{-1} of wet mass. Eggs were characterized by high concentrations of storage lipids (e.g. wax esters-steryl esters (WE-SE) and triacylglycerol (TAG)) and structural lipids (e.g. phospholipids). During embryogenesis, WE-SE and TAG decreased and WE-SE was exhausted at 4 hph. Together, the results provide previously undescribed early developmental stages for *A.vulpes* and will help focus restoration efforts concerning the feasibility for artificial propagation of *A.vulpes* in hatcheries as a restoration tool.

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NUTRITIONAL REQUIREMENTS OF FLORIDA POMPANO (*Trachinotus carolinus*) BROODSTOCK

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As the United States production of warmwater marine cultured fish increases, the demand for good quality seed is increasing. To satisfy that increasing demand, spawning quality and seed production success must be improved. Controlling the nutritional quality of broodstock diets and first-feeding larvae. Identifying the appropriate essential dietary nutrients, especially in serial spawners with short vitellogenic periods, would greatly improve not only the egg quality but also seed production. Comparison of the biochemical composition of eggs and larvae, at different stages, and patterns of use and conservation of nutrients during early embryo and larvae development, are often used to study nutritional requirements of warmwater marine fish broodstock. Florida pompano (*Trachinotus carolinus*) has been identified as a promising candidate for commercial scale aquaculture, but to date little information is available regarding captive broodstock dietary requirements. The present study aims to describe and compare lipid, fatty acid, and amino acid profiles in eggs and larvae from captive and wild Florida pompano throughout the reproductive season. The results to be presented will help evaluate the dietary fatty acids and amino acid requirements in captive broodstock in order to improve spawning and the production of high quality eggs.

BIOREMEDIATION POTENTIAL OF *Alitta brandti* (POLYCHAETE) TO UTILIZE NUTRIENT-RICH EFFLUENT FROM A MARINE RECIRCULATING SYSTEM

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Aquaculture produces excess amounts of waste discharge and utilizes large amounts of raw materials that potentially limit industry growth. The industry relies heavily on marine resources, mainly fish meal and fish oil, for use in formulated feeds. A strategy to increase resource efficiency in aquaculture is to utilize untapped waste streams to produce additional biomass from nutrient rich effluent, as well as those by-products from other feed industries. Deposit-feeding polychaete worms, *Alitta brandti* could be beneficial for waste bioremediation. The goal of this study is to evaluate if marine polychaetes can aid in the bioremediation of nutrients produced from the effluent of Sablefish, *Anoplopoma fimbria*.

An 8-week feed trial was conducted in a recirculating aquaculture system where individual worms of three different initial size classes (Weight Range: Small 0.2-1.8g, Medium 1.8-6.8g, Large 7-18g) were fed one of two effluent types (“Wet” or “Dry”) or a pelleted commercial control by Tetramin® (“Pellet”). A second experiment was conducted for a 4-week trial (initial worm sizes 0.1-2.5g) in which the wet effluent was enriched with either, feather, blood, or soybean meal, with additional supplementation of an additive *Schizochytrium* spp. and canola oil, or the pellet control.

After 8 weeks, the worms fed the control diet grew significantly larger ($P < 0.05$) than both effluent diets, although size was a significant factor (Figure 1). After 4 weeks, worms fed the soybean-enriched effluent grew significantly higher ($P < 0.05$) compared to the control (Figure 2). Our work suggests that *A. brandti* does have the ability to consume such effluents however, sufficient nutrient uptake is observed when effluents are supplemented with additional meal byproducts.

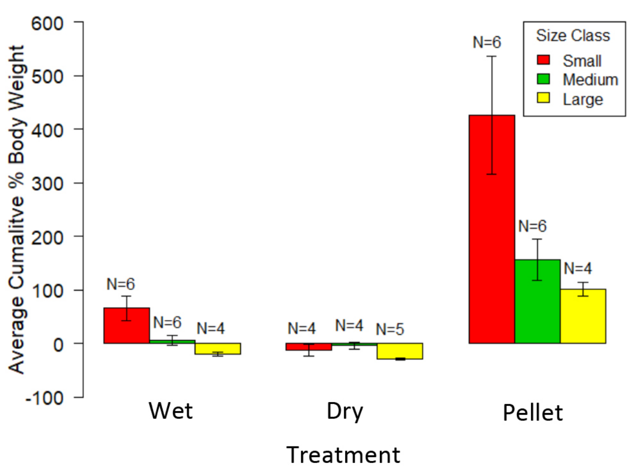

Figure 1: Growth performance amongst *A. brandti* in relation to effluent and control feed type and worm size. Error bars indicate standard error. (ANOVA, $P < 0.05$)

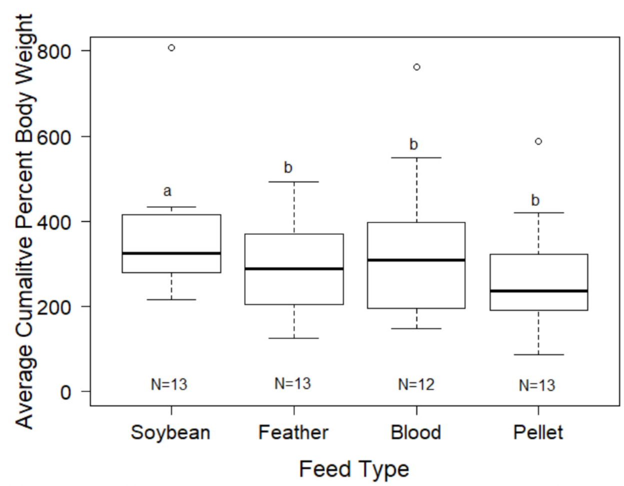

Figure 2: Comparing growth among formulated effluent feeds against the pellet control. Common letters denote non-significant differences. (ANOVA, $P < 0.05$)

GENE EXPRESSION PATTERNS IN DIPLOID AND TRIPLOID RAINBOW TROUT (*Oncorhynchus mykiss)* FED DIETS WITH ORGANIC OR INORGANIC ZINC

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In rainbow trout, zinc is among the most important essential micro-minerals involved in many biological processes. Zinc plays a role in oxidative stress, bone mineralization, and growth. The bioavailability of zinc depends largely on feed ingredients and their compositions. Plasticity of transition metals, such as zinc, is due in part to their ability to form complexes with nutrients in feed. Organic minerals have been observed to be more bioavailable to fish, resulting in a reduction in the amount required to be effective. This study examined gene expression response patterns in both diploid and triploid rainbow trout fed to satiation for 9 weeks with inclusion of either inorganic ($ZnSO_4$) or organic (Alltech) zinc in incremental levels (Zn_{63}, Zn_{123}, Zn_{183}) to an otherwise sufficient basal diet (Zn_{33}). Hepatic genes related to oxidative stress were analyzed and included superoxide dismutase (SOD1; SOD2), glutathione peroxidase (GPX1a; GPX1b1; GPX1b2), catalase (CAT), glucocorticoid receptor (GR), glutathione S-transferase (GST), liver X Receptor (LXR), and the zinc storage protein, metallothionein-A (MTA).

Results from this study revealed that genes related to oxidative stress were regulated by ploidy level and the dosage and type of zinc (Table 1). Organic zinc promoted higher levels of gene expression and increased susceptibility to a dose effect, supporting that organic zinc exhibits better bioavailability. Additionally, gene expression was more responsive to organic zinc in triploids than in diploids, suggesting that diploid and triploid trout have different zinc requirements. Based on these results, organic zinc appears to be more available, thus fish are more sensitive to it and should require less to meet the dietary zinc requirement. These findings also establish a relationship between dietary zinc and oxidative stress defense, indicating that adequate levels of dietary zinc are important for both fish health and growth performance.

Table 1: Hepatic gene expression (p-values).

Gene	Ploidy	Dose	Zn Type	Ploidy * Zn Type	Dose * Zn Type
SOD1	**0.012**	**0.002**	**<0.001**	0.154	**0.004**
SOD2	0.124	**0.020**	**<0.001**	**0.015**	**0.004**
GPX1a	**0.005**	0.071	**<0.001**	0.438	0.501
GPX1b1	0.089	0.098	**<0.001**	0.071	0.108
GPX1b2	0.299	0.714	**<0.001**	0.337	0.920
CAT	0.053	**<0.001**	**<0.001**	0.239	0.093
GR	0.125	**<0.001**	**<0.001**	0.652	0.382
GST	0.124	**0.002**	**<0.001**	0.764	0.800
LXR	0.075	**0.007**	**<0.001**	**0.009**	0.210
MTA	**0.005**	**0.004**	0.203	0.088	0.058

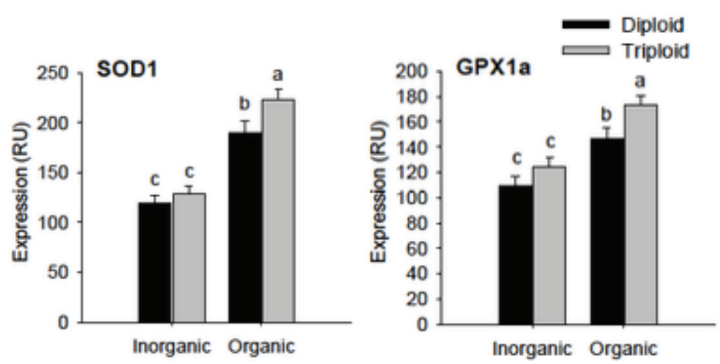

Figure 1: Gene expression by ploidy and type of zinc.

ORNAMENTAL FISH TRADE AND FARMING IN INDIA - STATUS AND PROSPECTS

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Ornamental fish trade in India can be categorized into two- export trade and domestic trade. The export trade is mainly constituted by the indigenous ornamental fishes of the Western Ghats and North Eastern Hills of India. India is blessed with a rich diversity of fresh water fishes in both these places. There are about 300 species of fresh water fishes in the Western Ghats alone of which more that 50% are potential ornamentals. In spite of the richness, contribution to the export trade is less than two percent due to various reasons. Whereas the domestic market mainly consists of exotic fishes viz., gold fish, angel, guppy, platy, molly, gourami etc. The domestic trade is fast developing in India since the beginning of 21st century. The total value of trade has increased from 550 million INR (7.8 million US$) to 3000 million INR (42.85 million US$) during the last decade. It is because of increasing number of aquarium hobbyists which is 2% of a total of 50 million (2015-16) middle income group house-holds (MIGHh) in country. A high growth of domestic trade was a mixed function of increasing number of MIGHh, change in behavior of Indian buyer's to spend a part of income on hobby, easy & cheaper availability of aquarium products and promotional effort by government. Moreover, most of the exotic ornamental fishes are tropical and especially southern part of India has congenial climatic conditions for their farming and production . Ornamental fish farming has become an employment opportunity for the rural population and thus helped to develop their socioeconomic status. The availability of varieties at retail aquarium outlets has also increased as many new exotic species of ornamental fish are bred locally in the country. Various aspects and case studies of the trade and farming will be discussed in the presentation. The domestic aquarium trade is projected to achieve a value of 11800 million INR (168.57 million US$) in next 5-6 years.

INDUCTION OF TRIPLOIDY IN WALLEYE *Sander vitreus* AND EVALUATION OF GROWTH DIFFERENCES BETWEEN TRIPLOID, CONTROL, AND HYBRID WALLEYE

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Walleye are native to most rivers and natural lakes in the Midwestern United States, as well as most of Canada and have been introduced through stocking into most man-made lakes due to their high value as both a sport and commercial species. Walleye propagation and stocking programs are a major focus of various fisheries management agencies; however, development of this species in the aquaculture industry has been limited. Induction of triploidy has long been used as a sterilization method across fish species and has proved a valuable tool used by management agencies to preserve genetic integrity of natural stocks. The objective of this study is to optimize production of triploid walleye and compare growth and survival to that of control and hybrid walleye in order to enhance the walleye aquaculture industry.

Gametes were collected from wild Maumee River walleye and sauger and transported back to the laboratory in Columbus, OH, where we conducted *in vitro* fertilizations. Three different pressure shocks applied at 4min post fertilization were evaluated for efficiency of producing triploid walleye: 1) 7000PSI, 40min duration, 2) 8000PSI, 30min duration, and 3) 9000PSI, 12min duration. Control and hybrid walleye were produced alongside. Fertilization rates differed between shock groups, with 65.6% in 7000PSI, 28.2% in 8000PSI, and 53.9% in 9000PSI, while fertilization rates for hybrid and control walleye were 28% and 23.1%, respectively. Mortality in the 7000PSI shocked group reached 100% at 10 days post fertilization (dpf), when the other groups were hatching. Triploidy in the 8000 and 9000 PSI shock groups were induced at rates of 68.8%, and 95%, respectively. At 18dpf, fish from control, hybrid, and highest surviving triploid group, 9000PSI, were stocked to aquaria, (260fish/tank) in duplicate, in a closed recirculation system maintained at 3ppt salinity. Fish were fed live *Artemia* nauplii as first food and algal paste provided turbidity. Fish were sampled after 10 and 17d of feeding. After 17d of feeding, the 2 aquaria for each group were combined and then randomly stocked to two 60L flow-through tanks (100 fish/tank). Fish continued to be fed live *Artemia*, until they were transitioned to dry diet at 40d of feeding. A subsample (n=10) of fish from each of the six 60L tanks were measured at 105d of feeding (122dpf). Mean weight and length data from 10, 17, and 105d of feeding are presented in Table 1. Fish continued to be grown and weight, length, and survival monitored.

Table 1: Mean ± st. dev. of body weight and total length of fish in each replicate tank from the experimental groups at 10, 17, and 105d of feeding.

Experimental Group	Days of Feeding					
	10		17		105	
	Weight (mg)	Length (mm)	Weight (mg)	Length (mm)	Weight (g)	Length (cm)
Triploid Walleye (9000 PSI)	**16.3 ± 0.4**	**14.1 ± 0.5**	**50.5 ± 4.6**	**19.8 ± 0.8**	**4.8 ± 1.4**	**9.4 ± 0.8**
Hybrid Walleye	**19.5 ± 2.6**	**14.4 ± 0.2**	**65.7 ± 0.5**	**20.7 ± 0.1**	**7.6 ± 2.0**	**10.6 ± 0.9**
Control Walleye	**21.6 ± 1.7**	**15.5 ± 0.0**	**53.8 ± 6.9**	**19.8 ± 0.5**	**5.1 ± 1.2**	**9.2 ± 0.5**

DEVELOPMENT OF A FISH MEAL REPLACEMENT DIET FOR JAPANESE WHITE TREVALLY *Pseudocaranx dentex* JUVENILES

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White trevally (*Pseudocaranx dentex*) is a novel marine finfish aquaculture species with a high market value that is being cultured commercially in Japan. Aquaculture nutrition research of white trevally has been little explored for the optimization of juveniles. This research evaluates the potential of soybean meal (SBM) as a replacement of fish meal (FM) and poultry byproduct meal (PBM) as a replacement for the flavor attractant krill meal (KM) in an effort to develop an environmentally sustainable and cost-effective juvenile diet for white trevally.

Three feeding trials were carried out at the Uragami Station, Aquaculture Research Institute of Kindai University, Japan. In trial 1, six iso-energetic diets were formulated; FM 560 g kg^{-1} diet (Control, diet C), and FM protein in diet C was replaced at 10 (S-10), 20 (S-20), 30 (S-30), 40 (S-40) and 50% (S-50) by SBM, and compared to a commercial reference. Fifteen juveniles (mean weight, ca. 42.7g) were randomly distributed into each of twenty-one 500L tanks, set in triplicate and fed twice daily until apparent satiation for 10 weeks. To further determine the optimal level of FM replacement by SBM in relatively small fish, in trial 2 seven iso-energetic diets were formulated, and FM protein was replaced at 30 (S-30), 35 (S-35), 40 (S-40), 45 (S-45) and 50% (S-50) by SBM. Another diet was designed by omitting binders carboxymethyl cellulose and guar gum from diet C, and referred to as C-CG. Thirty juveniles (mean weight, ca. 3.68g) were randomly distributed into each of twenty-one 300L tanks, set in triplicate and fed twice daily until apparent satiation for 6 weeks. In trial 3, diet C-CG from trial 2 was used as the control. An additional five iso-energetic diets were formulated as follows: FM protein from diet C-CG was replaced at 50 (S-50) and 70% (S-70) and KM from diets S-50 and S-70 was replaced by PBM and subsequently referred to as P-50 and P-70, respectively, and a FM-free diet (FMF). Fifteen juveniles (mean weight, ca. 31.8g) were randomly distributed into eighteen 500L tanks, set in triplicate and fed twice daily until apparent satiation for 8 weeks.

In trial 1, all SBM-based diets outperformed the control and commercial reference diets in terms of final weight gain and feed efficiency. The results of trial 2 revealed no significant differences between diets C and C-CG, indicating that binder was not the cause of poor growth performance of diet C in trial 1. Although there were no significant differences among SBM-based diets ($P>0.05$), S-50 exhibited the greatest final weight gain out of all treatments. In trial 3, there were no significant differences in the growth performance among SBM-based diets ($P>0.05$). While there were no statistical differences between diets C-CG and FMF, fish fed with diet S-70 showed significantly higher growth performance than those fed with both diets ($P<0.05$). The results of trials 1, 2 and 3 indicate that SBM has the ability to effectively replace FM up to 70% with the addition of KM, methionine, lysine, taurine and phytase supplementation. In addition, PBM has the ability to effectively replace KM in up to 50% FM replacement diets by SBM.

EFFECTS OF DIETARY SOYBEAN MEAL AND SUPPLEMENTAL TRACE MINERAL SOURCE AND LEVEL ON THE PRODUCTION PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN A CLEAR-WATER RECIRCULATING AQUACULTURE SYSTEM

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Antagonists in plant-based feeds can have major impacts on the availability of many nutrients to aquatic animals, but particular attention has been paid to essential nutrients that are more prone to antagonistic interactions. Inorganic trace minerals (TM's) in the form of oxides and sulfates represent the bulk of TM supplemented to animal feeds, but their bioavailability can be diminished by antagonists such as phytic acid and other minerals. The objective of this 6-week study was to assess the responses of Pacific white shrimp (PWS) to fish meal (FM)- and soybean meal (SBM)-based diets containing different supplementation levels of manganese, iron, copper, zinc and selenium from inorganic or organic sources.

Eight experimental diets were formulated to contain 35% crude protein and 8% lipid and different supplementation levels of the test TM's. Four diets (two FM- and two SBM-based) were supplemented with inorganic or organic TM's at 100% of typical commercial levels and two additional SBM-based diets were supplemented with the TM's at 50% of typical commercial levels. Two TM-unsupplemented diets and a commercial feed (CML) were used as controls for TM supplementation and diet performance, respectively. Each experimental diet and the CML were randomly assigned to four groups of 15 PWS juveniles (individual mean weight = 3.3 ± 0.09 g) stocked in 110-L aquaria operating as a recirculating aquaculture system at a salinity of ~ 25 ppt. Shrimp in each aquarium were fed four times daily at a fixed rate of 0.59 g/shrimp/day and feed amounts to each group were adjusted weekly based on survival. Statistically significant differences between dietary treatments were considered at $P < 0.05$.

Shrimp grew at a rate of 1.22 to 1.74 g/week to a final mean weight ranging from 10.4 to 13.7 g and survival ranged from 78 to 95%. The overall ANOVA indicated no effects of SBM inclusion, TM source and supplementation level on shrimp production metrics, whereas CML-fed groups grew slower and displayed higher FCR compared to shrimp fed some of the experimental diets. Contrast analysis on specific treatments showed that: i) in TM supplemented groups, FM-based diets outperformed SBM-based diets in terms of shrimp growth and FCR; ii) all TM supplemented groups performed similar to the respective TM unsupplemented control groups. Although these findings indicate that all experimental diets had adequate levels of TM's to support the production of PWS, overall FCR's were unexpectedly high (from 2.2 to 3.1) and the study will be rerun.

THE PRIVATE AQUACULTURE FISH HEALTH POLICY & REGULATION CRISIS IN THE US

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Once thought of as a noble supplement to wild seafood, in some sectors where there has been a long-standing commercial and/or tribal fishery for similar market species, farmed fish has been viewed as an unwelcomed competitor. This has resulted in fishermen allying with NGO's to fear-monger the risks of farmed fish to wild fish populations. A key risk that has been prominently featured is that of disease. Except for salmonids, across the US there is little federal oversight of fish diseases. It is more common for State resource agencies, with their stock enhancement programs, to be legally responsible for fish health regulations. While their long-standing experience with wild fish pathogens is to be respected, their policies and decision-making can often be lacking proper and rigorous risk assessment, transparency, inclusiveness, and accountability. Furthermore, cooperation between States for coordinated fish health policies is rare, causing for a patchwork of differing & independent regulations and applications across the US. This has had a negative effect on private aquaculture facilities who are often subject to expensive testing requirements, restrictions on stock movement, and outright stock destruction, without clear and agreed to justification. When the aquaculture industry has tried to push back against these policies it often encounters the precautionary principle/prove the negative stance. As these are resource agencies, edicts will give the wild fish the "benefit of the doubt" at the expense of the private sector. The welfare and growth of US aquaculture has been directly threatened by these policies. Examples will be given in the presentation with a list of potential solutions to what has become a crisis to US (and Canadian) aquaculture.

TARGETED MUTAGENESIS AS A RATIONAL APPROACH TO UNDERSTANDING AND CONTROLLING COLUMNARIS DISEASE

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Columnaris disease is a major problem for the aquaculture industry worldwide and results in substantial mortality and economic loss. The pathogenesis of the disease is poorly understood, and the existing control measures are ineffective. *Flavobacterium columnare* causes columnaris disease in wild, farmed, and ornamental freshwater fishes. Challenge experiments revealed that the type IX protein secretion system (T9SS) is required for virulence. When a core component of this system was deleted, the mutant was deficient in secretion and in gliding motility, and was avirulent towards zebrafish, rainbow trout, and channel catfish. The T9SS secretes many proteins. Analysis of cell-free spent culture fluid of wild type and T9SS mutant cells identified more than 50 proteins that are secreted by the T9SS. Among these were predicted adhesins, cytolysins, proteases, chondroitin sulfate lyases, and proteins involved in gliding motility and in iron uptake. We deleted genes encoding proteins secreted by the T9SS, including the motility adhesin SprB, chondroitin sulfate lyases, and proteases, to identify those critical for virulence. Cells lacking SprB, or cells lacking chondroitin sulfate lyases, were partially defective in virulence. In immersion challenges, the *sprB* mutant exhibited reduced mortality of zebrafish, and the chondroitin sulfate lyase mutant exhibited reduced mortality and reduced fin damage compared to fish exposed to a similar dose of the wild type strain. Mutants lacking individual proteases were as virulent as wild type cells, but a mutant lacking ten protease-encoding genes exhibited decreased virulence in zebrafish immersion challenges. The results suggest that multiple secreted proteins may be important for virulence. SprB facilitates attachment and movement of *F. columnare* along surfaces. It may be important for colonization of fish tissues and formation of the characteristic haystacks on infected gills. The *sprB* mutant formed non-spreading, non-rhizoid colonies on agar, and others have demonstrated a correlation between rhizoid colony morphology and virulence. Chondroitin sulfate lyases degrade chondroitin sulfate found in cartilage and connective tissues. Decreased levels of this enzyme resulted in decreased digestion of fins, a common site of *F. columnare* infections. Secreted proteases may digest proteins of the extracellular matrix and may contribute to the necrotic skin, gill, and muscle lesions associated with columnaris disease. *F. columnare* produces many proteases, and redundancy may explain the virulence of many of our single and multiple protease deletion mutants. Vaccination trials are underway to evaluate the immunizing potency of these mutants in zebrafish under controlled laboratory settings. Zebrafish survivors (previously exposed to □*sprB* mutant) exhibited modest resistance to infection by wild type cells. Future experiments aim to develop an effective vaccine and to design sustainable strategies to control columnaris disease in aquaculture.

THE USE OF DIPEPTIDE SUPPLEMENTATION AS A MEANS OF MITIGATING THE NEGATIVE EFFECTS OF DIETARY SOYBEAN MEAL ON ZEBRAFISH *Danio rerio*

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This study focused on the supplementation of health promoting dipeptides: carnosine (Car), anserine (Ans), and alanyl-glutamine (Ala-Glu). The dietary supplementation of these dipeptides has been shown to exhibit anti-inflammatory properties within the animal body. While the anti-inflammatory properties are known, little is known about their use in fish diets to mitigate the intestinal inflammation caused by dietary plant protein (PP). Therefore, the following objectives were included in this study: 1) To determine the effect of supplementation of Car, Ans, or Ala-Glu into PP-based feeds on growth performance of fish; 2) To determine if the supplementation of Car, Ans, or Ala-Glu affects the expression of genes associated with intestinal inflammation; 3) To assess if dietary Car, Ans, or Ala-Glu supplementation affects the expression of intestinal peptide transporter *Pept1*.

The study used zebrafish *Danio rerio* as a model species. At 33 dph zebrafish were randomly distributed into 15 (3L) tanks, with 50 fish in each tank. There were 5 treatment groups in this study. The first group (**(+) Control**) received a fish meal (FM)-based diet. The second group (**(-) Control**) received soybean meal (SBM) diet. The third group (**Ala-Glu**) was fed SBM-based diet, supplemented with Ala-Glu. The fourth group (**Car**) received SBM-based diet, supplemented with Car. The last group (**Ans**) was fed SBM-based diet, supplemented with Ans. All groups received their respective diets during 33-59 dph.

The results from this study show that the dietary supplementation of Car and Ala-Glu significantly improved the growth of zebrafish that were fed SBM diet. This study provides support for further research on how dipeptide supplementation can be utilized to improve fish performance on PP-based feeds. Intestinal gene expression and morphology results will be discussed in the oral presentation.

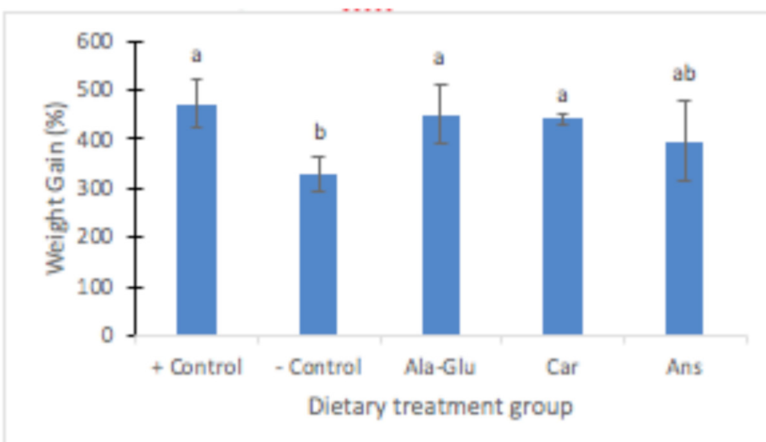

Figure 1. Weight Gain (%) during 33-59 dph.

NUTRITIONAL PROGRAMMING WITH PLANT PROTEIN USING LIVE FOOD AS A VECTOR IMPROVES DIETARY PLANT PROTEIN UTILIZATION IN LARGEMOUTH BASS *Micropterus salmoides*

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Nutritional Programming (NP) has been studied as a means of improving dietary plant protein (PP) utilization in different fish species. While NP has been traditionally done with dry feed during the juvenile stage, this study looked into using live feed for NP during the larval stage. The objective of this experiment was to determine the effect of NP with dietary PP induced during the first feeding of larval largemouth bass (LMB) *Micropterus salmoides* using live food as a vector on: 1) LMB growth performance; 2) expression of genes associated with inflammation and any morphological changes in the intestine; and 3) muscle free amino acid composition in LMB during its later life stages.

At 4 dph (days post-hatch), larval LMB were randomly distributed into 12 (50L) tanks, with approximately18 fish per liter. There were 3 treatment groups in this study. The first group **(LF-PP)** received soybean meal (SBM)-enriched *Artemia nauplii* from 5-25 dph, fish meal-based diet from 26-77 dph, and was challenged with SBM-diet during 78-121 dph. The second group **(FM-PP)** was not programmed during the live feed stage, received FM diet from 26-77 dph, and was exposed to SBM-diet during 78-121 dph. The last group **(+) Control** was not programmed during the live feed stage and received FM-diet throughout the whole trial (26-121 dph).

The results from this study showed that the programming of larval LMB with SBM-enriched live feed significantly improved weight gain in juvenile fish during the period of SBM-diet feeding compared to the non-programmed group (Figure 1). This study provides support for the use of live-feed enrichment with SBM for programming of larval stages as a means of improving the growth performance of aquaculture species fed with PP-based diets. The results on intestinal health status and muscle free amino acid composition will be included in the oral presentation.

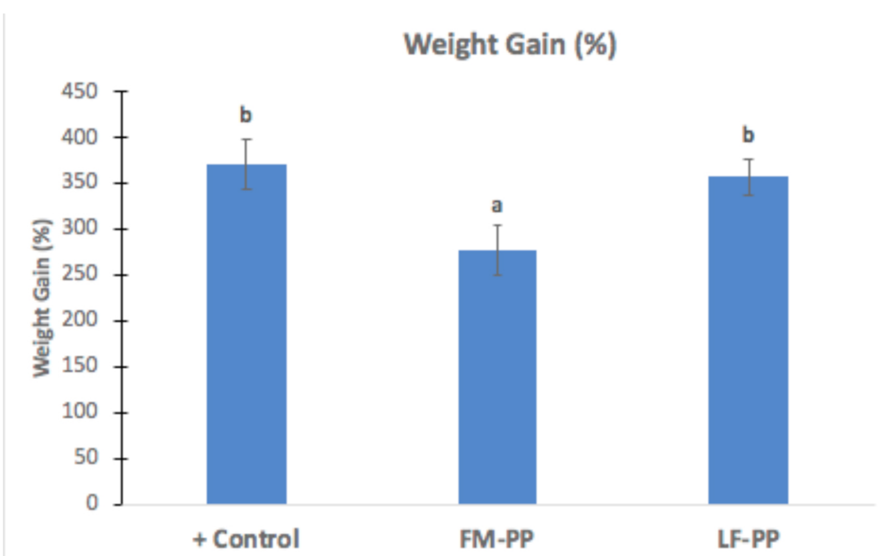

Figure 1. Weight Gain (%) during 78-121 dph (SBM-diet feeding).

CLEANER FISH RESEARCH AND PRODUCTION IN SUPPORT OF THE ATLANTIC CANADIAN SALMON AQUACULTURE INDUSTRY

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In 2018, the Newfoundland Aquaculture Industry Association (NAIA) through industry consultations identified Integrated Pest Management Strategies for Sea Lice Control as the number one industry priority for Atlantic Canada's finfish aquaculture sector. Potential losses in market value and resistance concerns related to prolonged reliance on any single therapeutant are key drivers that have prompted interest in the utilization of local cleaner fish species such as lumpfish, *Cyclopterus lumpus* and cunner, *Tautogolabrus adspersus*. A multifaceted research team at the Department of Ocean Sciences at Memorial University is conducting research on broodstock domestication and vaccine development as well as standardizing production protocols dedicated to developing this technology for industry use to assist in mitigating and controlling sea lice on Atlantic salmon.

Cleaner fish are fish that provide a service to other species by removing ectoparasites. The feeding behaviour of the cleaner fish is harnessed to create a natural defense for the farm. The success of this strategy depends upon the availability of high quality cleaner fish in sufficient numbers. Salmon farms could possibly require upwards of 10% or more of the salmon population per cage site. Culturing cleaner fish enables a continuous supply of high quality juveniles as demand requires. Cultured cleaner fish also have the added advantage of health screening and vaccination prior to deployment to reduce risk associated with disease transfer.

As the use of cleaner fish continues to produce favourable results globally the demand for these fish increases. Powell et al. (2013) estimates that up to 50 million lumpfish are required globally. The demand for these fish has increased substantially in Atlantic Canada over the past few years (Figure 1) and protocols have been developed and modified in an attempt to meet the demand.

This talk will highlight some of the protocols and technology developed over the past few years in an attempt to meet the demand for cleaner fish in Atlantic Canada.

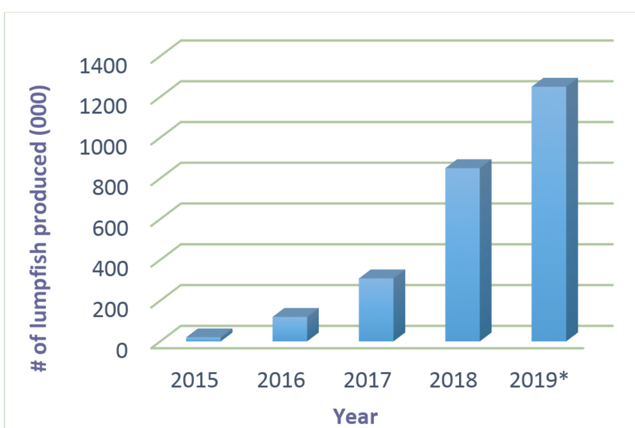

Figure 1: The number of lumpfish produced yearly at the Dr. Joe Brown Aquatic Research Building from 2015-2019.

*projected

THE RELATIONSHIP BETWEEN EMBRYONIC METABOLIC RATE AND JUVENILE GROWTH RATES IN RAINBOW TROUT AND THE PROTEOME UNDERLYING METABOLIC RATE OF EARLY EMBRYOS

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Recent work in several fish species suggests that metabolic rate is a stable trait of an individual and that embryonic or juvenile metabolic rate is predictive of later growth. Intraspecific variation in metabolic rate is also evident. Since the body temperature of poikilotherms is determined by the environment, metabolic energy is directed to locomotion, somatic growth, and reproductive development. In embryonic rainbow trout, which exhibit minimal locomotion and have not yet begun reproductive development, standard metabolic rate can be a proxy measure for somatic growth potential. Utilizing an in vivo cell viability assay recently adapted to estimate metabolic rate, based on the reduction of resazurin to resorufin by intracellular Nadh2, or by using a traditional oxygen consumption assay, embryonic rainbow trout were sorted by metabolic rate. The highest and lowest quintile groups were then reared in a commercial trout hatchery under standard conditions to assess differences in juvenile and adult growth rates related to embryonic metabolic rate. Additionally, individuals assessed for high and low metabolic rate at first cleavage (12 hours post fertilization, 6 ATUs °C) or at 8 days post fertilizations (96 ATUs °C) were used in a bottom up proteomics screen in order to identify the genomic and proteomic drivers of embryonic metabolism. This work helps establish the rainbow trout egg proteome and gives insights into the genomic drivers of metabolic rate in early embryos. The identification of mechanisms during embryogenesis that predict future growth potential may ultimately allow identification of robust molecular markers of egg quality.

THE ROLE OF AQUACULTURE IN REFUGIA CONSERVATION OF THREATENED AND ENDANGERED SPECIES

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The US Fish and Wildlife Service (USFWS) San Marcos Aquatic Resources Center (SMARC) has been contracted for many years to provide a refugium for petitioned, threatened, and endangered species. One of the unique aspects of our station is that we provide refugia for a broad variety of taxa including fish, plants, amphibians, mussels and other aquatic invertebrates. Our current refugia contracts include the Edwards Aquifer Habitat Conservation Program (EAHCP) and several Mitigation and Conservation Memorandum Agreements, such as with the Magellan and Kinder Morgan pipelines.

Refugia preserve the capacity for the covered species to be re-established should a catastrophic event, such as loss of spring flow or contamination, cause a loss of the wild population or threaten its critical habitat. These populations are vulnerable to extirpation throughout all or a significant part of their range. Establishing a refugia requires development and management of collection, husbandry, propagation, genetics, and reintroduction procedures. As part of the refugia program we also conduct research activities to expand knowledge of these species' biology, life history, effective reintroduction techniques, and more effective aquaculture and animal husbandry techniques. There is much discrepancy in the knowledge base of the species from fairly well known to almost nothing known for some of the invertebrate species. New aquaculture techniques are being developed that could be applied to similar species elsewhere in the United States and the world. For example, we are currently studying how to meet the nutritional requirements of an aquatic beetle that grazes on biofilms through artificial diet developments of 3D printed food sources and gut microbiome analysis.

The refugia model is becoming more important and utilized across the country as increasing pressures are put on our natural resources. Propagation of these rare species and their holding in aquaculture allow for studies by scientists that might not have a chance to interact with these species or similar ones in the wild. Effective aquaculture techniques for these species can be a resource to programs that are looking to supplement habitat preservation alone or where habitat preservation may be limited.

THE STATUS IN MEXICO OF COMMERCIAL PRODUCTION OF *Seriola rivoliana* BY KING KAMPACHI

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In the Americas there is an incredible emerging mariculture industry, which comes out due to the fact that there are different marine species for which it has been necessary to develop knowledge for each one of them and learn the specific management or "know how" for each species in several specific sites with a high range of temperatures and different environmental interactions.

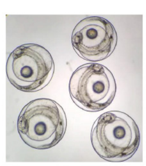
When I talk about an "incredible industry" I am talking about an industry with fertile soil for all enthusiasts who love aquaculture, particularly marine fish and that we have had the opportunity to try new farming methods, develop new technical protocols and challenge the premises that are held in the Salmon and Sea Bream and Sea Bass that are available in the Mediterranean to compete in the global market with species of high nutritional and economic value.

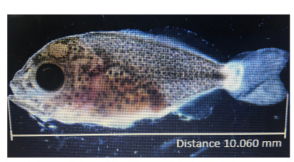
In the King Kampachi hatchery in La Paz, B.C.S., 18 months after launching, we have produced under controlled conditions nearly one million juveniles that have been stocked into offshore cages. To do this, we had to solve several bottleneck issues such as:

- Broodstock management to obtain high quality year-round without hormones;
- Egg incubation crashes;
- Post-hatch sinking larvae;
- First feeding mortalities;
- High quality rotifers in massive production;
- Improvement in deformity rates;
- High intake water temperature management (up to 30 Celsius);
- Specific pathogens with high temperature environments; and
- Fish welfare principles applied to *Seriola rivoliana*.

We reached our latest milestone in September 2019, when we were able to deliver a commercial juvenile batch (100,000+ juveniles transferred offshore) completely in a RAS system, during a summer when the water temperatures reached and sometimes exceeded 30 Celsius. At these temperatures everything changes in a hatchery: water quality, live feeds, and of course a high stress period that were an impediment to reaching an acceptable production of juveniles for production plans.

This milestone opened a new window for production in the toughest season of the year, giving us new opportunities to satisfy a high value fish market in Mexico and in the world.





USE OF CITIZEN SCIENTISTS TO MONITOR HARMFUL ALGAL BLOOMS, CHANGES IN ENVIRONMENTAL CONDITIONS AND ITS UTILZATION IN AQUACULTURE

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The negative environmental public health impacts from harmful algal blooms (HABs) have been noted recently in association with pet deaths, fish kills, and disease outbreaks in marine birds and mammals. Mounting evidence indicates global climate changes support increased frequency and geographic extent of HABs. The Phytoplankton Monitoring Network (PMN) is a NOAA research-based program utilizing volunteers to monitor phytoplankton species composition and environmental conditions. Volunteers are trained to identify general classes of phytoplantkon, including 14 known toxin producing species and 5 non-toxic species important for the aquaculture industry.

Since 2001, the network area includes coastal Atlantic, Gulf and Pacific waters. In 2014, NOAA and EPA collaborated to expand this citizen science program into freshwater habitats. Volunteers participate in hands-on research by collecting water samples, identifying species of interest, and entering data on a weekly or bi-weekly basis. Volunteers monitoring these sites represent public and private schools, colleges and universities, Native American tribes, state and national parks, aquariums, civic groups, shellfish growers and other non-governmental organizations.

The PMN was able to grow into a national monitoring program by use of various web-based tools such as an interactive web site and a geographic information system tool for data visualization and searchable database. NOAA scientists using internet teleconference capabilities and next generation digital microscopes give volunteer training sessions and workshops. This presentation will outline the use of these technologies and highlight the use of volunteer data in aquaculture settings.

IMMUNOLOGICAL EFFECTS OF BASILS GROWN IN AQUAPONICS ON VERTEBRATE (MICE AND FISH) SPLEEN CELLS IN-VITRO

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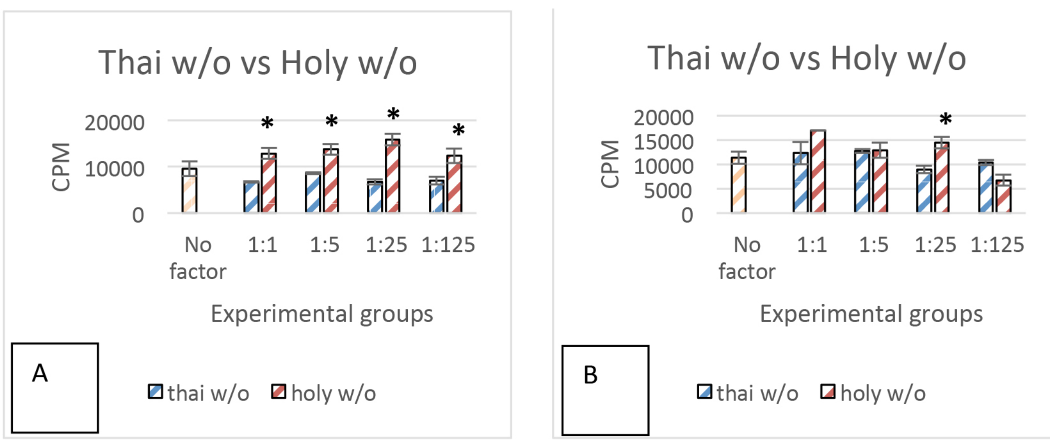
Experiments were conducted to investigate the immunological effects of two species of basils (Holy basil and Thai basil) grown in aquaponics system on the proliferation of spleen cells of fish and mice *in-vitro*. It was hypothesized that proliferation of spleen cells would be greater due to Holy basil extracts compared to the Thai basil extracts, since Holy basil has been known for its traditional medicinal effects. Basil leaf extracts were prepared at different dilutions (1:1, 1:5, 1:25, and 1:125) and were tested with and without Concanavalin-A (Con-A: a Phyto-mitogen) on the spleen cells of mice and fish (tilapia). Holy basil without Con- A showed more proliferation of spleen cells than Thai basil without Con-A of all dilutions in mice (P<0.05). On the other hand, in fish, Holy basils at dilutions 1:1 and 1:25 showed an increased spleen cell proliferation (P<0.05 for 1:25 only) compared to Thai basil at those dilutions only. The apparent results proved our hypothesis and showed that Holy basil extracts have prominent effects, compared to Thai basil, on the proliferation of spleen cells *in-vitro* for both mice and fish.



Figures. Spleen cell proliferation of Thai basil and Holy basil without Con-A at different dilutions for mice (A) and fish (B).

ARTIFICIAL PROPAGATION OF LAMPREY FOR CONSERVATION AND CONTROL

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There is a long history of artificial propagation of lampreys, with recent developments driven by the need for research animals and for recovery of imperiled species. Lamprey culture initially produced specimens for the study of evolutionary development in vertebrates. More recently, artificially propagated larvae have been used to improve identification methods for native lampreys, study invasive sea lamprey *Petromyzon marinus* in the Laurentian Great Lakes, provide animals for genomic studies, and for restoration and conservation.

Broodstock holding has indicated that adult lampreys tolerate extremely high densities when provided with cold, oxygenated water (Fig. 1). Fertilization and incubation experiments revealed that gamete contact times are very short and that embryos are resilient to low flow and poor water quality (Fig. 1).

Early larvae (Fig. 2) are also resilient to these factors and can tolerate abrupt changes in temperature and extended periods of starvation. However, they cannot survive sudden changes in water quality, excessive disturbance, and lack of beneficial microbial communities. These observations have led to more efficient and effective lamprey propagation and have yielded important information about the early life stage requirements of lampreys in the wild.

Further study is needed on a broader array of species to allow inter-specific comparisons of early life history. However, information from lampreys receiving the most attention to date (European river lamprey *Lampetra fluviatilis*, sea lamprey, and Pacific lamprey) indicates that culture and environmental requirements of the early life stages are remarkably similar, allowing generalization across lamprey species.

Fig. 1 Gravid adult female (left) Pacific lamprey (*Entosphenus tridentatus*) and developing embryos (right).

Fig. 2. Pacific lamprey prolarva.

WHY ASIAN SHRIMP FARMERS GROW PACIFIC WHITE SHRIMP *Litopenaeus vannamei*: A REMINDER OF HAWAII'S RECENT PAST

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In 1981, Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) was first identified on a shrimp farm in Hawaii causing mass mortalities in captive *Litopenaeus stylirostris*. IHHNV also was detected in a population of *L. vannamei* cultured at the same farm but this shrimp species appeared refractory to the virus. The high susceptibility of *L. stylirostris* to IHHNV and the relative tolerance of *L. vannamei* to this pathogen helped catalyze *L. vannamei* to be the dominant farmed species in the Western Hemisphere.

In 1984, the U.S. Marine Shrimp Farming Program (USMSFP) was formed as a congressional initiative to solve problems constraining the U.S. shrimp farming industry. The USMSFP consisted of member institutions including Oceanic Institute (OI) in Hawaii and the University of Arizona (UAZ). Because of the relative tolerance of *L. vannamei* to IHHNV, the USMSFP decided to commit resources to develop culture technologies for this species, including its domestication. Although IHHNV was not lethal to *L. vannamei*, infected populations exhibited Runt Deformity Syndrome resulting in growth suppression and cuticular deformities. It became apparent that IHHNV caused an economically significant disease in *L. vannamei* and that efforts to rid captive populations of this pathogen were critical to its domestication.

In 1989, OI and UAZ began developing the world's first Specific Pathogen Free (SPF) population of *L. vannamei* which was free of IHHNV and other pathogens. Offspring from SPF broodstock were evaluated at commercial farms in the U.S. and significantly outperformed non-SPF shrimp. OI began supplying SPF broodstock to U.S. farmers and by 1992 >95% of farms in the U.S. were stocked with offspring from these broodstock resulting in bumper crops from 1993-1995.

In 1994, OI started the world's first family-based breeding program to improve shrimp growth and survival, as well as tolerance to Taura Syndrome Virus which devastated U.S. farms in 1995. OI generated basic information about the quantitative genetics of shrimp breeding and distributed SPF, selectively bred shrimp to the U.S. industry. Between 2000- 2010, OI distributed >2.5 million shrimp to U.S. stakeholders, including Hawaii broodstock suppliers who developed their own robust breeding programs. Hawaii broodstock suppliers played a critical role in catalyzing a paradigm shift in Asian shrimp farming. Between 2003-2018, Hawaii broodstock suppliers provided ~ 5 million SPF, selectively bred *L. vannamei* broodstock to Asian shrimp hatcheries.

In 2000, Asian shrimp farmers produced an estimated 623,194 metric tons (MT) of black tiger prawn, *Penaeus monodon*, whereas production of *L. vannamei* was only 2,310 MT. By 2017, *P. monodon* production increased by 18% to 733,525 MT, whereas *L. vannamei* production skyrocketed by 158,500% to 3.66 million MT valued at $22.7 billion. This dramatic change is attributed, in large part, to the commercial availability of SPF, selectively bred *L. vannamei* originally developed by the USMSFP and commercialized by Hawaii broodstock suppliers.

REUSE OF HATCHERY EFFLUENT FOR SPAT BREEDING USING MEMBRANE PROCESS

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The aim of the study was to test the possibility of reusing an effluent from adult oyster breeding to feed an oyster spat with membrane process (ultrafiltration). The objective was two-fold: (i) treat a real hatchery effluent and (ii) use ultrafiltered water for juveniles.

A pilot able to treat 20 $m^3.d^{-1}$, equipped with hollow fiber membranes with a pore size of 0.02 μm, was continuously fed for two months with real effluents from oyster breedings. This discharge contained feces, pseudo feces and microalgae. Simultaneously, the treated water was used to feed an oyster spat (i.e. sensitive species).

For the reuse of treated effluent, growth performances of a spat (2500 oysters) were followed and compared to a control spat raised in the same conditions (quantity of food, temperature, flow) but fed with a control seawater filtered 1 μm and UV treated. Water quality of the two spats was controlled with bacteria and physico-chemical parameters measurements.

For effluent treatment, retention performances were controlled with total suspended solids (TSS) and bacteria measurements. Total bacteria and *Vibrio* bacteria concentrations, potentially harmful for shells concentrations were controlled.

For the treatment of effluent, TSS measurement highlighted a retention up to 99 % with a constant TSS concentration (>2 $mg.L^{-1}$) in permeate whatever the feed quality.

A total retention of *Vibrio* bacteria was obtained, the process offers a protection of shellfish toward these bacteria genus. Moreover, a lower total flora concentration is measured in ultrafiltred effluent than in control sweater (1 μm + UV). Ultrafiltration is efficient to treat effluents from oyster breeding. Hydraulic performances controlled on the duration of the tests demonstrated the sustainability of the process facing this effluent. Indeed, chemical cleanings were necessary every 12 h of filtration.

Ultrafiltered water produced was used for oyster spat breedings. Physico-chemical parameters, identical in two water qualities led to a similar spat growth for 2 months in the two water conditions (Figure 1).

To conclude, the study showed the efficiency of ultrafiltration to treat an effluent from oyster farm with (i) a removal of bacteria potentially harmful for oysters (ii) a removal of TSS and (iii) a resistance of the process facing an organic effluent. Moreover, this process produces a water with a quality adapted to hatchery nursery applications such as the growth of juveniles, a sensitive oyster stage of life. Ultrafiltration could be a solution to reuse effluents in shellfish farms.

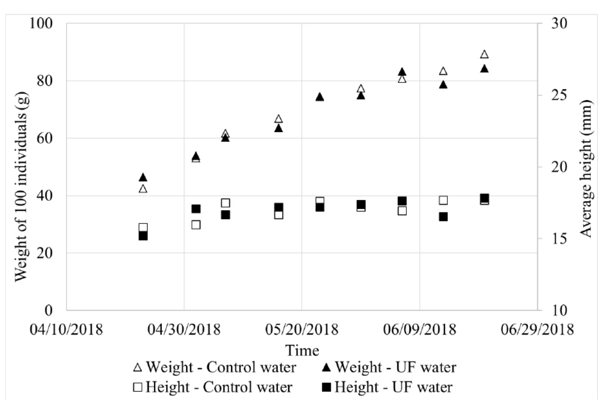

Figure 1: Evolution of spat growth

A PARTIAL SEQUENCE OF VITELLOGENIN ENABLES TO TRANSPORT AND ACCUMULATE FOREIGN PROTEINS INTO EGGS IN MEDAKA *Oryzias latipes*

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In this study, we developed a novel bioreactor system to deliver and accumulate foreign proteins in eggs using medaka fish *Oryzias latipes* with the aid of partial sequence of vitellogenin (Vtg). In teleost fish, Vtg, the hepatically generated precursor of egg yolk proteins, is secreted into the bloodstream and then taken up into eggs. Functional analyses of domains of Vtg were carried out with *in vitro* experiments and biochemical approaches. Therefore, the critical sequence of Vtg that enables the transportation of proteins secreted from the liver into the eggs *in vivo* remains unknown.

To apply the transport property of Vtg to the bioreactor, it is necessary to identify the smaller effective region precisely in the full length of Vtg, which is designated as the "Vtg signal" in this study. Based on the results of *in silico* analysis showing that medaka Vtg contains the secretory signal peptide (^{1}MRGLILALSLALVAANQ^{17}) and the receptor-binding region (^{178}HLSKTKDL^{185}), we predicted that the 300 aa N-terminal portion of Vtg ("Vtg signal") was sufficient to transport the foreign proteins produced in the liver into eggs. Then, we established two transgenic lines expressing the fused proteins including the Vtg signal and each reporter gene, *enhanced green fluorescent protein* (*EGFP*) or firefly *luciferase* (*LUC*)-fused *EGFP*, in the liver driven by a liver-specific *choriogeninH* (*chg*H) promoter. Each reporter signal was detected from the fertilized eggs spawned by the transgenic females, showing successful transportation of the proteins into the eggs with the Vtg signal (Figure. 1).

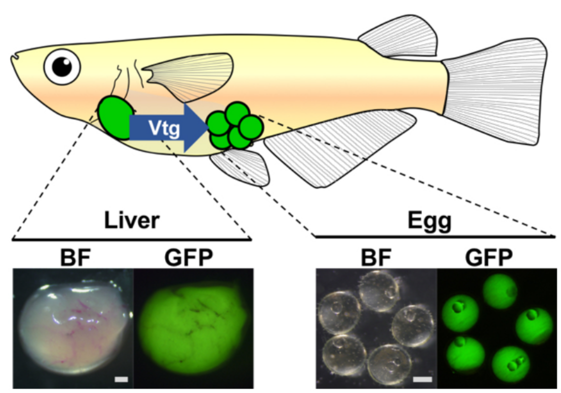

Figure.1 Fluorescent images of livers and eggs of sexually mature females in the transgenic medaka (*chg*H-*vtg signal*-*EGFP*). BF: bright field, GFP: GFP fluorescent image, scale bar: 200 µm.

NUTRACEUTICALS: FUTURE PROPHYLAXIS FOR FISH DISEASES?

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Aquaculture is rising in prevalence and importance in today's society with the increase in fish consumption and the limitations imposed by normal fishing methods. The world's population is rising and the subsequent demand for high protein sources of food is rising with it. A push for healthier sources of protein in industrialized nations is also increasing demand. Aquaculture offers a solution to these issues by providing a high protein food that requires less space to farm, a faster harvest time, lower cost to produce, and is less detrimental to the environment. Disease and mortality present a significant problem in that farmed fish are in crowded conditions leading to certain results. They are susceptible to contagious diseases that can spread rapidly in close quarters as well as their stress levels are increased in these unnatural conditions. This increase in stress over time leads to a reduction in immune response and therefore an increase in susceptibility. However, current aquaculture techniques involve the use of chemical drugs in order to reduce disease and mortality within the crops. In order to provide solutions to the problems of farming and the needs of the people without the use of potentially harmful substances many researchers are looking into the use of nutraceuticals in feed in order to decrease stress responses, increase immune responses, increase growth, and increase the nutritional value of farmed fish. This presentation will cover an overview of the use of different nutraceuticals and the beneficial results obtained from them.

THE POTENTIAL FOR CULTURE OF KYPHOSIDS AND OTHER HERBIVOROUS MARINE FINFISH

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Marine finfish aquaculture is dominated by carnivorous species, requiring diets high in proteins and oils, often fulfilled through wild-sourced fish products. Reliance on wild fish resources gives the impression that aquaculture is not sustainable, driving research (including over a decade of work by Kampachi Farms) to identify alternative ingredients and reformulate feeds for 'carnivorous' fish. However, herbivorous marine finfish do not require dietary fishmeal or fish oil, offering a direct means of circumventing these obstacles.

Kyphosids (aka nenue, chubs, rudderfish) are popular food fare among the Pacific Islands, and there are species native to much of the world. Research has shown *Kyphosus vaigiensis* (brassy chub) to be amenable to larval rearing, resist skin flukes, yield commercially attractive growth rates, and produce fillets with up to 28% lipid (by dry weight).

Initial spawns from Kampachi Farms *K. vaigiensis* broodstock in 2017 provided preliminary data for successful larval rearing. We have built upon this groundwork through 2019 having achieved consistent high-quality spawns by reorganizing cohorts, completing a successful larval run, developing algae-based pellet formulations, and collaborating with community fishponds to incorporate this species into their production.

Our 2019 grow-out studies of juvenile rudderfish confirm an attractive growth rate and FCR for production. The concluding blind taste test showed that quality fillets can be obtained from low-oil, fish-free diets with little discernible difference in flavor and texture. The successful culture of high-quality herbivorous marine finfish could provide a sustainable, low-cost option for fish farmers and seafood consumers.

INNOVATIONS IN FOOD PROCESSING TECHNOLOGIES TO PRODUCE SAFE AND QUALITY SEAWEED PRODUCTS

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Seaweed aquaculture makes up a significant portion of organisms cultured worldwide, ~28 million metric tons, with a value ~U.S. $10 billion. In addition to other uses (i.e. hydrocolloids and feed) for cultivated seaweed, *Undaria, Porphyra* and *Laminaria* are extensively used as food for direct human consumption. Seaweed is different from the land-based fruits or vegetables in many senses considering its complex physiology and composition. While the scientific has community already confirmed the positive effects of bioactive compounds in various types of seaweed in both raw and fresh form, the fate of the compounds in processed products and value-added products is yet to be established. So far, the focus of post-harvest processing and preservation has been on the drying or blanching and frozen products. There is limited study on the behavior, availability and release of bioactive compounds and heavy metals in the seaweed as it pass through various processing conditions.

Primarily, the existing processing conditions include drying of seaweed with hot-air medium. Different drying methods have been found to greatly affect the nutritional composition of the brown seaweed, *Sargassum hemiphyllum* (Chan, Cheung, & Ang, 1997). For example, Wong and Cheung (2001) reported that oven-drying was better than freeze-drying for the extractability and quality of proteins isolated from three subtropical brown seaweeds. The most commonly applied drying conditions, mainly in Asia, have longer drying time with higher throughputs. The target moisture content of the dried products varies from 8-15% wet basis. The educated consumers of today like to purchase a product with minimal processing and high quality. The existing processing and storage conditions don't provide any information on the fate and availability of bioactive compounds in the final products.

Besides, seaweed has not been tested to a number of advanced food processing technologies that are used for land-based food products. In addition to hot-air, these advanced technologies use microwave, pressure, vacuum etc. to develop high quality solid and liquid food products as well as ingredients. In this presentation, I will discuss on the available advance of food processing and preservation technologies that can be utilized for developing a number of food products and high value-added products from seaweed, mainly focusing on kelp (*Sachharina latissima*).

ECOLOGICALLY SUSTAINABLE NET PEN AQUACULTURE IN LAKE HURON, ONTARIO, CANADA

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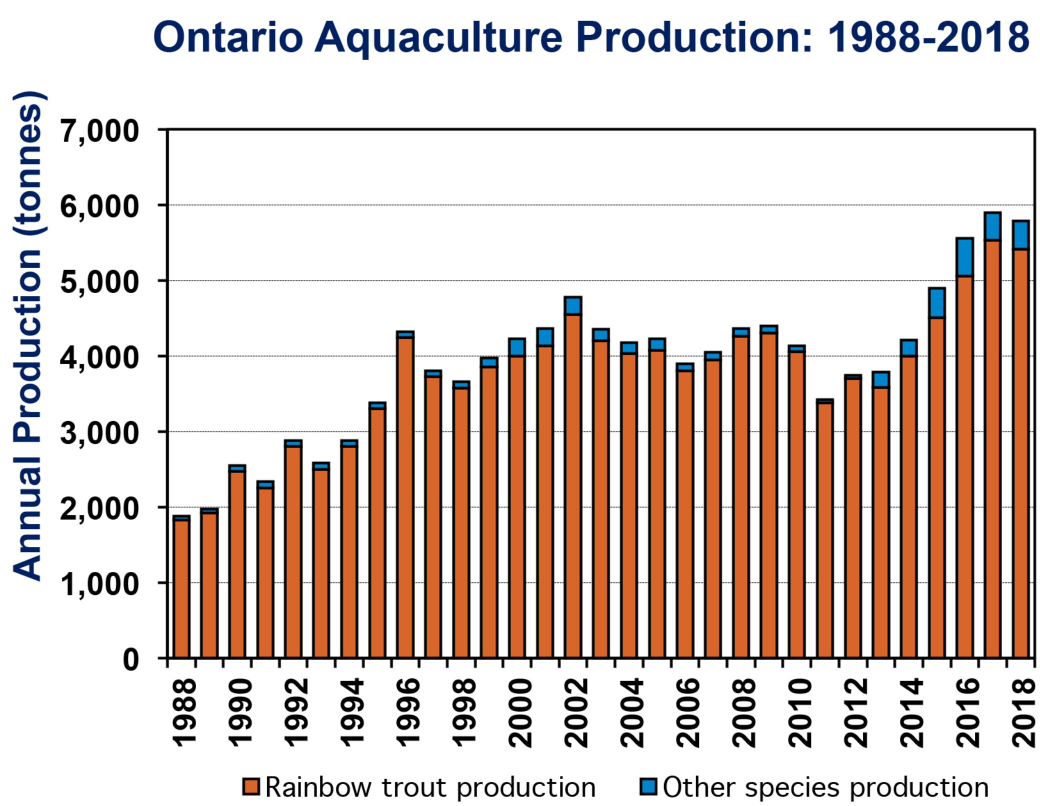
Net pen culture of Rainbow trout in the waters of Lake Huron has grown over the last three decades to provide significant economic and social value to Ontario. Those values are accrued by the public and by First Nations whom have increasing interests in the industry. With refinements in the regulatory framework for net pen aquaculture, there is impetus to expand production further. The history, current status, and future projections for the industry and the regulatory framework developed by the Province of Ontario and the related Federal regulations, which have been established to ensure the ecological sustainability of the sector, will be presented. The industry has adopted certification protocols which similarly support the economic and ecological sustainability of the sector. The development and regulation of freshwater net pen aquaculture has been based on an investment in science to support best culture practices to minimize environmental impacts.



HOST PARK: HAWAII'S PREMIER AQUACULTURE PARK ON HAWAII ISLAND

Gregory P. Barbour, Executive Director, NELHA

The Hawaii Ocean Science and Technology (HOST), administered by the Natural Energy Laboratory of Hawaii Authority (NELHA), is a unique incubator and technology park located in Kailua Kona, Hawaii. The park's abundant sunlight and sea water resources make it a prime location for aquaculture R&D and commercialization. NELHA also recently established an aquaculture incubator program which completed its first cohort in December 2019. An overview of HOS and its special resources will be presented along with how these special resources allowed for the development of unique world renown aquaculture products.

EDUCATING YOUTH THROUGH AQUACULTURE AND CREATING WORKFORCE PATHWAYS

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Aquaculture is the fastest growing sectors of food production worldwide, and is vitally important to obtaining sustainable food security in the future. However, in the United States aquaculture continues to grow at a sluggish pace, and is often misunderstood or completely unknown to many U.S. consumers.

In an effort to increase awareness for the next generation of seafood consumers, utilize a strong teaching tool, and create defined pathways into the aquaculture workforce many extension educators and academics are creating K-12 aquaculture programs and working closely with K-12 schools to help integrate aquaculture curriculums into the classroom.

The efforts that have been taking place in Michigan and the Midwest include the Youth Education in Aquaculture Initiative (http://ncrac-yea.org/), an effort funded through the North Central Regional Aquaculture Center and started by Lake Superior State University Professor Dr. Barbara Evans. Through this effort a network of schools is being mapped along with aquaculture resources such as hatcheries, private facilities, research centers, and more. The schools mapped through this effort are identified for having aquaculture integrated into their curriculum. This effort also includes a forum to allow networking opportunities between schools.

The efforts in Michigan go beyond mapping and also include curriculum creation, working directly with teachers to develop aquaculture programs and the Aquaculture Challenge, a competitive high school competition aimed towards engaging high schools in an integrative STEM and business program.

The efforts taking place in Michigan also are in line with national efforts that have begun to coordinate extension and Sea Grant work in K-12 aquaculture programing and workforce development across the United States and its territories.

This session will focus on these efforts and how they are leading to a clear and defined pathway into the aquaculture workforce.

Youth Education
in Aquaculture

STRUCTURE AND DYNAMIC OF BIOFOULING COMMUNITIES WITHIN AQUACULTURE AREA IN NORTHERN ADRIATIC SEA, CROATIA

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Biofouling communities are composed of sessile, suspension-feeding macrofouling organisms. Their composition and abundance are spatially and temporally variable, and are also determined by environmental factors and surface properties of collector substrates, such as: age, texture, depth, complexity, inclination and position in the water column. The intensive development of biofouling communities within aquaculture farms has a substantial detrimental impact on shellfish production due to physical shell damage, disruption of opening and closing of shellfishvalves, competition for food and space, reduction of water flow, and increased weight of aquaculture installations.

The current investigation was conducted between April and January within aquaculture areas of Lim Bay in the north-eastern part of Adriatic Sea and Pomer Bay.

Ropes with five replicates of clay tiles (12×12 cm) were placed next to mussels polyethylene (pergolari) mesh sleeves suspended horizontally from longlines, at depths of 1 and 5 meters from the surface.. Tiles were retrieved every three weeks, starting from May to January. Biofouling organisms were inspected immediately after collection of tiles. The most dominant biofouling species found in the Lim and Pomer Bay correspond to those previously observed in the Eastern Adriatic Sea (Fig. 1). Their variable horizontal and vertical distribution clearly reflects the complexity of ecological processes even within a small spatial scale.

Higher abundance of species found in Lim with respect to Pomer is probably related to the poor water circulation and consequently higher availability of nutrients within highly enclosed narrow and elongated fjord-like Lim Bay. On the other hand, Pomer Bay is deeper, more connected to the open sea and subjected to continuous exchange of water mass. The hydrographic conditions may have favoured more frequent occurrence of Tunicate species in particular *Botryllus*, *Phallusia mammilata* and *Diplosoma listerianum* within Pomer Bay.

Considering a great spatial variability and abundance of biofouling macrofauna species, further monitoring of their occurrence and recruitment peaks is required in order to choose an optimal deployment period for collectors and reduce negative effects of biofouling on aquaculture facilities and production.

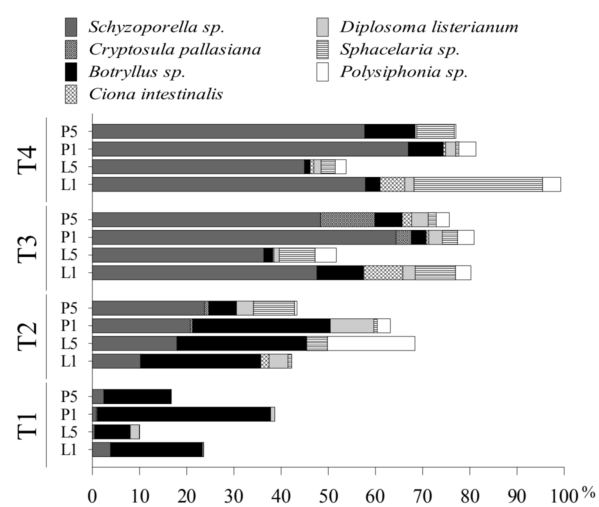

Figure 1. Average coverage of dominant species at both locations: Limski Kanal (L) and Pomer Bay (P): T1 = 15 June, T2 = 15 August, T3 = 15 November and T4 = 15 January.

STATUS OF *Macrobrachium rosenbergii* FARMING IN VIRGINIA 2019

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A combination of factors resulted in a drastic decline in freshwater shrimp pond culture in Virginia during the 2019 growing season. The factors included: more profitable alternative farm activities, sourcing juvenile shrimp for stocking, identifying feed supplies, labor, unfulfilled production levels, regulatory unknowns and marketing. Early industry expansion during the 2000s was due to encouragement by Virginia Cooperative Extension, Virginia Aquafarmers Network (VAN) and funding through the Tobacco Buyout Program. Educational workshops promoted best shrimp production management and stimulated interest among tobacco farmers evaluating alternative crops. Extension also provided stocking services to widely located producers in order to demonstrate success for the new crop, especially in tobacco growing counties. VAN also received economic stimulus grants to subsidized member purchase of feed. Shrimp production peaked in 2009 with two local shrimp nurseries and more than 25 production ponds varying in size from 0.05-1.0 ha generating annual sales in excess of $240,000.

In 2019 three major producers with 3 hectares of production ended operation. This followed the loss of multiple producers with more than 4 hectares of production after 2017-18. For economic reasons, some producers had to take off-farm jobs or expand existing tobacco production. Departure from shrimp was also due to farmer health/age, buyer-seller personality conflicts, poor pond drainage, labor, discouraging returns from minimal wholesale-market margins and low yields due to poor management (water quality, excess filamentous algae, wild-fish competition). The late commitment by traditional supplier to have available juveniles delayed several farmers from planning in time for pond preparation. A major feed supplier no long distributes sinking catfish pellets. Virginia State University is assessing alternative, less expensive nutrient sources to lower production costs to enable farmers to continue profitable shrimp production.

Presently, one producer remains in Virginia with the existing shrimp nursery supplying juveniles to producers in North Carolina.

As It Once Was

KEY CONSIDERATIONS IN FARM MANAGEMT AND HEALTH NUTRIENTS FOR HEATHIER SHRIMP

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Intensive shrimp culture in Vietnam as well as in Southeast Asia has proven postlarvae's quality, water-quality management and high-quality diets are all important for successful crops:

PL quality in term of daily growth rate is considered the most important indicator to shorten the culture period as well as to resulted in large-size shrimp. There is a big difference in growth rate with an average daily growth rate varies less than 0.2g, around 0.25g and over 0.35g coming from three different sources of the genetic PL.

Water quality management is focusing on low salinity during the first 45 days (7ppt – 10ppt), high alkalinity (120 -250 ppm), stable pH of 7.6 to 7.9, and high dissolved oxygen (average of 20 HP per 1,000 m^2) as well.

Nursery at high stocking density (1,000 PL up to 5,000 PL per m^2) in small lined tanks or lined ponds in indoor system has been practiced with premium hatchery diets (ranging from 45% up to 60% protein). Shrimp farmers also do top dressing with different healthy products, including vitamin/minerals, probiotics, organic acids, and immune-stimulators (nucleotides, β-glucan/Mannan, etc.). In addition, astaxanthin has been promoted by shrimp feed millers and also being top dressed at several farms for not only pigmentation but also for healthier shrimp.

VIETNAMESE PANGASIUS: PRODUCTIVITY GROWTH AND TRADE BARRIERS

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As other successful aquaculture species, Vietnamese pangasius producers have experienced a number of trade obstacles in major importing countries such as the U.S and EU. The U.S. was the first country where Vietnamese pangasius faced challenges as it was forced to change the name from catfish in 2002 and an anti-dumping duty was imposed in 2003. The second trade challenge occurred in the mid-2000s when pangasius was claimed to be unsafe and unsustainable in the EU market after the market share of Vietnamese pangasius increased significantly. The challenge has continued as in 2017 there were social media and social campaigns in the EU claiming that Vietnamese pangasius farming was polluting the Mekong Delta and it is produced in the dirty environment. Consequently, many large seafood distributors in Belgium, France, and Spain has suspended Vietnamese pangasius products. In the U.S., the stricter quality standards have been imposed, and as a consequence, only two Vietnamese exporters can currently ship the pangasius fillets to the U.S. market.

One would expect that all these trade challenges provide major impediments to Vietnamese pangasius producers. However, Vietnamese pangasius is a highly successful aquaculture species in terms of production growth and exports. While pangasius production in 1999 was only 69 thousand tonnes, it reached 1.42 million tonnes in 2018, equivalent to a 20 times increase. The main explanation is that productivity growth and improved production practices have improved competitiveness to such an extent that the effect of the import measures have not been the main barrier. This is amplified by flexibility with respect to which markets and product forms are exported, although the U.S. and the EU remain among the main markets.

In this paper we will provide an overview of the development in pangasius production and exports from the late 1990s. We will both show the impact of productivity growth and trade measures and campaigns. It is of particular interest to note that the effect of the trade measures and campaigns tend to be short-lived, and that the impact of the financial crisis in 2008 is significantly more severe as this reduced total demand.

QUANTIFYING SPERM PRODUCTION AND MORPHOLOGY FOR TWO SPECIES OF UNIONID MUSSELS

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Mussel diversity is declining, as more species are becoming extirpated or extinct at an alarming rate. While little can be done to bring back lost species, we must work on securing the fate of others that are threatened or endangered. This can be facilitated through gene banking and assisted reproduction to propagate these imperiled species. Unfortunately, limited information is available on freshwater mussel reproduction, especially as it relates to paternal impacts. Sperm quality follows a seasonal spawning period where cell characteristics change overtime. Thus, it is imperative to find the window of optimal cell health to further improve freshwater mussel reproduction. Our objectives were to quantify sperm production and morphology for two unionid mussels, *Ligumia subrostrata* and *Lampsilis straminea*, throughout the natural spawning season. Both species were held separately in two ponds in fifteen ~1' diameter mesh cages where temperature at the sediment-water interface was recorded with HOBO temperature loggers. Starting 29 Aug. 2018, male mussels (n = 5 for each species) were sampled every two weeks. Using a small syringe, sperm were collected directly from the gonad (Fig. 1A). Sperm density was determined for each male using a hemocytometer and a Nanodrop at 350, 600, and 700 nm. Sperm were fixed in 2.5% glutaraldehyde and examined using scanning electron microscopy (Fig. 1BC).

Sperm density for *L. subrostrata* ranged from 1.1×10^9 to 19.60×10^9 cells/mL with highest production from 26 Sept to 7 Nov (Fig. 1D). *L. straminea* sperm density peaked (20.02×10^9 cells/mL) on 13 Sept and declined thereafter (Fig. 1E). There was a positive relationship between hemocytometer counts and Nanodrop absorbance for both species ($R^2 > 0.94$). Sperm were uniflagellated and preliminary results for *L. subrostrata* and *L. straminea* showed mean head length and width were 3.28 ± 0.16 µm and 1.64 ± 0.11 µm and 3.41 ± 0.13 µm and 1.59 ± 0.13 µm, respectively. These seasonal cell densities and morphology parameters provide a standard for developing biotechnology for sperm collection and cryopreservation in freshwater mussels.

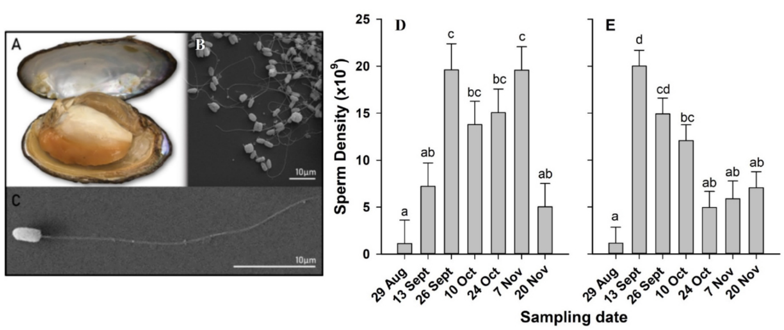

Fig. 1. L. subrostrata gonadal morphology (A) and SEM sperm micrographs (BC). Sperm density quantified throughout the spawning season for L. subrostrata (D) and L. straminea (E).

PHYSIOLOGICAL MECHANISMS REGULATING SPERM MOTILITY INITIATION IN EASTERN OYSTER, *Crassostrea virginica*

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Oyster aquaculture is expanding in the United States, where production has increased from $78 million to $192 million between 2008 to 2016. Many oyster farms rely on oyster seed produced at hatcheries that induce broodstock to spawn. Hatchery success can often be dependent on gamete quality. In order to improve hatchery production, a better understanding of oyster gamete biology is needed. Eastern oyster males and females release sperm and oocytes into the water column to be fertilized. Upon release, sperm is exposed to the external environment in which sperm motility is initiated. However, physical and chemical properties of the environment affect sperm motility performance, which is a key determinant for fertilization success. Thus, providing the optimal environmental conditions will maximize sperm activity and fertilization success. The objectives of our work were to determine the physiological mechanisms regulating sperm motility initiation in this species. Sperm swimming kinematics were evaluated by computer assisted sperm analysis (CASA), and curvilinear velocity (VCL) and percentage of motility were measured. Sperm were activated with artificial sea water (ASW) buffered to make a pH gradient from pH 6.5 to 10.5. Sperm were also activated across a range of salinities from 4 to 32 psu. ASW was tested with 0.5 to 3.5 mM EGTA to find the threshold of extracellular Ca^{2+} ions needed to initiate sperm motility. Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} free ASW and their respective channel blockers were used to elucidate ionic signaling involved in sperm motility initiation.

Results show that sperm VCL increased from pH 6.5 to 7.5 and peaked from 7.5 to 10 (Fig. 1A), while VCL peaked from 12 to 24 psu (Fig. 1B). Sperm VCL was highest with 0.5 to 2.0 mM EGTA and decreased from 2.5 to 3.5 mM EGTA (Fig. 1C). Compared to ASW, sperm motility was lower in Na^{+}, K^{+}, and Mg^{2+} free ASW significantly. Moreover, sperm motility initiation was suppressed in the presence of K^{+} and Ca^{2+} channel blockers. These results show that environmental salinity affects sperm motility initiation and indicate that sperm motility initiation is Ca^{2+}-dependent and require K^{+} exchange through plasma membrane. Our study provides insights into physiological mechanisms of sperm motility signaling in bivalves and provides valuable information to improve fertility in hatcheries and optimize cryopreservation protocols.

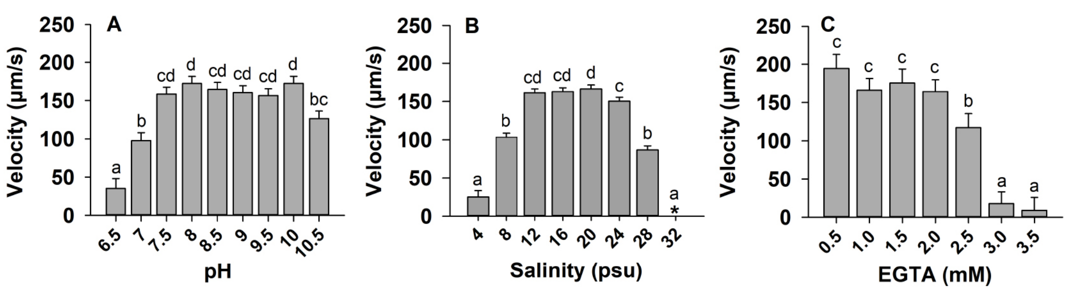

Fig. 1. Eastern oyster, C. virginica, sperm curvilinear velocity (VCL) from pH 6.5 to 10.5 (A), salinities from 4 to 32 psu (B), and EGTA, a Ca^{2+} ion chelator, from 0.5 to 3.5 mM (C).

SUGAR KELP (*Saccharina latissima*) CONCENTRATE FOR FISH FEED

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With the emergence of kelp aquaculture in the United States, there is an opportunity to use off-product plants in marine fish feeds. Off-product kelp is generally mature plants which are approaching senescence and unsuitable for human consumption. A feeding trial was conducted with sablefish (*Anoplopoma fimbria*), incorporating late season sugar kelp in plant based feeds at 2.5%, 5%, and 7.5%. Results demonstrated a significant increase in feed consumption with sugar kelp addition. Feed conversion ratio was reduced by kelp addition. 2.5% kelp and 5% kelp treatments initially increased growth before plateauing at 5% kelp addition.

Feed pellets extruded with 7.5% sugar kelp were harder and less soluble than the 0% kelp control feed. Feed extruded with 10% sugar kelp solids could not be produced with consistency and exhibited glassy properties. In an effort to increase the feed efficiency and protein availability of the kelp feeds, a process was developed using 0.3 M phosphoric acid in a 20:1 w/w ratio with dried sugar kelp powder to reduce the ash and alginate content and increase protein concentration. The acid treated sugar kelp is less hygroscopic and has more consistent behavior in the production of extruded feeds. Development of this process could create a market for low value sugar kelp as fish feed. Details of the processing and effects on pellet solubility will be described.

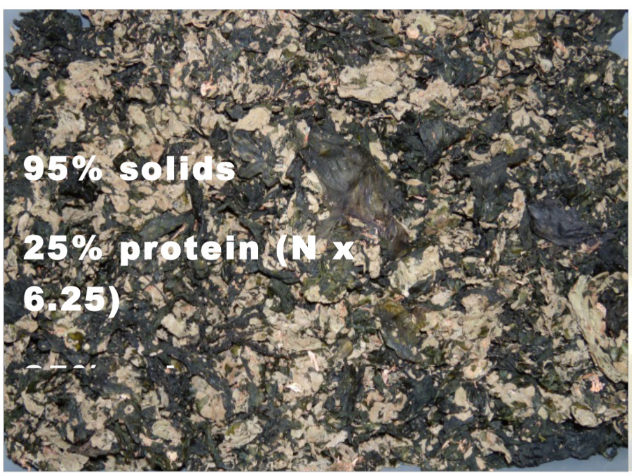

Figure 1. Dry late season sugar kelp.

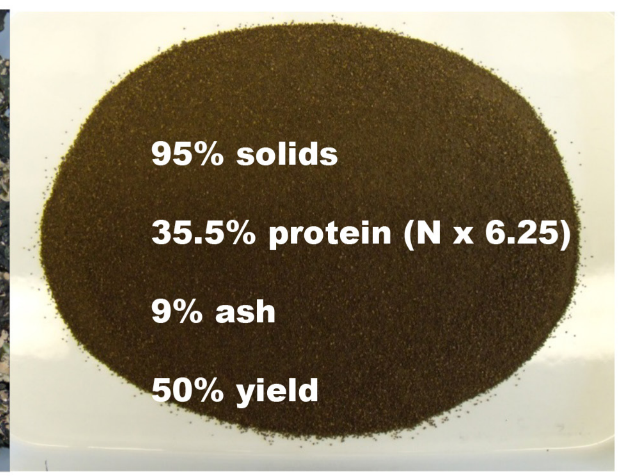

Figure 2. Sugar kelp concentrate processed with 0.3M H_3PO_4.

SOME EXOTIC FISH SPECIES IN NIGERIA AND RISKS TO BIODIVERSITY

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Exotic fish species or non-indigenous fish have become common in different parts of the world due to their use in aquaculture, improvement of local fisheries potential, recreation and sports, ornamental and hobby fisheries, as well as pest control. Indiscriminate transfer of fish species has been generating worldwide concerns.

Exotic species, despite possessing some attractive characteristics may introduce unhealthy competitions for food and habitat, prey on fish, introduce new diseases and parasites, or hybridize in the new environment. This study considers the ecological significance and risks the introduction of exotic species may pose to aquatic biodiversity as well as the ecosystem.

EXAMINING THE SOCIAL CARRYING CAPACITY FOR MARICULTURE DEVELOPMENT ON THE SOUTH CAROLINA COAST: ISSUES, FACTORS, VARIABLES, AND ATTRIBUTES INFLUENCING STAKEHOLDER PERCEPTIONS

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The expansion of shellfish mariculture presents a valuable opportunity to create and retain jobs while enhancing the local seafood supply and providing critical ecosystem services that benefit important nursery habitats because of its water purifying properties. Recent efforts to expand oyster production in South Carolina (SC) suggest that coastal landowner and waterway user opposition is the greatest threat to oyster farming because the infrastructure is often more visible to the public than clam mariculture. Understanding resident and visitor concerns and improving communications with these stakeholders is critical to the future of oyster mariculture development.

Underlying the potential opposition is the lack of information on the social carrying capacity for oyster mariculture on the SC coast. Social carrying capacity is the amount and type of use that an area can accommodate without unacceptably degrading cultural or community elements (i.e., view sheds and recreational behavior). The first phase of this project was qualitative research to identify issues, factors, variables, and attributes influencing stakeholder perceptions and attitudes towards the expansion of oyster mariculture. This information will then be used to develop a set of indicators that will serve as the basis for measuring social carrying capacity/societal acceptance of mariculture in Charleston and Beaufort counties in phase two. In total, 80 semi-structured field interviews were conducted with stakeholders in Charleston and Beaufort County guided by a script and questions approved by an industry research advisory group. Stakeholders interviewed included creek and riverfront property owners (n=20), recreationists (n=20), coastal business owners and managers (n=15), local and county government representatives (n=10), and tourists from outside Charleston, Beaufort and adjacent counties (n=15). Interviews were recorded, transcribed, and coded (open and axial) to identify underlying issues, factors, variables, and attributes of oyster mariculture. Here are representative quotes from those findings:

"I would say it does fit into the local culture. It's harvesting oysters. Commercially raised but we've been harvesting oysters here ever since there were people here, so yeah it fits in with the local culture."

"They're regulated, they're clean, and they're not going to get you sick (which I've done). I can see a lot of positives on the farmer side – and economically it's another place to make some money"

"Oysters filter the water – benefits, probably takes some of the pressure off the wild oysters. The supply is perhaps a more reliable source for consumption than the wild oysters."

"It shouldn't be in those areas where you have high public traffic of boating. I'm more concerned about oyster farming blocking or blocking access to recreational or other folk that do fish, ski, ride to look at birds…"

"I mean if it's taking up a smaller part of the waterway I don't think that that's personally a problem, but if it's taking up a large part I don't think that would be so cool."

DEVELOPMENT OF GENETICS-BASED SELECTIVE BREEDING OF STERILE KELP

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A major hurdle in the path of economically feasible kelp farming, at the scales needed to reshape crop markets, is the environmental concerns raised by the possibility of invasive species and trans gene flow; offshore farms are often close to marine protected areas, recreational areas and shipping channels. Trans gene flow is associated with many of our current terrestrial crops which are essentially non-native (e.g. wheat), being originally domesticated and improved in one region and then transplanted across the globe. New crops, including aquaculture crops, are held to more stringent standards to minimize the risks from invasive species and trans gene flow in order to protect native plants, and treasured natural or heritage populations. What potential solutions might allow domesticated genomically improved strains of kelp to be grown safely in natural areas? We propose to establish kelp strains with a completely sterile sporophyte stage. The haplodiplontic life cycle of kelp makes them remarkably well-suited for this kind of manipulation. The large sporophyte fronds are the generation that is harvested as a crop, but the microscopic haploid kelp gametophyte generation may be vegetatively propagated indefinitely in culture. We will identify natural alleles, i.e. recessive mutations (loss-of-function) in genes required for meiosis. Male and female gametophytes with these recessive sterile mutations can still produce gametes that can be crossed to produce fully sterile sporophytes (because gametes are produced by mitosis in the gametophyte generation, not meiosis). This allows for the fast, targeted selection of immortalized gametophytes.

COMBATING THE DETRIMENTAL MARINE ENVIRONMENT EFFECTS ON MOORING HARDWARE THROUGH DESIGN AND PROPER SELECTION OF MATERIAL BEST SUITED FOR AQUACULTURE INDUSTRY

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Throughout its lifetime, all mooring equipment is affected by various factors based on the surroundings. These environmental effects degrade the equipment over time, and could cause a significant failure to the given system without warning. This failure could be prevented through proper selection of the hardware material, as well as choosing the appropriate inspection guide designed for the rough seas.

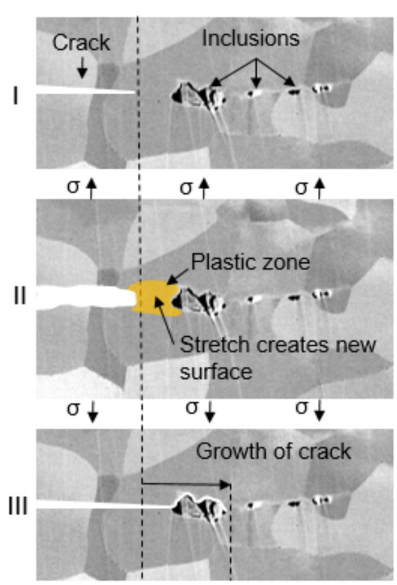
There are three main challenges when it comes to mooring in coastal areas: fatigue, corrosion and brittleness. All of these factors affect the steel properties, and their interaction affect the service life of the product. Fatigue, the most common problem present in any engineering application, is often overlooked. Fatigue failure could occur at a lower load than the intended working load limit (WLL). The following figure illustrates steps of fatigue.

- Step 1: Initiation of a crack in the material
- Step 2: Plastic zone forms during tension loading
- Step 3: Growth of crack at the end of cycle loads

Second, corrosion is the most obvious challenge in a marine environment. Seawater is a complex chemical system affected by concentration and access of dissolved oxygen, salinity, concentration of minor ions (e.g. Ca2+, Mg2+), biological activity and pollutants. These in turn are affected by temperature, depth, and ocean currents. Depending on the access of available oxygen, the steel will either corrode or be immune towards corrosion.

Lastly, brittleness is important to consider as the properties of material change when the temperature drops. These equipment are designed to elongate before they break, acting as a safety feature. The user can see that the product is being used incorrectly and can stop before catastrophic failure. However, as the temperature drops, steel passes through the stages where it becomes brittle and subject to early failure.

This paper will outline how we design against these three main environmental factors, especially in the extreme conditions that we commonly find in the aquaculture. To ensure the safety and longevity of the mooring equipment utilized in the net pens design, proper consideration of material selection, procurement and best- use practices are the utmost importance.



THE EFFECTS OF REGULATION ON SALMON AQUACULTURE STOCKS

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Oslo Stock excange is the main financial market for the world's largest salmon Aquaculture stocks. Most of the companies are based in Norway, but a few are also based in other countries, like Scotland and Faero Islands. Their operations are based in their home countries, but they also have international operations in eg. Chile and Canada. Consequently, most of the companies and their operations are regulated by the The Norwegian Aquaculture Act.

Some have also engaged in numerous voluntarily governance activities the last years, such as the implementation of a global ecolabel (Aquaculture Stewardship Council (ASC)). Coming into force January 1st 2006, The Norwegian Aquaculture Act and its regulations have devloped during the years, with many aimed to take into account environmental challenges.

This paper will investigate how the financial markets reacts to the introduction of these regulations. Since they are not voluntarily, the study will shed light on how industry broad compulsory compliance requirements is interpreted by the markets. Is the reaction different to foreign companies?

Using daily stock price data and issue date for regulations, we will identify any abnormal returns around the time of regulation issue. We will also distinguish these regulations to see what makes the most impact, either positive or negative, on share prices. The paper will contribute to understand how financial markets react to government regulations in an important industry with tremendous growth and many controversies. This should be of interest to equity owners, policy makers and managers.

HUSHED UP: PESTS, DISEASES, AND WEEDS IN ALGAL MASS CULTURE

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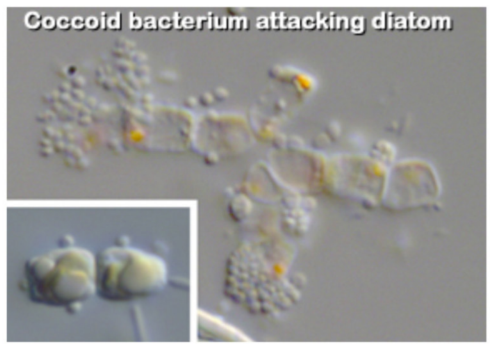
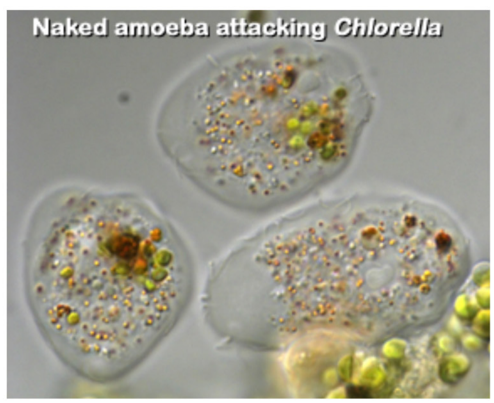
All crops on Earth suffer from pests, diseases, and weeds (PDWs), and algae in mass culture are not exempt. Pests and disease agents may be viruses, bacteria, protozoa of various types, fungi and fungus-like organisms, and micro-invertebrate animals.

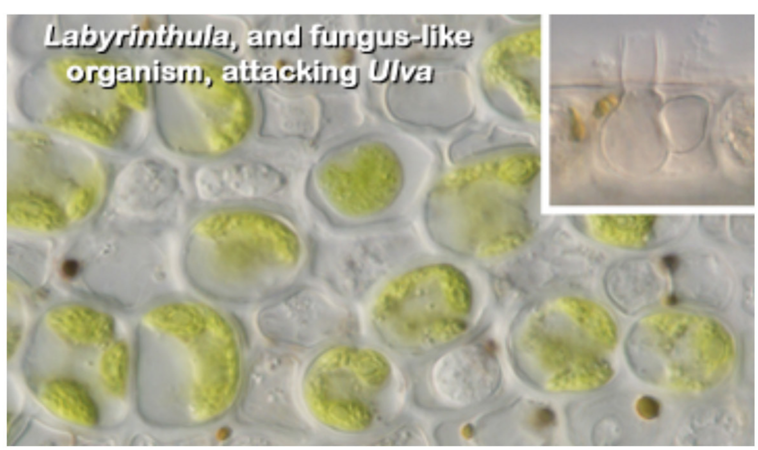
Arguably, the knowledge base for PDWs is inadequate to support the growth of algal biomass industries. Commonly, PDWs are "emerging", with the agents new to science, or poorly known and with little or no prior knowledge of their ability to attack algal biomass cultures.

Research support is meager. Effort is further impeded by the reluctance of the industry to discuss the issue, lest the result be backlash from consumers and investors, or loss of advantage *vs* competitors. Moreover, algae from mass culture may themselves become invasive weeds, with negative regulatory consequences for the industry. Release of a crop species from the PDWs that affect it in its native habitat may contribute to that species becoming troublesome.

Investigations likely to be helpful include those on PDW biodiversity, on assessing direct and indirect impacts of PDWs on crop species (including how these PDWs affect different life history stages of the same crop species), on the identification of PDW reservoirs in production and surrounding natural environments, and on discovering effective mitigation and non-dissemination strategies.







DETECTION AND GENOME RECONSTRUCTION OF TAURA SYNDROME VIRUS OF SHRIMP FROM DAVIDSON'S FIXED PARAFFIN EMBEDDED SHRIMP TISSUE

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Taura Syndrome (TS), caused by Taura syndrome virus (TSV), is an OIE-listed disease that has caused more than $2 billion losses to shrimp aquaculture worldwide since its emergence in 1992. TS is now prevalent in the Americas and Asia. Aquaculture Pathology Laboratory has an archive of Davidson's fixed paraffin embedded (DFPE) shrimp tissues blocks spanning across decades that represent a priceless biobank of information, and provide a durable and cost-effective ways of storing tissues. The objective of this study was to determine the utility of archived blocks as biological samples for pathogen detection and genetic studies, using TSV as a model. DFPE tissues present serious challenge in isolating high quality RNA due to the chemical modification, RNA hydrolysis by acetic acid, and cross linking of nucleic acids and proteins.

TSV infected tissue sections (~4 μm) (N=29) with Grades 3 and 4 levels of infection (on a Scale of 0 to 4) were taken from DFPE blocks from 2005. Total RNA was isolated using three commercially available kits to determine their suitability to yield high quality RNA (29 total x 3 kits = 87 samples). Upon some modifications, one of the three kits provided good quality RNA (average concentration ~250 ng/μl, average 260/280 value of 1.91) amenable to PCR amplification. TSV was successfully detected by conventional (231 bp) and real-time (72 bp) RT-PCR in all 29 samples. Sanger sequencing of representative amplicons confirmed TSV identity. Using Next Generation Sequencing, the complete genome of TSV was reconstructed for three RNA samples representing N=87.

This is the first study that demonstrates the utility of archived DFPE histological blocks for shrimp pathogen detection using TSV as model. This shows both health assessment and targeted pathogen screening in shrimp can be done using histological samples. The feasibility to reconstruct viral genome from archived DFPE blocks opens avenues for the discovery of novel pathogens in shrimp. This will also enhance our understanding of the evolution of shrimp pathogens that has a direct implication in disease management in shrimp aquaculture.

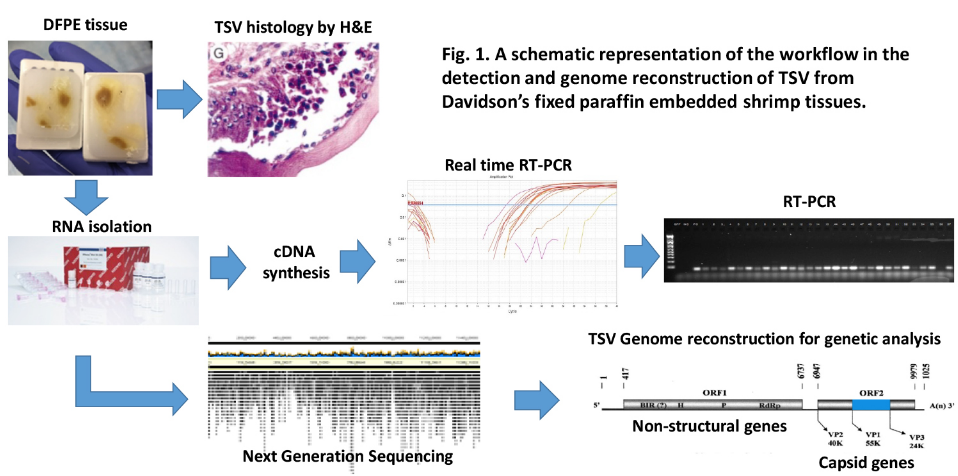

Fig. 1. A schematic representation of the workflow in the detection and genome reconstruction of TSV from Davidson's fixed paraffin embedded shrimp tissues.

DEVELOPING A SHRIMP DISEASE DIAGNOSTIC LABORATORY IN HAWAII

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Hawaii shrimp broodstock producers export more than half a million live shrimp annually to more than 15 countries. These countries require pre-shipment testing to prove that the shrimp are not infected with World Organization for Animal Health (OIE)-listed pathogens. In 2018, the Hawaii Department of Agriculture (HDOA) and the Center for Tropical and Subtropical Aquaculture (CTSA) provided funding to the University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources to create a diagnostic laboratory that would become U.S. Department of Agriculture (USDA) approved to perform testing for shrimp diseases that satisfies the requirements for exporting live shrimp.

The seven shrimp diseases that are currently listed by the OIE are the DNA viruses white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV); the RNA viruses Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV), yellow-head virus (YHV); the rickettsia-like agent causing necrotizing hepatopancreatitis (NHP); and acute hepatopancreatic necrosis disease-causing *Vibrio parahaemolyticus* (AHPND). The newly created University of Hawaii Animal Diagnostic Laboratory (UHADL) has the ability to test for all these pathogens using protocols that follow the OIE standards.

Key to obtaining approval from the USDA to perform disease testing for export purposes is the creation of and adherence to a quality management system (QMS). At the time this abstract was written, 66 standard operating procedures have been written. This QMS ensures consistent, accurate, and timely PCR testing and enables the UHADL to meet the needs of Hawaii's shrimp broodstock industry.

The laboratory has validated the real-time PCR protocols for WSSV, NHP, AHPND, IHHNV, IMNV, TSV and a conventional PCR protocol for YHV. The test methods utilized at UHADL comprise those that are endorsed and published by a recognized reference. A test validation and optimization were performed for each of the seven OIE-listed disease. The accuracy level, specificity, limit of detection and quantitation, precision and repeatability were determined for each protocol. The PCR assays for CMNV, MoV, LSNV, and PVNV are in the process of being developed.

PERCEPTIONS OF FARMED SEAFOOD IN THE FOODSERVICE COMMUNITY

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With funding from the National Sea Grant Program, New York Sea Grant and the National Aquaculture Association partnered to develop positive attitudes and perceptions about farm-raised seafood products in the foodservice community. By developing an appreciation of U.S. farm-raised seafood among educators, the project can reach thousands of culinary students who will become the new generation of celebrity chefs and food trend-setters.

Seafood is a daunting and confusing commodity with thousands of species farmed and fished in a variety of ways. However, most culinary schools do not offer a specific seafood course. To gain a better understanding of this sector and develop public relations strategies, the team constructed an in-depth survey to assess current attitudes, identify knowledge gaps and initiate conversations.

Initial results revealed that most educators realize that in order to provide seafood for future generations, we need to turn to aquaculture but there are concerns about sustainability, use of antibiotics, GMO products, environmental degradation, and impacts on wild stocks.

Trade school educators voiced concerns about the affordability of seafood. This severely limits the students' exposure to such products. Frozen seafood predominates with use of whole fish reserved for meat fabrication courses. If a local farmer provides product for the classroom, it is not only an opportunity to familiarize students with the product, but can also build markets and serve to develop a positive image of the farm.

Nutritional medicine is a trending topic in the foodservice community, however many educators are unaware of the recommendations of the Dietary Guidelines for Americans and the FDA advice for pregnant women. Additionally, many educators perceived farmed seafood as less flavorful and nutritious than wild products. The level of unawareness of the seafood HACCP program and other seafood safety programs was distressing.

Salmon was the most commonly used product in the classrooms surveyed. A concern for the entire seafood industry is how to expand the market beyond the usual top ten seafood choices. Familiarizing chefs with abundant, affordable and available species can help spread the word and acquaint consumers with non- traditional seafood options especially when those species are not part of the traditional menu. These efforts seek to help build connections between the U.S. aquaculture industry and future chefs.

BUILDING A FOODSERVICE EDUCATION TOOLBOX FOR FARMED SEAFOOD

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With funding from the National Sea Grant Program, New York Sea Grant and the National Aquaculture Association partnered to develop positive attitudes and perceptions about farm-raised seafood products in the foodservice community. To assist in those efforts, an effective and cost-efficient coalition of government, academia and industry, all focused on a common message is necessary. Extension educators play a pivotal role in this effort by sharing these resources with industry partners and others who can broaden their impact and use.

To develop a more complete understanding of our industry and to increase the outreach of the program, the team is conducting an industry survey. The goal of the survey is to better match growers with markets, identify appropriate public relations strategies, and help keep growers informed of opportunities to market and promote their products. Growers are urged to complete the survey on-line at https://bit.ly/2RgiHmE . A number of growers have already taken advantage of opportunities to participate in trade shows and other events to promote their products at no charge.

To help answer common questions about U.S. farm-raised seafood, a set of focus points with on-line references has been developed. Additional educational and promotional materials are available from New York Sea Grant and the National Aquaculture Association. These materials were designed to help educators answer questions outside of their area of expertise and assist in connecting producers to end users to successfully market U.S. farmed seafood.
Food writers want exciting stories that follow production from the grower to the chef who ultimately uses the product. To tell that story, a collection of photos, videos, anecdotes, and recipes is being compiled and will be incorporated onto a flash drive for distribution to writers, culinary educators, and others who can tell our story to a larger audience.

At the suggestion of industry, an industry speakers' bureau is being developed to assist producers in taking advantage of opportunities to talk directly with chefs, restaurateurs, and others in the foodservice industry. Many groups such as local chapters of the American Culinary Federation regularly host dinner meetings and welcome guest speakers.

Developing a connection between growers and individual chefs/culinary educators can not only increase direct sales, but can assist in developing marketing and public relations strategies. Extension and other industry educators can assist by informing growers of these opportunities, talking with local chefs and culinary associations, helping to develop materials that feature growers and chefs, and showcasing their local industry. We are seeking additional partners to move these efforts forward and help guide the creation of new and exciting resources for growers and culinary professionals across the country.

WHY CLEAN THEN DISINFECT – THE SCIENCE BEHIND AN EFFECTIVE C&D PROGRAM

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The efficacy of a disinfectant can vary greatly depending on the intrinsic qualities of the microorganisms as well as the chemical and physical environment, in which it is being used. Meticulous cleaning prior to disinfection decreases the microbial load and thus prepares the surface for disinfection. The objective of this paper is to highlight the importance of cleaning before disinfecting, which is a critical component of an effective biosecurity program. This will lead to a more informed decision when selecting which chemistry to use under challenging conditions.

Only surfaces that come into direct contact with the antimicrobial for the approved contact time will be disinfected. Hard water scale, organic material and biofilm can all act as a 'shield', which prevents the disinfectant from reaching its target. Cleaners are formulated to remove specific types of soils. Alkaline cleaners are best at removing proteinaceous or organic soils whereas, acidic cleaners are best at dissolving hard water scale. For example: using a toilet bowl cleaner (acidic) to remove dried on spaghetti sauce from dishes or a dish soap (alkaline) to remove soap scum and scale from a shower stall are not going to be very effective. Cleaners are also formulated around their functionality. Non-foaming cleaners are designed for applications where high levels of foam are undesirable, such as a tray wash machine. Foaming cleaners provide longer contact times, which help aid in the penetration of soil making them easier to remove. This is particularly relevant for vertical surfaces. Chlorinated cleaners assist in bleaching or whitening the surface. And lastly, neutral pH cleaners are used on sensitive or soft metals that may be susceptible to corrosivity from highly concentrated products.

Biofilm is a layer of microorganisms contained in a multilayer matrix starting with a monolayer and then developing into a microcolony. If the colonies are not separated or removed from the surface they anchor themselves more permanently to the surface and continue to grow. Additional dust, organic material and other pathogens get caught in the sticky matter becoming extremely difficult to remove. High pressure water alone cannot break the surface tension or the biofilm and a formulated detergent is needed to remove the biofilm prior to disinfection. The first and best option to prevent biofilm is keeping the surface clean and dry from the start.

Just because a surface looks clean, it does not mean it is clean of all pathogens. Cleaning is a critical aspect of effective Biosecurity programs. Selecting the right cleaner for the task will help break adherent films associated with scale and mineral deposits from iron and hard water and help reduce costs on labor and water usage. In doing so this will allow the registered disinfectant to work more effectively.

OPTIMIZING EARTHWORM POWDER AS SUPPLIMENTARY SOURCE OF PROTEIN-LYSINE IN FISH FEED FORMULATION FOR AFRICAN CATFISH (*Clarias gariepinus*) FINGERLINGS

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Lysine is one of the most important essential amino acid in fish feeds needed for optimum growth of fish. The combination of different feedstuff with suitable components of lysine can help to boost lysine content in fish feeds. Sources of lysine for fish feeds includes soybean waste, leucaena leucocephala leaves, fish meal and earthworm powder. The goal of this investigation is to enhance lysine content in fish feeds by incorporating different ratio of each chosen feed constituents. The investigation was conducted using central composite design (CCD) as statistical tool. The optimal lysine content was obtained at 25.00 %, 20.88 %, 10.00 % and 20.00 % of earthworm powder, soybean waste, leucaena leucocephala leaves and fish meal, respectively. The lysine content in formulated fish feeds was significantly affected by the composition of earthworm powder and fish meal in fish feed formulation. Earthworm powder and fish meal have contributed the largest portion of lysine in fish feed due to high lysine content which were 4.48 % w/w and 3.60 % w/w, respectively. The optimized fish feed shows high lysine content of 23.39 % w/w which doubles the lysine content in commercial fish feed (11.21 % w/w). The composition of fish feed obtained from this study can be used as guidelines for formulation of high lysine fish feeds for African catfish fingerlings.

GENOME-WIDE IDENTIFICATION AND EXPRESSION ANALYSIS OF SIX INSULIN-LIKE PEPTIDES WITHIN PACIFIC ABALONE (*Haliotis discus hannai*)

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Insulin family peptide members including insulin, insulin-like growth factors (IGFs) and insulin-like peptides (ILPs) play key roles in metabolism, growth, reproduction and lifespan. Mechanisms of insulin/IGF signaling (IIS) have been well-conserved over long periods of evolutionary time. We analyzed the transcriptome sequencing of Pacific abalone (*Haliotis discus hannai*) and identified six novel Abalone insulin-like peptides (AIPs). We characterized the genetic features, such as the genomic organization, gene length, protein length, and sequence similarities, as well as exon–intron structures of novel AIP genes. Additionally, we utilized RNA-seq data to further explore the expression patterns of AIPs, which may help uncover some vital functions of Pacific abalone. We performed quantitative real-time polymerase chain reactions (qPCR) in different tissues to observe their spatial expression patterns. The newly identified AIPs genes were expressed in diverse tissues. High level of AIP3 gene expression in the gill tissue and AIP2 gene expression in the mantle tissue may suggest that these genes function in osmoregulation, and ionic homeostasis. The genes identified in this study will lead to the development of markers for the identification of high-growth-rate abalone and female abalone.

EFFICACY OF *Ocimum gratissimum* (SCENT LEAF) POWDER AS ANAESTHETIC AND ITS EFFECT ON THE HAEMATOLOGY OF *Clarias gariepinus* JUVENILES

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Intensive nature of aquaculture have subjected farmed fish to a number of stressors due to handling proceedures and transportation from hatcheries to final stages. These stressors have most often been responsible for high mortalities recorded, hence hindering the growth of fish farming. Anaesthetics are used in aquaculture, fisheries and biological researches, as a way to minimize hypermotility during handling and transportation to reduce stress and mortality. Previous studies have reported the use of some plant materials to anaesthetized various fish species including *C. gariepinus* This studies aimed to investigates the efficacy of *Ocimum gratissimum* as anaethetic and determine its effects on some haematological parameters of *C. gariepinus* juveniles.

Fresh leafs of *O. gratissimum* were obtained from within the university campus, identified, air dry for 5 days and blended to powder. The experimental treatment were prepared from a stock solution of 2g in 10 litres of water (200mg/l) into five concentrations (0, 50, 100, 150 and 200mg/l) in 20 litres of water. Stages of induction and recovery were monitored and recorded using a stop watch. Blood was ollected by severing the caudal peduncle into Ethylene Diamine Tetra acetic Acids (EDTA) for the analysis of various hemaetological parameters using standard methods.

The result revealed that *O. gratissimum* caused anaesthesia which was concentration dependent. Induction time (min) reduced with increase in concentration while recovery increases as induction time reduces (Table 1 and Figure 1).

Haematological parameters showed some slight changes exspecially at higher concentration however some were not significant ($p > 0.05$). the mean values of red blood cells, haemoglobin, pack cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, basophile, eosinophile and neutrphile were decreasing with increased concentration of clove powder. Others such as white blood cells, platelets and lymphocytes increased with concentration (Tables 2 and 3). Fish exposed to 150mg/l cause induction shows that RBC, Hb and PCV were not significantly ($P > 0.05$) from those exposed to 50mg/l which were however not different from the control (0.0mg/).

The observed behavioural changes including initial hypermotility, hyperventilation, loss of equilibrium and no reaction to handling suggest the fish were immobilised (anaesthetized) by the plant material. This was in line with the reports of several researchers who used anaesthetics on fish during handling and transportation Slight changes in the haematological parameters reported in this study corroborates with the studies of many other researchers who have use plant materials as anaesthetic Minimal changes in RBC, Hb and PCV recorded on fish exposed to 150mg/l which were not different ($P > 0.05$) from the control but induces anaesthesia in 6.22 mins shows it is an ideal concentration. However, researchers have reported a 48 hours reversal to the haematological paramters of fish exposed to clove powder. Further research will be required to investigate the effects of *Ocimum* of the biochemical paramters of *C. gariepinus*.

Table 1: Induction and recovery time (min) of of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Concentration (mg/l)	**Induction**		Recovery	
	Induction 1	Induction 2	Recovery 1	Recovery 2
0	-	-	-	-
50	26.61 ± 2.34	-	-	2.82 ± 2.44
100	11.12 ± 1.54	15.26 ± 0.81	1.26 ± 1.38	4.43 ± 0.36
150	4.16 ± 2.36	6.08 ± 0.34	2.35 ± 2.14	6.76 ± 2.18
200	1.24 ± 4.56	3.22 ± 1.26	4.81 ± 0.19	11.67 ± 1.44
Mean with the same superscript are not significantly different at p< 0.05, Induction1 (loss of equilibrium), induction2 (deep anaethesia), recovery 1 (regain equilibrium), recovery2 (normal swimming).				

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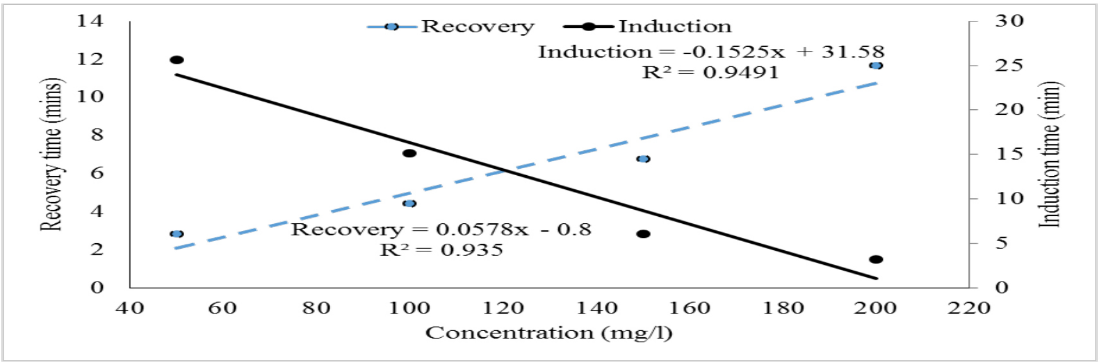

Figure 1: Relationship between Induction, Recovery time (mins) and Concentration (mg/l) of *O. gratissimum*

Table 2: The mean values of selected haematological indices of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Conc. (mg/l)	Haematological parameter							
	RBC (10^{12} cells/L)	WBC (10^{9}cells/L)	Hb (g/l)	Plt	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/l)
0.00	8.34±1.05^{a}	30.95±1.79^{c}	10.34±0.70^{a}	58.77±1.07^{c}	38.05± 3.20^{a}	66.30± 1.92c	21.17±1.92^{c}	30.84±1.28^{a}
50.00	7.06±0.08^{ab}	33.12±4.30^{c}	9.26±0.34^{ab}	61.82±1.71^{bc}	33.97±1.58^{ab}	67.88±2.49bc	23.38±0.58^{bc}	28.38±1.98^{ab}
100.00	6.82±0.70^{ab}	42.47±0.87^{b}	8.86±0.19^{bc}	70.63±1.46^{b}	30.32±1.27^{bc}	78.69± 5.73^{c}	24.60±0.65^{b}	27.87±2.22^{a}
150.00	5.93±0.90^{bc}	50.07±1.41^{a}	7.99±0.54^{bc}	77.00±3.05^{ab}	28.64±0.34^{bc}	93.76± 4.80^{a}	26.44±0.82^{ab}	26.59±0.78^{a}
200.00	4.80±0.33^{c}	52.87±2.36^{a}	7.31±0.80^{c}	98.14±4.93^{a}	26.17±1.34^{c}	102.52±4.53^{a}	28.70±1.28^{a}	25.31±1.08^{c}

Mean with the same superscript are not significantly different at $p< 0.05$, Conc.= concentration, PCV = packed cell volume , RBC= red blood cell Hb = haemoglobin, MCV =mean cell volume MCH= mean cell haemoglobin MCHC= mean haemoglobin concentration, WBC =white blood cell, Plt = platelet..

Table 3: The mean values of selected Differential white blood cell counts of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Conc. (mg/l)	Differential white blood cell count (%)				
	Neut	Lymp	Baso	Mono	Eosin
0	14.71± 1.92^{a}	56.25± 1.40^{b}	4.88± 0.20^{a}	5.99±0.32^{b}	8.07±0.17^{a}
50	13.74± 1.48^{a}	58.02± 1.49^{ab}	4.74± 0.36^{a}	7.01±0.14^{b}	7.66±0.44^{ab}
100	12.18± 0.60^{bc}	59.40± 1.78^{ab}	4.31± 0.31^{a}	7.92±0.53^{b}	6.61±0.36^{ab}
150	12.11± 0.83^{bc}	59.82±1.42^{ab}	3.95± 0.10^{ab}	9.08±0.05^{ab}	5.98±0.48^{ab}
200	10.79± 0.31^{c}	65.44±1.91^{a}	3.07± 0.77^{b}	10.27±0.85^{a}	4.14±0.42^{b}

Mean with the same superscript are not significantly different at $p< 0.05$, Conc.= concentration, Neut = neutrophil Lymp= lymphocytes Baso = basophil, Mono = monophil, Eosin = eosinophil,

CONTEXTUALIZING STEM FOR A NEW GENERATION OF ʻĀINA-CONSCIOUS LEADERS: USING GENETIC BARCODING TECHNIQUES TO IDENTIFY CONTEMPORARY CHALLENGES TO A NATIVE HAWAIIAN FISHPOND SYSTEM

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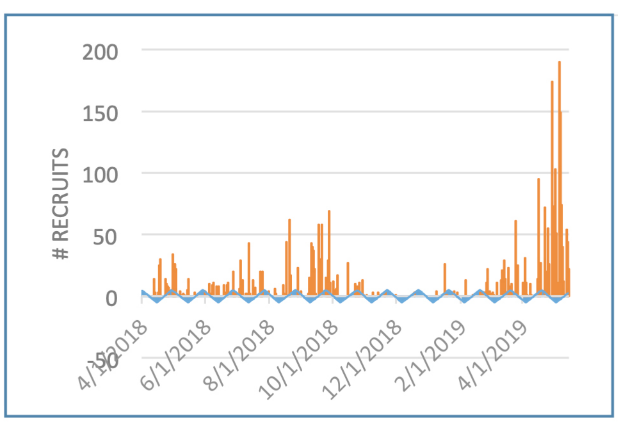
Loko iʻa (traditional Hawaiian fishpond systems) continue to function as managed enclosures for the recruitment and grow-out of fish. Waiāhole and Kapalaho are two of many loko iʻa located on the east side of Hawaiʻi Island that are being rehabilitated in collaboration with student and community groups. Kamehameha Schools has engaged students in the understanding of their ʻāina (lands) by supporting learning that honors the rich cultural heritage of Waiāhole and Kapalaho, and uses contemporary STEM (science, technology, engineering and math) techniques to deepen their understanding of the evolving challenges that these important places face.

Daily observational data from April 2018-2019 on the recruitment patterns of wild *pua* (juvenile mullet species) into the *loko iʻa* of Waiāhole and Kapalaho indicate year-long recruitment of 2-6cm fish into the *loko iʻa* (Fig. 1). The brackish waters of these *loko iʻa* support two mullet species, the native ʻamaʻama (*Mugil cephalus*) and the non-native invasive kanda (*Osteomugil engeli*), which are very similar morphologically at sizes below ~6cm. Increasing densities of kanda within the *loko iʻa* raise concern that competition for resources with the more desired ʻamaʻama could become a problem in the near future if kanda populations continue to grow.

Kamehameha Schools, in collaboration with the University of Hawaiʻi at Hilo, identified protocols for the extraction of genetic material from the CO1 gene of *pua* in the summer of 2019. Sequencing of unknown *pua* against known tissue from both ʻamaʻama and kanda affirms the ability of the protocols to successfully identify the species at their smallest of sizes. A single *pua* sample from each recruiting cohort exceeding ten or more individuals was taken beginning in January 2019 in order to inform the map of recruitment patterns (Fig. 1) with the corresponding recruiting species. Curriculum is being developed to bring the barcoding protocols into the 9^{th} grade classrooms to have students investigate and understand a real and community-relevant challenge to their ʻāina. We expect the preliminary data gathered with these students to be available in January, and will use this data to identify our next management steps with them.

Figure 1. Mullet Species recruits 4/2018 to 5/2019.



DETERMINATION OF OPTIMUM DIET FOR COMMERCIAL JUVENILE BURBOT *Lota lota maculosa* CULTURE

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Burbot (*Lota lota maculosa*) are the only true freshwater member of the gadiform (cod-like) fish and are a promising new species for cold water aquaculture. This "freshwater cod" produces a firm white fillet, the liver and roe are considered delicacies, and the skin can be manufactured into leather. Furthermore, burbot grow-out requires the same water quality parameters as trout production, and burbot have been found to be relatively refractory to many salmonid pathogens. Burbot culture can be incorporated into already existing trout production facilities, diversifying production for established cold water aquaculture operations. For commercial burbot culture to succeed, the optimum diet for the grow-out of this species must be determined. Two feeding trials were conducted at the University of Idaho to evaluate the effect of different dietary formulations on the growth of juvenile and sub-adult burbot, under intensive culture. Four dietary treatments were evaluated, Europa (commercial), Oncor (commercial), a formulated marine-like diet (Burbot 1; B1), and a formulated trout-like diet (Burbot 2; B2). Juvenile growth results indicated a difference in relative growth (RG; P=0.001), specific growth rate (SGR; P=0.001), and biomass gain (BG; P=0.001) among the marine blend dietary treatments, with the fish fed B1 and Europa performing better than the B2 and Oncor diets. This is indicative of a preference for marine-type diets during the juvenile life stage of burbot. Growth results also indicate a difference in protein efficiency ratio (PER; P=0.001) among the diets, with the fish fed Europa exhibiting the lowest protein utilization rate. Diet B1 was formulated with small inclusions of soy protein concentrate and wheat, given the close formulation similarities between B1 and Europa it appears that burbot can tolerate small inclusions of these plant products, although the commercial ingredient listing was not available. Sub-adult growth results indicate no differences in RG, SGR, BG. Thus, it appears that the sub-adult burbot are more tolerant of the rainbow trout-like formulation at this life stage While more research is needed, these results indicate that burbot may require a marine-type diet during the juvenile life stage, and could possibly be transitioned onto a trout-like diet at the onset of adulthood.

EFFECTS OF GRADED DIETARY *Hermetia illucens* INCLUSION LEVELS ON JUVENILE AND ADULT ZEBRAFISH GROWTH AND WELFARE

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For many years, aquaculture relied on the use of fish meal and oil as optimal ingredients, but presently they are no longer sustainable. Among several alternatives, the Black Soldier Fly (BSF) (*Hermetia illucens*) represents a very promising candidate, even if its inclusion in aquafeed still faces some problems because of its unbalanced fatty acids profile and chitin content. At this regard, the enrichment of BSF growth substrate with a potential polyunsaturated fatty acid (PUFAs) source represents a valid method to improve the insect's nutritional value. In the present study, according to a circular economy concept, land organic by-products (coffee silverskin) added with a 10% (W/W) *Schizochytrium* sp was efficiently bio-converted in a highly nutritious insect biomass which, in turn, provided a sustainable new ingredient for fish culture. The present study investigated the effects of diets including increasing levels of BSF meal (0, 25, 50, 75, 100% with respect to fish meal) during a six-months feeding trial performed on zebrafish (*Danio rerio*). Fish were sampled at 2 and 6 months and a multidisciplinary approach, including biometry, histology, gas chromatography, spectroscopy (FTIR), microbiota analyses and molecular biology was applied.

Results showed that insect's fatty acid profile can be improved by adding an adequate source of PUFAs in the growth substrate and that insect meal can be included in zebrafish diets up to 50% with respect to fish meal without significantly affecting fish physiology and development.

Higher inclusion levels negatively affected fish welfare and stress response

This study was funded by Ricerca Scientifica Cariverona, NUTRIFISH project N° 2017.0571

INSECT MEAL AND POULTRY BY-PRODUCT MEAL BASED DIETS DURING RAINBOW TROUT *Onchorynchus mykiss* CULTURE. FTIR IMAGING AND HISTOLOGICAL CORRELATIVE STUDY TO INVESTIGATE INTESTINE AND LIVER WELFARE

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Fish meal (FM) and fish oil (FO) represent the ideal ingredients for carnivorous fish diets due to their excellent nutritive properties, including high-protein content and adequate amino acid and fatty acid (FA) profile. Over the last decades, research has focused on testing different alternative and sustainable ingredients to be used in aquafeed, including insect meal (IM) and poultry by-products meal (PBM).

IM and PBM show several advantages such as a low environmental impact, high protein content and an adequate amino acidic profile.

The present study investigated the effects on intestine and liver welfare of IM (*Hermetia illucens*) and PBM based diets during rainbow trout (*Onchorynchus mykiss*) culture.

Specifically, six different dietary treatments were formulated: one control diet based on vegetable ingredients (C Veg); one fish meal based diet (C Fish); one diet with a 30% substitution of vegetable proteins with IM (IM130); one with 60% of substitution with IM (IM160); one with 60% of substitution with PBM (PBM160) and one diet combined with 50% PBM and 10% IM (PBM150+HM110). Feeding trials lasted until fish triplicated their initial weight. At the end of the feeding trial intestine and liver samples were collected and processed for histological and spectroscopic analyses. Fourier transform infrared (FTIR) spectroscopy is a fast, label-free analytical technique, which analyses the vibrational transitions induced on matter by the interaction with the electromagnetic radiation and is able to give detailed information on the macromolecular composition of non-homogeneous biological samples. Results showed that intestine did not show inflammatory events in medium and hind traits in none of the experimental groups apart a slight increase of mucous cell in medium intestine of trout fed diets including IM (IM130 and IM160). Liver histology showed differences among the experimental groups with a variable lipid accumulation in response to the different diets.

FTIR imaging analysis allowed to detect difference in gut mucosal layer composition and to characterize the distribution and quality of lipid in liver parenchyma. Moreover, to achieve more information, specific areas on the gut mucosa and liver parenchyma were carefully selected and the IR spectra extracted and analyzed. This procedure allowed to detect differences in the relative abundance of total lipids (LIP), fatty acids (FA), saturated (CH2) and unsaturated (CH) lipid alkyl chains, phospholipids (CO), glycogen (GLY), proteins (PRT), glutamate (GLU) and mucin (MUCIN). Finally the respective ratios in which each macromolecular class was represented were compared to the overall tissue biomass (TBM).

Founding was provided by Ager2-Sushin cod. 0112-2016.

CHARACTERIZATION OF INTERFERON (IFN)- INDUCED PROTEIN 35 (IFP35) IN ROCK BREAM (*Oplegnathus fasciatus*)

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Viral diseases are a considerable threat to cultures and natural fish populations. It is lead to significant losses in livestock and economic losses to the world aquaculture industry. Interferons (IFNs) and IFN-inducible proteins are responsible for the antiviral defense mechanisms of the innate immune response. IFN-induced protein-35 kDa (IFP35) is a cytoplasmic protein, and it can be translocated to the nucleus via stimulation. IFP35 has two tandem C terminus Nmi/IFP35 homology domains (NID) and containing an N-terminal leucine zipper motif. That domains are responsible for the IFP35 stabilization. IFP35 can stimulate apoptosis, and other cytokine-signaling pathways interact with CKIP-1 (casein kinase 2-interacting protein-1) and B-ATF (basic leucine zipper transcription factor, ATF-like).

Rock bream interferon-induced protein-35 (*RbIFP35*) homolog was identified, and its biological functions were characterized. rock bream complete cDNA of *IFP35* got from rockfish cDNA transcriptomic database. RbIFP35 protein characteristic and structural features were analyzed by several bioinformatics tools, such as ExPASy PROSITE, NCBI BLAST, and SignalP online server. The expression level of mRNA in rock bream was analyzed by qPCR technique. Conserved domain parts of the RbIFP35 were determined by using multiple sequence alignment tool, ClustalW. The phylogenetic tree analysis was constructed according to the Neighbor-joining (NJ) method by MEGA 5.0 software. The putative 3D structure of the RbIFP35 was constructed by I-TASSER server. *RbIFP35* cellular location was predicted by MultiLoc2 software. To measure the immune response of RbIFP35 on stimulant or pathogens, healthy rock breams were used to experiment. Each group of rock bream was injected with rock bream iridovirus, *Edwardsiella tarda*, *Streptococcus iniae,* lipopolysaccharide and polyinosinic: polycytidylic acid. Phosphate buffered saline (PBS) was injected into the control group. For each treatment, various tissues were collected from the challenged rock bream from different time intervals. Then total RNA was extracted from each tissue and did the qPCR assay. RbIFP35 Subcellular localization was performed using the rock bream heart cells with constructed pEGFP-N1/RbIFP35 plasmids.

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF P65 SUBUNIT OF NF-KB FROM REDLIP MULLET *Liza haematocheila*

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Nuclear factor (NF)-kappaB is a family of structurally related transcription factors which plays a central role in innate immune response by controlling gene network expression. All the members in the NF-κB protein family share a highly conserved DNA binding or dimerization domain called a Rel homology domain (RHD). The family can be subdivided into two subfamilies as “Rel” proteins and “NF-κB” proteins. RelA/p65, RelB, C-Rel are the members of “Rel” subfamily and p105 and p100 are the members of NF-κB subfamily.

The current experiment was designed to characterize homologs sequence for p65 subunit of NF-κB from red lip mullet. A nucleotide sequence for p65 was identified from mullet transcriptomic database. Bioinformatics tools and software were used in sequence characterization. Five redlip mullets were dissected and 12 different tissues including liver and spleen were collected. Groups of healthy red lip mullets were challenged with LPS, Poly I:C and *Lactococcus garvieae*. The spleen of fish (n=5) was sampled and pooled in 0, 6, 24, 48, 78 hours after the IP injection. mRNA extraction and cDNA synthesis were performed for tissue pools from both the studies respectively.qPCR was used to investigate the transcriptional modulation of *Lh*p65 gene in tissues of healthy fish and challenged fish.

The *Lh*p65 gene is consisted of 1899bp and predicted polypeptide sequence carries 632 AA. The predicted molecular weight of the *Lh*p65 subunit is 69.36kDa. In the phylogenetic reconstruction, Lhp65 cladded with its fish counterparts. Multiple sequence analysis indicated a significant similarity in the domain structure of *Lh*p65with other vertebrate sequences.

The intestine shows the highest expression of *Lh*p65 among other tissues. The digestive tract is one of the primary entering routes of pathogens. That may cause high expression in the intestine. *Lh*p65 is highly stimulated in spleen tissue by all three PAMP’s. Interestingly, continuous upregulation is observed in poly I:C and *L. garvieae* challenges. The population density increase of live *L. garvieae* in challenged fish may induce the transcription of p65 subunit. The viral mimic poly I:C may induce the transcription, in order to inhibit the viral replication by activating NF-κB target genes. However, the extensive investigation is required to designate the function of p65 in the fish immune mechanism.

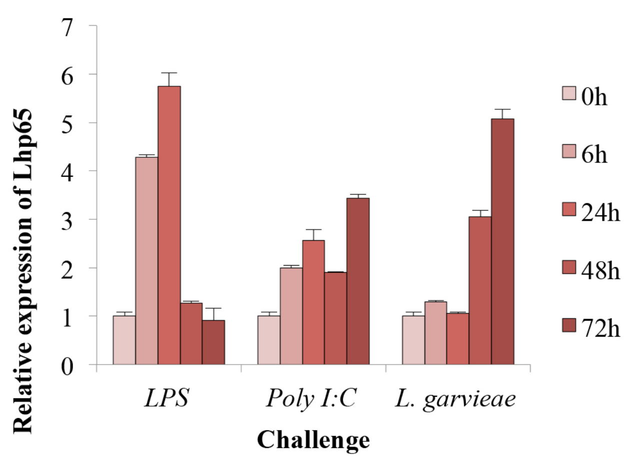

Fig.1 Relative expression of mullet p65 in spleen tissue at certain time points after different immune challenges

ESTABLISHING AN ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) BROODSTOCK

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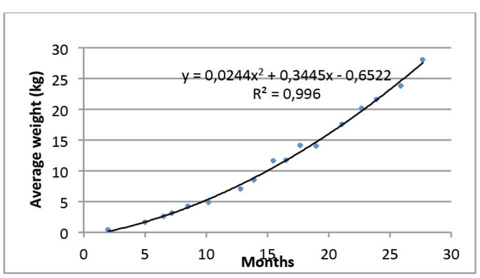
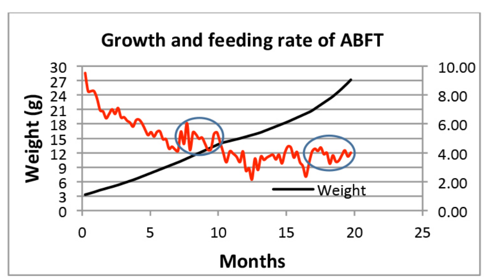
The Spanish Institute of Oceanography owns a facility to reproduce Atlantic bluefin tuna (ABFT). This facility was built in 2015 and in August 2017, some fingerlings born in captivity were moved to this installation, and some wild tunas were added two months later. In November 2019, this batch consists in 69 2^+ ABFT weighting 28 kg. Hopefully they can reach 40 kg at the beginning of June and spawn by the first time.

Fertilized ABFT eggs were collected from cages owned by PisciAlba and obtained larvae were cultured in IEO facilities. At the end of July 2017, some fingerlings (Cultured batch –CB-, 2 gr mean weight) were move to ICRA facilities and placed in a 1000 m^3 tank. At the end of September 2017 a total of 68 0^+ ABFT fingerlings (Wild batch –WB-, 414 g mean weight), were captured from the Mazarron Bay and placed in another tank in ICRA. 4 months later, 81 tunas coming from both batches were weighted (2.5 kg mean weight), tagged and place together in a 3500 m3 tank. During all the time, temperature ranged between 17 and 27ºC, and food consisted in bait, mainly, *Scomber scombrus* (47% of the total food) and *Clupea harengus (25%)* but also *Sardinella aurita*, *Engraulis encrasicolus* and *Scomber japonicus* (11, 10 and 7% respectively).

From February 2018 to November 2019, mortality has been 16.9% (10.9% in WB and 28.6% in CB) and tunas have grown to reach an average of 28 kg. Biomass in this moment is 1930 kg, which means a density of 0.55 kg/m^3. Feeding intake was high during the first six months (when tunas were smaller than 2.5 kg), but after this moment it has ranged between 2.5 and 7%, decreasing with the size of tunas and increasing slightly with higher temperatures (circles). Feeding conversion rate was quite stable during the period, averaging 13.8. According to forecast, at the end of next spring, average weight will be about 40 kg, with about 15 tunas greater than 50 kg. This means that they will be able to reproduce next summer if gonadal maturation develops properly.





EVALUATION OF GENETICALLY ENGINEERED *Camelina sativa* OIL AS A SOURCE OF DIETARY LIPID FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Farmed fish require a dietary source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), typically supplied by fish oil. With increasing production demands on the aquaculture industry with the growing human population and the dependency on wild fish, the continued use of fish oil is both environmentally and economically unsustainable. As a sustainable alternative, the oilseed *Camelina sativa* was genetically engineered (GE) to produce DHA and EPA at levels similar to fish oil. The present study evaluated the effectiveness of GE Camelina oil as a sustainable source of DHA and EPA in aquafeeds as a dietary lipid replacement.

Three experimental diets were designed: a fish oil control (FO), low (LCO) and high (HCO) levels of GE Camelina oil, and were fed to Rainbow Trout (initial weight 49.8 ± 11 g fish^{-1}) for 12 weeks. Muscle, liver, brain and eye tissue were sampled for analysis.

After 12 weeks, no significant differences were found in the final weights (178.5-197.8 g fish^{-1}) and weight gain (119.3-147.9 g fish^{-1}) of fish among treatments. Significant differences were found in feed conversion ratio of fish fed diets containing GE Camelina oil (FO = 1.10 ± 0.08 g fish^{-1}; LCO = 0.96 ± 0.08 g fish^{-1}; HCO = 1.38 ± 0.22 g fish^{-1}; $p = 0.029$). Significant differences were noted in the fatty acid profiles of experimental diets and tissues. In the diet, DHA and EPA content was higher in FO and HCO diets than in LCO, while 18:2n-6 (LNA) and 18:3n-3 (ALA) was higher in LCO and HCO diets than in FO. EPA in muscle tissue was higher in fish fed HCO and FO diets in comparison to LCO. DHA stored in muscle tissue was highest in fish fed FO diets. No significant differences were noted for DHA and EPA in brain tissue. Significant differences were noted in orange intensity and firmness (in fillets fed HCO diet in comparison to the FO control, however; other sensory properties of the fillets were not different. In conclusion, genetically engineered *Camelina sativa* was found to be an effective substitute for FO as a dietary source of EPA and DHA.

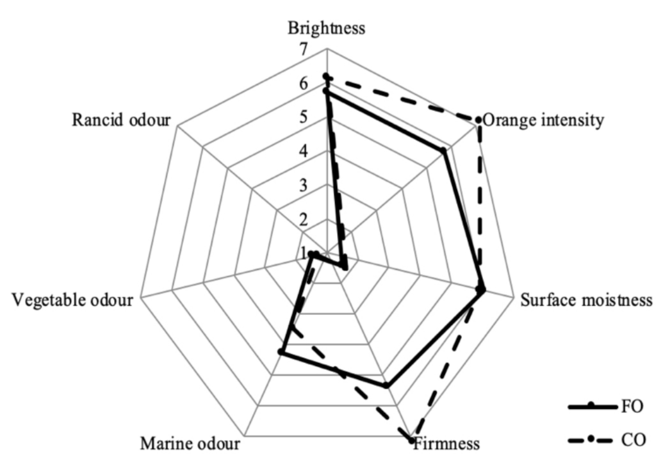

Figure 2.2: Radial plot of QDA sensory comparative analysis of week 12 Rainbow Trout muscle tissue sampled from FO and HCO diets.

EFFECT OF EXTREME STOCKING DENSITIES ON HYBRID CATFISH PRODUCTION AND WATER QUALITY IN EARTHEN PONDS

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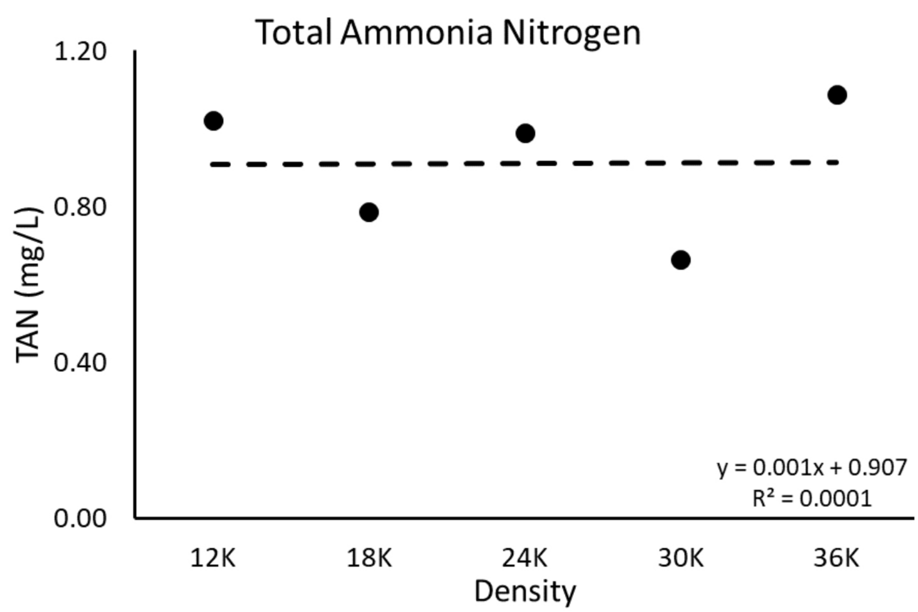
Earthen ponds used for ictalurid aquaculture have traditionally been regarded as phytoplankton-based systems, with much of the nitrogenous waste being assimilated by phytoplankton during photosynthesis. However, there is an upper limit to the phytoplankton population and once that limit is reached an excess of ammonia could accumulate. We designed this study to exceed the total ammonia nitrogen (TAN) uptake capacity of a standard catfish pond. We stocked a single ¼-acre pond with 6000, 12000, 18000, 24000, 30000, and 36000 hybrid (*Ictalurus punctatus* x *I. furcatus*) catfish per acre and analyzed feeding, growth, production, and water quality of these ponds using linear regression.

Catfish feeding and size decreased significantly as stocking densities increased, with fish at the 36K density consuming 17% less feed than the 6K density and weighing 21% less on average. Survival (%) and food conversion ratio (FCR) was not different among stocking rates and net production increased by 782 lbs/acre with every increase of 1000 catfish/acre. There was not an accumulation of TAN (mg/L) among the treatments. However, nitrite-nitrogen increased by 0.027 mg/L and nitrate-nitrogen increased by 0.101 mg/L for every increase of 1000 catfish per acre. Estimates of nitrification also increased significantly with higher stocking densities. These data suggest that with enough time and available nitrogenous waste, populations of nitrifying bacteria will become large enough to process excess ammonia and nitrite.



VARIANCE IN THE PHYSIOLOGICAL RESPONSES FROM THREE DIFFERENT STRAINS OF RAINBOW TROUT

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Our program has been selecting rainbow trout for enhanced utilization of plant-based feeds for nine generations. As this strain has been evaluated we routinely compare this selected strain against other domesticated lines of rainbow trout under vaired experimental conditions. This presentation will report in greater detail the physiological and nutritional changes documented for the ARS select line of trout and two other commercial lines. In nutritional studies our lab has verified that differences between the ARS selected fish and commercial control lines exist in nutrient uptake and retention, specifically in regards to amino acid transportation and metabolite levels detected in specific tissues (Fig 1). Very significant differences in growth was found between the strains on both the fishmeal and plantmeal based feeds but it was much greater when comparing between the lines when reared on the plantmeal feed. Furthermore, on the plantmeal feed the detection and development of enteritis was only found in the control lines when the fish were analyzed from two to eight months on the feed. Histochemical evaluation of the ARS select line versus control lines showed significant cellular and morphological differences. Significant differences were also found between the strains for resistance to specific bacterial and viral pathogens.

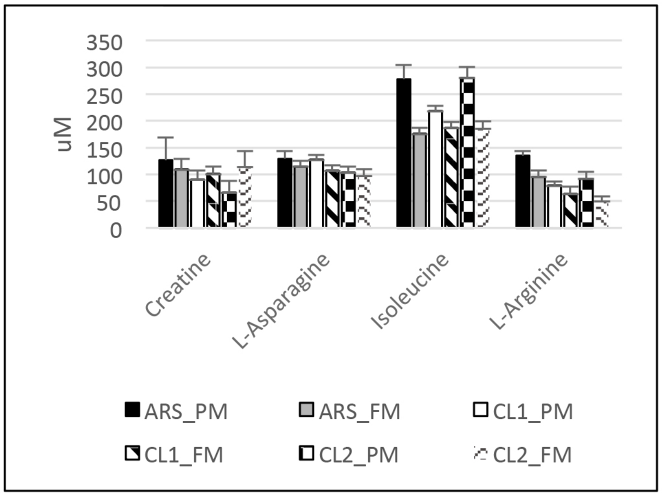

Figure 1. Metabolomic analysis of three strains of trout reared on either a fishmeal or plantmeal based feed.

MICROBIAL COMMUNITIES ASSOCIATED WITH MASS CULTURE OF COPEPODS *Apocyclops panamensis*, AND IMPACT OF PROBIOTICS ON *Vibrio sp.* REDUCTION

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Marine finfish aquaculture often suffers from mortality, diseases, malformation and low yield in larviculture stages, often due to the negative interactions between fish and opportunistic bacteria in the environment and live feed. It has been demonstrated that stable microbial environments, depends on the ratio of slow-growing k-selected microbiota to rapid-growing r-selected bacteria, which at higher ratio, the stable environment can improve the growth and survival of marine larvae. Production systems with unstable r-selected microbiota are more susceptible to pathogenic bacterial growth, while, in stable microbiota environments, the health of animals is promoted. In rearing conditions, microbes are introduced by feed, water and the larvae themselves. The bacterial population also depends on available organic materials in the system. Due to slow microbial maturation under normal rearing conditions, more pathogenic bacteria such as *Vibrio* spp. can generally be observed. Hence, understanding the host-microbe interactions can help decrease disease outbreaks, need for chemotherapeutants, and increase overall productivity.

Copepods are an increasingly important live feed in marine fish larviculture, adding to the list of potential bacterial vectors. Copepods play an important role in microbial loops due to their feeding habits, releasing of organic materials, and their chitin-based exoskeleton, providing an ideal substrate for bacterial accumulation and growth. Copepods can be colonized by bacteria, especially around oral, anal, intersegmental parts, and the intestine. Some research has been conducted on copepod microbial loading. However, as an emerging live feed in larviculture applications, there remains an unmet need to further understanding the microbial community dynamics during copepod mass production, which can affect copepod production, and fish larvae performance.

This study aimed to investigate and understand the impact of probiotics on copepod (*Apocyclops panamensis*) production, water quality, microbial community and *Vibrio* sp. reduction in mass production systems. The impact of probiotics, on copepod and water microbial changes were also studied.

THE NEUROTOXICITY OF SYNTHESIZED A PYRAZOLINE DERIVATIVE B8 COMPOUND ON RAINBOW TROUT ALEVINS *Oncorhynchus mykiss*

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4- [5- (3,4-Dimethoxyphenyl) -3- (4-bromophenyl) -4,5-dihydro-1H-pyrazol-1-yl] benzenesulfonamide (B8) compound was synthesized and rainbow trout juveniles were exposed to the B8 for 48 hours. Exposure doses were 0, 0.25, 0.5, 1 and 2.5 mg/L 1 ml of DMSO.

At the and of the experiments it was determined that 2.5 ppm B8 changed the critical swimming speed significantly ($p<0.05$). The effects of B8 on brain with respect to MDA Level (nmol / ml) and AChE Activity (EU / mg) were significant ($p<0.05$) also.

It is suggested that B8 should be investigated further for possible usage in aquaculture industry to produce healthy fish.

BIOFLOC TECHNOLOGY - FARMERS FRIENDLY CONCEPT TO INCREASE WHITE LEG SHRIMP (*Penaeus vannamei*) PRODUCTION IN THE OUTDOOR LINED POND

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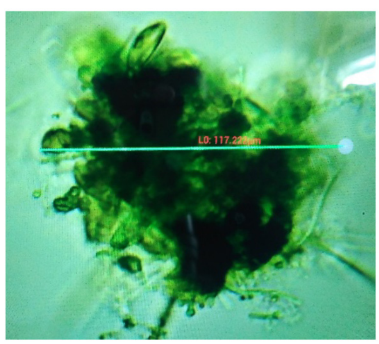
Aquaculture is one of vital sector to recompense food demand. Although, some improper intensified practices leads to diseases and effluent discharge, this circumstances would adversely affect culture species, target production and environment. To curtail this problem, sector should adopt right technologies. Among that, biofloc technology can fulfil the necessities of aquaculture industry without annoying environment. The present review explored the necessity of biofloc with special reference to farmers friendly technology to enhance the shrimp production

Shrimp (*Penaeus vannamei*) culture is highly profitable business, whilst risk based culture, it must be intensively cared to success the culture. During culture period, several problems aroused such as poor biosecurity, over/under feed input, sludge accumulation and lack of management paves water pollution which leads to disease occurrence. For example, those who are practicing normal shrimp culture in 3 hectare pond with high operational cost, instead of that, if would have been adopting biofloc technology in 1 hectare pond with less operational cost can get more production than normal culture practice.

Biofloc technology is the right choice to shrimp farmers. Intensive aeration (heartbeat) and supplementation of carbon source (heart) is the base of biofloc development. Biofloc has been continuously maintained by ideal carbon nitrogen ratio in the culture water. For example, 10 to 20ppm of carbon source added to neutralize the 1ppm of total ammonia in the culture system along with consideration of floc volume (15 ml/l), water coloration and climatic condition. While using this technology the following benefits can be obtained which enhance water quality and conserve water budget, more than 20% feed input/cost can be minimized, sludge can be recycled, more than 40% medicine cost can be minimized due to presence of beneficial bacteria which degrade the activity of pathogenic bacteria. Hence, biofloc technology is boon to aquaculture sector to increase the shrimp production without annoying environment. The remaining contents will be presented during conference.



Structure of biofloc

DIET AFFECTS THE GUT MICROBIOTA AND NUTRIENT ASSIMILATION PATTERNS OF GENETICALLY IMPROVED FARMED TILAPIA *Oreochromis niloticus*

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Nile tilapia, *Oreochromis niloticus* are the third most commonly farmed finfish species in the world, accounting for nearly 5% of the overall production of global aquaculture. In the past few decades much of the success of this species has been attributed to the development and distribution of Genetically Improved Farmed Tilapia (GIFT). Despite the ever-increasing availability of GIFT, the productivity of small-scale farming remains highly variable, particularly in developing nations. Commercial fish feed pellets can increase fish farm productivity, however, for small scale farmers these pellets are often inaccessible due to the high cost and farm remoteness resulting in many rearing fish using purely locally soured foods. Understanding the processes involved in maintaining gastrointestinal microbiota is vital because the composition of the microbial community significantly influences a host's growth and survival. This study used 16S rRNA gene sequencing and stable isotope analysis (SIA) to determine how a locally sourced diet affects the gastrointestinal microbiota and nutrient assimilation patterns of GIFT in Papua New Guinea.

A core microbiome consisting of three zOTUs (Fusobacteriaceae) accounted for an average of 52% of the relative bacterial abundance. Despite these similarities, GIFT fed commercial feed pellets were larger than those farmed using local foods. Diet was also found to significantly affect the microbial composition and heterogeneity of GIFT (Figure 1). SIA showed that carbon did not vary significantly between the treatments, however nitrogen was significantly enriched in fish fed a locally sourced diet (Figure 2). Understanding how locally sourced feeds affect the production of GIFT is an important step towards improving feeding practices, particularly for farmers with low financial capital.

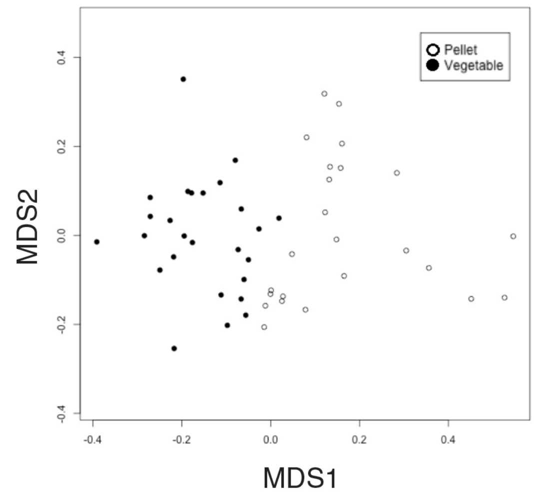

Figure 1: *Beta Diversity of the bacterial communities from the gastrointestinal tract of* O. niloticus *fed different diets*

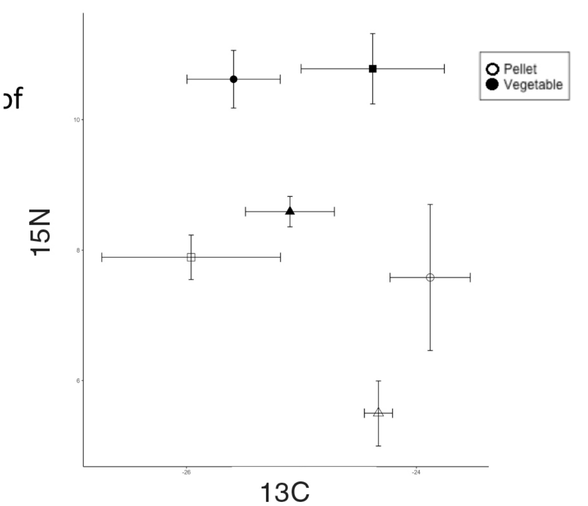

Figure 2: Stable isotope biplot (mean and standard deviation of δ13C and δ15N values) for O. niloticus *fed different diets*

OH SHUCK! WHAT HAPPENS IF EVERYTHING IS DEAD? EFFECTS OF CATASTROPHIC INVENTORY LOSS ON MARYLAND OYSTER-AQUACULTURE OPERATION NET PRESENT VALUE AND INTERNAL RATE OF RETURN

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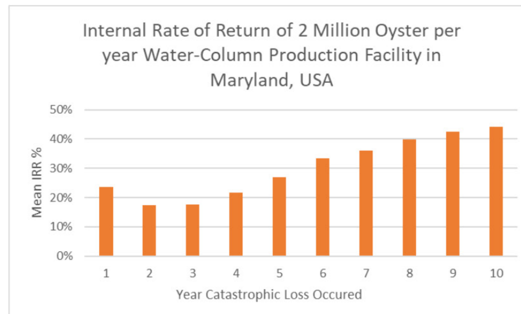
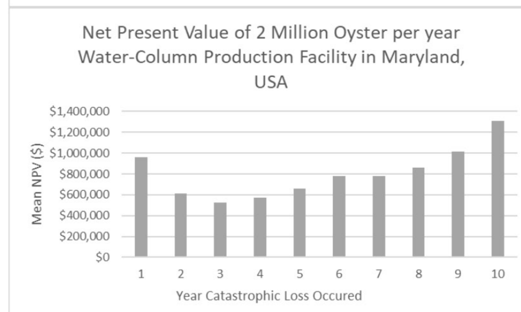
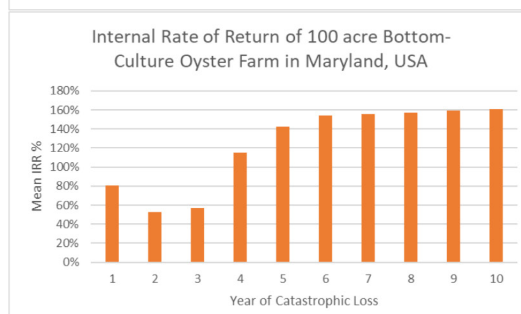
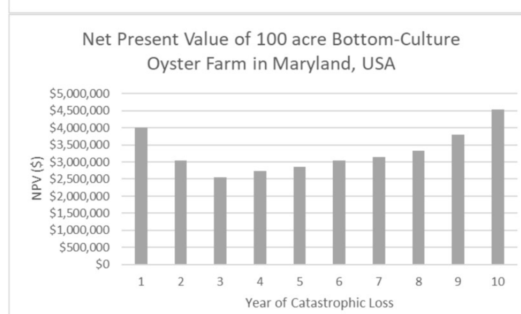
Oyster aquaculture in the state of Maryland has expanded at a rapid pace since leasing laws were revised in 2009. Until 2017, there had been very few catastrophic losses of oysters in aquaculture operations in Maryland. However, due to abnormally high rainfall since spring 2018, and the associated reduction of aquaculture oyster harvests, many growers have questioned how a catastrophic loss would influence the success of their oyster operations. Based on previous work, the authors modified oyster farm financial models to include a 100% inventory loss once during a 10-year period, then evaluated uncertainty in the estimates of net present value (NPV) and internal rate of return (IRR) of cash flows by using Monte Carlo analysis. Probability distributions were developed by interviewing oyster culture experts on key performance variables for bottom culture and water-column culture farms of different sizes. Model results suggest if a farm were to suffer a 100% catastrophic inventory loss, it would be better to occur during year 1 of the operation or in years 9 or 10 of the operation for a 2 million oyster per year water-column production operation. For a 100-acre bottom-culture operation, the lowest NPV occurred when the catastrophic loss occurred in year 3 while the lowest IRR occurred when catastrophic losses happened in year 2.









AN APPROACH TO DETERMINING ECONOMIC IMPACTS OF US AQUACULTURE

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Fisheries Economics of the United States is produced annually by the National Marine Fisheries Service and provides national and state level estimates of the total economic impacts of US seafood landings and imported seafood on the US economy. It does not contain an estimate of the impact of US aquaculturally produced seafood. As a demonstration, we took estimates of production and value for four aquaculture species: crayfish, salmon, oysters and clams, and using published production cost data and the same input/output model used for *Fisheries Economics of the United States*, and produced estimates of economic impacts. We make recommendations for improving the annual production and value estimates that are used for the input/output model, and for developing standardized industry surveys on production costs so that reliable impact estimates can be developed on an annual basis and included as part of *Fisheries Economics of the United States*.

Table 1. Summary of all impacts for marine aquaculture

Industry Sector	Direct	Indirect	Induced	Total
Growers				
Employment impacts (jobs)	5,337	1,518	2,267	**9,122**
Income Impacts (000 of dollars)	158,461	91,447	114,449	**364,357**
Output Impacts (000 of dollars)	393,998	317,217	366,678	**1,077,893**
Primary dealers/processors				
Employment impacts (jobs)	1,714	1,175	1,883	**4,771**
Income Impacts (000 of dollars)	89,634	67,441	95,048	**252,124**
Output Impacts (000 of dollars)	253,184	203,112	304,458	**760,754**
Secondary wholesalers/distributors				
Employment impacts (jobs)	2,184	1,257	1,387	**4,829**
Income Impacts (000 of dollars)	155,110	74,493	69,975	**299,579**
Output Impacts (000 of dollars)	207,367	217,020	224,447	**648,834**
Grocers				
Employment impacts (jobs)	3,035	324	668	**4,027**
Income Impacts (000 of dollars)	78,217	21,068	33,746	**133,031**
Output Impacts (000 of dollars)	89,120	56,955	108,056	**254,130**
Restaurants				
Employment impacts (jobs)	21,101	2,962	6,452	**30,516**
Income Impacts (000 of dollars)	442,835	180,086	325,799	**948,720**
Output Impacts (000 of dollars)	790,776	533,044	1,042,985	**2,366,804**
Harvesters and seafood industry				
Employment impacts (jobs)	**33,429**	**7,248**	**12,678**	**53,355**
Income Impacts (000 of dollars)	**924,256**	**434,537**	**639,019**	**1,997,811**
Output Impacts (000 of dollars)	**1,734,444**	**1,327,349**	**2,046,621**	**5,108,413**

REPLACEMENT OF FISH OIL BY A HIGH-DHA MICROBIAL OIL IN SALMON DIETS: EFFECT ON GROWTH PERFORMANCE, LIPID COMPOSITION AND GENE EXPRESSION

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The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential nutrients for farmed fish and for their human consumers. Fish oil is one of the main sources of these long-chain polyunsaturated fatty acids; however, due to an increasing demand for EPA and DHA and with a limited supply from wild fisheries, there is a need for alternative sources that are sustainable and cost effective for aquaculture. Here we investigated replacement of fish oil with a high-DHA, low-EPA oil extracted from single-celled thraustochytrids. This provided a unique opportunity to determine how these dietary fatty acids independently influence lipid metabolism and physiological pathways in salmonids.

A 16-week feeding trial was conducted with Atlantic salmon fed diets with a complete or partial replacement of fish oil with microbial oil. There was no significant difference in growth performance among the dietary treatments but we observed differences in lipid composition and gene expression. We investigated total lipid class and fatty acid composition in liver and muscle tissues using thin-layer chromatography and gas chromatography with flame ionization detection and mass spectrometric detection. Our results showed no significant differences in total lipids and lipid class concentrations among the dietary treatments for both tissues; however, significant difference were observed in proportions of omega-3 and omega-6 total lipid fatty acids and phospholipid fatty acids. In addition, there were differences in the phytosterol composition and in triacylglycerol and phospholipid molecular species. These results correlated with hepatic lipid metabolism biomarkers, although some levels were the same with high dietary DHA (high microbial oil) and high EPA (fish oil) indicating successful replacement of fish oil with microbial oil.

THE SOUND OF SILENCE; ENVIRONMENTAL BENEFITS OF SOLAR POWERED PUMP-OUT BOATS IN BRANFORD HARBORS

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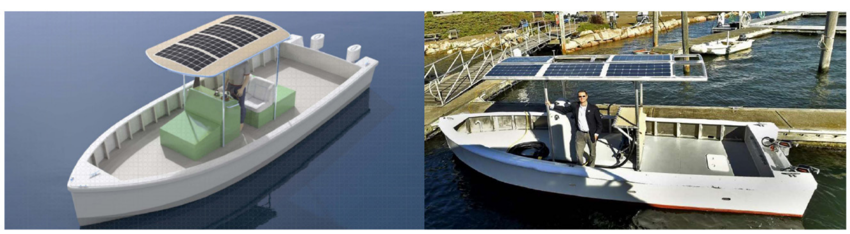
Sound pollution from boat motors is known to affect whales, crabs, and eels behavior and the physiology of fish embryos. Alleviating sound pollution is one management strategy that can affect marine environments positively. Recently, a solar-electric powered pump-out boat was developed that produces less sound and has a lower carbon footprint. This study will compare motors by examining the differences in motor noise using a hydrophone and examine the effects of these motors' noise on fish behavior and physiological (heart rate) responses in local blue and ribbed mussels.

Pollution has been known to affect all species and is commonly defined as the presence in or introduction into the environment of a substance or thing that has harmful or poisonous effects. We often see, smell, and can taste pollution and these abilities has shaped our definitions. The concept of 'hearing' pollution remains different as we often accept 'noise' as a life consequence.

The impact of sound pollution has been documented to affect mammals, fish and invertebrate species whenever it has been examined (Simmonds et al 2014; Merchant et al 2015). Weilgart (2007) documented the observed effects and responses to noise in marine mammals and found that effects of noise can range from mild responses (change in vocalizations, respiration, swim speed and foraging behavior) to lethal consequences (population displacement, elimination of population members and decreased reproduction). In fish, noise has been documented to increase the heart rate of juveniles (Jain-Schlaepfer 2018) and in invertebrates, noise has been documented to increase the heart rates of mussels, and increase shelter seeking and decrease the foraging time in *Carcinus maenus* (Williams et al 2015). Compared to other types of 'pollution' sound pollution has been little studied, yet has been documented to have negative effects on marine communities.



ACIPENSER IRIDOVIRUS-EUROPEAN (AcIV-E): EMERGING PROBLEM IN STURGEON FARMS?

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It's known that viruses are one of the main causes of mortality in farmed sturgeons: despite this, there's still little knowledge on etiological agents of these infectious diseases. Iridoviridae are viruses belonging to the Nucleocytoplasmic Large DNA Viruses (NLDV) known to cause diseases in sturgeons in North America. Several etiological agents have been isolated over the years in the USA: white sturgeon iridovirus (WSIV), Missouri River sturgeon iridovirus (MRSIV), Namao virus (NV). In Europe, iridovirosis has become an emerging issue for sturgeon farms. The etiological agent, called Acipenser Iridovirus-European (AcIV-E), has been identified in several sturgeon species (*Acipenser gueldenstaedtii*, *A. baeri*, *A. naccarii* and *Huso huso*). This work presents the data on AcIV-E monitoring in sturgeons farmed in northern Italy between January and August 2019.

Five farms in Northern Italy were sampled, for a total of 210 sturgeons analysed: 80 (38,1%) from Farm 1, 89 (42,4%) from Farm 2, 1 (0,5%) from Farm 3, 20 (9,5%) from Farm 4 and 20 (9,5%) from Farm 5. The subjects were necropsied under aseptic conditions and gill were sampled for AcIV-E DNA detection. A real-time PCR protocol targeting the Major Capsid Protein (MPC) gene described by Bigarré *et al*. (2017) was used to analyse the DNA previously extracted by a commercial kit.
The monitoring results are listed in table below.

Specific real-time PCR for MPC allowed to identify 68 (32,4%) positive sturgeons. Russian sturgeon *A. gueldenstaedtii* was the species with the greatest number of positive. Ten (4,8%) hybrid sturgeons were positive for AcIV-E: it must be considered that these fish are crosses with *A. gueldenstaedtii* (e.g. hybrids BAGU and GUBA), which appears to be the most susceptible species. Sterlet *A. ruthenus* was also positive to PCR: the low numbers sampled on this species need future investigation.

This monitoring allowed to clarify the risk represented by AcIV-E in Italian and European sturgeon farms, particularly for *A. gueldenstaedtii*, but the susceptibility of other sturgeon's species should be investigated. Complete viral genome sequencing, isolation on sturgeon cell cultures and identification of specific clinical signs will be critical points on which to plan future research.

Bibliography

Bigarré L., Lesne M., Lautraite A., Chesneau V., Leroux A., Jamin M., Boitard P.M., Toffan A., Prearo M., Labrut S., Daniel P. (2017) Molecular identification of iridoviruses infecting various sturgeon species in Europe. *Journal of Fish Diseases* 40:105–118.

	Pos	Neg	Tot
Acipenser gueldenstaedtii	55	69	124
A. naccarii	-	20	20
A. baeri	-	20	20
A. ruthenus	3	7	10
Huso huso	-	7	7
A. transmontanus	-	6	6
Hybrids	10	13	23
	68	**142**	**210**

BACTERIAL INFECTIONS IN ITALIAN STURGEON FARMS: EIGHT YEARS OF MONITORING

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The increase in the demand for caviar has encouraged a rapid expansion of intensive sturgeon farming over the last twenty years. Likewise, to the increase in productions, the onset of infectious diseases has raised. Although it's known that viral infections represent the major cause of mortality in sturgeons, even bacterial diseases can be an important source of economic losses. Here we present data of a health monitoring aimed to characterize bacterial infections occurred in Northern Italy sturgeon farms between 2009 and 2017.

Sturgeon production for aquaculture has increased worldwide. In Italy since the nineties, it's very important mainly for caviar production. Despite this, there is limited information about diseases that affect sturgeon farming also in this country. Accordingly, in this study we describe cases of bacterial infection during a monitoring campaign carried out from 2009 to 2017. We analyzed samples from Russian sturgeon *A. gueldenstaedtii*, n = 725, Siberian sturgeon *Acipenser baerii*, n = 103, Adriatic sturgeon *A. naccarii*, n = 10, Sterlet sturgeon *A. ruthenus*, n = 16, White sturgeon *A. transmontanus*, n = 2, and Beluga sturgeon *Huso huso*, n = 2 from Northern Italian fish farming for a total of 858 samples. The subjects were necropsied under aseptic conditions and evaluated for the presence of lesions such as wounds, bleeding or other pathological alterations. Collection of samples for bacteriological exam was taken from kidney and brain using first isolation media (Columbia Blood Agar or TSA). The colonies grown after 24-72 hours of incubation at 22±2°C were selected, cloned in selective media and identified by biochemical tests using API galleries (API 20E and 20 NE, bioMérieux). Phenotypic bacteria identification was confirmed by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technology on VITEK MS system (bioMérieux, France). The bacterial exam was positive in 297 individuals (34,61%) while the remaining 561 (65,39%) showed no bacterial growth. The isolated bacterial species were: *Aeromonas hydrophila* (37,71%), *Plesiomonas shigelloides* (17,17%), *Aeromonas sobria* (10,77%), *Pseudomonas* spp. (6,06%) *Acinetobacter* spp. (3,42%) and others in small percentage belonging to *Citrobacter* spp., *Yersinia ruckeri*, *Aeromonas salmonicida*, *Vibrio* spp., *Flavobacterium* spp. and *Chryseomonas* spp. No Gram+ bacteria have been isolated. The presence of many bacterial species implied a mixed infection of these fish, and hemorrhages are observed in all bacteria-infected fish, but external clinical signs are not pathognomonic for any isolated bacteria. The most isolated bacteria appearing low pathogenic. But even so, sometimes fish are predisposed to systemic gram-negative facultative bacterial infections resulting from handling trauma or adverse growth, and viral diseases. This knowledgebase is important as it will help create a bacterial profile of sturgeons reared in Italy.

Mycobacterium pseudoshottsii IN FARMED RED DRUM *Sciaenops ocellatus*: ISOLATION AND HISTOLOGICAL FEATURES

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Mycobacterium pseudoshottsii is a slowly growing photochromogenic mycobacterium closely related with *M. marinum*, well known as fish and human pathogen. *M. pseudoshottsii* was reported for the first time in 2005, during an acute case of mycobacteriosis in wild striped bass *Morone saxatilis* in Chesapeake Bay (Maryland and Virginia, USA). After this first case, the species was isolated in USA in several other fish and locations (New York Bight, Rhode and Corsica rivers). Other cases of mycobacteriosis caused by *M. pseudoshottsii* have been reported in farmed marine fish (*Seriola quinqueradiata*, *S. dumerili*, *S. lalandi*, *Epinephelus septemfasciatus*) in western Japan. The first case in Europe involved a European sea bass *Dicentrarchus labrax* farm in southern Italy.

In this work, the first case of mycobacteriosis caused by *M. pseudoshottsii*, in farmed red drum *Sciaenops ocellatus* is reported.

Twenty-one red drum from a farm in South-East Italy were previously analysed in September 2018: only the culture was performed, because fish were frozen. In June 2019 a second sampling in the same farm was performed: 15 fish were collected for culture and histological evaluation. The animals were necropsied and splenic and renal miliary nodules were detected. Portions of gills, liver, spleen and kidney were sampled for histology and portions of spleen and kidney, were collected for the isolation of nontuberculous mycobacteria in solid media (Löwenstein-Jensen and Stonebrinks medium). The tissues for histopathology were processed by standard paraffin wax techniques, stained with haematoxylin-eosin (HE) and Ziehl-Neelsen (ZN) acid-fast staining.

Slowly growing acid-fast bacilli were isolated in 7 fishes (1 in 2018, 6 in 2019) and subjected to DNA amplification by PCR and Sanger sequencing of *16S rRNA*, *hsp65* and *rpoB* genes. Biomolecular analysis leading to the identification of isolates as *M. pseudoshottsii* after comparing the sequences obtained with those present in public database (GenBank).

Microscopically, lesions were detected in all organs analysed. In spleen and kidney, the lesions were so severe that the normal organ architecture appeared completely destroyed. The lesions were characterized by multiple disseminated necrotizing granulomas. The lesions displayed large amounts of acid-fast bacilli.

To our knowledge, this is the first report of mycobacteriosis in red drum caused by *M. pseudoshottsii*.

The severity of the episodes described, suggests the need for constant health monitoring to clarify the role that this mycobacterium can play for Mediterranean farms. Further investigation will be needed to better understand the risk for other fish species and for humans, in relation to the proven pathogenicity of species like *M. pseudoshottsii* (e.g. *M. marinum*). Another critical point to clarify will be the way of entry of this mycobacterium in the Mediterranean Sea by use of biomolecular techniques.

THE RISK OF WIDESPREAD OF POTENTIAL ZOONOTIC PARASITES TROUGH ORNAMENTAL FISH TRADE: THE CASE OF *Centrocestus formosanus*

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The ornamental fish trade is growing worldwide and Southeast Asian countries exporting numerous fish species for European countries. Exported fish is susceptible to several parasite species, including the digenetic trematode *Centrocestus formosanus*. With this study we present the results of a health monitoring focused on *C. formosanus* detected in ornamental fish imported in Italy during 2018 and 2019.

Generally, major losses in aquarium industry are caused by viral, bacterial, and fungal infections. However, metazoan parasites also can cause economic losses. In many cases, mortality may not be caused directly by parasite infestation, but by secondary infections. *Centrocestus formosanus* (Trematoda: Heterophyidae) is a digenetic trematode. This parasite has been found in the gill of numerous fish species especially from several Asian countries. The life cycle of *C. formosanus* is complex. The definitive host is a piscivorous birds or a fish-eating mammal, while the first intermediate host is a snail. Several fish species can act as second host. Infection of *C. formosanus* induces pathological changes in the gill filaments that envelops the metacercarial cysts. Gill infection results in structural and functional damage caused by a hyperplasia of the epithelial cells and fusion of the secondary lamellae. Infections are associated with mortality, decreased feeding rate and increased susceptibility to bacterial diseases. In the last years an increasing number of diagnostic tests for the detection of fish pathogens permit to improve sanitary control. *C. formosanus* is a potential zoonotic parasite that could cause epigastric pain and indigestion accompanied by occasional diarrhea. Several cases of human cases have been reported in Asia.

During 2018 and 2019 we performed a health monitoring on 46 batches of ornamental fish species imported from Singapore, Thailand and Israel with the aim to detect *Centrocestus formosanus*. Gill were investigated under a microscope to check the mature metacercariae (with X-shape excretory bladder). A total of 8 examined ornamental fish (17,40%) were infected with metacercariae of *C. formosanus*. The highest prevalence was found in *Carassius auratus*, followed by *Xiphophorus* spp. and *Colisa lalia*. The role live fish trade in the in the translocation of new parasites in sites previously free is a problem of major concern worldwide. For this reason, it is important to adopt quarantine and monitoring measures to avoid public health issues. This study highlights the necessity to improve the veterinary inspection on ornamental fish to avoid the widespread of exotic parasites, especially in *Carassius* spp. species used to supply lakes and domestic aquarium.

IS FISH QUALITY HEALTHIER IN AQUAPONICS?

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Aquaponics is an emerging holistic technology that integrates recirculating fish farming system with high value vegetables production. There is an information gap on the quality of fish produced in US aquaponics as compared with the imported fish quality produced in the aquaponics and conventional aquaculture systems. Our study was conducted with Tilapia as a test fish to determine and compare the fish quality in terms of nutrient density, proximate composition, protein and amino acids, fatty acids, and heavy metal contents. Tilapia produced in our aquaponics experiment and commercially available fish products from different sources collected from ethnic stores and supermarkets were processed and analyzed for macro (carbon, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur), micro-nutrients (iron, manganese, copper, zinc, boron, molybdenum, and silicon), and heavy metal (aluminum, lead, nickel, and chromium); amino acid profile (Hyp, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, Hyl, His, Lys, Trp, and Arg); and fatty acid profile (saturated, mono- and polyunsaturated). Results showed that aquaponic fish products were nutritionally enriched and balanced, fortified with essential amino acids, and high in mono- and polyunsaturated fatty acids than that of the imported and commercially available fish products in the market. The macronutrient density Ca / (K+Na+Mg) was higher in the Tilapia produced in aquaponics as compared to the imported and other sources. The P:Ca and N:P, N:S, and P:S were more balanced. The heavy metals contents were significantly lower in the aquaponic fish products. Based on our results, it is concluded that aquaponic fish product quality is much better for public health when compared with the commercially available imported fish products produced under highly intensive aquaculture systems.

AN AQUAPONICS INDUSTRY SURVEY

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Aquaponics is viewed as a sustainable technology that allows producers to grow fish and plants in a healthy, pesticide-free environment. Much of the interest is at the hobbyist level for backyard gardening but other interest groups include educators and commercial producers. Aquaponics serves as a useful platform to teach a variety of academic subjects in a real-world context. In other words, a living laboratory that increases student engagement and participation. Others have an interest in scaling up this technology to create a primary source of income. While there is tremendous interest in aquaponics as a sustainable agricultural production practice, questions remain about the commercial viability this technology has in the real world.

In spite of the interest in aquaponics, there is little published data available about the status of the aquaponic industry as a whole. Supporting groups like extension, research, education and public agency personnel need this information to improve their understanding of this developing industry and create support programs and materials for practitioners. To that end, a survey was developed to generate a 'snapshot' of the current status of the aquaponics industry, discover trends based on previous surveys, and emerging issues needing immediate- and long-term attention.

The survey was developed using the online survey software, Qualtrics. The survey format included an introductory set of questions that all participants answered, which helped to develop comparisons among the groups. At the end of the common questions, participants were asked to self-select into interest groups that align with their own aquaponics experience. The self-selection led to more individualized questions that gathered relevant information for the three main stakeholder groups – hobbyist, educators, and commercial producers. The survey concluded with a common set of socio-demographic questions. The survey was administered to participants from social media aquaponic interest groups, professional society groups, and email list serves.

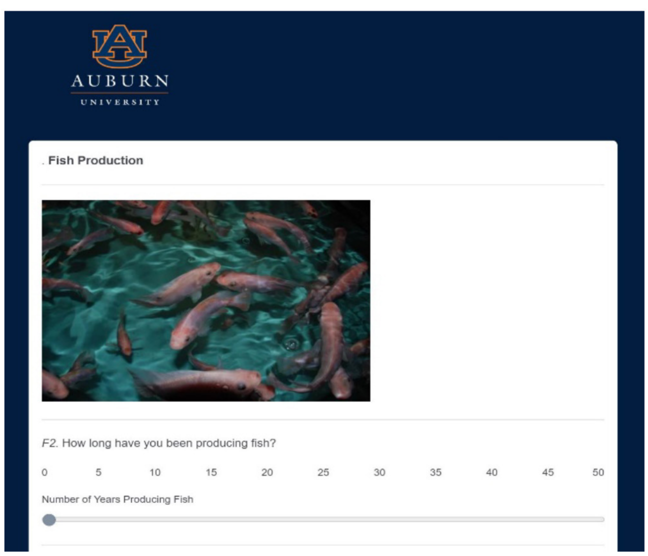

Figure 1. A screen capture of part of the aquaponics survey.

PERFORMANCE OF AQUACULTURE EFFLUENT FOR VEGETABLE CROP PRODUCTION IN OUTDOOR RAISED BEDS IN ALABAMA

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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 three common vegetable crops – eggplant (*Solanum melongena*), bell pepper (*Capsicum annuum*), and tomato (*Solanum lycopersicum*). 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.

The first trial was conducted to gauge the production capabilities and variability within the raised beds using bell pepper and eggplant. Soil tests were conducted and raised beds were prepared with the appropriate amount of fertilizer for the production cycle, with the exception of nitrogen, which was applied through the aquaculture effluent. Produce biomass data was collected as fruits reached marketable size. Labour and input data were collected to produce an enterprise budget.

The second trial used a determinate, hybrid variety of tomato called Celebrity to assess the differences in production when using three different nutrient delivery techniques. Treatments included a side-dressed granular fertilizer program, fertigation with water soluble nutrients, and aquaculture effluent.

Figure 1. Raised beds with tomatoes grown using aquaculture effluent.

THE EFFECT OF NUTRITIONAL PROGRAMMING ON GUT MICROBIOME IN ZEBRAFISH *Danio rerio*

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Nutritional programming (NP) is looked at as a promising approach that can counteract the negative effects of dietary plant protein (PP) by introducing PP to fish in the early developmental stages and enhancing PP utilization later in life. However, the mechanism of NP is still unclear. The objective of our study was to assess the effect of NP on the gut microbiome in zebrafish *Danio rerio*.

At 4 days post hatch (dph) zebrafish larvae were randomly distributed into 12 (3 L) tanks, 157±16 larvae per tank. The study included four treatment groups: 1) A positive control group that received a fishmeal diet (FM) throughout the entire trial (+ control); 2) A negative control group that received PP diet throughout the entire trial (-control); 3) A NP group that received dietary PP during the larval stage followed by FM-based diet during the juvenile stage and PP diet again during a PP challenge in the grow-out phase (NP-PP); and 4) A FM-group that received FM-based diet and was challenged with a PP diet during the grow-out phase (NP-FM).

During the PP challenge the NP-PP group achieved the highest weight gain compared to the (-) control and NP-FM groups. At the end of the study, there were no significant differences between groups in relative abundance (RA) of the overall gut microbiome. However, some differences were detected in RA of particular phyla. The RA of *Bacteroidetes* was significantly higher in (-) control compared to the (+) control but not different with the other groups at 26 dph. Similarly, the RA of *Planctomycetes* was found higher at 26 dph in the (-) control group compared to all other groups. The study showed that NP does not affect the overall gut microbiome in zebrafish but certain phyla are influenced by continuous dietary PP exposure throughout fish life.

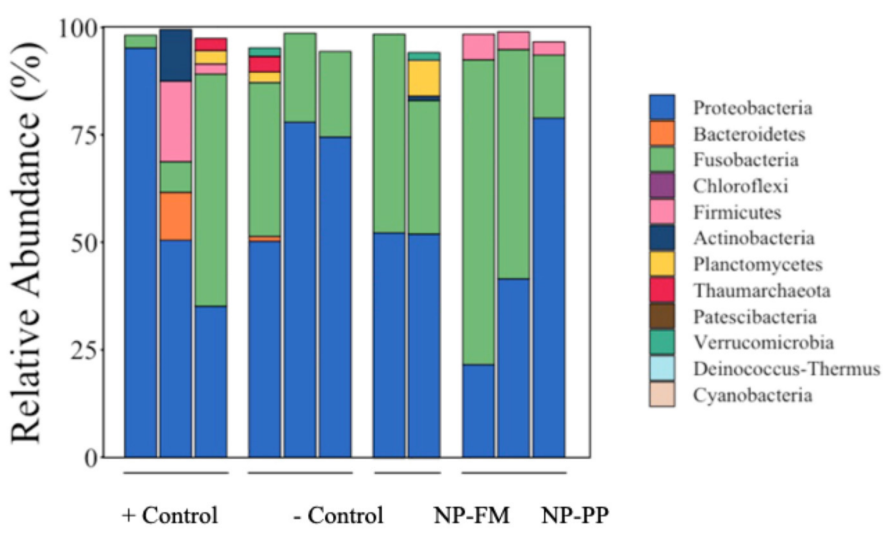

Figure 1. Relative abundance of intestinal microbial Phyla within samples from final weight

ADSORPTIVE NITRATE REMOVAL FROM AQUACULTURE WASTEWATER USING BIOCHAR AND ZEOLITE

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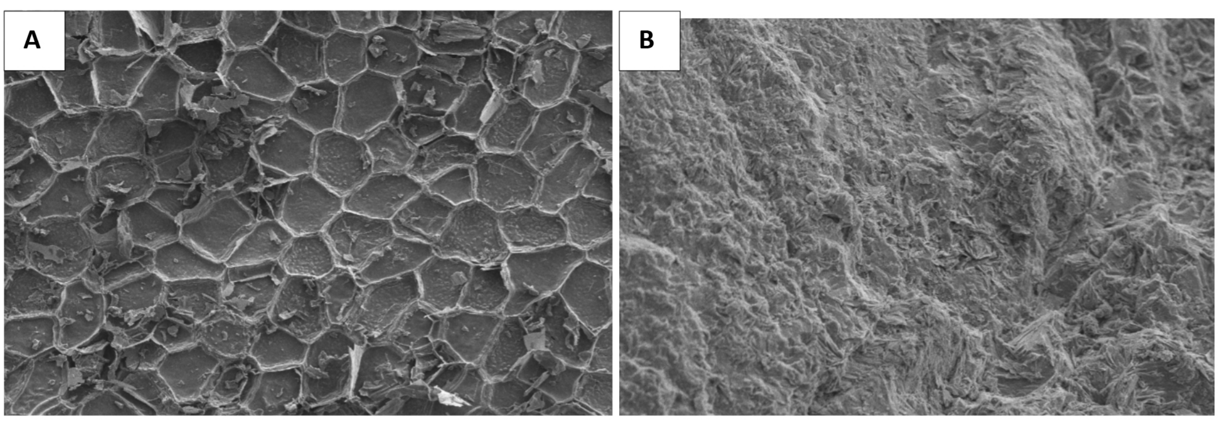
Removing toxic nitrogen compounds such as nitrate from aquaculture effluent is critical to recirculating aquaculture and important to enhancing local water quality. This project focused on alternative media for nitrate removal, specifically biochar and zeolite. Nitrate removal from aquaculture wastewater by adsorption is a vital process in this regard. In the present study, pine-derived biochar and zeolite were used as adsorbents for nitrate removal from synthetic aquaculture wastewater. The adsorbents were characterized via Brunauer–Emmett–Teller (BET, for determining specific surface area), Fourier Transform Infrared (FTIR, to test the presence of functional groups), and Scanning Electron Microscope (SEM, to study the surface morphology). Closed reactors were used, with mixing at standard laboratory conditions. The effects of different parameters on nitrate adsorption capacity of biochar and zeolite were studied. Measurements of pH, nitrate concentration (50, 120, and 200 mg/L), adsorbent dosage, and particle size of adsorbent were recorded. The experimental data were fitted to adsorption kinetic models (pseudo-first-order, pseudo-second-order, and Avrami) and adsorption isotherm models (Langmuir and Freundlich). The best fit model for each experiment will be presented. The results obtained are expected to reveal the optimum values of pH for nitrate adsorption, adsorbent dosage, and the maximum nitrate adsorption capacity of both the adsorbents. This would provide critical data for improved engineering design of denitrifying bioreactors using alternative media. Additionally, preliminary results suggest economic advantages of the use of these media could favor their use in some areas. This would open-up opportunities for development of best management practices.



100 µm

Fig: SEM of the surface morphology of the adsorbents: (A) biochar and (B) zeolite.

INTEGRATED MULTI-TROPHIC AQUACULTURE WITH THE CALIFORNIA SEA CUCUMBER *Parastichopus californicus* AND THE PACIFIC OYSTER *Crassostrea gigas*: INVESTIGATING CAGE DESIGN ELEMENTS FOR HOLDING JUVENILE SEA CUCUMBERS AND MAXIMIZING WASTE CAPTURE

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The California sea cucumber (*Parastichopus californicus*) has been identified as an ideal species for Integrated Multi-Trophic Aquaculture (IMTA) due to its nutrient recycling capability and high market value. The overall objective of the study was the design of a cage that effectively contains juvenile sea cucumbers in a way that maximizes extraction of large-particulate waste (*i.e.* faeces/pseudofaeces) within an IMTA system with Pacific oysters (*Crassostrea gigas*). Because of the sea cucumber's morphology and behaviour, small mesh sizes are required for effective containment, which could restrict the flow of farm particulates to the sea cucumbers, hence reducing overall IMTA system efficiency. That factor, as well as animal behaviour and habitat preference, were carefully considered when developing new suspended cage designs. High Flow™ oyster grow-out trays (L × W × H: 56.25 × 56.25 × 21.25 cm, mesh size: 1.2–3.0 cm) were chosen as the basic cage since they are commercially available, relatively cheap, and because, at present, there are no commercial cages designed specifically for sea cucumber culture. Three basic cage designs were tested: (1) unmodified High Flow™ oyster cage with lid ("Unmodified"), (2) High Flow™ cage with fine mesh (mesh size: <1 mm) on the sides and lid ("Fine Mesh"), and (3) High Flow™ cage with fine mesh (mesh size: <1 mm) on the sides, with no lid, and an encircling mesh fringe (mesh size: 250 μm) along the top rim to potentially restrict emigration ("Fringe"). Each of these cage designs incorporated either no oyster shell or oyster shell (weight: 2 kg) as a substratum for the juvenile sea cucumbers, for a total of six cage types.

These six cage treatments (*n*=5) were tested at a Pacific oyster (*Crassostrea gigas*) farm where they were suspended beneath rafts from May to Nov. A control Fine Mesh cage with oyster shell was established at a reference site 320 m away. Containment and growth of the sea cucumbers were monitored over time. Oyster faeces/pseudo-faeces deposition and total organic matter (TOM) in each cage were also examined. The effects of oyster shell presence/absence were non-significant for the parameters studied and the values below reflect combined treatment means (*i.e. n*=10). The Fine Mesh cages contained significantly more sea cucumbers (mean ± SE: 77.1 **± 7.9%)** than the Unmodified (21.4 ± 5.4%) and Fringe (28.6 ± 4.8%) cages. The Fringe cages at the oyster farm contained significantly more sea cucumbers (38.7 ± 6.3%) than the ones suspended at the control site (20.0 ± 2.7%). All three cage types had significantly different sediment retention and TOM rates: Fringe cages (3.93 ± 0.96 g m^{-2} day^{-1}, 0.37 ± 0.10 g m^{-2} day^{-1}, respectively), Unmodified cages (1.60 ± 0.60 g m^{-2} day^{-1}, 0.15 ± 0.06 g m^{-2} day^{-1}), and Fine Mesh cages (0.12 ± 0.03 g m^{-2} day^{-1}, 0.03 ± 0.01 g m^{-2} day^{-1}). Absloute growth rates (g d^{-1}) of sea cucumbers in the fall (Oct./ Nov.) were negative and not significantly different among cage types. Absolute growth rates in the summer (Aug./Sep.) were postive with those in the Fine Mesh cages (0.01 ± 0.02 g d^{-1}) being significantly lower than those in the Unmodified (0.22 ± 0.05 g d^{-1}) or Fringe (0.19 ± 0.05 g d^{-1}) treatments. Our results indicate that there is a trade-off between waste capture/sea cucumber growth and sea cucumber containment efficiency, dictated by cage mesh size.

DEVELOPMENT OF HIGH VIGOR SHRIMP USING HIGH THROUGHPUT SHRIMP GENOTYPING

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The use of DNA based markers in genetics programs has been widely adopted in many agricultural industries. When using DNA markers in a shrimp selection program, it is relatively easy to quantify survivals and determine genetic distance from specific shrimp or families in actual production environments. The main limitation when applying this technology to shrimp genetic programs is the time and cost to do many samples. Therefore, the real benefit this technology offers has been limited.

In 2019, API installed a High Throughput DNA Genotyping System that allows API to genotype thousands of shrimp samples daily using a fully automated system. This new high throughput genotyping system allows APi to get the full benefit of conducting a DNA based shrimp genetics program. The Author will give a brief overview on the API genetic program and how we are maximizing the potential of this new technology. The Author will present actual production data reflecting the genetic improvements that have been experienced to date. Shrimp broodstock companies have generally focused on marketing one or two lines of production shrimp, ie. a fast growth line and a disease resistant line. Using this new high throughput genotyping technology, it is now possible to develop custom selected shrimp lines for particular production systems and or environments.

THE ROLE OF ACOUSTICS IN SHRIMP FEEDING ACTIVITY

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Passive acoustics monitoring (PAM) has become an increasingly popular tool to study shrimp feeding activity, as "click" sounds are produced by the mandibles to shred the food. Consequently, automated feeding has been developed according to the specific acoustic signals for different species, culture phases and management strategies. For this work we evaluated the acoustic characterization in different size classes of the white shrimp *Litopenaeus vannamei* when feeding on commercial diets.

The sound emission (clicks) was analyzed in different size classes during the nursery (0.04, 0.5 and 1g) and growout phases (4, 8 and 12g). For the acoustic recordings (30 minutes), 25 and 13 shrimp of each size class (nursery and growout, respectively), were maintained in two rectangular plastic tanks (50 L) covered with acoustic foam and a hydrophone placed centrally (Aquarian Audio AS-1, 16 bit / 96 kHz). The shrimp were fed *ad libitum* with a commercial pelleted diet (38% crude protein; 5.2 x 2.4 mm and 48 mg pellets). A total of 30 clicks were analyzed per recording file during the feeding activity to determine the frequency band (low, high and peak; kHz), maximum power (dB) and duration (ms; milliseconds) of the sound.

The duration of the clicks was similar regardless of the shrimp size, but the other acoustic variables differed significantly among the size classes (Table 1). Shrimp started to produce sound with 0.04g, but the clicks have a significantly lower frequency (21.6 kHz) than other size classes and a higher frequency (>48 kHz) that is not captured by the acoustic equipment. Overall acoustic data indicated that larger shrimp (growout phase) produced sounds with lower frequency band and higher power than nursey shrimp. Furthermore, studies are underway to evaluate the acoustic response of different diets during the feeding activity of *L. vannamei*. These results may provide a useful information to improve PAM feed management in shrimp.

TABLE 1. Mean (± SD) acoustic variables of the different size classes of *L. vannamei* during the feeding activity.

Size (g)	Low Freq (kHz)	High Freq (kHz)	Peak Freq (kHz)	Max Power (dB)	Duration (ms)
0.04	21.6±3.6 a	>48*	33.0±7.2 a	85.9 ±3.4 d	3.1±0.9
0.5	12.0±3.7 b	>48*	33.4±6.9 a	99.4±6.20 bc	3.5±0.7
1	6.9±0.1 c	44.9±3.4 ab	18.9±9.1 b	94.5±8.3 c	3.1±0.6
4	4.3±1.4 cd	47.3±1.1 a	15.1±6.7 b	111.9± 4.5 a	3.0±0.4
8	3.9±1.4 cd	46.3±2.5 a	11.6±4.7 b	111.1±6.6 a	2.8±0.9
12	2.5±1.9 d	42.1±4.9 b	11.0±4.2 b	106.8±5.5 ab	3.2±0.4

abcd Values with different letters in the same column are significantly different ($P < 0.05$).
* Higher than the maximum frequency recorded by the equipment

SOUND EMISSION OF *Macrobrachium rosenbergii* DURING FEEDING ACTIVITY

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The study evaluated the sound emission of the giant freshwater prawn Macrobrachium rosenbergii during feeding activity in captivity, as well as described the sound generation mechanism and main associated acoustic variables.

The structure responsible for sound emission was identified based on simultaneous audio (Aquarian Audio AS-1, 16 bit / 96 kHz) and video (Sony HDR-XR100, 1980:1080 pixels) recordings during the consumption of feed pellets. Two animals (28.2 and 33.5 g) were maintained in two glass aquaria (48 x 23.8 x 38 cm) for the acoustic characterization of feeding activity.

The animals were fed two pellets (38% crude protein; 5.2 x 2.4 mm and 48 mg) and recordings lasted around 10 minutes. This procedure was repeated until obtaining recording with perfect visual framing. The acoustic parameters (minimum, maximum and peak frequency, maximum power and sound duration) of 18 sound pulses were analyzed.

The giant freshwater prawn emits click sound when closing the mandibles to shred the pellets, as verified in marine shrimps Litopenaeus vannamei and Penaeus monodon. Data were pooled as the acoustic parameters were similar between the two animals. The mean values (± SD) for the acoustic parameters were: minimum frequency of 3.8 ± 0.6 kHz, maximum frequency of 44.7 ± 3.2 kHz, peak frequency of 10.1 ± 4.2 kHz, maximum power of 113.4 ± 6.8 dB, and sound duration of 7.8 ± 1.3 ms. The results suggest that passive acoustics can be used to monitor feeding activity of M. rosenbergii.

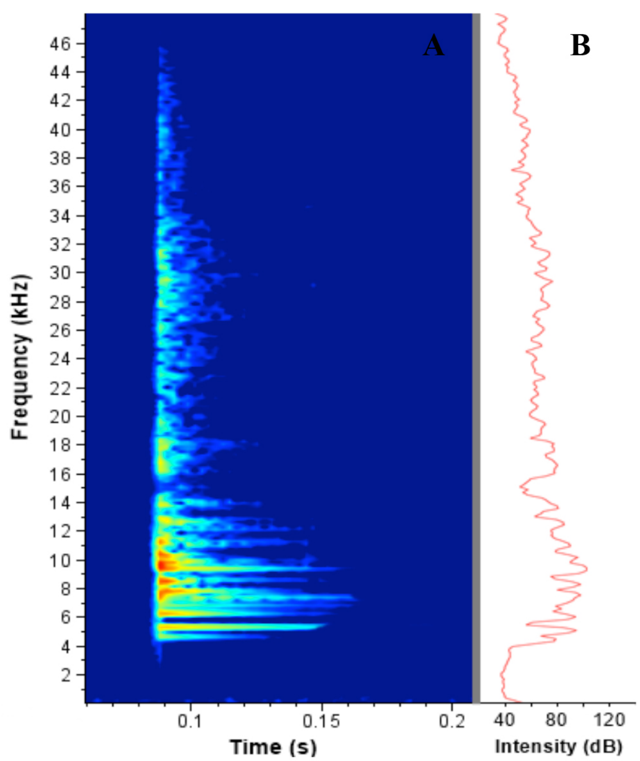

Figure 1. Isolated click spectrogram (A) and sound spectra (B) of *Macrobrachium rosenbergii* during feeding activity.

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

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Statistics has a preponderant role in the formation of competencies of students and citizens, since it allows us to analyze variables, design experiments, improve predictions, and provide elements for the decision-making process in different situations (Batanero and Godino, 2005). However, different investigations have found that statistics are used incorrectly, and apparently elemental concepts (such as the mean, median and mode) are not understood, which is why it has been suggested, among other activities, to develop "Learning from cooperative work with projects or activities of exploratory analysis of student data in the statistical class, supported by recent learning theories that highlight the value of social interaction and discourse in the construction of knowledge" (Batanero and Godino, 2005).

This project is currently undergoing and has as objective to design didactical materials for teaching and learning the topic of "Measures of central tendency" in the area of statistics for students in grade nine. The methodology for developing this material follows the heuristic principles of the Realistic Mathematics Education (RME): reinvention through progressive mathematization, didactical phenomenology, and self developed or emergent models. Data generated from an aquaculture production project will allow contextualize statistical problems for the grasping of statistical concepts. The contextualization of statistics in relation to the aquaculture sector considers the importance of this production sector for the economy of the region (Huila, Colombia) in which the project is developed.

The possibility of contextualizing statistical tools for decision making in a real environment is the starting point for establishing a link between students with their economic environment, and with the reality of the region where they live. The contextualization of mathematics and statistics in relation to the aquaculture sector follows the importance of this production sector to the economy of the department of Huila, Colombia. This region produces 44% of the country's total aquaculture production (SIOC, 2016). More than 50% of total tilapia production in Colombia occurs in the Betania reservoir with a production of 30,000 metric tons (Pulido et al. 2015).

EFFECTS OF HIGH OXIDIZED FISH OIL ON GROWTH AND LIPID METABOLISM OF RICE FIELD EEL *Monopterus albus*

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Oil is important for lipid metabolism and fish growth. The unsaturated fatty acids in oil are readily susceptible to autoxidation and rancidity, following by the oxidative and rancid products like peroxides and malondialdehyde. These products are toxic to fish, and shows greatly negative impacts on nutrient metabolism and further lead to excessive lipid deposition. The present study was conducted to the effects of high oxidized fish oil levels and supplementation of apple polyphenol (AP) and taurine (TA) on the growth performance, antioxidant and lipolysis of rice field eel.

Fish oil was heated up to 70 °C with constant aeration and POV was monitored every 4 h until it reached 600 and 800 meq/kg values. Control group fed with basic diet, and POV600 and POV800 groups respectively fed with diet containing 600 and 800 meq/kg oxidized fish oil, respectively. Based on the diet of POV600 group and POV800 group, the diet of AP and TA groups was supplemented with 0.5% apple polyphenol or 0.2% taurine, respectively. Each group has 3 replicates capes with a density of 100 eels in per cage, which was placed in a natural water environment.

The results showed that: the liver cells of eels fed high level oxidized oil are obviously degenerated together with numerous lipid vacuoles (Fig.1), meanwhile the intestinal structure was incomplete and the number of goblet cells decreased sharply following by a large number of vacuoles (Fig.2); the oxidized oil could affect lipolysis by AMPK in liver through AMPK-ACC-CPTI and AMPK-lipin-PPARα-CPTI pathways. The results of this study established an important theoretical foundation for the study of AMPK-mediated lipid catabolism.

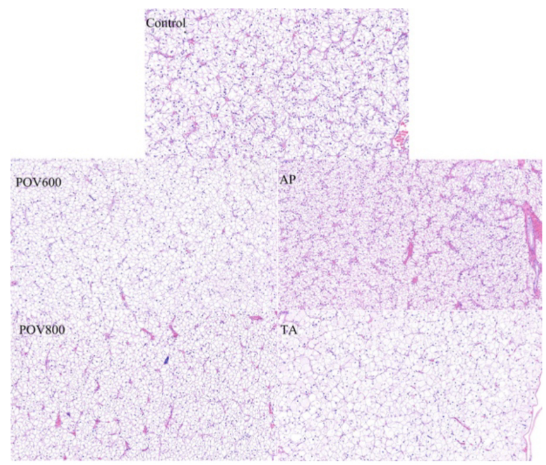

Fig. 1 Liver tissue of rice field eel (×40 multiple)

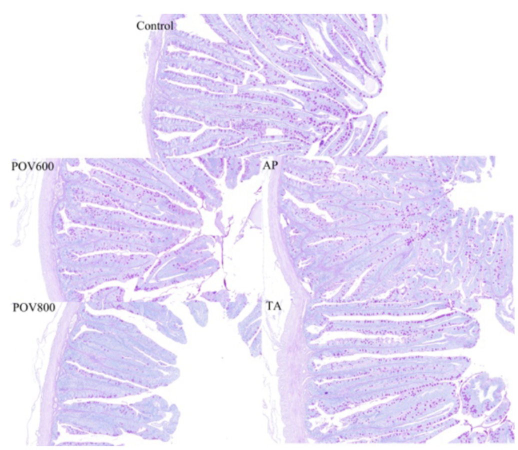

Fig.2 Intestine morphology of rice field eel (×20 multiple)

SULPHATED POLYSACCHARIDES EXTRACTED FROM SEAWEED ON THE SKIN-MUCUS IMMUNITY OF RAINBOW TROUT

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In pursuing a more sustainable aquaculture the prevention of disease becomes an important goal. Skin-associated lymphoid tissue (SALT) has the mucus as the most significant innate response mechanism. Due to this effect of mucus, pathogens are immobilized before they can adhere to or make contact with epithelial cells. Interest in the use of marine macroalgae in animal production is increasing, mainly due to the wide range of biological activities attributed to it, such as immunomodulatory, antioxidant and antiviral properties. Using the vast sustainable marine resources of the coast of Brittany (France) and in collaboration with several research bodies, Olmix has been able to isolate, identify and concentrate sulphated polysaccharides from the cell wall of macroalgae that have different activities to support the general health of animals. Some of these isolated polysaccharides include activities such as immunomodulation, mucin secretion and the strengthening of intestinal barrier functions.

The study was carried out at indoor race-way system of a commercial fish farm located in São Paulo Brazil for 11 weeks (78 days). Fish were assigned to 2 treatments with 2 replicates (100 fish/tank), totalizing 400 fish. Two iso-nutritive diets, Control and Test (commercial product based on combination of green and read algal extracts), were formulated to differ only on the inclusion of 0.3% of macroalgae polysaccharides. Fish were fed twice daily at 9:00 am and 4:00 pm *ad libitum*. Average water temperature and dissolved oxygen were 14 °C and 7,3 mg/L, respectively. Skin mucus samples were collected at week 4 and 11. The functional activity of complement was assessed by hemolytic assays (n=8/treatment) using sheep red blood cells as targets. Mucus samples (50 µL) were incubated at room temperature for 25 min with 2% sheep blood (25 µL), cold-PBS (75 µL), the supernatant was collected, and absorbance read at 405 nm. Lysozyme activity was determined in skin mucus (n=8/treatment) using a turbidimetric method incubating 10 µL mucus sample in 200 µL suspension of *Micrococcus lysodeikticus* in PBS (0.2 g/L) at pH 6.8, absorbance read at 450 nm. Rainbow trout weight were at day 1: 89.8 g ±1.9g, at week 4: 189.2 g ±6.1 and at week 11: 338.5 g ±7.5. Hemolytic activity was improved in macroalgal sulphated polysaccharide fed trout and increased at week 11 compared to week 4 for both groups. Lysozyme activity was not significant affected neither treatment of sampling week. These results highlight the potential effects of macroalgal sulphated polysaccharides on improving the innate immune response of skin-mucus in rainbow trout.

ALGO-CLAY COMPLEX ON NILE TILAPIA PERFORMANCE UNDER MYCOTOXIN POLICONTAMINATION

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In recent years, the awareness of mycotoxin-related issues in the aquaculture industry has been raised again, mainly due to the increasing inclusion levels of plant meals in aquafeeds. Most mycotoxin exposure reported general clinical manifestations due to mycotoxin ingestion are related to a reduction in growth performance and in some cases alteration of blood parameters or immunosuppression. To the best of our knowledge, this is the first experiment to investigate the effects of aflatoxins, fumonisins and deoxynivalenol combined in diets for Nile tilapia.

The aim of this study was to evaluate the efficacy of algo-clay complex (ACC) on the toxicological effects on performance on Nile tilapia (*Oreochromis niloticus*) fed mycotoxin policontaminated diet for 30 days. Fish were assigned to 3 treatments and 4 replicates (15 fish/tank) in an indoor recirculating system. The fish were fed an isonutritive diet formulated according to the NRC (2012), using corn, soybean and meat meal, and vitaminic and mineral premixes. Raw material and experimental diets were screened for the presence of mycotoxins (aflatoxins, zearalenone, deoxynivalenol, fumonisins, diacetoxyscirpenol, T-2 Toxin and ochratoxin A) and nothing was detected. Experimental diets differed only by the mycotoxins and/or ACC inclusions: T1-Control; T2-Policontanation and T-3 Policontamination+ACC. The mycotoxin policontaminated diets included aflatoxins 50 ppb, fumonisins 1000 ppb and deoxynivalenol 2000 ppb all combined. ACC was included at 2 kg/T in diet T3. Body weight was recorded at day one, fifteen and thirty during the feeding trial obtained by weighing each fish individually.

The exposure to mycotoxin policontaminated diet impaired the fish growth by 6% after 30 days meanwhile the inclusion of 2 kg/T of ACC mitigated toxic effects on body weight (p= 0.0268). These results highlight the potential efficacy of algo-clay complex to mitigate the toxicity of aflatoxins, fumonisins and deoxynovalenol combined on performance of Nile tilapia.

Body weight of the Nile tilapia fed diet containing mycotoxins and/or supplemented with ACC

	Day 1		Day 15		Day 30	
Treatments	Body weight, g	C.V. %	Body weight, g	C.V. %	Body weight, g	C.V. %
Control	7.79	8.0	12.84	1.6	19.72 a	15.7
Policontamination	7.84	8.5	12.35	1.8	18.14 b	16.2
Policontamination + ACC	7.85	8.0	12.66	2.8	18.53 ab	15.2
p-value	0.8773		0.1054		0.0268	

A COMMERCIAL BLEND OF PREBIOTIC FIBER, OREGANO, THYME, CINNAMON ESSENTIAL OILS AND *Yucca schidigera* (ONE CURRENT™) SUPPLEMENTED FEED INCREASED CHANNEL CATFISH FINGERLING GROWTH AND ENHANCED DISEASE RESISTANCE

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Weight gain and health are determining factors in the success of catfish production. After a three-month pond study, fish that were fed ONE Current™ (a commercial feed additive by Strong Animals®, a brand of Ralco®, containing a proprietary blend of oregano, thyme and cinnamon essential oils, Actifibe® Prebiotic and *Yucca schidigera*) test diet demonstrated significantly greater weight gain than fish fed a control diet. When transferred into tanks and immersion exposed to *Edwardsiella ictaluri*, fish that were fed the test diet demonstrated significantly higher survival than fish fed the control diet.

Intestinal innate immune cells are essential for communicating with commensal bacteria and killing gut pathogens. Therefore, leukocytes were isolated from the gut and anterior kidney tissues of test diet fed fish and from fish fed the control diet, and characterized using monoclonal antibodies for dendritic cells, neutrophils, cytotoxic cells and macrophages. When visualized by flow cytometry, there was no significant difference between the number of cell types from fish fed the test diet or the control diet. However, when incubated with bacteria, macrophages and cytotoxic cells from fish fed the test diet phagocytosed or bound significantly higher numbers of bacteria than the same cell type from fish fed the control diet, indicating these cells were more efficient at phagocytosing and binding bacteria than cells from the control diet fed fish. Significantly higher reactive nitrogen species (RNS) production and significantly higher lactate dehydrogenase activity (LDH) were also demonstrated by adherent leukocytes from the test diet fed fish.

Gut morphology underlies nutrient absorption. A greater surface area of the gut will lead to greater nutrient absorptive capabilities. Histological examination of the gastrointestinal tract demonstrated significantly greater mucosa, submucosa and lamina propria height after month 2, and greater villi height and width after months 2 and 3 in the fish fed the test diet. Higher RNS and LDH production, and higher bacterial phagocytosis by macrophages and binding by cytotoxic cells from test diet fed fish contributed to higher survival. Based on the findings of this study, use of ONE Current™ supplemented feed in catfish production increases growth, improves health, and minimizes infectious disease losses.

THE VELELLA EPSILON PROJECT: PIONEERING OFFSHORE AQUACULTURE IN THE SOUTHEASTERN GULF OF MEXICO; CHAPTER 2 – PROJECT PERMITTING, STAKEHOLDER OUTREACH, AND PUBLIC SCOPING

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The Velella Epsilon Project is an extension of previous projects (Velella Beta-test and Velella Gamma Project) which demonstrated small-scale offshore marine fish culture in the waters of Kona, Hawaii. The Velella Epsilon (VE) Project will adapt these technologies to Gulf of Mexico (GOM) waters, while pursuing two simultaneous efforts: (a) permitting and deployment of a research-scale, demonstration net pen in Federal waters, and in tandem, (b) navigating the commercial permitting process to obtain a commercial offshore aquaculture permit in the GOM, while documenting this effort in a Manual for Aquaculture Permitting Pathway (MAPP).

The VE Project focuses on a small, pilot-scale (single net pen) aquaculture system where up to 20,000 almaco jack (kampachi; *Seriola rivoliana*) fingerlings would be reared for approximately 12 months in Federal waters approximately 40 miles west southwest of Sarasota, Florida. We expect to yield approximately 17,000 fish (85% survival rate) with a final fish size of approximately of 4.4 lbs/fish. We anticipate an estimated final maximum harvest weight of 74,800 pounds [lbs] whole weight. These fish will be landed in Florida, marketed, and sold to state- and Federally-licensed dealers, in accordance with state and Federal law.

The VE Project will lay the groundwork for wider acceptance of commercial aquaculture in the GOM region by: (1) Serving as a platform for the promotion of rational aquaculture policies and demystification of the industry, by providing a working net pen example to politicians, constituents, journalists, and other influencers of policy or public perceptions, as well as the local community; (2) Increasing public awareness of, and receptivity towards, offshore aquaculture and the need to culture more seafood in U.S. waters, by providing public tours of the offshore operation, including (possibly) snorkeling inside the net pen, and fee fishing; (3) Acting as a demonstration platform for data collection of water quality, potential benthic impacts, and marine mammal and fish stock interactions resulting from offshore aquaculture in the GOM; and (4) Providing local recreational, charter, and commercial fishing communities with evidence of the benefits of aquaculture, through the fish attraction device (FAD) effects of the project, and by documentation of fish aggregation and fishing boat activity around the VE Project.

Chapter 2 – Project Permitting, Stakeholder Outreach, and Public Scoping - will walk us through the second year's experiences and achievements of multiagency collaboration and coordination; fishing industry and seafood stakeholder outreach; and public hearing engagement events.

THE VELELLA EPSILON PROJECT: PIONEERING OFFSHORE AQUACULTURE IN THE SOUTHEASTERN GULF OF MEXICO; PRELIMINARY PERCEPTIONS AND CONCERNS RESULTING FROM A STAKEHOLDER ENGAGEMENT WORKSHOP

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Stakeholder outreach and engagement is the core foundation for addressing and achieving the primary objects identified for the VE Project to serve as a successful demonstration project. As such, workshops and other venues undeniably serve as a critical process for commencing a productive narrative regarding the perceptions and concerns of offshore aquaculture among diverse interest groups, let alone garnering the social license necessary for conducting offshore aquaculture research, demonstration, or commercial operations. Kampachi Farms participated in a workshop aimed at conducting high-level discussions for identifying such perceptions among a diverse set of interest groups including recreational and commercial fishers, retail and wholesale seafood businesses, research and commercial aquaculturists, fisheries scientists, economists, and state and Federal regulators. Discussion themes included broodstock sourcing, genetics, feeds, fishery impacts, disease, market competition, economics, research, regulations, and permitting.

UPDATED PLAN TO IMPROVE ATLANTIC SALMON AT THE NATIONAL COLD WATER MARINE AQUACULTURE CENTER

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The USDA-ARS National Cold Water Marine Aquaculture Center (NCWMAC) in Franklin, ME has been supporting the U.S. coldwater marine aquaculture industry for the past sixteen years by developing a genetically improved North American Atlantic salmon. The St. John River (SJR) strain was chosen because of fast growth, certification of North American (NA) origin, and widespread utilization by industry. Objectives of the program are to: 1) develop a selection index for carcass weight, fillet color, fat content, and sea lice resistance, 2) evaluate and validate the usefulness of incorporating genomic information into the salmon breeding program, and 3) evaluate the usefulness of a lumpfish (*Cyclopterus lumpus*) selective breeding program.

Our selected and unselected (control line) Atlantic salmon are evaluated with the assistance of industry partners in net pens to simulate commercial conditions. We have observed an increase in growth by approximately 15% for each generation while survival has been similar between groups of fish. With the increase in land based aquaculture systems in the U.S., other strains of Atlantic salmon such as the Gaspe strain may also need to evaluated. Efforts are being made to determine whether the SJR strain will grow as well in recirculating aquaculture systems as the Gaspe strain, which has been selected for growth in land based systems. There is no information regarding genotype by environment (G×E) interactions among different genetic lines of Atlantic salmon and this information is important to producers who have interest in identifying genetic lines that are best suited for their production environment and management style.

Efforts have been initiated to generate genomic research resources for the NCWMAC breeding program. We used high coverage whole genome Illumina resequencing for SNP discovery in 80 NA Atlantic salmon individuals from three aquaculture stocks that are propagated by the NCWMAC. Incorporating genomic selection into our population of Atlantic salmon should enhance the efficiency of our selective breeding program.

Lumpfish nutritional requirements and reproduction techniques are currently being assessed. Developing sustainable methods to culture lumpfish will be another important tool to mitigate the effects of sea lice in Atlantic salmon grown in net pens. Research accomplished during the proposed project plan will result in the development of genetically improved Atlantic salmon for release to U.S. producers.

CORTISOL AFFECTS GROWTH RATE, FEED CONVERSION EFFICIENCY, NUTRIENT DIGESTIBILITY AND METABOLIC RATE IN JUVENILE RAINBOW TROUT *Oncorhynchus mykiss*

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The elevation of plasma cortisol levels in fish associated with stress is known to reduce growth rates by mobilizing fuels for energy-demanding processes, resulting in a higher cost of living. It has been shown that routine metabolic rates are higher in cortisol treated rainbow trout compared to sham treated control fish, and it was proposed that cortisol was responsible for the mobilization of lipid stores to fuel this additional energy requirement. The present study was conducted to assess how chronically or periodically elevated plasma cortisol affects nutrient uptake and utilization and metabolic rates.

Rainbow trout (mean weight 115.1g) were reared at 15°C in modified Guelph type digestibility tanks of 189 l volume at a flow rate of 40 l per hour. Six treatment regimes were performed in triplicate. Fish were fed identical commercial trout diets twice daily at a daily ration corresponding to 1.4% of tank biomass over a period of 9 days during which all feces were collected to calculate ADCs. Two of the treatments were injected i.p. with 5 μl g^{-1} of 6 or 12mg ml^{-1} cortisol dissolved in liquefied coconut oil (30°C), to obtain a dose of 30 (LC) or 60μg (HC) cortisol per gram of fish. These cortisol treated fish were compared to a sham (S) treatment that was injected solely with coconut oil as well as a true control group (C). An additional group was kept at 22°C as a thermal stress treatment (TS), while the last group was subjected to a daily stress event (DS) by chasing them with a stick for one minute. Following the digestibility trial, maximum metabolic rates (MMR) and standard metabolic rates (SMR) of the fish were assessed by intermittent respirometry.

Cortisol did not affect feed intake, but cortisol treated fish showed significantly lower specific growth rates and higher feed conversion ratios compared to all other treatments (Fig. 1). This reduced performance was not observed for daily stressed fish, highlighting that the observed effects are rooted in the chronically elevated cortisol levels rather than recurrent stress events. The relative mass of digestive tissues was reduced in cortisol groups in relation to all other groups, while SMR was elevated in fish of both cortisol treatments and significantly affected by cortisol. Apparent digestibility coefficient of lipid and protein was reduced in cortisol treated fish with significantly lower ADC values in the high cortisol group compared to all other treatments. The decreased lipid and protein utilization can be one explanation for the lower growth rates observed in the cortisol treated fish. Another explanation may be the increased SMR in cortisol treated fish. Together with reduced feed conversion abilities, this observation supports the theory of a higher cost of living for fish facing chronically elevated cortisol levels.

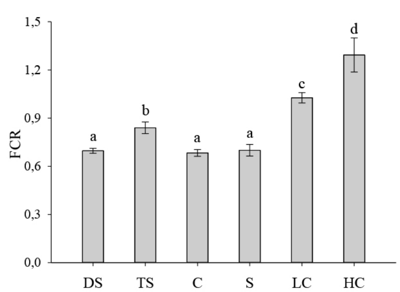

FIGURE 1. Feed Conversion Ratio (mean ± SD)

DEVELOPING A LUMPFISH RESEARCH PROGRAM AT THE NATIONAL COLD WATER MARINE AQUACULTURE CENTER

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In the summer of 2016 the USDA ARS National Cold Water Marine Aquaculture Center (NCWMAC) started investing the potential of breeding lumpfish (*Cyclopterus lumpus*). The goal of the program was to establish spawning and husbandry techniques in the US to allow for the use of lumpfish as cleaner fish in the US salmon aquaculture industry. The center spawned fish for the first time in the spring of 2017 and successfully hatched eggs in the spring of 2018. The program has faced several challenges including disease outbreaks due to the collection of wild broodstock animals and difficulty in maintaining a consistent supply of potential broodstock.

In 2018, the NCWMAC began collaborative efforts with both the University of New Hampshire (UNH) and the University of Maine Center for Cooperative Aquaculture Research (CCAR) to expand research efforts in the US on this species. In particular, the consortium is looking at nutritional studies on lumpfish and improving commercial scale husbandry techniques. One of the primary goals of the program is to rapidly translate research findings from studies at the NCWMAC and UNH into a commercial scale breeding program at CCAR.

THE ECONOMIC VALUE OF CLEANERFISH

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Sealice is considered one of the main environmental issues challenging the sustainability of salmon farming. Regulations to limit this externality have led farmers to apply several different treatments, including mechanical, chemical and pharmaceutical treatments as well as preventative measures such as fallowing and using cleanerfish (Fig. 1). Some of the treatments may negatively affect salmon production, such as mechanical sealice removal and chemical treatment. Thus, preventative alternatives, such as cleanerfish, are increasingly used to control the sealice stock within the farm location and keep it from reaching the regulatory threshold. This paper investigates the economic value of cleanerfish by estimating the likelihood of avoiding the sealice regulation threshold.

Specifically, using an ordered logistic regression, the probability of being over the sealice limit within a month is estimated, conditional on the number of cleanerfish and other control variables. In addition, the loss in biomass related to the farms being over the limit provides a measure of revenue forgone. Results indicate the use of cleanerfish reduces the need for other palliative treatments by 4%. Moreover, salmon biomass lower when the location is over the sealice limit. This suggests farmers incur monetary losses not only related to the necessary treatments, but also in revenue forgone by the decreased biomass.

The estimates suggest a reduction of 7-20% in biomass. Thus, for a location with a standing biomass of 1261 t per month and using around 2000 cleanerfish, the value of cleanerfish in order to reduce palliative treatments and the biomass loss related to that is around 2 NOK/kg of salmon.

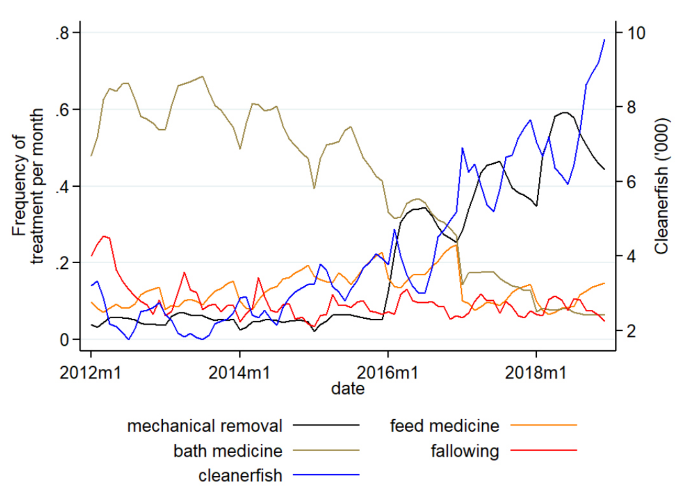

Figure 1. Sealice treatments (in weeks per month per farm location) from January 2012 to December 2018. Source: Barents Watch.

THE EFFECTS OF POPULATION DENSITY AND ENVIRONMENTAL COMPLEXITY ON AGGRESSIVE BEHAVIOR OF ZEBRAFISH

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Environmental complexity has been demonstrated to improve laboratory rodent wellbeing and enhance research data. Relatively little is known with regards to the type of environmental complexity that might be useful for zebrafish (*Danio rerio*). It has been well documented that suboptimal environmental conditions can subject fish to chronic stress and distress, cause abnormal behavior, and add additional research variables. The lack of enrichment contributes to zebrafish aggression and the formation of dominance hierarchies. Dominant fish differ significantly from fish subjected to subordination through greater access to food, shelter and mates; immune functions; metabolism; susceptibility to toxins, reproduction, and stress. See table below. Such dramatic differences may easily lead to confounding research outcomes due to the unfavorable impact observed on fish welfare. Therefore, this study aims to explore a model to evaluate the impact of environmental enrichment/complexity on aggression, a behavior that can be measured and to develop an environmental enrichment structure that can increase complexity of the environment, and be easily cleaned, stored, and incorporated into large facility husbandry practices as the figures below outline.

Preliminary findings indicate enrichment appeared to have decreased aggression, but further trials are required to prove significance. Furthermore, the study demonstrated that zebrafish reared in an enrichment free environment preferred the environmental enrichment structure, which was also influenced by dominance status within the groups of 2 to 4 fish. Further trials are currently being conducted to acclimatize the fish to the enrichment structure and provide a longer testing intervals to help develop statistical significances for the study.

	Dominant Zebrafish	Subordinate Zebrafish
Resources	Greater access to food, shelter, mates	Decreased access
Immune Function	Normal	Suppressed
Metabolism	Normal	Increased
Growth	Higher body mass	Reduced growth
Susceptibility to toxins	Normal	Increased
Reproduction	Higher gonadosomatic index (GSI)	Delayed sexual maturation
Stress	Greater HSI	Higher plasma cortisol levels

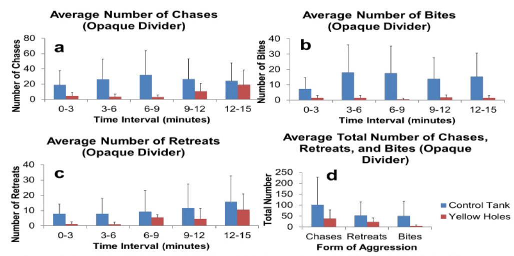

Figure 6. Aggression measured in Number of Chases (a), Number of Bites (b), Number of Retreats (c), and Total Number of Chases, Bites, and Retreats for Control Tank (blue) and Yellow Holes Tank (red) during a 15 minute observation period after 24 hours of isolation with an opaque divider (*n*=4).

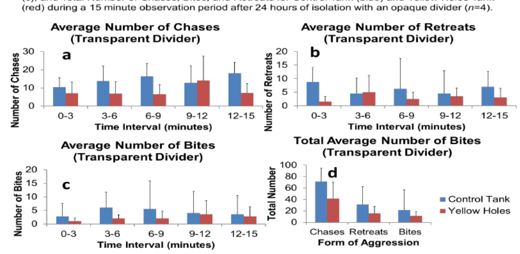

Figure 7. Aggression measured in Number of Chases (a), Number of Bites (b), Number of Retreats (c), and Total Number of Chases, Bites, and Retreats for Control Tank (blue) and Yellow Holes Tank (red) during a 15 minute observation period after 24 hours of isolation with a transparent divider.

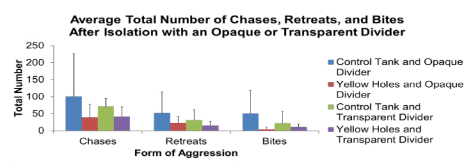

Figure 8. Aggression measured in Number of Chases (a), Number of Bites (b), Number of Retreats (c), and Total Number of Chases, Bites, and Retreats for Control Tank (blue) and Yellow Holes Tank (red) during a 15 minute observation period after 24 hours of isolation with a transparent divider.

EFFECT OF DIFFERENT DIETS ON THE LARVAL DEVELOPMENT OF SEA CUCUMBER *Holothuria arguinensis*

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Sea cucumbers fisheries becoming commercially exploited due to the increasing demand in Asian markets. The resulting unregulated exploitation has led to the collapse of several natural stocks. In this context, aquaculture represents an alternative to meet the demand, reducing pressure on wild stocks and at the same time allowing the restocking of local populations. Sea cucumbers have great potential to be reared in aquaculture and have been shown to have a valuable application in integrated multi-trophic aquaculture systems (IMTA). *Holothuria arguinensis* Koehler & Vaney, 1906 is a potential species for aquaculture in Europe. However, there are very few studies on nutrition during the larval development of this species. The present study was undertaken in order to examine the effect of three diets in the larval development, growth, survival and settlement success of *H. arguinensis*

Holothuria arguinensis broodstock were maintained in a recirculating aquaculture system (RAS) with sediment. To induce the spawning, individuals were first transferred and maintained for 24 hr in substrate-free spawning tank, to void their gut contents, followed by thermal stimulation to induce gamete release. Larvae were fed with three diets: *Rhodomonas baltica* and *Phaedactylum tricornutum* (Diet A); *R. baltica and Skeletonema marinoi* (Diet B)*; R. baltica* and *Chaetoceros calcitrans* (Diet C). The experiment was conducted in 50 L fiberglass tanks, 3 tanks were used for each diet. Water samples were taken daily after the first day to monitor larval development. The mean length and width of 15 larvae were register during the planktonic phase and after settlement. Juveniles number was estimated by counting the juveniles in three substrates: pvc tubes, transparent plastic plates and ceramic tile.

H. arguinensis larvae increased in size, reaching the mid auricularia stage on day 7 post fertilization, with a higher mean length and width in larvae fed with the Diet C, 543.24 μm and 301.06 μm, respectively ($p < 0.001$). Doliolaria stage presented a significantly higher length (699.64 μm) and width (475.78 μm) in individuals fed with Diet B ($p < 0.05$). Pentactula stage followed the same pattern, significantly higher lengths and widths were observed in larvae fed with Diet B and at this stage, survival was also higher in animals fed with Diet B (6.25%) than with the Diet A (2.76%) or Diet C (2.76%). Although, no significant differences were observed in the mean length of juveniles ($p > 0.05$) fed with the three diets, width was significantly higher in *H. arguinensis* juveniles fed the Diet A (263.74 μm; $p < 0.001$). The highest juvenile number was observed with the Diet A (54.19%), mainly in the pvc tube, followed by Diet B (31.46%) with also a higher number of juveniles in this subtracte. Diet C presented the lowest number of juveniles (14.35%). In this study, new mixtures of microalgae were used to rear *H. arguinensis* larvae until the juvenile stage. Diet B, *R. baltica* and *S. marinoi*, appear to be advantageous during the latest stages of larval development and Diet A lead to a higher number of settled juveniles. Considering high commercial value, ecological role and its potential use in IMTA, we assume that the development of effective diets for the different stages of *H. arguinensis* larval development would be extremely valuable to the aquaculture industry.

EVALUATING THE PRACTICAL APPLICABILITY OF AQUACULTURE AND GONAD ENHANCEMENT IN THE SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816)

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Gonads of *Paracentrotus lividus* are considered a delicacy and the increasing market demand depleted several wild stocks. In this context, sustainable echinoculture can play an important role for supplying commercially gonads and also for local restocking. Nevertheless, the success of this activity largely depends on the development of valuable hatchery techniques, establishment of adequate rearing conditions and nutritional balanced diets for the different life cycle stages.

This aquaculture research is part of a multidisciplinary project called "Ouriceira Aqua: Aquaculture and Enhancement of Gonad Production in the Sea Urchin *Paracentrotus lividus*", which launched in July 2017, funded by the European Commission under the Operational Programme MAR2020. The general goals of this project are to improve the aquaculture of the sea urchin *P. lividus* using innovative approaches for broodstock conditioning, larval development, growth and optimization of gonadal yield. The project seeks to evaluate the potential rearing of this species, contribute to develop technological aspects that are still bottlenecks and increase gonad yield in adult sea urchins in recirculating aquaculture systems. In this context, firstly the project intended to achieve the gonads' maturation to produce viable larval rearing and perform larval nutrition experiments in order to optimize the survival rate and settlement success, and then improvement of the somatic growth with diets that can promote faster growth. Enhancing roe of the sea urchin *P. lividus* were also investigated using artificial diets and manipulating zootechnical parameters, in order to obtain gonads with the quality demanded by consumers: larger, with desirable colour, texture and flavour.

Regarding the potential aquaculture of *P. lividus*, results showed advances in fertilisation protocols, improvement of larval production and settlement with new mixtures of microalgae and improvement of juveniles' growth with the developed extruded diets. Regarding the enhancement of gonads, some jellified diets with selected vegetables and new formulations of extruded diets, that were not previously used, can improve the productive process and gonad quality. The effects of these diets have been studied to determine which provide the factors that can influence the marketability of sea urchins. In this way, insights and results from the Ouriceira Aqua project will be analysed and discussed.

Acknowledgements
This project has the financial support of Operational Programme MAR2020 through the project 16-02-01-FMP-0004: Ouriceira Aqua: Aquaculture and Enhancement of Gonad Production in the Sea Urchin (*Paracentrotus lividus*). Also, this study had the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2019 granted to MARE - Marine and Environmental Sciences Centre.

IS IT LEGAL TO SELL SEAFOOD AS 'ORGANIC' IN U.S. MARKETS?

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Organic food products are a substantial and growing market segment, but the U.S. has not established any organic standards for seafood. As a result, U.S. aquaculture producers may seek to be certified under a foreign organic standard and to advertise that status on their products. While these certifications and sales do not appear to violate foreign laws, this study identifies potential liability for entities selling aquaculture products as organic in U.S. markets under federal and state organic products law, federal food labeling law, and state unfair competition and consumer protection laws.

- Organic aquaculture label claims appear to violate the Organic Foods Production Act (OFPA), although the USDA has indicated that it will not enforce against such labels as long as they do not suggest that the product is USDA-certified. However, California's organic program does explicitly prohibit the sale of organic aquaculture and could face enforcement action there.
- Canadian law does not prohibit advertisement of products based on its organic certification, and its new organic regulations allow properly-certified organic aquaculture products to bear the Canadian Organic Seal even when exported.
- Food product labeling must comply with the Lanham Act, which currently appears to raise the potential for liability with respect to organic claims on seafood. These claims could give rise to claims by competitors under an unfair competition theory.
- State unfair competition and consumer protection laws could apply to organic aquaculture claims. Plaintiffs have asserted such claims in a variety of states, and courts have allowed them to proceed in the context of personal care products. While uncertain, it is possible that courts would also allow similar claims for organic seafood.

These findings suggest that entities marketing "organic" seafood may face a variety of legal challenges to the sale of their products. As a result, growers and markets selling organic-labeled seafood products must carefully weigh the risk of liability against the benefits of making organic claims.

ASSESSING GUT TRANSPORT OF METHIONINE AND LYSINE IN FAST-GROWING RAINBOW TROUT

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Future growth of aquaculture depends on the discovery of alternative sources of protein to feed fish. Plant-derived proteins lack sufficient levels of two essential amino acids, methionine and lysine, for fish dietary requirements. We examined differences between a genetically selected line of rainbow trout that grows more rapidly on a plant protein diet when compared to a non-selected line. We hypothesize genetically selected rainbow trout uptake these two limiting amino acids more efficiently in their gut via more numerous amino acid transporters as compared to non-selected fish. Amino acid uptake and total amino acids were assessed over time in plasma using [^{13}C] and [^{15}N] stable isotopes of methionine and lysine measured by LCMS. Amino acid transporter expression in the gut was assessed using qPCR. Results show amino acid uptake in rainbow trout selected for growth on a plant-protein diet is greater than the uptake observed in non-selected fish. Quantitative PCR of amino acid transporters in the proximal intestine also show increased expression stimulated by lysine over time consistent with levels amino acid of uptake.

CHARACTERISTICS OF THE FISHING AND ENVIRONMENTAL PERCEPTION OF THE FRESWATER PRAWNS' FISHERMEN IN ITAPEMIRIM RIVER, ESPÍRITO SANTO, BRAZIL

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We aimed to understand the relationship between freshwater prawns' fishermen and the environment in the Itapemirim River, southern region of Espirito Santo-Brazil, in order to identify the fishing characteristics, traditional knowledge about use of traps, the level of environmental perception and the knowledge about the species that inhabit the river. The results of this work can be supporting the creation of effective public measures for the conservation of fisheries resources, not only based on biological concepts but with the possibility of contribution from the fishing community.

We conducted semi-structured interviews with fishermen from two different regions across the Itapemirim River: the river head and the region that is near from the estuarine area. The required number of people involved in fishing activities was reached through the "Snowball" method. For that, 16 questions were elaborated, being 6 closed and 10 subjective ones contemplating the environmental characterization, use of fisheries resources and economical aspects of the fishing. The interviews were documented in audio and video form along with notes in the interview script. This work happened after our studies about the occurrence of freshwater prawns in the Itapemirim River, when we found six species of freshwater prawns: *Macrobrachium jelskii*, *Macrobrachium carcinus*, *Macrobrachium acanthurus*, *Macrobrachium olfersii*, *Palaemon pandaliformis* and *Potimirim brasiliana*.

We observed two different fishermen group. Both of the groups use traps and sieve to fishing. In the headland region, amateur fishermen fish freshwater prawns aiming to get live bait for recreational fishing. This group showed low environmental perception when compared with the community that lives near the estuarine area. This is due possibly to non-dependence on fishing for family maintenance. In the region near estuarine area, the community usually fish freshwater prawns to sell for human consumption. The animals sold represent a large part of family income. In the estuarine region, the relationship between man and fishing is greater, with greater experience and proximity to the environment, as well as the financial and food dependence on products from this environment. Due to this dependence, they present a broad knowledge about the biotic and abiotic components that make up this ecosystem, enabling them to identify flood and low river seasons, reproductive and migratory periods, ecological relationships, behavioral habits of aquatic organisms, as well as other aspects characteristic of the region. However, of six species of shrimp that occurs in the Itapemirim River, only 3 (*M. acanthurus*, *M. carcinus e M. olfersii*) were recognized through photos by fishermen being these as species with higher economic values cited.

THE EFFECTS OF HANDLING TYPE ON THE LEVELS OF *Vibrio vulnificus* AND *V. parahaemolyticus* IN NORTH CAROLINA CULTURED OYSTERS

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The routine handling of oysters is a common industry practice for off-bottom oyster farmers in order to produce a high-quality food product. These handling practices expose oysters to elevated temperatures, permissible for the growth of *Vibrio parahaemolyticus* (*Vp*) and *V. vulnificus* (*Vv*). Farmers can re-submerse their oysters in the water following routine handling, allowing the elevated levels of *Vibrio* to return to ambient levels before harvesting for raw consumption. In the past, our group has examined the effects of gear type, handling type, and season on re-submersion. However, these studies were only performed in two growing areas within Alabama waters, leaving open the question of geographic variation. With re-submersion being a relatively new topic in seafood safety, a similar study was performed on a farm in Cedar Island, North Carolina using floating cages in 2018-2019. In this study, the effects of two common types of handling (tumbling and refrigeration) were examined on *Vibrio* levels in oysters both initially and after re-submersion. Both tumbling (tumbled, T; or not, NT) and refrigeration (refrigerated overnight, R; or not, NR) in four different experimental treatment combinations (TR, TNR, NTR, NTNR) were tested during a 24-hr removal from the water. Then, the recovery of levels of *Vp* and *Vv* levels during a 14-day re-submersion period was examined. A set of non-treated oysters that remained submerged throughout the study was tested to monitor ambient *Vibrio* levels over time. The preliminary data from 2018 suggest similar effects of the handling types on *Vibrio* levels as seen in a previous study in Portersville Bay, Alabama (2016-2017), with the non-refrigerated treatments experiencing significant increases in *Vibrio* levels. The data show that all *Vibrio* types recovered to ambient levels within the oysters after 7 days of re-submersion regardless of handling type, similar to the results of the Alabama studies. Final data from the four trials performed in 2018-2019 will be presented at the meeting.

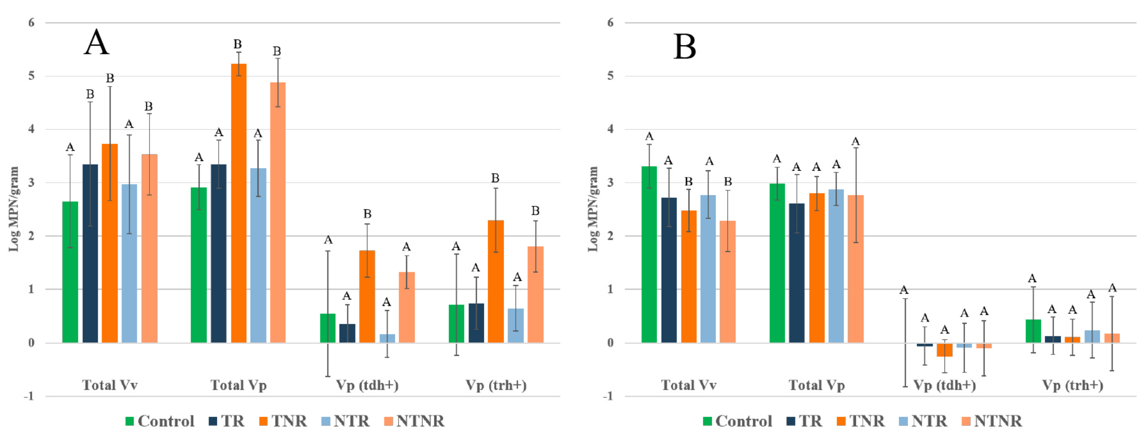

Figure 1. Levels of *Vibrio* types after the treatments were applied (A), and after 7 days of re-submersion (B). Each bar represents the average of samples from the two trials in 2018 (n=6) taken for each treatment type (control, tumbled and refrigerated (TR), tumbled and not refrigerated (TNR), not tumbled and refrigerated (NTR), and not tumbled not refrigerated (NTNR).

GENOMIC RESOURCES FOR *Seriola* FARMING IN THE U.S.: GENETIC AND GENOMIC TOOL DEVELOPMENT FOR *Seriola dorsalis* and *Seriola rivoliana*

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It is expected that *Seriola* species will be among the first marine fish permitted for offshore culture in the United States. In order to benefit the expected growth of *Seriola* culture, the Southwest Fisheries Science Center – in collaboration with Iowa State University – has started developing genomic resources for the California Yellowtail, *Seriola dorsalis*, and the Almaco Jack, *Seriola rivoliana*. These efforts have included genome assemblies, genetic diversity studies, parentage and spawning dynamic analyses, transcriptomic studies, sex-marker development, and genome-wide association studies to identify markers or genomic regions associated with deformities and improved cold tolerance. These genomic tools can be used to improve *Seriola* farming through enabling broodstock selection, which utilizes variation existing in wild populations, or through the more efficient selective breeding. A project is also ongoing to apply the Offshore Mariculture Escapes Genetic/Ecological Assessment (OMEGA) model to simulate farm scenarios being considered for *Seriola dorsalis* off the coast of Southern California. This predictive model was designed to evaluate genetic impacts associated with farmed fish escapement. By utilizing the OMEGA model, researchers, management agencies, and the aquaculture industry will be able to better mitigate the risk of escapees from offshore culture facilities, and more effectively plan responsible culture operations (including breeding program design) that may be acceptable in a given scenario.

Our progress, to date, on these projects will be briefly described in this presentation, along with a short discussion of some of the collaborative projects that have come out of the biennial *Seriola* Workshop held at the Southwest Fisheries Science Center.

TAPPING INTO LOCAL FOOD SYSTEMS FOR AQUACULTURE: WHAT SMALL PRODUCERS NEED TO KNOW

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This study examined the prospects of marketing aquaculture products through food hubs in the Midwest. The addition of aquaculture products in food hubs could translate into the expansion of aquaculture production. However, engaging food hubs requires an understanding of factors that could incentivize them to supply seafood in order to facilitate economically sound production and marketing decisions. This study also explored various business models for processing services in shared-use and commercial kitchens in an attempt to explore processing and value-added product development opportunities for the aquaculture industry to ensure their sustainability and profitability. We surveyed select food hub and shared-use kitchen businesses in Illinois and Indiana, with emphasis on the quality of information to derive.

The response rate for the questionnaire was 31.3% (10 out of 32) from both food hubs and commercial kitchens in Indiana and Illinois. Seafood is not a common product handled by food hubs and commercial kitchens because of the barriers created by licenses and certifications requirements. There is a lack of information concerning the cost and benefits of aggregating and distributing seafood, and also processing available seafood to food hub operators.

The common business models for food hubs are non-profit, cooperative and for profit. The average number of years of operation is 5.5 years with some co-ops being in operation for 20 years. With the exception of one food hub, that has a meat processing facility, all the other food hubs only aggregate and distribute with not processing services. For food hubs to commit to processing and marketing locally produced seafood, producers need to meet supply frequency and quantities. Food hubs tend to have contract obligations with their institutional buyers and therefore require consistency in supply from producers.

The 3 commercial kitchens in our sample are all for-profit. The kitchens have been in operation for about 3.5 years, on average. The commercial kitchens require membership before a producer or supplier can use their facility. They provide space to process and prepare mainly produce for the market, and some kitchens provide additional services like training in kitchen and food safety, entrepreneurship, culinary education; packaging, labeling, pricing and deep cleaning services after the tenants have finished using the kitchen. Commercial kitchens get revenue from membership and rental fees. All 3 kitchens will allow the processing of seafood in their facility.

Even though the kitchens are certified for commercial food processing activities, each tenant is required to be certified (e.g. Food Safety manager certificate) in order to use the facility. Some kitchens also require liability insurance from their tenants while they are using the facilities. Food processors must also follow good manufacturing practice and have a Hazard Analysis and Critical Control Point (HACCP) plan.

ON-FARM EVALUATION AND MONITORING OF DIFFERENT MANAGEMENT STRATEGIES EMPLOYED BY A COMMERCIAL BAITFISH FARM TO CONTROL *Microcystis*

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Arkansas is the leading producer of baitfish in the United States. Arkansas baitfish farmers in recent years have reported increasing problems related to blue-green algae. Blue-green algae blooms, such as *Microcystis* species, can have deleterious effects on baitfish production including odors, toxins, reduction of seining efficiency, and fish mortality. Attempts to control blue green algae populations have become of paramount importance for the baitfish industry, and management strategies include the use of chemicals such as copper sulfate, copper chelates, potassium permanganate, among others.

In order to assess the efficacy of different management strategies employed by commercial baitfish farms in Arkansas, monitoring of microalgae populations and water quality were performed during early Spring through late Fall 2019 on a commercial farm in Lonoke County, Arkansas. Six commercial baitfish production ponds (4.0-7.1 hectares) were monitored by Extension personnel on a weekly basis. Water quality parameters that were measured for each pond included temperature, pH, total ammonia nitrogen, nitrite nitrogen, nitrate, and phosphorous. Microalgae counts were performed via microscopic examination. The impact of different management strategies and treatment regimens employed by the cooperating farm on pond water quality and microalgae will be presented.

EXPERIENCES OF SHRIMP CULTURE *Litopenaeus vannamei* IN CONDITIONS OF LOWER SALINITY, USING PRECISION AQUACULTURE

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The shrimp production increase in Ecuador has been doubling in the last 5 years, hoping to close 2019 with more than 620,000 MT exported.

The growth of production has been mainly due to the implementation of technology in culture management: reduction of production cycles (with use of raceways and nursery), better nutrition and technology in feed (use of extruded feed), implementation of aeration and automatic feeding, as well as precision aquaculture.

With the implementation of precision aquaculture and the improvement of productive indicators, it has generated confidence in investments to efficiently grow shrimp in low salinity conditions (less than 10 ppt).

Table 1: Production indicators: Higher Production Efficiency Index (PEI) and Lower cost / pound shrimp

Company	n	Density (i/m2)	Harvest weight (g)	FCR	Survival (%)	Weekly growth (g)	Days of culture	Biomass (lb/ha)	PEI	Cost/pound of shrimp
A	5	20.9	23.4	1.4	53	1.8	90	5329	0.7	1.3
B	48	14.2	23.5	1.5	71	1.4	113	5156	0.7	1.4
C	24	17.3	21.0	1.4	65	1.9	81	5171	0.8	1.2
D	10	13.9	20.7	1.4	60	1.6	91	3796	0.8	1.5
E	6	24.5	19.4	1.5	61	1.4	95	6282	0.6	1.2

PEI = ((weekly growth (g) x survival (%) / FCR) / 100

Table 2: Production indicators: Lower Production Efficiency Index (EIP) and Higher cost / lb shrimp

Company	n	Density (i/m2)	Harvest weight (g)	FCR	Survival (%)	Weekly growth (g)	Days of culture	Biomass (lb/ha)	EIP	Cost/pound of shrimp
F	12	23.5	16.8	1.7	52	1.3	88	4254	0.4	1.6
G	34	21.6	20.9	2.1	47	1.5	97	4572	0.4	1.7
H	120	23.8	21.0	1.8	52	1.4	105	5470	0.5	1.5
I	9	13.3	18.9	1.1	47	1.3	105	2532	0.5	1.6
J	43	27.2	16.5	1.8	52	1.0	111	4820	0.3	1.5
K	4	17.0	23.8	1.3	42	1.3	134	3696	0.4	1.6
L	18	19.9	19.1	1.5	59	1.3	110	4728	0.5	1.4
M	6	16.5	21.1	2.4	61	1.5	99	4631	0.4	1.8

Cost /pound of shrimp = Total production cost / Shrimp production biomass (lb)

THE BUSINESS CASE FOR UTILIZING SEAWEED AQUACULTURE AS A EUTROPHICATION MITIGATION TOOL

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A growing human population coupled with climate change is exacerbating the spread and impact from organic matter over-enrichment (eutrophication)[1]. Despite promising techniques and tools, our current actions are proving insufficient. This mounting issue requires accounting for multiple stressors[2], including nutrient loading. Adequate management of nutrient loading requires a holistic set of proven tools that prevent both point source and non-point source pollution, and mitigate eutrophied areas. In this study, we map seaweed aquaculture's potential nutrient uptake in highly impacted estuarine environments. Using the Gulf of Mexico as a case study, we go one step further to make a business case for seaweed aquaculture as an additional mitigation tool.

Seaweed aquaculture has long been proposed as an additional nutrient pollution mitigation tool, but implementation has been stymied by a lack of market access, technology, and political will. However, increasing global demand for seaweed[3], recent advancements in open-ocean farming techniques, and efforts to streamline permitting[4], provide a new opportunity to simultaneously maximize financial and ecosystem benefits of seaweed aquaculture through strategic spatial planning around eutrophied areas.

In our preliminary analysis adapting values from Xiao et al., 2017[5], we projected the N and P uptake from farming the Gulf of Mexico. If we were to farm 5% of the Gulf of Mexico's 2019 dead zone[6], 435 km^2, up to 558 tDW seaweed could be produced, removing 20 tN km^{-2} yr^{-1} and 2.64 tP km^{-2} yr^{-1}. One step further, using the Connecticut Nitrogen Trading Program as an example, this amount of N could result in \$225,300 t km^{-2} yr^{-1} of sales for the estimated N removal[7]. While these calculations are a best case scenario, seaweed aquaculture offers a promising method of subsidizing nutrient mitigation.

Total for China (2014)		
Seaweed Production[16]	2.00	million t DW
Seaweed Area[16]	1,250	km^2
N concentration*	3.76 ± 0.92	% DW
P concentration*	0.47 ± 0.19	% DW
N removal	$75{,}371 \pm 18{,}423$	t N year^{-1}
P removal	$9{,}496 \pm 3{,}875$	t P year^{-1}
Per km^2 of Seaweed Farm and Year		
Seaweed Production	1,604	t DW
N concentration*	3.76 ± 0.92	% DW
P concentration*	0.47 ± 0.19	% DW
N removal	60.31	t N km^{-2} year^{-1}
P removal	7.60	t P km^{-2} year^{-1}
N input**	3.38	t N km^{-2} year^{-1}
P input**	0.06	t P km^{-2} year^{-1}
Seaweed Farm N Footprint area	17.8	km^2 of coastal ocean removed of N inputs km^{-2} of seaweed farm
Seaweed Farm P Footprint area	126.7	km^2 of coastal ocean removed of P inputs km^{-2} of seaweed farm

Table 1 Total nutrient removal by seaweed aquaculture in China *and the nutrient removal capacity of Chinese seaweed farms km^{-2}. The seaweed farm N and P footprint area refers to the km^2 of Chinese coastal waters receiving nutrient inputs.*

1. van Beusekom J.E.E. (2018) Eutrophication. In: Salomon M., Markus T. (eds) Handbook on Marine Environment Protection. Springer, Cham
2. Breitburg, Denise, et al. "Declining oxygen in the global ocean and coastal waters." *Science* 359.6371 (2018): eaam7240.
3. Allied Market Research, 2018
4. FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals.
5. Rome. Licence: CC BY-NC-SA 3.0 IGO. pg: 145
6. Xiao, Xi, et al. "Nutrient removal from Chinese coastal waters by large-scale seaweed aquaculture." *Scientific reports* 7 (2017): 46613.
7. Turner, R. Eugene, and Nancy N. Rabalais. "2017 Forecast: Summer Hypoxic Zone Size Northern Gulf of Mexico." *Louisiana State University, Website PDF article. Accessed December* 11 (2017).
8. Kim, Jang K., George P. Kraemer, and Charles Yarish. "Use of sugar kelp aquaculture in Long Island Sound and the Bronx
9. River Estuary for nutrient extraction." *Marine Ecology progress series* 531 (2015): 155-166.

THE EFFECT OF DIETARY 17A-METHYLTESTOSTERONE ADMINISTRATION ON SECONDARY SEX COLORATION IN ADULT FEMALE ROSY BARBS AND DWARF GOURAMIS

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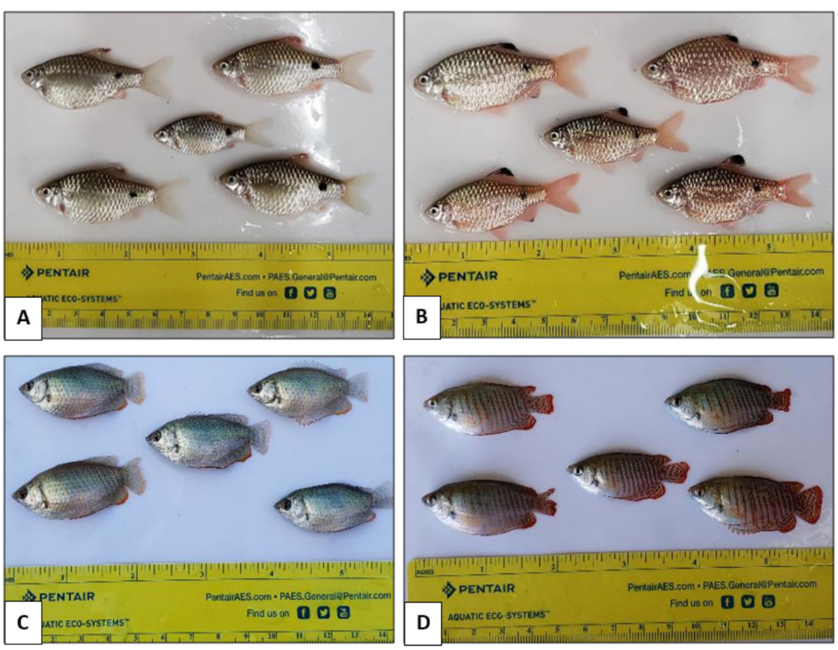
Sexual phenotype is highly plastic in teleosts and controlled by a variety of species-dependent factors. In aquaculture, sex ratios are often manipulated to produce desirable phenotypic traits. In ornamental aquaculture, male fish are more valuable in many sexually dimorphic species due to more ornate coloration. These traits may be influenced after sexual differentiation by increasing the circulating androgen levels. This study investigated the effects of dietary 17α-methyltestosterone (MT) administration on adult female Rosy Barbs and Dwarf Gouramis. A panel of judges evaluated male secondary sex coloration weekly during and after prolonged MT feeding. Growth parameters, survival, and gonadosomatic index (GSI) were assessed at the end of the feeding regime. In both species, none of the MT diet concentrations affected survival and all resulted in coloration changes, highlighting both the efficacy and safety of MT administration. In Dwarf Gouramis, MT diets caused pronounced color changes and reduced their GSIs at all dietary concentrations. In Rosy Barbs, MT diets induced the expression of male coloration, but generally did not reach the color intensity of naturally occurring males and did not result in differences in GSIs. This study suggests that post differentiation androgenic treatment may be a viable option to induce secondary male sex coloration in ornamental species.



Photographs representing typical coloration patterns of Rosy Barbs after 11 weeks of control (A) or 240 mg MT/ kg feed (B) administration and Dwarf Gouramis after 9 weeks of control (C) or 240 mg MT/ kg feed (D) administration.

ROLE OF CHANNEL CATFISH CLCA IN FISH BACTERIAL DISEASES

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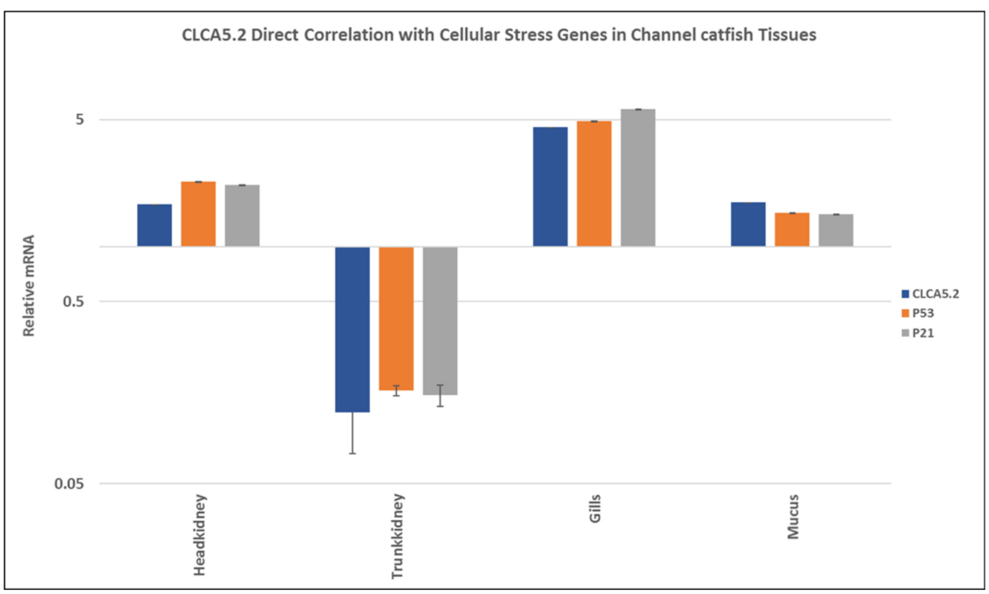
The stringent regulations on the use of antibiotics call for new solutions to treat bacterial infections. Mucus is the first line of defense that fights pathogens in fish. Mucus consists of humoral factors that include metalloproteases that act as antibacterial peptides. In this study, we investigate the Channel catfish CLCA gene family that is secreted in the fish mucus.

CLCA genes are well conserved across species, including fish. CLCAs are expressed at cell-cell junctions of epithelial cells and required for maintenance of epithelial integrity. There are three different forms of CLCA variants in zebrafish. The catfish CLCAs have never been explored. We have identified a catfish EST similar to zCLCA5.2 isoform, which is 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. For example, CLCA1 is found in mucus granules and mucus barrier of airways and regulates the level of mucus secretion. CLCAs are Zn+2 metalloproteases in other species, and this metal-binding motif is highly conserved across species.

Interestingly, we find that cCLCA 5.2 expression is significantly higher in gills and mucus, which is directly correlated with stress-inducible genes P53 and P21. We speculate that cCLCA5.2, like other CLCAs, is a Zn^{+2} dependent metalloprotease expressed at cell-cell junctions of gills. Although we dint see high expression in skin, we did evidence its presence in the mucus collected from skin. Therefore, we speculate that cCLCA5.2 in the mucus is a potential antibacterial peptide that can fight catfish pathogens.



A MULTIDISCIPLINARY APPROACH TO INVESTIGATE BIOLOGICAL EFFECTS ON INTESTINE PHISIOLOGY AND APPETITE STIMULUS IN RAINBOW TROUT *Onchorhincus mykiss* FED DIETS WITH GRADED LEVELS OF INSECT MEAL AND POULTRY BY-PRODUCT MEAL

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One of the most critical issues that threatens the sustainability and the further development of intensive carnivorous species aquaculture is its dependency on aquafeed ingredients such as fishmeal (FM) and fish oil (FO). Due to ecological and economic implications, the desirable goal of the feed industry is to replace these ingredients with more sustainable ones. Over the last two decades plant proteins and vegetable oils have been widely used in aquafeed, because they are readily available on the feed market and cost-effective. However, ”veggie” diets show some disadvantages, possibly affecting fish welfare and are often in direct competition with human nutrition. Recently, research has been focused on insect meal (IM) and poultry by-product meal (PBM) as ingredients for aquafeed formulation. These ingredients have a low environmental impact and show proper nutritional qualities for fish culture. The present study investigated the physiological effects on these new ingredients (*Hermetia illucens* meal and PBM) during rainbow trout (*Onchorynchus mykiss*) culture. Specifically, six different dietary treatments were formulated: one control diet based on vegetable ingredients (C Veg); one fish meal based diet (C Fish); one diet with a 30% substitution of vegetable proteins with IM (IM130); one with 60% of substitution with IM (IM160); one with 60% of substitution with PBM (PBM160); one combined diet with 50% PBM and 10% IM (PBM150+HM110). Feeding trials were carried out until fish triplicated their initial weight. A multidisciplinary approach was applied to study the dietary effects on intestine immune/inflammatory response and on appetite stimulus. Beside a traditional histological and biomolecular approach, Fourier Transform Infrared Spectroscopy (FTIR) was adopted as a new methodology to characterize the macromolecular composition of intestine mucosa. Histology on intestine did not shown inflammatory events in medium and hind traits in none of the experimental groups apart a slight increase in mucous cells in trout’s medium intestine fed IM diets. FTIR analysis on intestine samples provided interesting information on mucosal layer composition of the different experimental groups and showed as the nutrients transport in the intestine varied in relation of the experimental diets. RT-PCR showed an increase in the expression of genes involved in the activation of immune system related genes in medium intestine of C Fish, PBM130 and PBM160 groups. Moreover, an increased expression of genes involved in the inflammatory cascade was observed in medium intestine of C Veg. No significant differences among the experimental groups were detected about signals involved in appetite. These results suggest that the tested alternative ingredients are suitable for trout culture.

The project was founded by Ager2-Sushin cod. 0112-2016.

ENHANCING FRESHWATER PRAWN (*Macrobrachium rosenbergii*) LARVICULTURE WITH *Centella asiatica*

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Centella asiatica L. (Gotu Kola) is a green leafy vegetable commonly found in Asia and is gaining importance due to its high nutritional values and beneficial carotenoids. This study was done to investigate the potential of *C. asiatica* as an enrichment in the diets of freshwater prawn, *Macrobrachium rosenbergii*, larvae to improve larviculture. Triplicate groups of *M. rosenbergii* larvae (n=750) were fed different feeding formulation consisting of *Artemia* nauplii and egg custard. Enrichment of 100 ml of *C. asiatica* extract were used in this study. Control treatment were fed non-enriched diets of *Artemia* nauplii and egg custard, treatment B consisted of non-enriched *Artemia* nauplii and *C. asiatica* enriched egg custard and treatment C consist of *C. asiatica* enriched *Artemia* nauplii and egg custard. Larvae were fed three times a day at 0800 h (egg custard), 1300 h (egg custard) and 1700 h (*Artemia* nauplii). All experimental diets were tested at stage 4 larvae. The number of days for larvae to reach stage 4 for all treatment were comparable at day 6. Stage 5 to post larvae stage (stage 12) onwards, larvae fed comprehensive enriched diets were observed to metamorphosis rapidly compared to control treatment. Larval stage index (LSI) recorded more than 80% of freshwater prawn larvae metamorphosis to post larvae (PL) in treatment C at day 29 compared to control at day 35 (p<0.05). The survival was significantly improved (p<0.05) in larvae fed enriched diets where the highest mean survival was recorded for treatment C (67.9%) and the lowest in control (49.4%). The results demonstrate that supplementation of *C. asiatica* in the diets of freshwater prawn larvae provided nutritional advantages and cost effective solutions by improving the larviculture period and survival, under the experimental conditions that were employed here.

INOVATING METHODS FOR STAGGERING KELP OUTPLANTINGS BY MODIFYING SEED STRING GROWING CONDITIONS

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Kelp farming has the potential to economically diversify coastal communities of Alaska while offering potential ecosystem services, including carbon sequestration and mitigating eutrophication. Our research is focused on identifying methods to control early life stages of kelps, including *Saccharina latissima* and *Alaria marginata* in order to optimize kelp seed production. Storage of seeded string could be used for staggering kelp outplanting from the kelp hatchery to ocean farm providing more flexibility and enabling more control of timing in the seeding and outplanting process. As part of this research we are addressing two questions.

1) How can we slow gametophyte growth and reproduction in a hatchery setting?
2) How does storage in conditions that stall gametophyte growth and reproduction affect the viability of the seed string when outplanted?

To evaluate question 1, we tested removing iron from the culture medium or filtering out blue light, varying light intensity and temperature. We found that low temperature is most effective at delaying both gametogenesis and slowing gametophyte growth subsequently is the best candidate condition for seed storage. We also found gametophyte growth to be highly dependent on the month of seeding (Fig. 1). Following these results, we approached question 2 by growing kelp seed string under a positive control and four storage treatments comprised a combination of low temperatures and varying nutrient conditions.

Preliminary results suggest one storage treatment (at low temperature and full nutrients) was successful. We extended seed storage for an additional 35 days beyond the positive control without adverse effects to sporophyte density and length at the time of outplanting and up to 3 weeks after outplanting. Our results provide a storage method for hatcheries that will give more flexibility and the ability to stagger kelp outplanting from the same sporophyte brood stock.

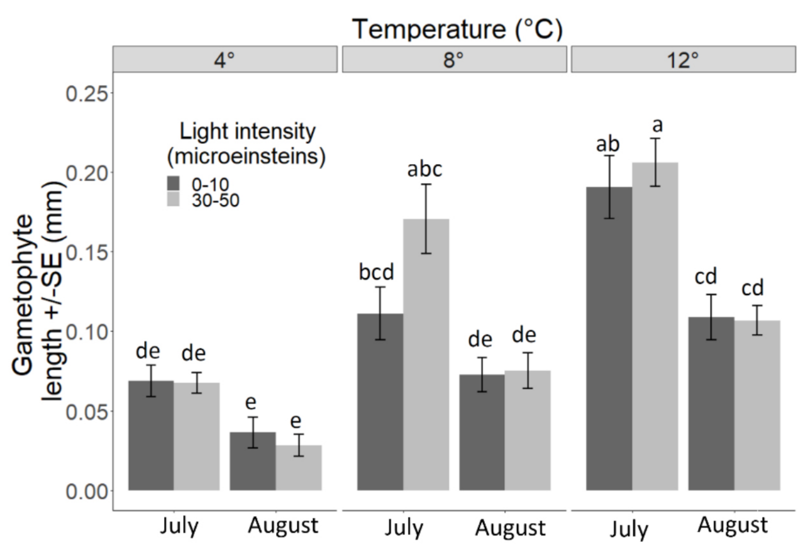

Figure 1. Gametophyte length under different temperature and seeding months. Letters denote statistically similar groups.

USE OF A RECIRCULATING HYDROPONIC BIOREACTOR FOR CLEANING CONTAMINATED WATER FOR USE IN AQUACULTURE

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Clean water is a limiting resource in many parts of the world. Not only is the availability of clean water detrimental to people's survival, but it is limiting to the expansion of aquaculture in many places. In addition, many water sources that are or could be used for aquaculture, such as treated effluent, contain various contaminants that can have potentially hazardous effects on humans and the environment. These contaminants include heavy metals, pharmaceuticals and industrial compounds, bacteria and nitrogenous waste, among other pollutants. Some of these compounds, are referred to as "emerging contaminants" because the effects are not yet known or fully understood. Even water that appears clean to the naked eye can contain significant concentrations of contaminants.

To clean water of these emerging contaminants and other pollutants, a recirculating hydroponic bioreactor was proposed, designed and assessed for its ability to remove contaminants and clean water for use in aquaculture. It was chosen for its relatively cheap cost to build and operate compared to other water treatment options.

In order to give a real world assessment, hydroponic bioreactors were used to clean up tertiary effluent from a wastewater treatment plant in Tucson, Arizona. Results showed that after five days of recirculation in the bioreactors, there were significant reductions of all measured contaminants in the water as well as complete removal of ammonia. Based on performance, a larger-scale bioreactor could be built to treat contaminated water to provide a new or improved water source for fish culture.

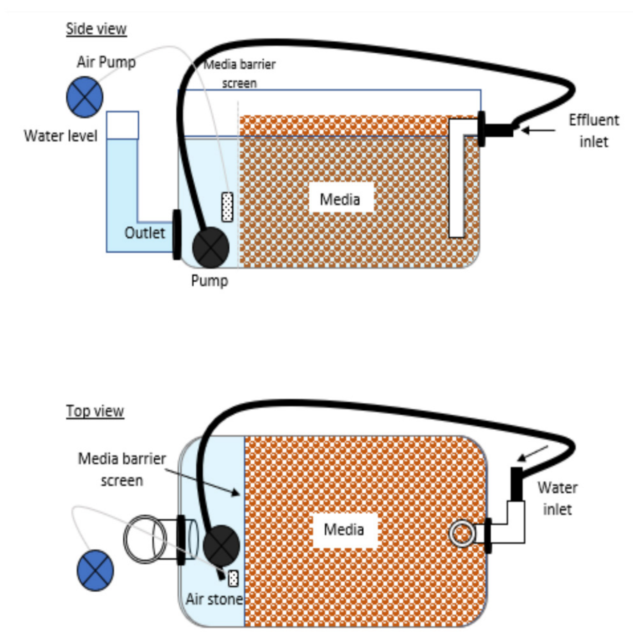

Figure 1. Design schematic of recirculating hydroponic bioreactor

ADVANCED ENGINEERING REDESIGN OF THE AQUAPONICS GREENHOUSE AT THE UNIVERSITY OF ARIZONA WITH A DECOUPLED AND COUPLED SYSTEM

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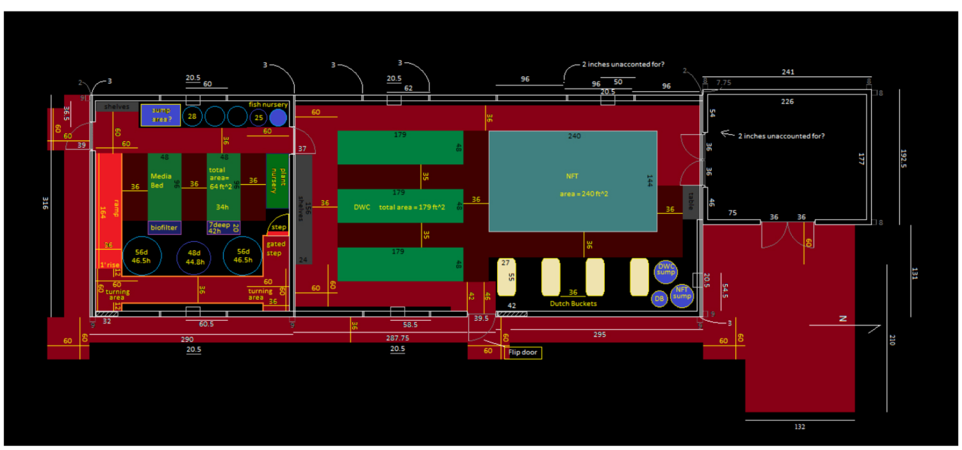
The University of Arizona has become a world leader in research, teaching and extension regarding controlled environment agriculture (CEA). Up to this point, CEA at the University of Arizona has included and focused on sensor technology and computer-controlled greenhouse production, vertical farming, space life support agriculture systems, and hydroponic plant production.

Aquaponics, an alternative and sustainable form of plant production with fish, has largely been driven by hobbyists and small-scale growers and until more recently has not been part of CEA. However, it is increasing in both popularity as a potential way to grow plants commercially. The idea here was to redesign the 200-m^2 aquaponics greenhouse at the University of Arizona to showcase intensive aquaponics systems including a coupled version which would be suitable for mid-scale production and a decoupled aquaponic system to model how aquaponics can be scaled for large-scale commercial production.

The redesign included multiple sensors and controls to optimize plant production. The fish culture systems or RAS systems included advanced filtration for intensive fish production. In addition, the decouple aquaponics system included an anaerobic bioreactor to maximize nutrient availability to plants from the fish waste. The goal for the redesign is to demonstrate how aquaponics can be viable on a commercial scale as an option for efficient and sustainable plant production. In addition, an alternative design plan was considered with complete ADA accessibility and compliance to demonstrate agriculture to an inclusive population.



AUTOMATIC FEEDERS SCHEDULE AS A TOOL FOR IMPROVEMENT OF SHRIMP (*Litopenaeus vannamei*) PRODUCTION SYSTEMS IN PONDS

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Shrimp are regarded as slow feeders with preference for many small meals. Various studies have shown improved growth and production as the number of feedings increases. With the move to automated feeding systems the number of feedings can be greatly increased and labor reduced. Technological development has presented shrimp industry with an increasing number of on-demand (satiation) feeding systems. Building on previous research, a 90-day pond trial was designed to test shrimp productivity under different feeding schedules and rations. The production trial was conducted in 16, 0.1 ha outdoors ponds, stocked at a 35 shrimp/m^2, and fed a 35% protein soy-optimized feed. Standard ration for timer-feeders (SRTF) was calculated based on a standard ration (SR) which is based on an expected weight gain of 1.3 g/wk, a feed conversion ratio (FCR) of 1.2, and a weekly mortality of 1.5% during growout period. All ponds were fed the same amount of feed for the first 30 days after which four treatments were initiated including SR160 Daytime, SR160 Nighttime, and SR175 24 hours which were fed using automatic timer-feeders, and a fourth treatment utilized on-demand AQ1 acoustic feeding system (AQ1). The first three treatments were feeding increasing percentages of SR equally until Day 75, Day 30-45 fed SR130%, Day 45-60 fed SR145%, Day 60-75 fed SR160%. The 24h treatment ration was increased to SR175% for Day 75-90 and the other two stayed at SR160%. Preliminary one-way ANOVA and Student-Newman-Keuls test results presented in Table 1 indicate that AQ1 System application resulted in statistically higher feed inputs which resulted in higher yields. However, on-demand feeding system did not result in larger shrimp nor higher weekly weight gain that shrimp fed SR 160 during Daytime. Timer-feeder treatments with nighttime and 24 hours feeding schedule had lower feed input than daytime as consequence of providing meals during the period of the night in which oxygen availability is typically lower, consequently meals were skipped more often to avoid critically low oxygen levels. Further analysis will be performed and presented at the conference.

Table 1. Response of Pacific white shrimp to different feed management protocols in ponds

	IndW (g)	Survival (%)	WtGain (g/wk)	Yield (kg)	Feed Input (kg)	FCR
SRTF 160 Daytime	26.13^{ab}	69.15	2.01^{ab}	625.38^{b}	641.67^{b}	1.03
SRTF 160 Nighttime	24.81^{b}	69.55	1.91^{b}	602.94^{b}	613.58^{b}	1.01
SRTF 175 24h	24.56^{b}	71.77	1.89^{b}	615.90^{b}	617.71^{b}	0.99
AQ1 System[1]	29.65^{a}	77.00	2.28^{a}	800.63^{a}	790.1^{a}	0.99
P-value	0.0500	0.4123	0.0500	0.0057	<0.0001	0.8951
PSE[2]	1.1200	3.2054	0.0862	30.8977	13.9822	0.0355

[1] n=3 due to electrical issue
[2]PSE – Pooled Standard Error
n=4

COMPARING DIFFERENT FEEDING PROTOCOLS FOR IMPROVEMENT OF SHRIMP (*Litopenaeus vannamei*) PRODUCTION IN SEMI-INTENSIVE RECIRCULATION

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Multiple studies have reported improved growth, productivity and potential for reductions in labor costs when multiple feedings are achieved though automatic feeding systems. Technological development has presented shrimp industry with an increasing number of on-demand (satiation) feeding systems as well as traditional timer-feeders which are simple, relatively inexpensive and a reliable tool for many farmers. Building on previous research that showed improvement in production when increasing from 2 to 6 feedings/day, this trial aimed to test shrimp productivity under different feeding schedules and rations. An 11-week growth trial was performed in 32, 750 L tanks in green water recirculation greenhouse system, stocked at 30 shrimp/tank (35 ind/m^2) , and fed a 35% protein soy-optimized feed. Water in tank systems was obtained from a semi-intensive shrimp production pond. All tanks were hand-fed the same feed ration four meals a day for the first 3 weeks after which a set of 8 treatments was designed to compare growth performance at different feeding schedules and feed levels. Five treatment were fed during day time hours from 7am-7pm with different number of feedings per day. One treatment was fed at night from 7pm-7am and two treatments fed the daily ration over 24 hours. Standard ration (SR) was calculated assuming a doubling of weight weekly until reaching 1.3g then calculated on an expected weight gain of 1.3 g/wk, a feed conversion ratio (FCR) of 1.2. Feed inputs were increased to 130% SR for weeks 4-5, 145% SR weeks 6-7 then 160% for weeks 8-11. One treatment (SR 175 24h) gradually reaching 175% at week 7 and the SR175 Day only 175% during the last two weeks. All treatments were fed using belt feeders using lines of feed, except 4 meals per day treatments which were hand-fed. Data presented in Table 1 is result of one-way ANOVA and Student-Newman-Keuls test. Further statistical analysis will be conducted and its results will be presented in the talk.

Table 1. Response of Pacific white shrimp to different feed management protocols in tanks

	IndW (g)	Survival (%)	Wt Gain (g/wk)	Final Biomass (g)	Feed Input (g)	FCR
4 Meals SR 160 Day	15.89[a]	92.53[a]	1.43[a]	439.85[ab]	570.9[a]	1.32
6 Meals SR 160 Day[1]	16.00[a]	78.87[b]	1.44[a]	379.27[a]	570.9[a]	1.52
8 Meals SR 160 Day	16.88[ab]	86.68[ab]	1.52[ab]	438.75[ab]	570.9[a]	1.31
12 Meals SR 160 Day	16.82[ab]	86.65[ab]	1.52[ab]	437.85[ab]	570.9[a]	1.32
6 Meals SR 160 Night	15.92[a]	86.65[ab]	1.44[a]	414.20[ab]	570.9[a]	1.40
12 Meals SR 160 24h	16.81[ab]	86.68[ab]	1.52[ab]	436.55[ab]	570.9[a]	1.33
12 Meals SR 175 24h	18.41[b]	88.35[ab]	1.67[a]	485.83[b]	627.1[b]	1.33
6 Meals SR 175 Day	16.76[ab]	87.53[ab]	1.51[ab]	439.90[ab]	585.0[c]	1.34
P-value	0.0488	0.2001	0.0440	0.042	<0.0001	0.2562
PSE[2]	0.366	1.951	0.033	12.296	0	0.04

[1]n=3
[2]PSE: Pooled Standard Error
n=4

ON-SITE RAPID COLUMNARIS IDENTIFICATION

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A research study was initiated to investigate the potential of a portable handheld real time PCR device, Biomeme Two3™ (Smith-Root, Vancouver, Washington), to detect eDNA of the bacterial pathogen *Flavobacterium columnare,* the causative agent of columnaris outbreaks on commercial catfish farms. Columnaris disease, caused by the bacterial pathogen *Flavobacterium columnare*, causes more losses to the US catfish industry than any other disease. Columnaris disease is transmitted horizontally from fish to fish, is highly contagious, and outbreaks are commonly associated with poor environmental conditions. Mortality patterns vary from a few fish every day to several hundred fish a day depending on the conditions. Water samples were collected from four different regions of eighteen ponds and from two different locations. Initial trials revealed that high concentrations of algae interfere with the handheld PCR devise resulting in inaccurate results. Modifications to the tests enabled more accurate readings. Readings from the handheld device were verified using qPCR techniques on an Applied Biosystems machine. Development of a correlation between columnaris bacteria counts in the water and columnaris outbreaks in fish would enable farm managers to devise management protocols that would reduce catfish losses due to columnaris disease. The study is currently on-going, and results will be presented.

ESTABLISHMENT OF PRIMARY CELL CULTURE FROM DIPLOID CRAPPIE

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Crappie, from the genus *Pomoxis*, are North American, warm water Centrarchids. Both species in this genus, the black crappie (*Pomoxis nigromaculatus*) and the white crappie (*Pomoxis annularis*), are popular and are of significant importance to the sportfishing and recreational fishing industries. Recently, the use of the hybrid, a first-generation cross between the black crappie male and white crappie female, has gained popularity. The hybrid has shown superior growth traits and has been known regularly outgrow the parental species. The hybrid also has greatly reduced reproductive capacity. This is considered an advantage by pond owners, as uncontrolled reproduction and overpopulation is a problem encountered with both parental species. Black and white crappie do not readily hybridize in the wild, and due to the villiform nature of the testes, males have to be sacrificed to obtain milt for in-vitro fertilization and hybrid production. The production of xenogeneic white and black crappie broodstocks would enable the production of hybrids by natural pond spawning. In xenogenesis, primordial gem cells (PGCs) from a donor are extracted and implanted on the gonadal ridge of a sterile host. The implanted PGC will colonize and produce germ cells from the donor in the gonad of the host. The aim of this study is to develop and characterize a new cell culture protocol for developing a xenogeneic triploid crappie. Primary cell culture protocol development in triploid crappie is underway and the results will be presented.

USDA RESEARCH SUPPORT FOR MARINE FINFISH AQUACULTURE

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USDA research support for marine finfish aquaculture is delivered through intramural and extramural programs at the Agricultural Research Service (ARS) and the National Institute of Food and Agriculture (NIFA), respectively. These programs have the overall mission of supporting research that delivers technologies that improve domestic aquaculture production efficiency and product quality while minimizing impacts on our natural resources. ARS conducts research under the ARS National Program for Aquaculture at 10 different locations through 14 projects performed by 50 ARS scientists and in funded collaborations with 12 cooperating institutions. NIFA provides support for aquaculture research, technology development and extension programs through formula grants, competitive funding opportunities, or Congressionally directed grants including support of the Regional Aquaculture Centers, the Aquaculture Special Research Program, and up to 14 competitions for which aquaculture researchers are eligible to apply. Many of these programs in ARS and NIFA are able to support marine finfish aquaculture.

In the 2019 NOAA publication Fisheries of the United States, harvests from U.S. commercial capture fisheries were reported for over 85 individual marine finfish species. These species, for which markets already exist, are excellent candidates for developing aquaculture industries that will meet increasing demands for seafood through domestic production. In addition, there is aquaculture potential for many other species that have markets that are too small to quantify, resulting in a combined list of over 100 native candidate species. Furthermore, non-native finfish species that are imported for U.S. consumption are candidates for domestic aquaculture production in land-based closed containment systems. USDA agencies will seek to develop partnerships in support of optimizing production efficiency for promising marine finfish, provide information and technologies that facilitate expansion of domestic aquaculture in state and federal marine waters where permissible, and support research that enhances land-based production systems.

A DIET ENRICHED WITH TRYPTOPHAN CAN SUPPRESS POST-SHIPMENT STRESS IN *Amphiprion ocellaris*

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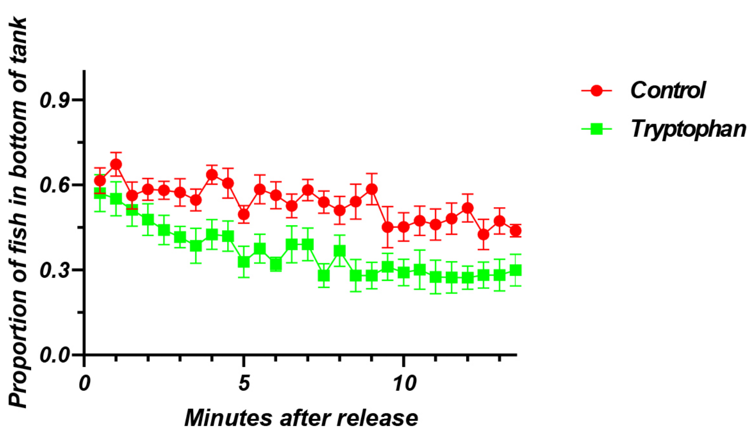
Post-shipment stress is a major cause of mortality in the trade of ornamental fish. During transit, fish are subjected to a number of stressors, including deteriorating water quality, crowding, and confinement stress. Dietary supplementation with tryptophan has been shown to reduce crowding stress in fish. In this experiment, we studied the effects that a conditioning diet enriched with tryptophan had on post-shipping stress in the marine clownfish, *Amphiprion ocellaris*. Stressed and anxious fish prefer swimming in the bottom of a tank, sometimes remaining immobile. We hypothesized that fish fed a diet rich in tryptophan would show less post-shipment stress. A total of 332 juvenile clownfish were divided into ten 76L tanks. All the tanks were connected to our multi-tank 1,113L system. Five tanks were designated as diet A and five tanks were designated as diet B. The experimenters did not know which of the two diets was enriched with tryptophan. The fish were fed their designated diets for a period of seven days. On day 9, approximately 15 fish from each tank were bagged. Each bag was filled with one liter of water and several liters of oxygen, and placed in a covered box at room temperature for 24 hours. On day 10, fish were released into a 38L holding tank following a five-minute acclimation period. After the fish were released, their behavior was recorded with a video camera for 15 minutes. The fish vertical position in the tank was observed every 30 seconds. During the first two minutes of recording, a majority of the fish in both treatment groups stayed in the bottom 1/3 of the tank. Afterwards, more fish in the tryptophan-enriched diet left the bottom of the tank to explore the tank compared to fish in the control diet. During the last two minutes of observation, there were more fish in the bottom of the tank in the control group ($p<0.001$). Thus, fish fed a diet enriched with tryptophan exhibited less anxiety and stress when moved into a new tank after spending 24 hours in a shipping bag. Considering that 48 hours had elapsed from the time the fish was fed to the time the observations were made, the effects of tryptophan on the stress axis seem to be consistent. Thus, tryptophan-enriched conditioning diets should be considered to minimize shipping and post-shipping mortalities in the marine ornamental industry.



ANALYSIS OF EFFECTS OF ENVIRONMENTAL FLUCTUATIONS ON THE MARINE MYSID CULTURE AND ITS DEVELOPMENT AS A FOOD SOURCE FOR FINFISH

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Mysids are among the most important food items for animals in aquaria and that support fisheries, but information on their performance in controlled culture systems is still limited. We reared the marine mysid *Neomysis awatschensis* in a controlled laboratory system, and measured its growth, 20–hydroxyecdysone (20E) levels, molting, and survival in response to environmental fluctuations in temperature, pH, and salinity, and inferred their potential associations based on annual field sampling. The 20E levels were significantly elevated during the postnaupliod stages, and even higher levels of 20E were maintained in the adult stages than in the nauplioid stages. Values of growth parameters (i.e. total length and the lengths of the antennal scale, expod, endopod, and telson) and 20E levels were higher during a 40-day period at 25 °C than at other temperatures, with shorter intermolt intervals, although morality was also increased. Among the surviving mysids, the number of newly hatched juveniles produced was higher for females exposed to 20 °C than that in other groups. Relatively higher growth and survival rates were measured at salinities over 25 practical salinity, while lower salinities under 15 practical salinity significantly reduced growth and survival. The number of newly hatched juveniles was lower at salinities under 15 practical salinity compared to those over 20 practical salinity. Overall, low temperature and salinity reduced mysid reproduction and the maintenance of the second generation. In the case of pH variation (pH of 7.0–8.0), there were no significant effects on growth and the number of newly hatched juveniles, although the survival rate was slightly lower and the 20E level fluctuated at a pH of 7.0. We believe that these associations between growth and environmental conditions can provide crucial information for optimizing mass mysid culture for a reliable food source in aquaculture and fisheries.

TILAPIA PRODUCTION IN BRAZIL: AN ANALYSIS OF EMERGING POLES

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Tilapia is the most farmed fish in Brazil, its production continues to expand being consumed almost entirely by the domestic market. The recent drawback mechanism and environmental release laws for production in areas of the Amazon biome are two key elements in expanding and sustaining Brazil's exports. Based on the Global Value Chain (GVC) approach. This research aims to analyze two emerging tilapia poles in Brazil, comparing them with other poles that have existed longer in the country.

First, we defined a series of indicators corresponding to the six dimensions of the GVC (i.e. input-output structure; governance; updating; local institutional context; industry stakeholders) capable of establishing a comparison between the poles. As many of the elements of GVC's analysis are essentially based on qualitative data, we use the fuzzy logic to consolidate these indicators in quantitative terms.

In the first trimester of 2019 we collected data from the youngest Brazilian poles of Boa Esperança (Northeast) and Serra da Mesa / Cana Brava (Central West), as well as six others scattered in other regions of country, the dataset totaled a sample of 569 production units. Finally, we calculated the indicators and aggregated them spatially, which gave us a deep profile of each pole.

As a result, we identified at the producer level a set of important elements for the structuring of emerging poles, most of them related to the access to and availability of financial resources, infrastructure and input networks. In addition, in relation to the older poles, we identified other important factors to facilitate their definitive entry into global chains.

BACTERIOPHAGE THERAPY TO REDUCE PATHOGENIC VIBRIOS IN MOLLUSCAN SHELLFISH AQUACULTURE

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Minimizing the effects of vibrios in molluscan shellfish is a formidable task. From larval mortalities caused by pathogens like *Vibrio coralliilyticus* (Vc) and *Vibrio tubiashii* (Vt) to human pathogenic vibrios, like *Vibrio parahaemolyticus* (Vp) and *Vibrio vulnificus* (Vv) in market shellfish, new innovative approaches are needed to alleviate outbreaks. Larval oyster mortalities in the US have reached unprecedented levels with the largest East Coast hatchery experiencing major losses in 2019 due to unexplained mortality events. West Coast hatcheries continue to experience sporadic mortality events that have limited the availability of seed oysters needed for commercial aquaculture operations. Many outbreaks have been associated with Vc, which is more pathogenic to shellfish at elevated seawater temperatures. Interventions to reduce vibrios in shellfish have become more urgent as vibrio pathogenicity in the environment appears to be increasing. Technological advancements, like the development of bacteriophage-based methods to reduce Vc and Vt in hatcheries, are showing great promise, while the application of bacteriophage (phage) therapy against vibrios is expected to reduce pathogenic vibrios in market shellfish.

We isolated and characterized from Hawaiian seawater phages that have broad specificity toward eight strains of Vc. A three-phage cocktail reduced larval oyster mortalities by over 80% in hatchery trials. The cocktail had no negative effects on larval development or motility. Commercialization of this cocktail is being sought.

During a survey of Delaware Bay oysters, we frequently isolated phages against potentially dangerous, pandemic strains of Vp from three harvesting sites. Characterization of phage isolates revealed tailed and non-tailed phages. These phages are earmarked for short-term treatments in phage therapy trials of market-sized oysters in a depuration-like system. Phage-based technologies are offering new avenues to enhance shellfish hatchery and commercial oyster operations.

FIGURE 1. Electron micrographs of phages against Vc (left) and Vp (right)

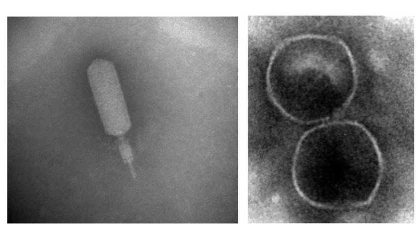
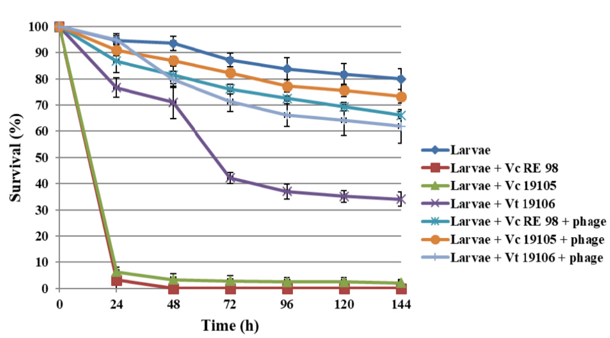


FIGURE 2. Survival of larval oysters from Vc and Vt strains using phage therapy



TEMPERATURE EFFECT ON OXIDATIVE STRESS AND DEPURATION EFFICIENCY OF DIFFERENT BIVALVE SPECIES PRODUCED IN AQUACULTURE

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Consumption of bivalve mollusks may represent a risk to human health. Depending on the microbiological load, bivalves require depuration before commercialization, to ensure food safety. Production areas (e.g. in EU countries) can be classified in A, B or C, based on *Escherichia coli* load, which must not exceed 230, 4600 and 46000 most probable number (MPN) of *E. coli* per 100 g of flesh and intravalvular liquid, respectively. Usually, depuration last for 24 h in recirculating systems (equipped with filters, UV sterilizers and temperature control systems - water chillers), using the same water parameters for all purified species. However, due to physiological specificities, it might be relevant to adapt water parameters, namely temperature, to the physiological requirements of each species, in order to optimize the depuration process. We aimed to test the effect of temperature on depuration efficiency, cellular energy allocation and oxidative stress and damage of clams (*Ruditapes decussatus*) and razor clams (*Solen marginatus*) produced in class C areas.

Bivalves, harvested in Algarve, south Portugal, were depurated during 24 h in 250 L modular depuration systems equipped with a filtration system and a UV-c unit (25W UV-c, 6.000 μW.s/cm^2), testing 4 temperatures: 10, 15, 20 and 25 ºC. Samples for microbiological and biochemical analyses were performed at 0 h (arrival) and 24 h (depuration period). Microbiological load, antioxidant defenses (catalase, glutathione S- transferases, and total glutathione), oxidative damage (lipid peroxidation and DNA damage) and cellular energy allocation were evaluated.

Bivalve 24 h depuration process (diminution of *E. coli* levels to legal commercialization values) was successful in all tested temperatures. However, *R. decussatus* performed better at 20 ºC (<18 E. coli/100 g) and *S. marginatus* between 10 and 15 ºC (69 and 62 *E. coli*/100 g, respectively).

No changes were observed on antioxidant defenses. *R. decussatus* seemed to have a higher range of temperature tolerance, with DNA damage increasing only at 25 ºC, whereas temperatures above 20 ºC caused increase in *S. marginatus* DNA strain breaks. Regarding energy consumption, *R. decussatus* presented a higher activity of electron transport system than *S. marginatus*, although no significant changes in energy consumption were observed due to water temperature (Fig. 1). This study highlighted the relevance of considering species-specific requirements to improve bivalve depuration process and food safety.

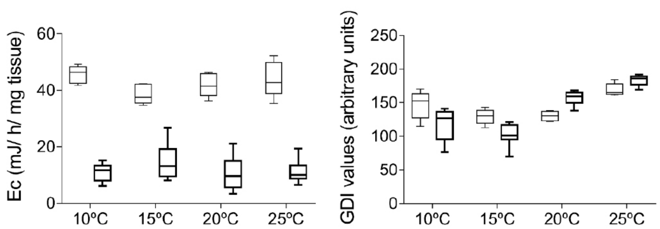

FIGURE 1. Energy consumption (Ec) and DNA damage (GDI) of *Ruditapes decussatus* (grey line) and *Solen marginatus* (black line) after 24 h depuration under different temperatures.

EVALUATION OF THE PRESENCE OF MICROPLASTICS IN COMMERCIALLY IMPORTANT FISHES IN NORTHWESTERN MEXICO: AN ALTERNATIVE FOR THE SUSTAINABLE MANAGEMENT OF FISHERIES

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The microplastics in the marine environment are described and reported since 1970. However, Thompson in 2000 was the one who first coined the descriptive term for these, the same that denominated as those particles or fragments of plastics < to 5 millimeters of diameter. Since then, research in the world has increased significantly, predominantly in controlled aquaculture and laboratory. The most recent studies of microplastics have conducted in fish, water, and sediment.

Nowadays, it is possible to observe contamination by plastics in practically all the oceans of the world; this includes the most remote areas known to humans.

The objective of the present study was to examine the presence of microplastics in the digestive tracts of the most commercialized marine fishes species for human consumption in the northwestern part of the Mexican Pacific.

Four quarterly samples were carried out, during an annual cycle, obtaining a total of 720 organisms of six different species, captured using artisanal fishing techniques. Also, were examined some samples of water and sediment from the caught area.

In the laboratory, the intestines of the caught fishes were analyzed, applying 30% hydrogen peroxide (H2O2) to disintegrate the organic matter. Residues filtered on cellulose paper (Whatman number 40), then the filter was dried at 50 ° C and the microplastics identified. In the case of water and sediment samples, an adaptation of the technique described above made, adding zinc chloride (ϱ = 1.5 g / l) as an extra reagent to obtain particles through buoyancy.

Of the six species of fish analyzed, three of them presented contamination by microplastics. The size of these contaminants varied from 0.33 mm to 4.8 mm in diameter. The most frequently found microplastics were Nylon fragments, followed by pieces of plastic bags (polyethylene) and particles derived from PET containers. The present study shows a relationship between the types of microplastics in marine fish's intestines and those plastics observed in sediment. For this reason, it suggested that; Sediment contamination is a risk factor for the health of the ichthyofauna present in the studied area.
Keywords: Microplastics, Marine fishes, water, sediment.

OXIDATIVE STRESS AND HISTOPATHOLOGICAL CHANGES IN COMMON GOLDFISH WHEN EXPOSED TO MICROPLASTICS

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Plastics are inextricable to the everyday lives of most people around the world, leading to substantial plastic waste. When plastics are inappropriately disposed of, they end up in natural waterways that eventually degrade into smaller pieces, known as microplastics (MPs) that range from 1 to 1,000 µm in size. Initially, it was believed these pose little to no danger to fish. However, increasing research is showing MPs can adversely affect energetic status, digestive enzyme activity, reproduction, oxidative status as well as the internal organs. The aim of this study was to examine the effects of increasing MP exposure, in the form polyvinyl chloride (PVC) at 0, 0.1 or 0.5 mg/L on the oxidative status and histological changes in goldfish, *Carassius auratus*, after 4 days.

The MPs were confirmed to contain no heavy metals, polyaromatic hydrocarbons, polychlorinated biphenyls, or phthalates. Approx. 10% were <40 mm, 50%<140 mm, and 90%<310 mm. MPs were directly added to the water. Each treatment was triplicated with 5 fish in each and the study duration was 4 days. Aeration was provided, which was sufficient to keep the MPs in suspension throughout the study. After 4 days, the blood was drawn to measure the hemoglobin and hematocrit while the gills and liver/intestine were removed fixed for later histological examination. Meanwhile, the gills, liver and brain were sampled for oxidative parameters.

Based on histological examination of the intestine, MPs were clearly present in the goldfish exposed to 0.1 or 0.5 mg/L. Despite their presence, damage was not observed nor to the gills. In contrast, the liver showed some classic instances of inflammation (lipofuscin-like material and white blood cell infiltrations) and vacuolization (Fig. 1). This was supported with preliminary analysis indicating the liver exhibited oxidative stress. However, hematocrit, hemoglobin or viscerosomatic indices were unaffected.

Clearly, MPs are not harmless to fish, based on the adverse histopathological changes under environmentally relevant concentrations. Due to the ubiquity of MPs in aquatic systems, further research should examine any adaptive strategies fish might have to cope with such challenges.

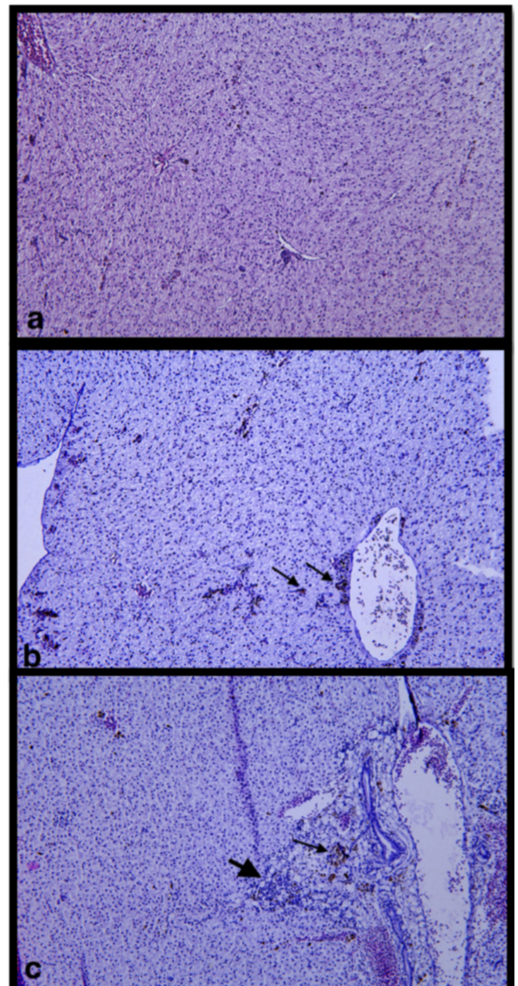

Fig. 1: Liver histology of goldfish in the control (a), 0.1 MP/L (b) or 0.5 mg/L treatments. Livers in 0.1 mg/L showed lipofuscin (arrow), which was also observed at 0.5 mg/L along with white blood cell infiltrations (arrowhead)

TRANSLOCATION OF INGESTED MICROPLASTICS IN TILAPIA AND POSSIBILITY OF HABITUATING TO SUCH INGESTION

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It has been estimated that trillions of plastic particles are currently floating on the surface of the oceans and can degrade to form microplastics (MPs) that range in size from 1 – 1000 µm. The ingestion of MPs can alter digestive enzymes and cause severe histopathological damage in some fish species. This could be due to their physical presence via translocation, but has only been shown in a few studies. The aim of this study was to fed tilapia MP contaminated feeds (at 0.1 or 1.0%) and the fish were sampled at 2 week intervals for plasma biochemistry, intestinal digesta short chain fatty acids (SCFA), potential presence of MPs in the liver, gills and muscle as well as liver histopathology, trypsin activity and HSI.

Diets (isontirogenous/isolipidic) were formulated to meet the requirements of tilapia. MPs were added at expense of cellulose. Treatments were triplicated with 10 juveniles (23 g) in each and fed twice daily to apparent satiation. After 2 weeks, fish were sacrificed, sampled and measured for the above parameters, and this was repeated after another 2 weeks.

At both weeks, feed intake significantly decreased in the 0.1 and 1.0% MP treated diets. Fish often repeatedly expelling and chewing the food. MPs were detected in the gills, liver and muscle, indicating translocation. Liver histopathology revealed severe damage that included hemorrhaging and extensive necrosis (Fig. 1). Both liver trypsin activity and HSI were also significantly lower along with intestinal digesta SCFA. After another 2 weeks, these parameters normalized to the control with the liver only showing some localized instances of inflammation. MPs in the liver also decreased.

These findings have been facilitated by excreting MPs, as the number of translocated MPs in the liver decreased, but the exact mechanism(s) for this finding should be explored further.

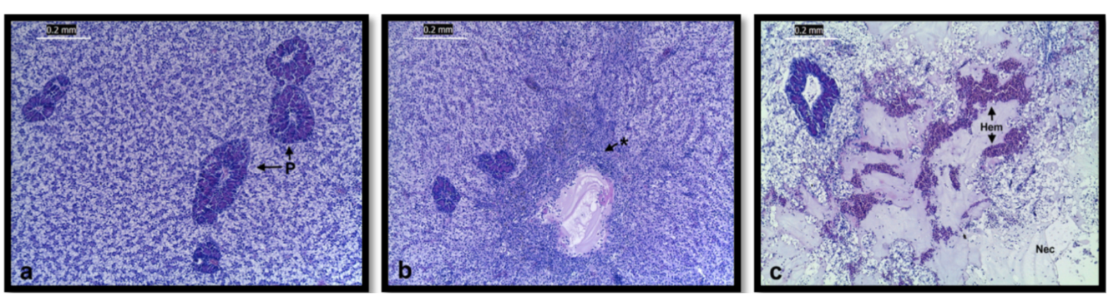

Fig. 1: Histopathology of fish in control (a), 0.1% (b) or 1.0% (c) dietary treatments after 2 weeks. Liver in 0.1% showed substantial inflammation (asterick) while at 1.0%, there was extensive damage that include hemorrhaging (Hem) and necrosis (Nec).

TIMING OF PUBERTY IN ATLANTIC BLUEFIN TUNA - THE ENDOCRINE APPROACH

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Over the past few decades, aspects relating to reproductive potential of Atlantic bluefin tuna (ABFT) have attracted growing public and scientific interest. This is largely due to the implications of impaired propagation on the species' stock recovery. Nonetheless, determining the indicators of the exact timing of puberty (= first sexual maturity) in ABFT and the capacity of a population to seasonally spawn and produce viable fry, remain elusive.

In this regard, a recent European study that closely followed captive-reared ABFT undergoing first sexual maturity, has successfully established a novel endocrine criteria for differentiating sexual-immature from sexual-mature specimens. The growth parameters recorded for captive ABFT juveniles were consistent with the length-weight relationship established for wild Mediterranean ABFT stocks. In addition, the histological analyses of the gonads indicated advanced sexual maturation in ABFT captive males compared to females. However, no robust conclusion could be drawn addressing whether the observed puberty in 3-year (3Y) ABFT males is a general feature also occurring in wild populations, or a phenomenon induced by the culture conditions.

Measurements of the two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are the pivotal regulators of gonadal development and gamete maturation, revealed a clear discrepancy in the pituitary FSH to LH ratios in immature vs. mature ABFT. A 3-fold higher FSH to LH ratio characterized the 2-year (2Y) sexually immature fish, which contrasted to the <1 ratio in the other two groups consisting of (a) 3Y pubertal males and immature females and (b) fully mature adults. Moreover, a similar FSH/LH ratio benchmark has been reported in mammals and humans. In these studies, the intra-pituitary mechanism that prepares the gonadotrope cell population for puberty is represented by an increase in the storage of LH accompanied by a comparable decrease in the storage of FSH and a subsequent decrease in the FSH to LH ratio. Clinical studies that monitored circulating gonadotropin levels in children undergoing pubertal development revealed that the FSH to LH ratios, higher or lower than 1, reliably discriminate between pre-pubertal and early pubertal groups, respectively. Interestingly, both western and eastern ABFT stocks exhibit comparable age and size at sexual maturation, which undermines the previous assumption that western ABFT mature at a much older age than eastern fish.

In summary, the useful FSH/LH criteria for screening sexual maturity among ABFT populations exemplifies the potential of the endocrine approach to identify clinical biomarkers for assessing a vast array of reproductive traits. Following the same rational, future studies combining new advances in genetics and endocrinology may further our understanding of the interplay between energetic balance, growth and maturation in ABFT. As a result benchmarks could be determined enabling discrimination between ABFT populations that successfully spawn and those who fail to complete their reproductive cycle. Such criteria will, ultimately, help resolve the controversy of "skipped spawning phenomena" among western ABFT stocks.

PERFORMANCE OF LARGEMOUTH BASS *Micropterus salmoides* FED SOYBEAN MEAL-BASED DIETS SUPPLEMENTED WITH GLYCINE, PREBIOTIC, AND NUCLEOTIDES

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Production of food size largemouth bass (LMB) has received more attention in recent years driven by the demand from Asian markets in north America while production in China has jumped from 150 thousand MT in 2013 to over 450 thousand MT in 2017. Commercial feeds for LMB still appear to contain high levels of costly fish meal (FM); whereas recent studies have shown that most of the dietary FM can be effectively replaced by plant-protein ingredients. Supplementation of nutritive or non-nutritive compounds to plant-protein based diets may have significant effects on the production performance and/or health status of LMB. Based on literature information and due to the lack of evaluations in LMB, the objective of this 10-week study was to investigate the potential effects of supplemental glycine, a commercial prebiotic (GroBiotic®-A; GBA), and a nucleotide mix on the production performance, antioxidant status, and intestinal micromorphology of LMB fed soybean meal (SBM)-based diets.

Six experimental diets (a FM-based and five SBM-based diets) were formulated to contain 42% crude protein (CP) and 12% lipid. Four SBM-based diets (GLY, GBA, NCTDs, and COMB) were supplemented either with glycine (2%), GBA (2%), the nucleotide mix (0.15%), or all three supplements combined (respectively). Twenty feed-trained LMB (6.0 g/fish) were stocked in each of 24, 110-L glass aquaria operating as a recirculating aquaculture system. Each experimental diet was randomly assigned to four groups of fish and fed to apparent satiation twice daily. Statistically significant differences between treatments were considered at $P < 0.05$.

Survival of LMB in the study averaged 98.8% and was unaffected by diet. Final weight of the fish ranged from 48 to 61g and weight gain from 712 to 913% of initial weight. Within SBM treatments, growth of LMB fed the GBA, NCTDs and COMB diets was outperformed by that of fish fed the GLY diet. While a similar growth performance of LMB was observed between GLY and SBM-based control treatments, GLY was the only dietary treatment that did not differ from the FM-based control group (FM-C). Although no improvements in FCE were observed within SBM-based treatments in response to the test ingredients, no differences were found among GLY and FM-C fed groups. Our results showed a slightly but positive effect of supplemental glycine in improving growth performance and FCE of LMB when dietary FM is almost completely replaced (reduced from 44% to 4%) by SBM. Results for intestinal micromorphology and antioxidant status of LMB fed the experimental diets will be presented.

ON-FARM TANK SYSTEMS DEPLOYED ON COMMERCIAL SHRIMP FARMS: A LOW BUDGET APPROACH THAT WORKS FOR EXTENSION DEMONSTRATION

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There are many different approaches that can be implemented when carrying out on-farm Extension demonstrations in aquaculture. The design and approach used by Extension professionals is often governed by the particular problem or question that needs to be addressed as well as budgetary constraints within Extension programs. West Alabama is home to a unique inland marine shrimp industry that uses a low salinity artesian ground water (1 – 11 g L^{-1}) source to fill earthen ponds for semi-intensive production. While shrimp farmers use the same aquifer to fill their ponds, the ionic composition and salinity of the water source is quite variable. For the last 15 years, research, demonstration, and technology transfer have been carried out in west Alabama using low budget on-levee tank systems that can be set up and operated by Extension personnel on commercial shrimp farms using this unique water source (Fig. 1). In addition to using these tank systems to help answer a number of pressing questions faced by the inland shrimp industry they have been used to test new diet formulations and feed management regimes on commercial farms. Collectively, these Extension demonstrations on shrimp farms have resulted in 10 refereed journal articles as well as a number of popular and Extension publications since 2005. While carrying out on-farm Extension demonstrations can be challenging, Extension professionals must increasingly use innovative approaches to carry out relevant and practical on-farm technology transfer via traditional and non-traditional demonstration techniques. A historical view of west Alabama demonstrations using on-farm tank systems will be discussed; including funding sources for Extension work, on-farm Extension trials with limited availability of university full-time equivalents (FTEs), and coordination with farmers.

Fig. 1. On-farm Extension demonstration at a commercial shrimp farm in Boligee, Alabama.

THE MONITORING OF ABANDONED, LOST AND DISCARDED FISHING GEAR THROUGH CITIZEN SCIENCE: A GLOBAL APPROACH

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Marine debris has been substantially increasing over the past decades and most of it is made of synthetic material. Current estimates available for annual plastic mass emissions into the ocean are mostly accounting for terrestrial sources but do not include an important component of the plastic mass budget found in marine waters; fishing gear. Indeed, there is relatively limited information available on the levels of abandoned, lost and discarded fishing gear (ALDFG) on a yearly basis worldwide. However, we are aware that the amount, distribution and effects of ALDFG have risen substantially over recent decades with the rapid expansion of fishing effort, fishing grounds, and the transition to synthetic, more durable and more buoyant materials used for fishing gear. For this reason, there is a need for more robust estimates of the amount of ALDFG generated each year to add this important component to annual plastic mass emissions into the ocean. Here, we present a citizen science project where volunteers from around the globe collected information on ALDFG in different fishing harbors. Using the seven most commonly types of fishing techniques around the world, 16 volunteers from 14 countries helped collecting information using standardized surveys. Preliminary results will be presented here along with the method used and some of the challenges encountered.

INTERACTIVE DIETARY EFFECTS OF CARBOHYDRTATE AND LIPID LEVELS IN THE PRESENCE OR ABSENCE OF BILE ACID ON LARGEMOUTH BASS (*Micropterus salmonides*) PERFORMANCE

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Fish oil is the preferred source of oil in fish feeds as it is high in ω -3 fatty acids like EPA and DHA. However, fish oil is a finite resource that needs to be reduced or replaced in fish feeds by using sustainable alternatives. Carnivorous species like largemouth bass (*Micropterus salmonides*) are metabolically adapted to low dietary carbohydrate (CHO) utilization, thus, diets containing more CHO can reduce their growth performance. Therefore, the goal of this study was to induce higher CHO utilization in largemouth bass by reducing lipid inclusion in the diets and assessing whether dietary bile acid (BA) supplementation could enhance lipid utilization. Hence, three variables were the subject of study: lipid and CHO inclusion level and bile acid supplementation. A total of 8 isonitrogenous (40% crude protein) diets were formulated: 4 diets containing 9% lipid (low, L) and the other 4 diets having 12% lipid (high, H); both 9% and 12% combined each of the following diets: 1- low CHO (20%) + 0% BA; 2- low CHO (20%) + 1.5% BA; 3- high CHO (30%) + 0% BA; 4- high CHO (30%) + 1.5% BA. A total of 480 fish initially weighing 6.0 g average were randomly distributed into 24 tanks (20 fish per tank) and fed each respective diet for 8 weeks.

Results show that performance of fish was highest in diet 7 (H-CHO + L-lipid + BA), which was statistically similar to diet 1 (control) (Fig 2). Feed conversion ratio was unaffected by dietary treatments, whereas feed intake was significantly affected.

Liver histopathology showed that the combination of H-CHO (30%) and H-lipid (12%) (diet 2) led to extensive vacuolization and granulomas. However, H-CHO (30%) and L-lipid (9%) supplemented with BA mitigated these adverse effects. Genes expression profile related to carbohydrate and lipid metabolisms are being analyzed in the liver and muscle, which will be presented. Overall results indicate that lipid can be reduced by increasing the inclusion of CHO in the presence of BA supplementation for largemouth bass diet.

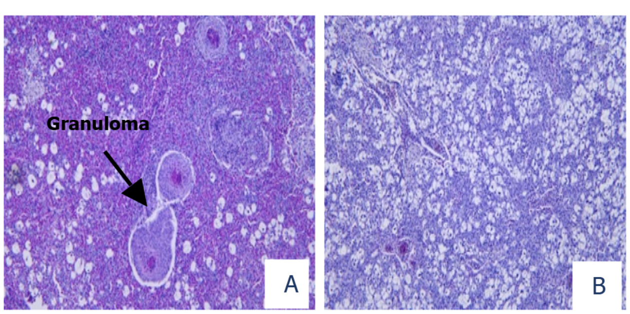
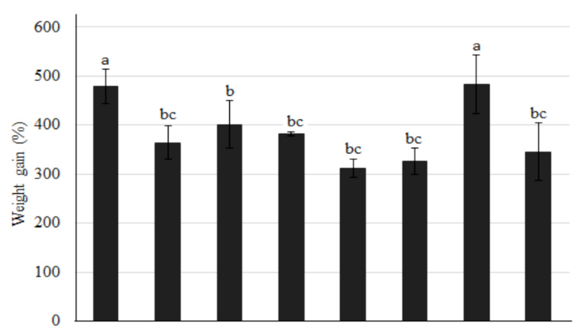


Figure 1: Liver histology (Magnification 400X) (A) H-CHO, L-lipid with no bile shows granulomas (B) H-CHO + L-lipid + BA mitigated granulomas.



CHO	L	H	H	L	L	H	H	L
Lipid	H	H	L	L	H	H	L	L
Bile	-	-	-	-	+	+	+	+

Figure 2: Results of growth performance

THE ROLE OF FOOD VALUES, TRUST IN REGULATORY AGENCIES AND PRO ECONOMIC PERCEPTIONS OF AQUAULTURE IN GRANTING SOCIAL LICENSE TO OPERATE

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Purpose: The purpose of this paper is to examine variables related to trust in aquaculture within the context of social license to operate.

Design: This study uses a random sample survey of Wisconsin residents on their perceptions of aquaculture at a critical time point in industry development within the state. Analysis was conducted using OLS regression, with variables entered step-wise in assumed causal order.

Findings: We find that a preference for gmo free/organic foods is negatively related to trust in aquaculture. In contrast, a preference for locally grown foods, trust in government agencies (i.e. the USDA, FDA), the perception that aquaculture supports jobs in Wisconsin, and the belief that aquaculture is good for Wisconsin are positively related to trust in aquaculture, and collectively account for the largest portion of explained variation in trust among respondents. Identifying as a female, conservative, and a respondent's perception that s/he has greater environmental knowledge are also positively related to trust, but explain less variation overall.

The lack of a direct or indirect relationship between trust and perceptions that aquaculture reduces pressure on wild fisheries, and that aquaculture is a sustainable source of fish, is one of the more interesting findings of this study. Implications of this will be discussed

Implications: Results imply that communication emphasizing pro-economic benefits of the industry, local sourcing, and government regulation of aquaculture may be effective in emphasizing trust in aquaculture among members of the public. Strategic implications of this will be discussed.

Dependent Variable: Trust in Aquaculture	
	Std. Coeff.
Demographics	
Age	-.08
Gender (female=high)	.15***
Race (Caucasian=low)	-.02
Education	-.05
Income	.01
Incremental R^2	*1.2*
Political Ideology	
Conservative ideology	.15**
Liberal ideology	.01
Incremental R^2	*1.2*
Perceived Knowledge	
Aquaculture	-.03
Environmental	.13*
Incremental R^2	*1.4*
Food Values	
Locally grown	.12*
GMO free/organic	-.18***
Incremental R^2	*4.4****
Trust in Regulation	
Government agencies	.43***
Incremental R^2	*22.3****
Environmental Perceptions	
Reduces pressure on wild fisheries	.02
Sustainable source of fish	*.01*
Incremental R^2	*2.5**
Pro Economic Perceptions	
Supports Wisconsin jobs	.14**
Good for Wisconsin	.17***
Incremental R^2	*4.8****
R^2	***37.6*****
*** p≤.001; **p≤.01; * p≤.05	

WHY WE NEED FINFISH AQUACULTURE AND NOAA'S RESOURCES FOR HELPING DEVELOP IT

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Despite recent growth in the shellfish and seaweed sectors, finfish aquaculture in the United States has remained stagnant over much of the last couple decades. At the same time, the US consumer is eating mostly finfish. Of the top 10 seafood items consumed by Americans in 2017, seven were finfish, two were crustaceans and one was a mollusk. The top 10 items have accounted for 84-97% of total US consumption over the last 12 years. Aquaculture's contribution to the top ten averaged over the last two years (2016 and 2017) was 57% by weight; about half from shrimp and half from four fish (Salmon, Tilapia, Pangasius and Catfish). Very little of this was from US production and almost none from marine fish production. Consumption data from 2005-2017 was used to compute the per capita dose of long chain omega-3 fatty acids (LCn-3FA, EPA + DHA) delivered to the average American through seafood. A minimum dose of 250 mg EPA+DHA/d is recommended by numerous health organizations including the American Heart Association. Seafood is the primary natural source of LCn-3FA's and is the dominate source consumed by humans. During the period, the top ten have provided between 72-84 mg/d per capita dose to the average American. Aquaculture provided an average of 55% of the per capita dose of LCn-3FAs in 2016 and 2017. Farmed Salmon provided the largest contribution to American's LCn3FA dose with 29% of the total. To reach dietary recommendations for LCn-3FAs we need to greatly increase our consumption of seafood of all types, however <u>farmed fish are the most promising option to significantly increase LCn-3FA consumption by Americans due to their popularity with consumers, relatively higher concentrations of LCn-3FA's relative to other genera, and the ability to increase the amount of LCn-3FA's in farmed fish through dietary manipulation</u>. How much of this will be produced domestically?

NOAA has a long history of investing in finfish development. For example, NOAA grant programs (Sea Grant, SK, and SBIR) have supported most of the researchers in this session. NOAA labs provide some key infrastructure (e. g. hatchery and feed manufacture) and NOAA products aid in site selection and permitting (e. g. Aquamapper, MarineReports, and other marine spatial analysis and modelling tools/services). Based upon the lack of growth in the industry over the past decades, this has not been sufficient. To that end we are assessing how we can best use our limited finfish aquaculture resources to increase sustainable production of the farmed fish our population desperately needs.

HOW COLLABORATION CAN DRIVE INDUSTRY CHANGE – THE OPPORTUNITIES AND THE CHALLENGES

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More than 37% of the Earth's land surface is reserved for agriculture, producing almost all of the food we eat. The IPCC's Special Report on *Climate Change and Land* (August, 2019) demonstrates how fragile and decreasingly productive that land is. Rapid and large-scale change to the food system is needed to provide nutritious food to a burgeoning global population, without further damaging natural resources.

Recommendations such as those from the EAT Lancet Report, are encouraging us to move towards a plant-based diet. However, due to socio-economic, cultural and access factors, meat will remain in people's diets. We need to consider the most sustainable and nutritious meat options, and that includes seafood.

The oceans cover 70% of the planet, yet provide just 5% of the protein we eat. If responsibly managed, a blue revolution can provide eco-friendly and nutritious food while alleviating pressure on land and protecting ocean biodiversity. The blue revolution isn't a vision for the future; it has begun. Aquaculture provides more seafood for human consumption than wild capture, and demand is growing. If done right, aquaculture can support healthy oceans, healthy people and a healthy planet.

The industry recognizes that if it is to expand to meet demands in a sustainable manner, a step-change decrease in environmental impact is needed. Achieving such radical change requires a new perspective on food production, and a group of salmon farmers, representing approximately half of the global industry, has been trialing a new approach.

The Global Salmon Initiative (GSI) uses collective problem-solving to drive environmental improvements across the entire industry, not just the top performers. This CEO-led change model is unlike any other in the food sector, employing a system of pre-competitive collaboration guided by four key principles: transparency, collaboration, responsibility and innovation.

GSI focusses regional and global efforts on environmental and social priorities. By combining expertise from within the group as well as from NGOs, external private and public sector partners, the group believes it is easier and faster to innovate and improve – and is seeing results.

Working on the topics of certification, biosecurity, feed sourcing and transparency and public perception, the GSI has been running for 6 years, and this session we will look at how the GSI has implemented its model of collaboration, what progress it is demonstrating, and what challenges the group continues to face. In addition, we shall discuss what is needed to transfer this model to other sectors and how wider collaboration is crucial for the whole aquaculture sector to reach its potential in a blue revolution.

IMPACT OF EXOTIC AFRICAN CATFISH *Clarias gariepinus* (BURCHELL, 1822) ON THE FISH DIVERSITY OF KADAMBA TANK, TAMIRABARANI RIVER BASIN, THOOTHUKUDI, TAMILNADU

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Human activities such as industries, agriculture, aquaculture etc., always impact the ecosystem particularly ecological biodiversity. Riverine ecosystems in India have been suffering from heavy human intervention, which leads to declined fishery of native fish species. Exotic fish species introduction in aquaculture, inadvertently found in native aquatic ecosystem, threats to endemic fishes and native fish fauna. African catfish, *Clarias gariepinus* was introduced to India from Bangladesh which is cultured in northeastern states (West Bengal and Assam) and southern state (Andhra Pradesh), together with the Indian Major Carps. Serious loss to the carp in such mixed culture ponds led farmers to switch over to monoculture of african catfish. The present study was conducted in Kadamba tank (known as half-sea), Tamirabarani river basin, Thoothukudi, Tamilnadu for the period of 12 month (June 2015 to June 2016), to understand the impact of introduced exotic fish species on its ecological diversity.

Fish samples were collected by gill netting and cast netting. The collected specimen were fixed in 10% formalin for further studies. Present study recorded 61 fish species, categorized under 8 orders viz., Cypriniformes (34 species), Perciformes (9 species), Siluriformes (8 species), Cyprinodontiformes (5 species), Anguiliformes (2 species), Beloniformes (1 species), Clupiformes (1 species), and Synbranchiformes (1 species). Among 61 species, 5 were endemic to Western Ghats, 6 were threatened, 5 were vulnerable, 1 was endemic to the Tamiraparani riverine system and 6 were exotic. The exotic fish species recorded during the study were, Tilapia (*Tilapia mossambica*), common carp (*Cyprinus carpio*), grass carp *(Ctenopharyngodon idella*), Mosquito fish (*Gambusia affinis*), African catfish (*Clarias gariepinus,* and Armoured sucker catfish (*Pterygoplichthys disjunctivus)*. A survey was conducted among the local fisher folk indicated decline in native fish species at some stations of the lake with increase in catches of the African catfish (*Clarias gariepinus*). This increase in catches of African catfish at several locations in the lake indicated negative impact on the native fish diversity.

This is the first report about Kadamba tank at Tamiraparanei river basin. Since, *Clarias gariepinus* has spread into natural water bodies, affecting native fish species by way of genetic impact, disease introduction, and catastrophic ecological impacts such as predation, competition and environmental modification. Scientific studies on the impact of this species on the native fish biodiversity in the Kadamba tank and Tamirabharani river system are very scarce. The present effort could initiate more studies in the future that could bring insights into the emerging issue and prompt needed precautionary measures by management of native biodiversity. Further analysis and clarification will be presented in the conference.

ONTOGENY OF OSMOREGULATORY STRUCTURES AND SALINITY TOLERANCE IN ATLANTIC CROAKER, *Micropogonias undulates*

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Atlantic croaker is a popular live bait for many game fish in the Northern Gulf of Mexico. Aquaculture of this species could supply for demand, particularly during the fall and winter seasons when the availability of wild caught croaker in the desired size range for bait is limited. Hatchery culture is currently performed at 30 psu, consistent with conditions encountered by wild croaker larvae during their first few weeks of life. This protocol is challenging in areas where high salinity water is not readily available such as the Mississippi Gulf coast. The purpose of this study is to examine the ontogeny of salinity tolerance and assess the potential for low salinity culture during the larval period.

Broodstock used in the experiment were conditioned at 30 psu and induced for final maturation and spawning using LHRH slow release implants. Newly hatched larvae were stocked in 6 1,000-L tanks for larval rearing at a stocking density of 30 larvae/L. Larval culture was initiated at a salinity of 30 psu. Salinity was gradually lowered to 15 psu starting at 22 dph for 3 larval tanks (LS group) while the remaining 3 tanks (HS group) remained at 30 psu until 68 dph. Groups of 30 larvae were sampled at 1, 5, 12, 18, and 25 dph and directly transferred for salinity challenge to 1-L beakers filled with 2, 10, 20, or 30 psu water(3 replicate beakers per treatment). Beakers were assessed for larval mortalities every 6 hours for up to 3 days, or until all larvae died.

Survival rates through larval rearing did not differ significantly between the LS and HS groups ($p = 0.44$). Larvae were longer in the LS group beginning at 5 dph. There was a significant effect of age, salinity, and the interaction between these two factors on survival duration post transfer during salinity challenges (Fig. 1). Older larvae (12-25 dph groups) survived longer. Survival duration was shortest at 2 psu and longest at 10 psu (iso-osmotic conditions). The transfer of 25 dph larvae to 10, 20 or 30 psu led to similar survival durations. While these results suggest that larval viability may be improved at 10 psu, the actual survival in culture tanks may be reduced due to negative buoyancy at that salinity. Further study of buoyancy and feeding at low salinity is warranted. Larvae from the study were processed for immunohistochemistry to reveal chloride cells through detection of the Na/K-ATPase enzyme and describe their distribution and abundance in different tissues and organs.

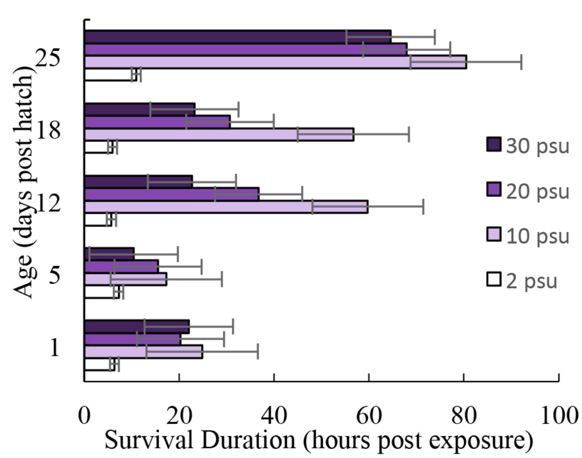

Fig 1. Survival duration (hpe ± SE) for individual larvae in abrupt salinity challenge.

NON-B DISRUPTION IN THE WITH SHIMP *Litopenaeus vannamei*

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In a previous study, the frequency of three different trypsin phenotypes of *Litopenaeus vannamei* in aquaculture was described. Phenotype CBA was the most abundant, followed by phenotype CB, but phenotype CA decreased its frequency and was not found in shrimps after 45 days of culture (Fig 1).

The conclusion was that shrimp with phenotype CA inexplicably die under culture conditions (Aguiñaga-Cruz et al 2017). The question was if the process of shrimp aquaculture exerts excess pressure on the CA organisms, will they die? or will they die no matter where they grow? To answer this question, the trypsin phenotype frequencies in 60 breeder shrimp of a natural population were analyzed to predict the trypsin phenotype frequency in the next generation. Then, a total of 1000 *L. vannamei* shrimp within the F1 generation of the same natural population weighing 2, 3, 5, 7 and 15 g were collected for 5 months, and the trypsin phenotype frequencies were recorded. The results indicate that the F1 is out of Hardy–Weinberg equilibrium, and the absence of phenotype CA in the natural population is similar to that in aquaculture (Fig 2). In conclusion, the process of aquaculture does not exert pressure on the CA phenotype; such mortality is associated with another phenomenon not yet understood and deserving of further examination. At the moment, we know that the mortality occurs when shrimp inherit a trypsin codominant allele monomorphic called A, which act as lethal gene, and thus, we named the phenomenon "NON-B disruption".

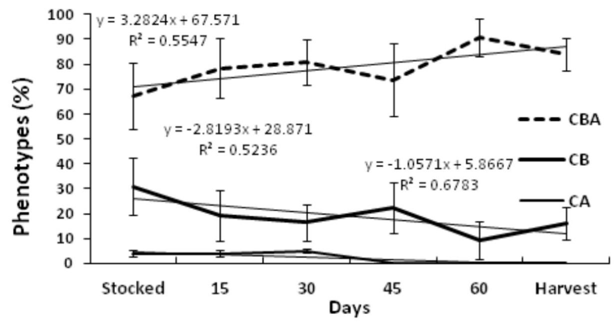
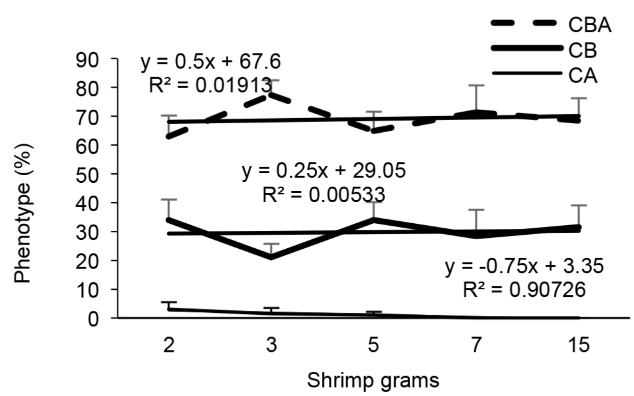


Fig 1. Trypsin phenotypes distribution in Aquaculture

Fig 2. Trypsin phenotypes distribution in a natural population



A SIMPLE MODEL FOR MEASURING PRODUCTION PERFORMANCE IN LABORATORY AND SHRIMP FARMING

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Shrimp production depends on indicators that allow producers to make decisions about the management of one or multiple variables that promote a good production performance rate. Indicators such as the weekly growth (WG), survival fraction (SF), and feed conversion rates (FCR) are important in aquaculture for decisions making process. However, although the application of indicators individually represents a useful and common practice, the combination of some of them to obtain additional information can be a powerful tool for making better decisions.

The goal of this work was to analyze a simple model to measure the production performance rate (PPR) FCR [(WG x SF)/ FCR = PPR], so that it can be used to improve shrimp farming practices. To analyze the model, two trials were performed: a feeding laboratory experiment testing 22 commercial feed types, and the output production results of 42 commercial farms under their common operating conditions. The results showed the convenience of the PPR model. In the laboratory experiment, different feeds led to significant differences in PPR, making it possible to distinguish among feeds with high, regular or low performance (Fig1). In commercial farms, the results were less clear, but it was possible to attribute low, regular and high levels of PPR to differences among farm management practices (Fig 2). Our results lead us to advocate for the use of PPR to evaluate shrimp culture production using simple measurements of weekly growth, survival, and FCR.

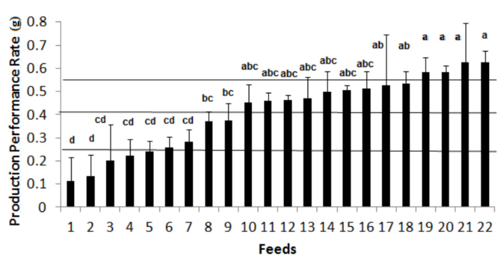
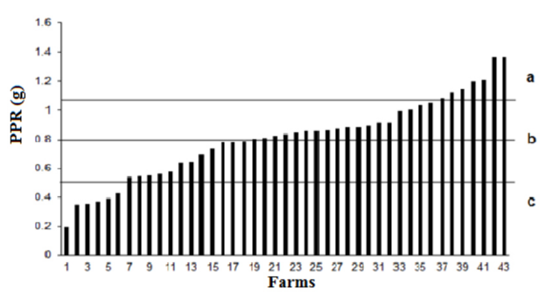


Fig 1. Production Performance Rates obtained by testing different feeds for shrimp cultured in a laboratory experiment.

Fig. 2. Production Performance Rates in different shrimp farms.



EFFECTS OF DIFFERENT LEVELS OF DIETARY PROTEIN AND LIPIDS ON GROWTH PERFORMANCE AND DIGESTIVE ENZYMES OF RABBITFISH (*Siganus rivulatus*), REARED IN WELL WATER

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Rabbitfish *(Siganus rivulatus)* is a potential candidate for warm water marine aquaculture production. Until now, rabbitfish aquaculture has not produced on a commercial scale. That may be due to lack of knowledge of its nutritional requirements. Moreover, inadequate protein and lipid levels in the diet lead to an impaired growth. Thus, the determination of the suitable ratio of protein to energy in fish diet is important. Accordingly, the purpose of this study was to determine optimal protein to lipid ratio and its effect on growth performance and digestive enzymes of *S. rivulatus*.

A 2×2 factorial feeding trial was carried out. Four semi-purified diets containing 2 protein levels (30% and 40%) and 2 lipid levels (6% and 12%) were formulated and fed to triplicate groups of Rabbitfish (*S. rivulatus*) juveniles (0.32 ± 0.16 g /fish) for 56 days.

Fish fed on P40L12 and P40L6 diets gave the best final weight (FW), weight gain ratio (WGR), daily gain index (DGI) and specific growth rate (SGR), followed by P30L6 and P30L12 (Table 1). The dietary levels of protein and lipids have been well reflected on the digestive enzymes of fish (Table 2). The highest value of protease has been recorded at P40 with both lipid levels followed by P30L12. Fish fed on P30L6, P30L12, P40L6 to P40L12, respectively, showed an increase in lipase upward. The highest result of amylase has been detected in P30L6 followed by P30L6 and P40L6. The highest value of glutamate dehydrogenase has been given at P30L6 and then the results were plateaued.

Table 1. Effects of dietary protein and lipid levels on growth performance of *S. rivulatus*

Diet		IW (g)	FW (g)	WGR (%)	DGI (%)	SGR (% day^{-1})
Protein	**Lipid**					
P30	L6	0.33± 0.02	1.55± 0.07^b	375.7± 0.16^b	0.73± 0.04^b	2.79± 0.01^b
	L12	0.33± 0.01	1.85± 0.06^b	461.8± 34.60^b	0.91± 0.05^b	3.08± 0.11^b
P40	L6	0.32± 0.01	2.57± 0.01^a	713.5± 14.42^a	1.34± 0.01^a	3.74± 0.03^a
	L12	0.30± 0.00	2.31± 0.01^a	666.5± 6.97^a	1.20± 0.01^a	3.64± 0.01^a

Table 2. Effects of dietary protein and lipid levels on digestive enzymes of *S. rivulatus*

Diet		Protease (U mg^{-1}prot)	Lipase (U mg^{-1}prot)	Amylase (U mg^{-1}prot)	GDG (U mg^{-1}prot)
Protein	**Lipid**				
P30	L6	28.00± 3.00^c	9.21± 0.67^d	0.46± 0.02^b	11.98± 0.07^a
	L12	45.00± 3.00^b	12.31 $±1.05^c$	0.62± 0.03^a	9.10±0. 10^b
P40	L6	90.00± 2.00^a	16.22 $±0.64^b$	0.45± 0.01^b	9.86±0. 10^b
	L12	92.50± 2.50^a	18.93 $±0.19^a$	0.34± 0.05^c	8.90±0. 08^b

Glutamate dehydrogenase (GDG)

DETECTION OF A PARASITIC MICROSPORIDIA ISOLATED FROM FARMED AND WILD SHRIMP IN NORTHWEST MEXICO

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The presence of pathologies in aquatic crops as well as in fisheries is a serious problem for the aquaculture industry. Microsporidiosis is a disease caused by an obligate intracellular parasite corresponding to the Microspora phylum, and has gained great relevance due to the severe damage and economic impact on cultured and wild captured shrimp. The aim of this project is to identify the microsporidium that causes milk shrimp muscle in farmed and wild shrimp in Northwestern Mexico through histological and molecular analysis.

Shrimp samples (8 g average weight) were collected with signs of milk abdominal muscle (Fig. 1) in farms and fishing regions of Southern Sonora, Sinaloa and Nayarit. The analysis performed at the Aquatic Health Laboratory (Laboratorio de Análisis de Sanidad Acuícola) of the Instituto Tecnológico de Sonora, included histopathology (Hematoxylin-Eosin) with fixed whole organisms to determine the cellular damage of the infection in different shrimp tissues; with endpoint PCR and sequencing, the genus of the associated microsporidia was determined.

The incidence of infected shrimp (milk muscle) appeared in farms of the three states, but in captured wild shrimp was detected only in Sonora and Sinaloa. An abundant invasion of spores (2 μm) in abdominal muscle tissue, and a lesser presence in the intestine, hepatopancreas and pleopods was determined by histopathology (Fig. 2). The infected species correspond to *Penaeus vannamei* and *P. stylirostris*.

Fig.1.- Infected shrimps with white and milk abdominal muscle collected from Nayarit.

Initially, DNA from infected tissue samples was used to amplify the 18S rDNA gene by PCR. 1,200 bp fragments were obtained and sequenced. The microsporidia identification corresponds to *Perezia sp*. (previously *Pleistophora sp*.), according to the Genebank database of NCBI. Another PCR analysis with high quality DNA and specific primers CDS (Cotton Shrimp Disease) were performed. The amplified products (440 bp approximately) confirm the presence of *Perezia sp*. microsporidia in analyzed shrimps.

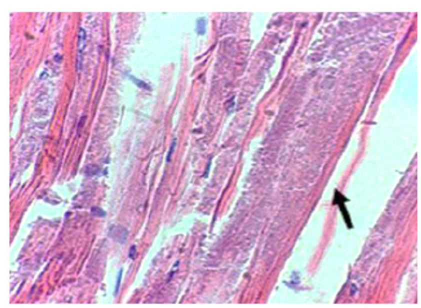

Fig.2.- Histological sections of muscle invaded by microsporidia spores (40X), HE staining.

The high degree of damage caused by this parasite through tissue invasion is an important factor to consider to improve biosecurity, diagnosis and health strategies for aquaculture and fisheries.

MARINE TELEOST ORTHOLOG OF CATALASE FROM *Amphiprion clarkii*: MOLECULAR PERSPECTIVES AND ENZYMATIC BEHAVIOR WITH RESPECT TO ITS POTENT ANTIOXIDANT PROPERTIES

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Catalase is an important antioxidant enzyme that virtually exists in all oxygen respiring organisms. It protects the organism against various oxidative stress by eliminating excessive hydrogen peroxide (H_2O_2) generated in the cell and thereby maintaining the correct redox balance in the cells. Two types of catalases have been identified from organisms as classical Fe heme enzymes and catalase -peroxidases which are engaging in different mechanisms. Despite the main function of ROS scavenging ability, several evidences are available for the intervention of catalases in host immune responses according to previous studies. *Amphiprion clarkii* is a so-called anemone fish and in the wild it will always try to find an anemone that can protect it from predators. This fish is one of the most commonly kept anemone fishes among saltwater aquarists. In this study we have characterized catalase from *Amphiprion clarkii* (ClCat) and determined the antioxidant activity of the purified recombinant protein against H_2O_2.

The deduced amino acid sequence of ClCat was identified and it consisted with an open reading frame (ORF) of 1581 bp which codes a protein with 527 amino acids. The molecular weight of ClCat was predicted as 60 kDa with a 7.25 theoretical isoelectric point (pI) followed by an instability index of 26.37. Following the in-silico predictions, ClCat consisted with highly conserved proximal active site signature (64-80) and proximal heme-ligand signature (354-362) which resemble the typical catalase characteristic domain signatures.

The recombinant protein of ClCat (rClCat) was purified using pMAL protein fusion and purification system. Following the functional assays, increased peroxidase activity was obtained with the increment of the rClCat concentration. The potential protective ability of rClCat against oxidative DNA damage was determined by the thiol mixed-function oxidation (MFO) assay. The relative activity of the rClCat was evaluated using a range of temperature and pH conditions in order to determine the favorable reaction conditions of its enzyme catalytic activity. According to the plotted graphs, the highest residual activity for rClCat was obtained within 30 °C to 50 °C of temperature range while optimum pH was obtained at pH 7. These results indicate that the rClCat has detectable peroxidase activity against H_2O_2 and protective activity against DNA damage while exhibiting significant activity towards range of temperature and pH conditions.

MOLECULAR AND FUNCTIONAL INSIGHTS OF PEROXIREDOXIN 3 (HaPrx3) FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*: EVALUATION OF ITS ANTIOXIDATIVE ACTIVITY AND INNATE IMMUNE RESPONSES

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Peroxiredoxins (Prxs) are cysteine dependent antioxidants that play a vital role in regulating the levels of peroxides, peroxynitrite and organic hydroperoxides. Peroxiredoxin 3 (Prx3) belongs to the typical 2-Cys Prx family and thioredoxin like super family with thioredoxin fold. The present study describes the identification, characterization and functional analysis of prx3 (HaPrx3) from big belly seahorse (*Hippocampus abdominalis*). The cDNA with the 726 bp open reading frame encoded 241 amino acid protein with 26.20 kDa molecular weight. HaPrx3 shared the highest similarity and identity with the protein sequence of *Oplegnathus fasciatus* (Striped beakfish) and according to the phylogenetic analysis HaPrx3 clustered within the branch of teleost.

The highest *HaPrx3* mRNA elevation was observed for the ovaries following blood, brain and testis among fourteen tissues of seahorse. The *HaPrx3* transcripts in blood and liver tissues were showed significant upregulation for the immune challenge with lipopolysaccharide (LPS), polyinosinic:polycytidylic acid (poly I:C), *Edwardsiella tarda* and *Streptococcus iniae* displaying their immune defense against pathogenic infection. Furthermore, recombinant HaPrx3 (rHaPrx3) was showed dose dependent DNA protection ability against the reactive oxygen species generated from the metal catalyzed oxidation system. The rHaPrx3 was able to reduce the nuclear fragmentation and apoptotic body formation in the H_2O_2 treated cells. In summary, these findings justify the redox potential during the oxidative stress conditions and innate immune responses of HaPrx3 in *Hippocampus abdominalis*.

INSIGHTS INTO THE REDOX REGULATORY FUNCTIONS OF GLUTAREDOXIN-1 FROM BLACK ROCKFISH (*Sebastes Schlegelii*) WITH MOLECULAR AND EXPRESSION ANALYSIS

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Glutaredoxins (Grx) are versatile disulfide oxidoreductases with CXXC motifs that belong to the thioredoxin fold super-family. They catalyze the reduction of protein disulfides, glutathione (GSH) and protein mixed disulfides via coupled reactions with NADPH, GSH and glutathione reductase. As these redox proteins serve as an inevitable member for the redox homeostasis, it is implicated as a cellular protector against oxidative stress conditions. Further, glutaredoxins involve in iron homeostasis and bind labile 2Fe–2S clusters. The present study was carried out to characterize rockfish glutaredoxin-1 (*SsGrx1*) in molecular, transcriptional insights and ultimately examine the functional aspects. Recombinant *SsGrx1* (*rSsGrx1*) was used to perform the insulin disulfide reduction assay. Transient transfection method and WST1 assay was used to study the *SsGrx1* cell survival functions in FHM cells upon H_2O_2 exposure.

Amino acid sequence analysis indicated the presence of specific motifs and amino acids required for the GSH binding, including $T^{69}V^{70}P^{71}$ motif, $G^{83}S^{84}D^{85}$ motif, K^{20} and Q^{58}. The phylogenetic tree unveiled that all the Grx family members were emerged from a single ancestor and affirmed the evolutionary conserveness of *SsGrx1* with the other Grx1 counterparts. *Larimichthys crocea* was identified as the evolutionary closest member. *SsGrx1* and other vertebrate Grx1 showed higher sequence homology, especially the two catalytic cysteine residues and the TVP motif for GSH binding were 100% conserved among the counterparts. Tissue distribution analysis confirmed an ubiquitous nature of the *SsGrx1* basal expression in naive rockfish tissues with the highest expression found to be in kidney. Immune challenge experiment showed a significant up-regulation of the *SsGrx1* mRNA expression in blood and gills with the injection of LPS, *S. iniae* and poly I:C. During the functional studies, *rSsGrx1* was able to reduce the insulin disulphide bonds in a concentration dependent manner. Further, the cell survival assay against H_2O_2 exposure, exhibited a significant FHM cell survival compared to the negative controls.

The presence of the amino acids and motifs including the TVP motif, K^{20} and Q^{58} implies the importance of the GSH mediated redox activity which was affirmed by the conservation in multiple sequence alignment. The tissue distribution of *SsGrx1* was found to have a wide range in the rockfish tissues illustrating its ubiquitous functions in different kind of cells and tissues. The significant upregulation of the *SsGrx1* after immune challenge denotes the immune relevance of *SsGrx1* to prevent the pathogenic attack. The functional studies revealed the *SsGrx1* function in insulin disulfide reduction and cell survival against H_2O_2 induces oxidative stress. Collectively, the results obtained in this study plausibly suggest the immune relevance of *SsGrx1* and the relevance for oxidative protection.

ANALYSIS OF CAROTENOIDS IN THE PIGMENTATION OF THE SNAPPER SPOTTED ROSE *Lutjanus guttatus* (Steindachner, 1869) GROWN IN CIRCULAR FLOATING CAGES

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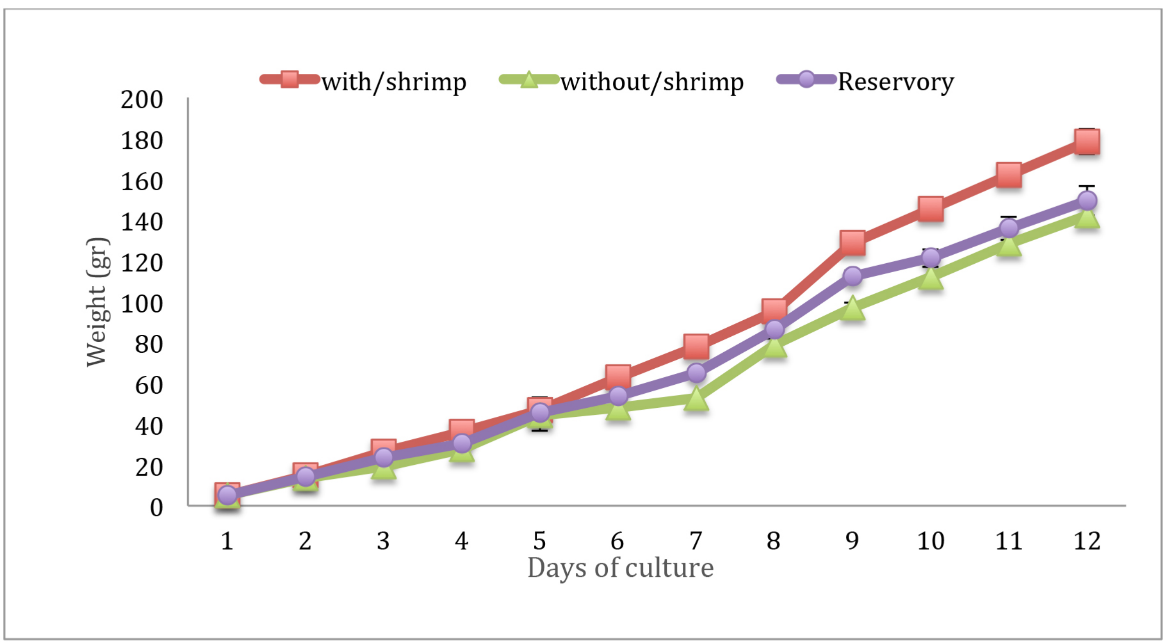
The spotted rose snapper *Lutjanus guttatus*, is the second most important snapper of a commercial in the Mexican Pacific Ocean. Natural stocks have pigmentation problems when they are taken to captivity, which are transferred to larvae that lose commercial value due to the low skin color. The bioassays were designed to provide the following diets: 1. Commercial diet + shrimp head (triplicate), 2.- Commercial diet (triplicate) and 3.- Fish fed with natural foods. Temperature, salinity, pH, dissolved oxygen, nitrites, nitrates, ammonium and phosphate were monitored. Biometric analyzes were performed every 10 days to calculate the supply of food rations. In 180 days of cultivation the daily growth (2.02 gr) and survival (73%). The shrimp feed/head treatment was the one that showed a better performance in the culture as well as in the pigmentation of the skin with carotenoids, at the beginning of the culture (2.44 ± 1.01) and, at the end of the culture (17, 31 ± 6.57) *vs* without a shrimp head starting the culture (3.60 ± 1.53) and at the end the culture (7.49 ± 3.26). The results indicate that adding by-products such as shrimp head to red-skinned fish such as snapper *L. guttatus* increases skin pigmentation.



PHYTOPLANKTON AND OTHER MICROALGAE IN AQUACULTURE PONDS AND THEIR IMPORTANCE IN HAWAIIAN FISHPONDS

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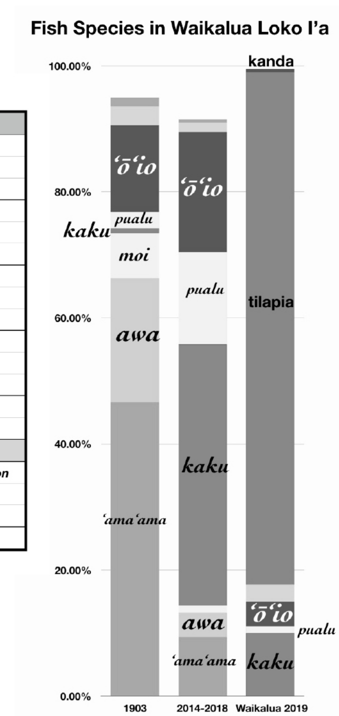
Restoring and maintaining the few remaining ancient Hawaiian fishponds are important for preserving this nearly 1,000 year old technology. The current problem of low recruitment and production of the keystone species, striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*), reflects on the deterioration of Hawaii's nearshore fisheries. Hatchery production is possible but regardless of the source of the fry the Hawaiian fishponds must have a balanced ecosystem with phytoplankton and other microorganisms to support the growth of these filter-feeding and grazing herbivores. Microscopic observations of water samples and sediments from Waikalua Loko I'a indicates an extremely low population of desired feed organisms. The standing biomass of fish is estimated to be around 10 grams/square meter. The importance of these factors in the biological restoration of Hawaiian fishponds or loko i'a will be discussed.

Loko i'a that are currently in production have been in continuous production since they were originally built. Even when left unmanaged they are subject to the inflows and outflows of tides and currents. Pua or fish fry are attracted to the estuarine environment within the pond walls or kuapa. After reaching a certain size they may not be able to swim out of the pond due to the 1/2" grate (makaha) that blocks the entrance of the channel (auwai). The striped mullet (ʻamaʻama) and milkfish (awa) are two species that traditionally represented the health of a loko i'a. They enter the pond as fry during different seasons. Mullet fry (pua ʻama) enter ponds during the winter months. Milkfish fry (pua awa) enter during summer months. Other species enter during their own seasons.

Species of Waikalua Loko I'a

Native Species	Scientific Name
Aholehole (flagtail)*	*Kuhlia sandvicensis*
ʻAmaʻama (mullet)	*Mugil cephalus*
Awa (milkfish)*	*Chanos chanos*
Awaʻaua (ladyfish)	*Elops hawaiensis*
Kaku (barracuda)	*Sphyraena barracuda*
Lae (leatherback)	*Scomeroides lysan*
Moi (Pacific threadfin)*	*Polydactylus sexfilis*
ʻOʻopu (goby)	eleotrid/goby species
ʻOʻopu hue (puffer)	*Arothron hispidus*
Opae (shrimp)*	*Palaemon debilis*
Oʻio (bonefish)	*Albula glossodonta*
Paʻopaʻo (golden trevally)	*Gnathodon speciosus*
Pualu (surgeonfish)	*Acanthurus blochii*
ʻUlae (lizardfish)	*Synodus* spp.
Introduced	Species
Tilapia (black-chin tilapia)	*Sarotherodon melanotheron*
Kanda (summer mullet)	*Osteomugil engeli*
Toau (blacktail snapper)*	*Lutjanus fulvus*
*not collected in June 2019 sampling	



Nursery systems are important in modern mullet and milkfish production. Post-larval fry are grown to fingerlings or larger sizes before being placed in growout ponds. Nursery tanks or ponds are prepared to receive fry. Wherever possible an effort is made to slowly acclimate the fish to their new home environment. The appropriate live feeds and commercial feed are fed until the fry have fully transitioned to the formulated diet. What is often overlooked is the microbial biofilm that develops on tank walls and along the bottom of ponds. As in aquaponics systems waste products from feed digestion are taken up by algae and other microorganisms resulting in a microbial community that often serves as supplemental food for the fry. The nutritional value of this microbial ecosystem, when properly managed, can be important for its contribution to fish growth and health. Such studies have been done in shrimp production systems. It is reasonable to relate a similar importance of microbial communities and biofilm to herbivores and omnivores in loko i'a.

THE DECLINE OF HAWAII'S MULLET FISHERY AND THE IMPACT ON LOKO I'A

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Prior to western contact ancient Hawaiian fishponds (loko i'a) were operated with minimal management. Restocking of ponds occurred naturally, each species entering the pond according to their own season. The estuarine environment attracted species that sought that habitat for food and protection. The nearshore fishery has changed drastically since the first western contact in 1778. There was very little written documentation on the health and structure of Hawaii's fishery until John N. Cobb produced "The Commercial Fisheries of the Hawaiian Islands in 1903". That report serves as the basis for assessing the changes in Hawaii's nearshore fisheries with its current status.

In 1948 the Territory of Hawaii's Division of Fish and Game (currently Division of Aquatic Resources or DAR) began maintaining annual catch reports of all commercial fishing activities throughout Hawaii. "Commercial Marine Landings Summary Reports" are currently available online from 1997 through 2018. Recreational and subsistence fisheries are not monitored so those impacts cannot be assessed. The nearshore fisheries for species reared in loko i'a has changed since 1903 and those changes mirror the fish population in Waikalua Loko I'a.

Sampling of Waikalua Loko in June 2019 generated a list of species. The barracuda (kaku) was the dominant predator. Herbivores (`ama`ama, awa) dominated the fishery and ponds in 1903. In 2019 the pond is still dominated by herbivores; the non-native tilapia and kanda. Fishery data (2014-2018) on these species indicate that the kaku is present in high numbers relative to the more desirable `ama`ama and awa. This suggests that kaku will continue to enter Waikalua Loko in high numbers. As the top predator they remain trapped and feed on all species. Tilapia have become established in Waikalua Loko I'a with survival mechanisms that allow them to proliferate in spite of predation by kaku and other carnivores.

EXTENDED INCUBATION FEEDING PROTOCOL AND HYPERSALINE ACCLIMATION OF LARVAL SPOTTED SEATROUT *Cynoscion nebulosus*

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Spotted seatrout (*Cynoscion nebulosus*) is a popular sport fish and highly targeted by anglers on the Texas coast. To offset pressures by anglers and natural events, Texas Parks and Wildlife Department implemented a stock enhancement program in which hatchery-reared fish are released into the wild to augment natural stocks. One of these hatcheries is located on a hypersaline lagoon resulting in fish often being challenged by hypersaline conditions in outdoor rearing ponds. The objectives of this study were to determine: 1) a feeding protocol for use during extended incubation that produces high survival and growth rate of spotted seatrout larvae, and 2) whether gradual hypersaline acclimation during extended incubation leads to increased growth and survival of larvae when subjected to hypersaline conditions.

Two feeding trials (12 days each) were conducted to evaluate rotifer enrichments used in the diet of larval spotted seatrout. Treatments in both feeding trials (100 larvae/60 L tank, 4 rep./trt.) included Algamac-3050®, Easy Dry Selco®, Ori-one®, and Rotigrow Plus®. After the feeding trials, a salinity experiment (T=17 days, 100 larvae/60 L tank, 4 rep./trt.) was conducted in which larvae were subjected to hypersaline (50 ppt) conditions at 3, 6, 9, and 12 days post hatch (dph). Initial salinity was 35 ppt. Starting at day 3, salinity was raised by 1 ppt per day for all treatments not yet at 50 ppt.

In both feeding trials, survival was significantly ($P<0.05$) affected by dietary treatment. In the first trial, Algamac-3050 had significantly higher survival (75.2±6.3%) than the other three treatments, whereas in the second trial, Algamac-3050 (83.2±2.8%) and Easy Dry Selco (79.8±6.0%) had significantly higher survival than the other two treatments. In both feeding trials, larvae in the Algamac-3050 treatment exhibited significantly higher growth (i.e., length, weight, specific growth rate, and percent weight gain) than any of the other treatments. Based on these results, Algamac-3050 was selected for use in the salinity trial.

In the salinity trial, survival was significantly affected by treatment, with larvae exposed to hypersaline (50 ppt) conditions at 3 dph having lower survival (8.2±5.8%) than the other three groups. Survival improved significantly when fish were incubated until 6 (52.5±7.0%) or 9 (65.0±7.7%) dph, although there was no improvement in survival between 9 and 12 (65.2±7.1%) dph. Growth parameters followed the same trend as survival.

The results of this study suggest that larval rearing of spotted seatrout in hypersaline conditions may be improved by increasing the incubation time beyond three days, and by gradually acclimating larvae to a higher salinity during this extended incubation.

NEW DEVELOPMENTS IN PROGRAMS FOR DAY-1 AND ADVANCED TRAINING & EDUCATION IN AQUATIC VETERINARY MEDICINE

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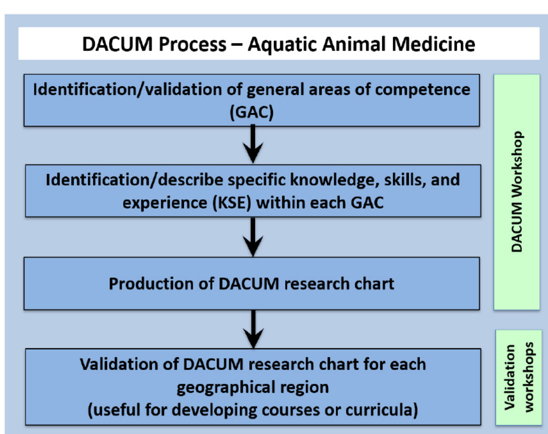
With increasing impacts of diseases on aquaculture production in all countries, the need for a well-trained aquatic veterinary workforce (including veterinarians and para-veterinarians – veterinary technicians or nurses, and non-veterinary "aquatic animal health professionals") has become a global imperative. While a number of International and National veterinary organizations have, or are developing processes to determine, evaluate, harmonize and accredit veterinary education throughout the world, two organizations are focusing on the educational needs of aquatic veterinarians and veterinary curricular and continuing CEPD programs throughout the world – the World Aquatic Veterinary Medical Association (WAVMA), and the International Partnership on Aquatic Veterinary Education (i-PAVE).

While preliminary surveys of veterinary schools throughout N. America and Europe suggested that there many have a number of aquatic veterinary courses, no veterinary school includes all subject-matter identified in WAVMA's Aquatic Veterinary Certification Program (CertAqV). This Program identified 9 core (or Day-1) general areas of competency (GAC) that are needed to practice aquatic veterinary medicine (equivalent to the knowledge, skills and experience required of individuals receiving a veterinary degree). These cover clinical and non-clinical subjects specifically focused on aquatic species and industries, and include: 1) Aquatic Environment and Life Support Systems; 2) Taxonomy, Anatomy & Physiology; 3) Husbandry and Industries; 4) Pathobiology & Epidemiology of Aquatic Animal Diseases; 5) Diagnostics and Treatment of

Aquatic Animal Diseases; 6) Clinical Veterinary Experience and Client Communications; 7) Public Health, Zoonotics & Seafood Safety; 8) Legislation, Regulations, and Policies; and, 9) Principles of Aquatic Animal Welfare. Since 2010, >110 individuals from >27 countries have been certified to be competent in all 9 GACs, and ~70 more are currently being evaluated.

To build on these efforts, i-PAVE embarked on a multi-year project to verify and validate that all GACs in the WAVMA CertAqV Program equally apply to any country, using a "DACUM" Process (**D**eveloping **A** **C**urricul**UM**; Fig 1), which has been used elsewhere for analyzing many occupations and validating that curricula meet the needs of a profession. Preliminary results of DACUM workshops in N. and S. America, Europe, Africa, and the Asia-Pacific, using veterinarians actively engaged in aquatic veterinary medicine for a number of years, indicate that ~215 specific tasks will fulfill all the GACs identified in the WAVMA CertAqV Program. Importantly, all of these GACs and related tasks can be covered in a single 6-week course, should a veterinary school desire. Furthermore, fulfilling the WAVMA CertAqV requirements prepare individuals for Board Certification in aquatic veterinary medicine.



EXTENDED INCUBATION FEEDING PROTOCOL AND HYPERSALINE ACCLIMATION OF LARVAL SPOTTED SEATROUT *Cynoscion nebulosus*

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APPLICABILITY OF THE MICROFRAGMENTATION TECHNIQUE TO PROPAGATE CORALS IN A FISHERFOLK COMMUNITY IN THE PHILIPPINES

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The implementation of coral nurseries is a highly prevalent method of coral restoration in the Philippines. These nurseries primarily focus on fast-growing branching corals and do not typically include massive corals. This is due to the slow growth rate and morphology of these corals. Microfragmentation is a relatively new technique that has displayed positive results for massive coral propagation. This method utilizes small fragments of corals (≈1-3cm), which are mounted on discs and placed in nurseries. Once the fragments reach appropriate size, the fragments of a single coral (i.e., genotype) are transplanted to a restoration site and placed in groups 2-4cm apart. Over time the fragments fuse into larger corals. This enables the corals to share resources and reach size of sexual maturity sooner in comparison to traditional nursery methods. Though microfragmentation is becoming more practiced, there is no scientific literature specifically focused on this method in the Philippines. This study aimed to determine if: 1) genotype significantly influences growth, 2) the individual fragment of each genotype accounts for differences in growth, and 3) this method is viable in a fisherfolk community with minimal technology in the Western Visayas of the Philippines.

A total of five coral heads (genotypes) of the lobe coral *Porites lobata* were fragmented with hammer and chisel by several volunteer fisherfolk. The fragments were cultured from April-August 2018 in an *in-situ* fixed-leg nursery at a depth of approximately 2m. Maintenance was performed approximately weekly and measurements of growth (i.e., volume change) were conducted monthly utilizing an Olympus Tough TG-870 camera and ruler while freediving. The fragments were then transplanted to a selected restoration site and monitored for one month.

A hierarchical Bayesian log-linear regression model indicated that 71.5% of growth (volume change) variation was attributed to coral genotype and 18.1% of growth variation was attributed to the individual fragment, with some individual fragments growing particularly slower or quicker. Overall, the average monthly increase in coral fragment volume was 30.1% per month (95% CI 21.7-38.7%/month) with the greatest monthly increase for a genotype at 50.8% per month (95% CI 23.5-66.1%/month).

It is not surprising that coral genotype had significant influence on growth rate. The growth variation among fragments of the same genotype could be attributed to individual differences and the fragmentation method used, which could have caused more stress to some fragments than others. Though high growth rates were documented for the lobe coral, the location and shallow depth of the nursery required significant maintenance, which may not be feasible in a remote fisherfolk community.

TEXAS OYSTER CULTURE: ARE WE THERE, YET?

John Scarpa*, William Balboa, Brad Lomax, and Joe Fox

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In Texas, Hurricane Ike (2008), the Deepwater Horizon disaster (2010), and extreme salinity depressions from rain events (2015 and 2016) reduced harvest and purchase of wild oysters. These natural and human-induced events spurred the discussion of oyster culture in Texas, as well as further assistance with oyster restoration. To assist the industry and environment The Oyster Resource and Recovery Center in Palacios, Texas, was proposed and will receive support from Bucket 1 of Deepwater Horizon disaster fines allotted to Texas. The project will be initiated when contractual agreements are executed between the federal government, the Texas state agency handling the RESTORE funds (www.restorethetexascoast.org), and our institutions.

Approximately two years ago, discussions with agency, non-profit organization, and oyster industry personnel, noted that a state regulatory framework for oyster culture did not exist and questions about community acceptance were unknown. This past year, the 86th Legislature of Texas passed House Bill 1300 (HB1300) and Senate Bill 682 (SB682) that gave Texas Parks and Wildlife Department (TPWD) and TPW Commission the authority and requirement to establish a program governing "cultivated oyster mariculture" by September 2020.

In the prior year, eight public presentations were conducted to determine if there were potential unknown user conflicts and if the public had solutions for these conflicts. A survey was given before a presentation on bivalve aquaculture, which included potential conflicts, and then completed after the presentation period. Meeting size ranged from 5 to 51 individuals as determined by those that returned surveys; total number of surveys obtained was 244. A majority (67.6%) were generally familiar with aquaculture. Before the presentation, a majority had a positive opinion of aquaculture (65.1%) and a positive opinion of bivalve aquaculture (62.6%) as compared to a negative opinion of aquaculture (3.8%) or bivalve aquaculture (2.5%); the remainder indicated no opinion or mixed opinion. After the presentation, the proportion of respondents indicating a positive opinion of aquaculture rose to 89.8% and to 89.3% for bivalve aquaculture. The proportion of respondents still indicating a negative opinion to aquaculture decreased slightly to 3.3%, whereas it decreased to 0.9% for bivalve aquaculture, with the remainder still indicating no opinion. The information developed during this project should aid TPWD as it establishes a program governing off-bottom, mid-water, and surface culture of oysters in Texas waters. Texas oyster culture may not be there, yet, but should be by late 2020 with production occurring in 2021.

The research project data noted here resulted from Gulf States Marine Fisheries Commission award ACQ 210-039-2017-TAMU to JS; the presentation and survey were reviewed by the TAMU-CC Institutional Review Board.

AN EVALUATION OF SEX REVERSAL RECIPES FOR FEMINIZATION OF MALE LAKE TROUT *Salvelinus namaycush* AND FUTURE DEVELOPMENT OF A YY MALE BROODSTOCK

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Lake Trout populations in western U.S. waters have been shown to have adverse impacts on native or desirable fish species and are often targeted for manual removal. The population in Lake Pend Oreille, Idaho has been successfully suppressed to levels that have permitted the rebound of the primary forage population and more recent use of the same approaches in Yellowstone Lake Wyoming have allowed native Yellowstone cutthroat there to begin rebuilding. Despite these apparent successes, annual suppression costs on these two waters has recently ranged from 1-2 million annually, amounts far above those available for successful Lake Trout eradication in most other western waters. Alternative or complementary methods that could be used to control or completely eradicate Lake Trout populations are needed. YY Males, which are created in the hatchery by feminizing XY males then crossing them with normal XY males, comprise another approach to eradicating undesirable populations. If stocked YY Males survive and reproduce with wild females, it could eventually drive the wild population sex ratio to 100% males, at which point the population would be unable to reproduce and be eradicated after stocking ceased. A sizeable YY Male Brook Trout broodstock has been developed and is currently being field evaluated by stocking in 16 Idaho waters. The present study documents the first step in companion efforts to develop a YY Lake Trout Broodstock for similar wild Lake Trout eradication evaluations. Lake Trout eggs were obtained on 16 October, 2016 by spawn crosses at the Story Fish Hatchery in Wyoming, transferred to the Grace Fish Hatchery in Idaho and placed in Heath trays. Newly hatched fry (n= 120) were transferred to 24 individual 3.75 gal pots and treated with one of 12 treatments. Treatments tested included immersion (200-400 microg/L), top-coated feed for 97 d beginning at first-feeding, and immersion-feed combinations involving both Estradiol and Estrone as the feminizing hormone. Growth and survival were monitored periodically and compared to controls. Numbers were culled to 35 fish per tank to prevent overcrowding. A total of 840 fish averaging 82.3 mm were removed from the 24 pots in June 2017, PIT tagged and reared in a common garden raceway until sex could be readily determined visually. Mean growth rates (in length and weight), mortality rates, and observed sex ratios following necropsy for all treatment and control groups will be reported and next steps in development of a YY Lake Trout broodstock discussed.

KEY FACTORS INFLUENCING POST-RELEASE SURVIVAL OF HATCHERY-REARED JUVENILE SNOOK *Centropomus undecimalis*

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The common snook (*Centropomus undecimalis*) supports a valuable recreational fishery in southwest Florida, but mass mortality events due to cold stuns and red tide suggest stock enhancement is needed to aid population recovery and adaptive management of the fishery. To guide effective marine fisheries enhancement with common snook, timely estimation of post-release survival is required, but this has been particularly challenging to measure for estuarine and marine finfishes. We apply novel principles for release activities of hatchery-reared juvenile common snook in southwest Florida to guide timely estimation of post-release survival, focusing on the influence of season, in-hatchery conditioning, and release site acclimation and habitat.

Over the last 4 years, Mote Marine Laboratory has employed replicate release designs to stock >15,000 juveniles tagged with passive integrated transponder (PIT) tags during experimental release efforts. These hypothesis-driven release experiments were conducted in four tidal-creek systems monitored with marine-adapted PIT tag antenna arrays that provided recapture histories capable of being analyzed with Cormack-Jolly-Seber mark-recapture models.

Models suggest that the success of snook stocking efforts are primarily dependent on the specific location and time at which individuals are released. In particular, the availability of nursery habitat at release sites may be a critical factor influencing post-release survival in southwest Florida because important habitat has been lost to coastal development and shoreline hardening. Yet, the use of variable tag sizes, pre-release acclimation to habitat, and predator-exclusion cages during release also influenced rates of survival and detectability of hatchery-release fish. The highest survival rates were observed for individuals released in the lower reaches of the tidal creek systems in the spring, indicating these areas may provide ideal release sites for juvenile fishes at that time. Short-term differences in survival among the first four weeks after release primarily influenced the overall impact of the stocking activity, and these survival rates were consistently identified after one year of monitoring. The application of these novel release activities to aid adaptive-management can identify optimal release sites, times, and protocols that will promote adaptive management of stock enhancement programs and maximize the impact on receiving populations.

KING KAMPACHI: AN OVERVIEW OF THE LARGEST *Seriola rivoliana* OPERATION IN THE WESTERN HEMISPHERE

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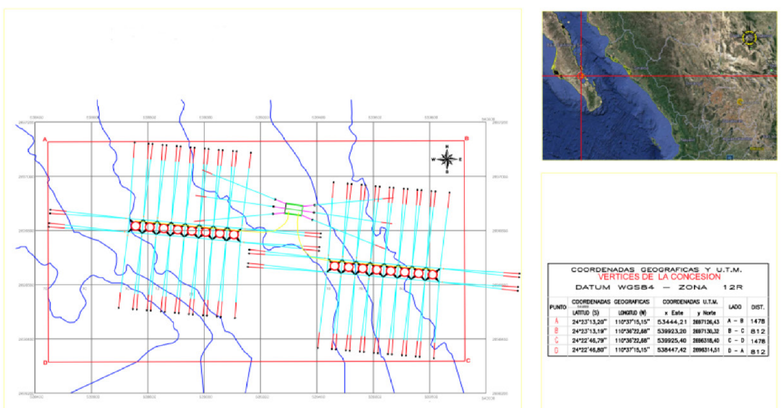
In and about La Paz, B.C.S. México, now operates the largest *Seriola rivoliana* farm in the Western hemisphere: King Kampachi (projected 2020 harvest: => 1,200 metric tons). We broke ground for our pilot hatchery in mid-January 2018, began our first run in late April, and made our first sale--to one of the top 20 restaurants in the world--in mid-October, 2018. By April, 2019 our product could be found in restaurants across the United States and Mexico, and in early June of the same year we became the first producer in the world to receive ASC certification in our species.

This presentation will give an overview of the project, from broodstock to market, including the major financial and operational milestones achieved, as well as some of the myriad innovations our team has developed in almost every aspect of our operations.



THE DELICATE BALANCE OF AQUACULTURE AT A PUBLIC AQUARIUM

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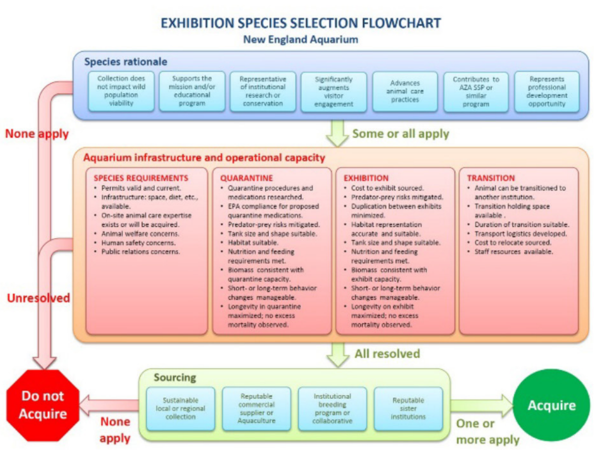
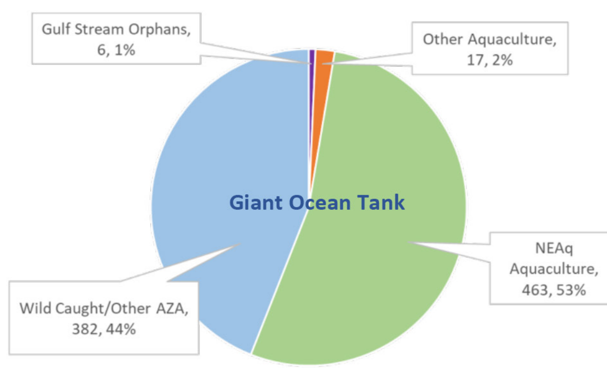
New England Aquarium, in collaboration with Roger Williams University, is at the forefront of developing rearing techniques for select marine fish species in our exhibits. We are sharing our processes, culturing methods, and aquacultured fish with other aquariums to promote sustainable practices and reduce the impact on wild populations.

To maximize limitations on time, resources, and space, the Aquarium has developed a sustainable collections strategy which includes a Collections Management Committee and Live Foods & Larval Program. Once the exhibit collection plan has been vetted through the committee, key exhibit species are evaluated as potential candidates for aquaculture. These species are assessed using a set of criteria for inclusion, which includes: animal welfare concerns with regards to collecting practices, reducing resources and carbon footprint associated with shipping and transport, species that are considered short-lived or high rotation in NEAq exhibits, and species that are important to NEAq and/or the public aquarium industry.

Initial efforts to collect eggs from several multi-species exhibits proved difficult to control and some species were mistakenly reared, diverting space and resources from key focus species. This led us to switch attention to broodstock populations, which gave us full control over nutrition, light cycle, and egg collection and avoid future accidental rearing efforts. Ongoing work to catalogue eggs collected from exhibits for DNA sequencing will help identify species' eggs in future collections from multi-species exhibits.

The New England Aquarium has made strides in culturing marine species and live foods for our collections in the past few years under our Larval Program, including 6 species' first. This presentation will detail the methods, mishaps, and successes of aquaculture at a public aquarium.





CHARACTERIZATION OF CEMENT PROTEIN DISTRIBUTION IN THE ACORN BARNACLE *Amphibalanus amphitrite*

Janna N. Schultzhaus*, Christopher M. Spillmann, Chenyue Wang, Dagmar H. Leary, Chris R. Taitt, Kenan P. Fears, and Kathryn J. Wahl

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Acorn barnacles are major marine fouling organisms whose success is largely due to their ability to adhere to diverse substrates via a sub-micron thick proteinaceous adhesive layer. Although the identities of several proteins in the adhesive are known, one outstanding question concerns their spatial distribution throughout the organism. This information would be valuable in terms of understanding where these proteins are synthesized, how they are transported to the substrate interface, and their distribution and function throughout the organism.

To address these questions, we utilized custom polyclonal antibodies against two prominent proteins in the adhesive layer, AaCP19-1 and AaCP43-1, and characterized the immunostaining patterns in histological sections via confocal microscopy. Positive immunostaining was observed in separate and distinct acellular tissue linings near the inner shell (Figure 1).

To confirm these proteins were located in areas other than the substrate interface, we performed proteomics on formaldehyde fixed paraffin embedded (FFPE) sections as well as tissue collected from freshly dissected barnacles (Table 1). Both proteins were identified at an enriched level in samples where the adhesive layer was clearly present (FFPE Adhesive), but were also found in samples removed from the interface.

Our results indicate these two proteins, once thought to be unique to the adhesive layer, are located in other regions of the barnacle and likely have additional physiological functions.

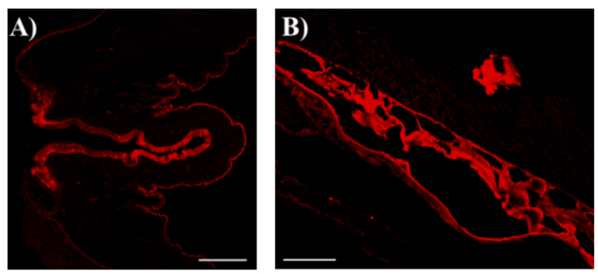

FIGURE 1. Immunostaining profile of A) α-AaCP19-1 and B) α-AaCP43-1 in tissue lining the inner shell. Scale bar = 100 μm.

TABLE 1. Proteomics results for FFPE and fresh tissue. PSM = peptide spectral matches; Score = Mascot probability score.

	AaCP43-1			AaCP19-1		
Proteomics Results	**PSM**	**Peptides**	**Score**	**PSM**	**Peptides**	**Score**
FFPE Adhesive 1	128	25	4420	16	12	78
FFPE Adhesive 2	97	21	2954	10	8	60
FFPE Top 1	31	10	190	7	7	48
FFPE Top 2	25	6	159	14	9	63
FFPE Top 3	29	5	212	11	7	63
FFPE Middle 1	28	10	155	12	10	85
FFPE Middle 2	42	19	272	19	12	114
FFPE Middle 3	16	3	150	8	6	47
Fresh Top 1	5	5	39	10	6	69
Fresh Top 2	11	10	62	16	12	114
Fresh Top 3	12	9	72	8	7	52
Fresh Upper Shell 1	9	8	66	10	6	65
Fresh Upper Shell 2	7	7	32	11	9	59

IDENTIFYING MOLECULAR MARKERS ASSOCIATED WITH RESILIENCE TO OCEAN ACIDIFICATION IN THE EASTERN OYSTER

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The eastern oyster (*Crassostrea virginica*) is found in environments with variable salinity, turbidity, oxygen, pH, and temperature ranging from the Gulf of Mexico all along the Atlantic coast of North America. The eastern oyster is able to have a broad geographic distribution and live in a wide variety of environments because of physiological plasticity, evolutionary adaptation to specific environments, or both. Natural genetic variation is a valuable source of resilience to changing environments. The evolution of resilience to climate related stressors depends on the standing stock of genetic variation. The oyster genome is highly polymorphic and has many genes related to environmental stress. The high polymorphism means that there is high gene diversity and variation that is a prerequisite for adaptation to changing environments.

In this study, we investigated if resilience to ocean acidification in the eastern oyster is genetically dictated. Adult oysters were spawned and larvae were reared in ambient conditions ($p\mathrm{CO_2}$ of ~600 ppm) and acidified conditions with elevated $p\mathrm{CO_2}$ ($p\mathrm{CO_2}$ of ~ 1200 ppm) immediately upon fertilization. Samples for RNA and DNA processing were collected for gene expression and SNP profiling. A time 0 sample was taken before larvae were moved to $p\mathrm{CO_2}$ treatments. A second sample of larvae was collected after they were in $p\mathrm{CO_2}$ treatments for 96 hours, and a third sample of juveniles was collected after metamorphosis. High-throughput SNP profiling methods (ddRADSeq) was applied to DNA samples. Frequency shift of SNPs was contrasted between sampling times and $p\mathrm{CO_2}$ conditions to identify resilience-associated genetic variants (Fig 1). RNASeq was performed on RNA samples to identify differentially expressed genes in the larval and juvenile oysters in different $p\mathrm{CO_2}$ treatments. The hope is to provide information to breed populations of oysters able to withstand ocean acidification.

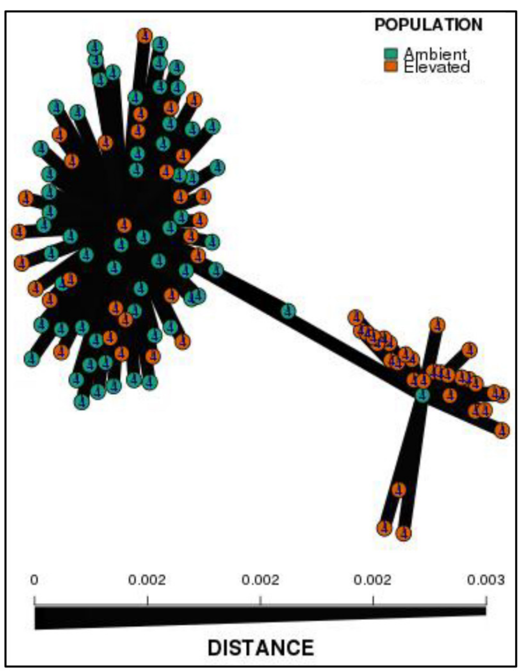

Figure 1. Differences in SNPs of juvenile oysters reared in ambient and elevated $p\mathrm{CO_2}$ treatments.

5 WAYS SUBMERSIBLE PENS CAN INCREASE YOUR FARM VALUE

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Submerged production systems have become a prominent production method for marine aquaculture, mainly for farmers looking to escape storms and the rough wave environment found at the surface of exposed locations. Submerging pens and grids is proven to be an effective way to reduce damage to system components and has opened new high-energy sites for production, but there are additional significant reasons to submerge pens and infrastructure.

Having access to water above and below a thermocline can save whole crops of fish. The University of New Hampshire is producing steelhead trout near the Isles of Shoals, NH. They are having a lot of success but are seeing high mortality during the summer months due to heat stress. They are looking at submerging their pens by 15m to get their fish below the thermocline and into water 4°C cooler which would alleviate the mortality associated with the high summer temperatures. By using submersible pens, the acceptable range for farming salmonids in US waters may be expanded from mid-Maine to Massachusetts on the East coast and from Washington State to the California bight on the West coast (figure 1).

Harmful algal blooms (HABs) typically occupy the top 5 – 15 meters where light can penetrate. HAB events caused massive mortality in Norway during the summer of 2019 and are expected to be more prevalent in the future according to climate change models. Submerged pens can offer protection from these events by moving fish below the most dangerous depths.

Multiple sources of value creation will be explored during this presentation, including using submersible pens and technology that take advantage of vertical current profiles, reduce parasites, and decrease visual impacts, vandalism and poaching.

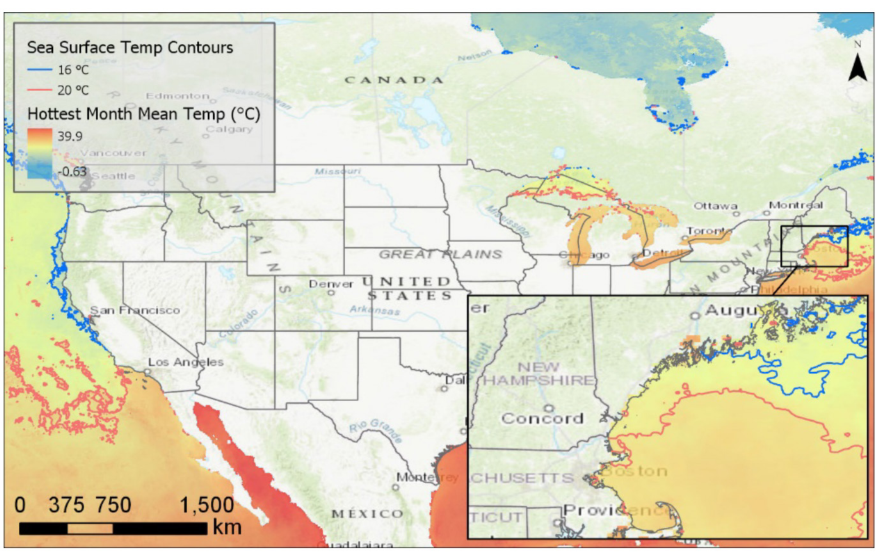

Figure 1: A simple model showing the potential expansion of suitable areas for salmonid production by submerging pens below a 4°C thermocline.

GROWTH HORMONE REGULATES INTESTINAL NUTRIENT TRANSPORTERS IN MOZAMBIQUE TILAPIA *Oreochromis mossambicus*

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Tilapia, one of the main finfish cultured worldwide, exhibit excellent growth in captivity. In vertebrates, including fish, the endocrine system orchestrates the production and release of hormones by integrating sensory information with an array of physiological functions in nearly every organ. Growth is largely regulated by growth hormone (GH), which is secreted by the pituitary gland. Once in circulation, GH can bind to its receptor (GHR) in target tissues, such as muscle, liver and intestine, to initiate physiological responses that include cell proliferation and differentiation, nutrient uptake, and protein synthesis. The intestine is the primary site for the uptake of nutrients required for growth. Proteins that transport oligopeptides, amino acids, sugars, water, and ions facilitate the uptake of nutrients across the intestinal epithelium. Little is known, however, on how GH affects intestinal nutrient uptake.

Our laboratory has developed a model for investigating whole-organism effects of GH by employing the Mozambique tilapia (*Oreochromis mossambicus*). In this model, we surgically remove the pituitary gland (hypophysectomy; Hx) and replace GH via intraperitoneal injections. With this approach, we showed that GHR expression in muscle, liver, and intestine of Hx fish was dramatically reduced following hypophysectomy; an effect that was recovered by replacement with GH. Next, we showed that GH stimulates the expression of intestinal PEPT1, PEPT2, and GLUT2. These results indicate that GH is a key regulator of peptide and sugar transport in the intestine of tilapia. Alternatively, NKCC2 and SLC7 were suppressed by Hx and unaffected by GH; thus the expression of the $Na^+/K^+/Cl^-$ co-transporter, and amino acid transport is seemingly under the control of a pituitary factor other than GH.

[Supported by HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01) and NSF (IOS-1755016 and -1755131)]

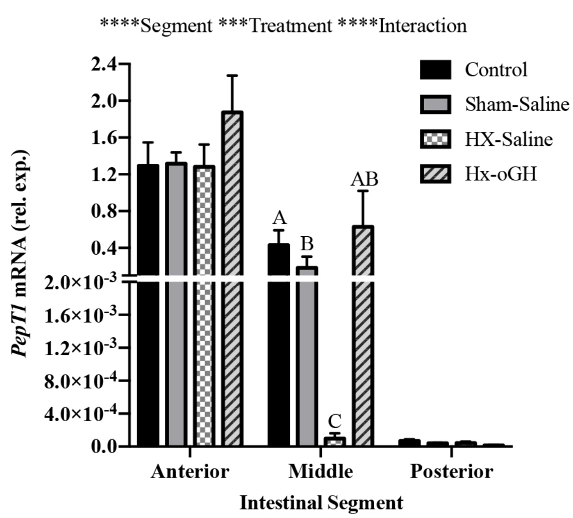

Fig. 1: Effects of hypophysectomy (Hx) and GH replacement (Hx-oGH) on intestinal mRNA expression of PEPT1 in Mozambique tilapia.

TO PATHOGEN DETECTION AND RNAi ANTIVIRALS IN SHRIMP BREEDING

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Disease is the biggest unknown risk in Shrimp aquaculture, with a growing list of commercially devastating disease-causing pathogens around the world. Pathogen detection and screening has been fundamental to understand these pathogens, determine how to manage them during culture and develop successful Shrimp breeding programs. In recent times, the use of RNAi antivirals (double-stranded RNA injected into broodstock) has also started to play a role in developing pathogen free breeding stocks. This presentation will describe some of the key ways in which pathogen detection, screening and RNAi antivirals are playing a role and being used in Shrimp Breeding and shrimp production globally. A short introduction to a high-throughput disease screening platform now on the market, Shrimp MultiPath, will also be given.

POPULATION GENETICS AND TAXONOMIC SIGNATURES OF WILD TILAPIA IN JAPAN, BASED ON MITOCHONDRIA DNA CONTROL REGION ANALYSIS

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The successful introduction of tilapia to Asian and South American countries was because of its adaptiveness to new environments under different environmental conditions to foster outstanding ecological and physiological variations making successful invaders of ecosystems throughout the tropical and subtropical regions. However, the cohabitation of other tilapia species with the Nile tilapia (*Oreochromis niloticus*) causes the reduction in population sizes, genetic diversity, and perturbation of the other species. This study aimed to establish the level of genetic integrity, population diversity, and population biogeography of wild tilapia species found in Japan. The mtDNA control region sequences generated fifty-two distinct haplotypes indicating significant differences of genetic variability in the entire data set for which 78.87% and 21.13% respectively represent the shared and singleton or private haplotypes.

The analysis of molecular variance (Table 1) showed high variation within populations except for taxonomic units indicating a significant genetic structure among the 11 populations analyzed.

Our results indicated that the introduction of *O. niloticus* contributed significantly to genetic differentiation among populations in only a few generations showing signals of mtDNA introgression across the species boundary of *O. niloticus* subjecting the genetic integrity of previously introduced species to jeopardy. The presence of private alleles, restricted gene flow among populations, and low levels of genetic exchange between species indicated that the taxonomic signatures captured in our data is represented by a network of relatively same genetic units that are geographically restricted.

However, clustering of biogeographic regional populations restricted by distance and the Pacific Ocean allowed us to define the population genetic structure as a mapping of taxonomic and genetic diversity rather than distance, therefore, for genetic conservation purposes, we propose an urgent need for a countrywide genetic assessment of tilapia species thriving in Japan as some populations indicated minimal or no genetic pollution of some taxonomic signatures.

PROGRAMS AT THE US FOOD AND DRUG ADMINISTRATION TO MONITOR UNAPPROVED CHEMICAL AND DRUG RESIDUES IN AQUACULTURE PRODUCTS

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Aquaculture, both domestically and internationally, poses unique production problems not encountered in wild-caught fisheries. Aquaculture producers may use veterinary drugs or general-purpose chemicals that can have a negative effect on the cultured animals, the surrounding environment, and cause food safety risks. The potential immediate and long-range human health consequences may include hypersensitivity reactions, toxicity-related reactions, possible carcinogenic effects, and increasing antibiotic-resistant microorganisms.

All chemical substances and aquaculture drugs used in food producing animals in the United States require approval under Section 512 of the Federal Food Drug and Cosmetic Act. Unapproved chemicals or veterinary drugs used to treat diseases are considered unsafe new animal drugs, and not allowed in seafood products at any level. For example, the use fluoroquinolones, an unapproved veterinary drug poses the risk of increasing antibiotic resistant bacteria with the potential for serious human health consequences from untreatable infections.

To protect consumers, the FDA has monitoring programs in place to ensure both imported and domestic aquaculture seafood products are free from potentially harmful drug residues. The Agency's approach to controlling unapproved chemical or drug residues in seafood is regulatory in nature with emphasis on intelligence gathering, selective sampling and compliance follow-ups.

DIRECT UTILIZATION OF SUGARCANE BAGASS AS AN ECONOMIC CARBON SOURCE FOR BIOFLOC CULTURE SYSTEM TO ENHANCE SHRIMP (*Litopenaeus vannamei*) GROWTH UNDER SUPER INTENSIVE REARING CONDITION

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Aquaculture technologies have been developed over the last decade to increase productivity and reduce cost of fish and shrimp farming. Among different approaches, Biofloc technology (BFT) used as a promising technology to advance shrimp farming economically and environmentally. Biofloc technology is an efficient way to manage water quality conditions for cultured species by optimizing carbon and nitrogen concentrations in shrimp rearing system. BFT is a symbiotic process that includes confined aquatic animals, heterotrophic bacteria and other microbial species in the water to enhance animal growth. In the current study, we have utilized sugarcane bagasse as an economic carbon source to feed *L. vannamei* larvae for 90 days in a BF based system under two different shrimp densities (12 PLs/litre and 16PLs/litre). Water parameters and shrimp measurements were monitored on biweekly basis. Thence, water parameters during the experimental period were within the acceptable range for shrimp culture, as water temperature ranged from 27.91 to 28.76± 0.87 C, dissolved oxygen from 5.66 to 6.19± 0.52mg/L, pH from 7.14 to 7.25± 0.12, and salinity from 27.77 to 29.01± 1.25 ppt. Expectedly, total ammonia Nitrogen (TAN), Nitrite (NO2-N), nitrate (NO3-N) and phosphorus (PO4-P) concentrations have been increased with stocking density in control tanks, meanwhile interestingly, the values were significantly low in BF treated tanks compared with the control tanks. On the other hand, total suspended solids (TSS) have been increased with biofloc treated tanks than control ones. Furthermore, shrimp growth was higher in terms of growth and survival (SR%) (12BF and 16BF) than that fed the control diet (12C and 16C). Also, the mean shrimp weight (FBW) was significantly higher at biofloc treatments in both stocking densities compared to the control ones. Finally, enhanced shrimp growth, water quality and TSS in BF treated tanks were associated with a significant decrease in the percent of "total vibrio count/total heterotrophic count" in both densities in BF tanks than those in the control counterparts. This may refers to the flourish beneficial bacterial community under BF treated tanks.

Genuinely, in our study we have utilized a BF system-based Sugarcane bagasse as a direct food source to feed *L. vannamei* larvae. Sugarcane bagasse as an economic carbon source could not only enhance the heterotrophic bacterial community that was conjugated with enhanced water quality parameters of TAN, NO2-N, NO3-N and PO4-P but also enhance shrimp growth under intensive rearing. Results of our study strongly recommend using Sugarcane bagasse in intensive farming of *L. vannamei*.

Acknowledgements: The research was performed within the "Development and Research Application of bioFloc Technology for increasing shrimp production in Egypt (EGY-DRAFT)" project, which is financially supported, by the Science & Technology Development Fund (STDF), Ministry of Scientific Research, Egypt. (Agreement No. 25305, Reintegration Grants). The authors are grateful for all the support.

ARE TERRESTRIAL PLANTS THE SOLUTION TO SUSTAINABLE AQUAFEEDS?

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The transition of aquafeeds from fishmeal and fish oil to alternatives has been in progress since the early 2000s. A wide variety of products are being investigated including microbial proteins, fish and animal processing waste, insect meal, algae and terrestrial plants. Terrestrial plant products appear to be the most promising alternatives due primarily to the quantity of protein and oil required. Concurrent with the shift toward alternative feedstuffs, there has been a growing awareness of the potential environmental effects of both aquaculture and agriculture, and a desire to make aquaculture "sustainable." In 1990 the U.S. government passed a law (U.S. Code, title 7, Section 3103) that defined sustainable agriculture and aquaculture. Sustainability is site-specific and over the long-term is required to:

(1) Satisfy human food and fiber needs.
(2) Enhance environmental quality and the natural resource base upon which the agriculture economy depends.
(3) Make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls.
(4) Sustain the economic viability of farm operations.
(5) Enhance the quality of life for farmers and society as a whole.

An excellent paper (Aquaculture Research 2007 38, 551-579) contains a review of some "sustainable" plant products that may provide potential feedstuffs and improve aquaculture sustainability. This paper did not contain a definition of sustainability, as there were no conventionally produced crops that were certified as sustainable in 2007. Constance (2010 Sustainability 2 (1) 48-72) wrote "because the concept of sustainability is deeply contested, agribusiness is able to exploit the ambiguity surrounding the definition of sustainable and exercise power in attempts to frame sustainable agriculture in their favor." It will be difficult to claim that intensive aquaculture is sustainable without sustainable feeds. Although numerous feedstuffs are being examined, soy appears to be the leading candidate to provide a majority of the protein in aquafeeds. In 2013 soybeans, grown in the USA, were certified as being sustainably produced. My presentation will examine the current U.S. stainability law, an ecological definition of sustainability, and the certification process in general.

CHARACTERIZATION OF NK-LYSIN ANTIMICROBIAL PROTEIN GENES, AND THEIR ACTIVITIES, IN RAINBOW TROUT

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The physiological control, and number, of antimicrobial peptides (AMPs) is not well understood in finfish. Based on the new rainbow trout genome assembly, we identified 6 new saposin-like AMP genes in rainbow trout. Protein sequence alignments and *in silico* modeling show that the proteins encoded by those genes belong to the Nk-lysin AMP sub-family (termed: Nkl 1, 2, 3, 4 and Nkl-like a & b). Transcriptomic data show that expression of nkl1-nkl4 mRNAs occurred in many tissues. By contrast, the nkl-like a & b mRNAs are mostly expressed in immune-related tissues. The effects of various aquaculture stressors, and a disease challenge (*F. psychrophilum*; Fp) in rainbow trout, were examined using RNA sequencing. Abundances of nkl1, nkl2, nkl4, and nkl-like a were downregulated by high-temperature and salinity stress, and nkl3 and nkl-like b were downregulated by high-temperature. In the Fp challenge study, abundances of nkl3, nkl4, nkl-like a and nkl-like b, were significantly affected by genetic line (resistant vs non-resistant) and treatment (PBS or Fp), which were further verified by qRT-PCR with spleen tissue sampled at 4 post-challenge time points (0 - 144 h). This work represents an initial characterization of these Nkl proteins in rainbow trout, with ongoing *in vitro* work to characterize how these AMPs affect flavobacterial pathogens, their biofilms, and survival and replication of novirhabdoviral pathogens. Understanding the distribution, regulation and bioactivity of these AMPs may enable rational design of approaches to reduce infectious disease in commercial aquaculture.

PROGRAM FOR THE INTEGRATION OF MACROALGAE MARICULTURE PRODUCTION INTO PREEXISTING SALMON AQUACULTURE OPERATIONS IN PRINCE WILLIAM SOUND, ALASKA

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There is significant interest in macroalgae aquaculture in Alaska, where such production promises to provide industry with products for use as food items, animal feed, organic fertilizer, biofuels, cosmetics, plastic alternatives and other sustainable products. Macroalgae production also shows promise for mitigating against the negative impacts of ocean acidification (OA) due to its ability to absorb carbon dioxide. Because macroalgae also absorbs nutrients, co-cultivation of macroalgae alongside other cultured marine species (such as finfish and shellfish) can help to recycle waste emanating from cultured production while further buffering these organisms from the corrosive effects of OA. Research has shown that OA can have negative impacts on juvenile pink salmon (*Oncorhynchus gorbuscha*) following early seawater entry, including significant alterations in olfactory responses such as anti-predator and homing behavior. Further, research has also shown that ocean waters receiving significant glacial melt – as is the case for much of Prince William Sound (PWS) – are more susceptible to OA. As PWS glaciers continue to melt at an accelerated and unprecedented rate, previous projections for the Sound's trend towards an increasingly acidic state may prove to be conservative, with disastrous implications for local fishery resources, and especially for the area's pink salmon hatchery programs.

The presentation seeks to further the discussion surrounding potential opportunities for kelp mariculture production in conjunction with preexisting PWS salmon aquaculture operations in such a manner that will benefit the area's existing salmon fisheries while strengthening associated and dependent industries and communities through diversification of available product forms. The presentation will describe the potential for a mutually beneficial relationship between the area's commercial fishing industry and Alaska Native entities through the development of a robust and sustainable local mariculture industry, with an initial focus on large-scale production of macroalgae in the vicinity of salmon hatchery saltwater netpen rearing operations. The presenter asserts that macroalgae production in concert with PWS salmon hatchery production provides the area's fishery stakeholders with a rare win-win scenario where large-scale macroalgae production provides a valuable economic incentive while also providing an ecological service for the benefit of all fishery resources in the area, including both wild and hatchery salmon in PWS.

Wally Noerenberg Hatchery, PWS. Photo courtesy of The Cordova Times.

DEVELOPING CULTURE METHODS FOR LARVAL PACIFIC LAMPREY: ENERGETIC DEMANDS

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As a First Food for the Confederated Tribes of the Umatilla Indian Reservation, Pacific lamprey holds a deep cultural significance. Artificial propagation is a tribal restoration focus, both to replenish declining wild populations and gain a greater understanding of this species.

In culture, oxygen availability can be limiting, especially after larvae are fed oxygen-consuming yeast and fish food. Therefore, it is crucial to understand oxygen consumption rates of the two age classes in culture (2018 and 2019 spawn), and in conditions with or without food. A goal for artificial propagation is release into the wild, so it is vital to understand how energy demands and fitness of propagated larvae compare to wild-caught larvae.

Oxygen consumption was measured in static chambers with optical oxygen probes. The resulting data was compared between age classes, between fed and unfed groups, and between lab-propagated and wild-caught larvae. We hypothesized that oxygen uptake would increase with size and with feeding due to specific dynamic action and yeast consumption and would drop back down after feeding. We also hypothesized that the energy demands of wild lamprey and lab-propagated lamprey would mirror each other.

Larvae from the 2019 spawn have low respiration rates. However, upon feeding the combination of yeast respiration and mostly specific dynamic action of the lamprey significantly increases oxygen consumption. Larvae return to their normal consumption rate in 24 hours. Larvae from the 2018 spawn had higher weight-adjusted respiration rates even before feeding (Fig. 1). This shows that oxygen is vital when feeding. Wild and lab propagated lamprey had similar oxygen demands (Fig 2). This indicates that lab-reared lamprey may be adequate surrogates for wild fish.

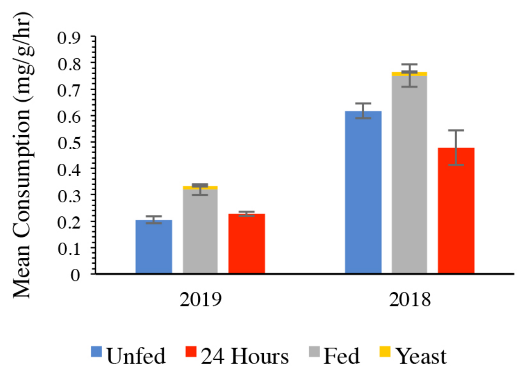

Figure 1. Mean oxygen consumption (mg/g/hr) of unfed, fed, yeast and fish 24 hours after feeding (± SE) (n=8) of larval Pacific lamprey from the 2019 and 2018 spawn.

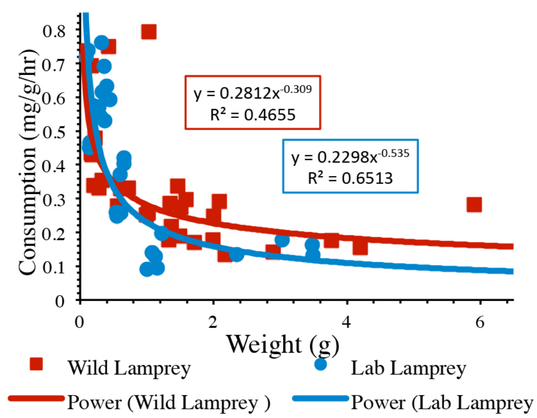

Figure 2. Comparison of oxygen demands of wild (n=30) and lab propagated lamprey (n=30).

REFINING ANESTHESIA METHODOLOGIES FOR HOGFISH (*Lachnolaimus maximus*): THE FIRST STEP TOWARDS LARGE SCALE PRODUCTION

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Hogfish (*Lachnolaimus maximus*) are a high demand recreational and commercial foodfish, targeted by both recreational and commercial fisherman along the Gulf Coast of Florida and the Atlantic Coast. Monandric protogynous hermaphrodites that can reach 90cm in length, hogfish are commonly targetedby spearfishermen in Florida. Declining catch rates and high demand make this species a good candidate for aquaculture. Currently there are no comprehensive data on aquaculture methods for this highly desired foodfish. The University of Florida has collected Hogfish via hook and line, netting, and trapping to develop broodstock and aquaculture protocols for this species. Groups of fish are kept in recirculating systems that are either temperature controlled or non-controlled, with some groups set up as harems (1M:>3F) and others as grow-out groups.

Behavioral observations are recorded daily for each broodstock tank. Harem groups have been established in 5 tanks, while other tanks contain smaller fish for growth rate studies. Harem group tanks are photothermally controlled. Volitional spawning is expected within peak breeding season for hogfish, January through April. If volitional spawning fails to produce progeny, hormonally induced spawning will commence to ensure larval reproduction.

The first step to ensuring successful hormonally induced spawning is concrete data on anesthesia protocol, necessary for hormonal injections and reduction of stress during transport and minor surgical procedures. The anesthetic agents Tricaine-S and Aqui-S 20E (INAD #11-741) will be tested on individual fish at or near the size of broodstock animals to determine effective dosages. Fish (n=40) will be exposed to one of the following: 100, 125, 150 mg/L for Tricaine-S, and 5, 10, and 20mg/L for Aqui-S 20E. Up to five fish per treatment dose will be used to obtain adequate statistical power. Anesthetic and dose effectiveness will be based on the time required for anesthesia induction, level of anesthesia achieved according to a common scheme (Sedation, Anesthesia, Surgical Anesthesia, and Death), and recovery time. An effective dose will allow handling of broodfish for 30-60 seconds without adverse effects and can be applied to any future situation where Hogfish are to be handled and kept alive.

RECENT ADVANCES IN AQUAPONICS IN CANADA

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Aquaponics is an example of integrated production system where fish, plants and microorganisms form mutualistic relationships similar to natural ecosystems. It is a significant departure from the paradigm existing in current agriculture based on monoculture. The aquaponics microbiota plays a pivotal role in forming those relationships. Among many other benefits, aquaponics allows to recirculate the same water indefinitely without necessity to discharge. Alberta became a leader in aquaponics technology in Canada with the research starting in early 2000s at Crop Diversification Centre South in Brooks and Lethbridge College. This research resulted to the first zero-waste aquaponics facility built in Brooks in 2005 due to the incorporation of the aerobic bioreactors. The bioreactors convert solid waste into soluble fertilizers, which can be recycled back to the system. Two research grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) received in 2013 and 2015 allowed to further improve the technology. The conditions of the fermentation process in the aerobic bioreactor have been optimized to achieve the efficient conversion into the soluble minerals (Khiari at al 2018). Another improvement was the development of biochar as a stable bio-filtration medium to remove finer suspended solid particles in the range 1-50 micron from fish effluent. Biochar can be produced from renewable sources, such as woodchips, straw, rice husk and coconut coir, as a result of anaerobic pyrolysis. The biochar as a growing and filtration medium in soilless culture has been developed in Alberta since early 2000s (Savidov 2013). The trickling filter can be used as a secondary water treatment after removal of larger suspended particles using more conventional methods, such as drum filters or passive settlers. The larger biochar pores can be populated by bacteria converting this material into a very efficient and affordable biofilter for conversion of fish-toxic ammonium to plant preferred nitrates. A recent study has been conducted with bamboo biochar to optimize the flow rate and the thickness of the biochar for removal of the suspended solids. Both aerobic bioreactors and biochar filters can revolutionize aquaponics and fish farming and make it more competitive with traditional technologies based on monoculture. Aquaponics has a potential to become the first food production system, which generates zero waste.

RESILIENT AQUAPONIC FOOD PRODUCTION IN TEMPERATE CLIMATES USING A CONTROLLED ENVIRONMENT GREENHOUSE AND A VARIETY OF ENERGY ASSETS

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Santa Fe Community College has been a leader in education and training for Aquaponic food production since 2013. Focusing on food production, as well as, energy and water conservation, several commercial production systems have been installed growing a variety of fish and plant species. A 12,000 ft^2 climate-controlled greenhouse was completed in 2019 and commercial hydroponic and aquaponic systems have been installed to train students as the future workforce. Water use minimization through recycling of nutrient solutions is integral to all cultivation systems. Safe food production (Good Agriculture Practices) is a major focus of the program and all students are trained and certified in safe food-handling. Small-scale, night radiant cooling trials are being conducted and show promising results for cooling water without the use of high energy demand chillers. Additionally, through the Office of Electricity (U.S. Department of Energy), a lithium-ion battery has been installed, coupled with the campus solar energy power grid, that will increase the resiliency of the greenhouse operations and shift peak load thereby lowering operational power costs. Once operational the "nanogrid" greenhouse will have the ability to operate independent of local utilities.

CONTINUED BIOMASS OPTIMIZATION OF ALGAL TURF SCRUBBERS FOR AQUACULTURE AND BIOFUEL APPLICATIONS

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Algal turf scrubbers (ATS) have been widely used since their invention for efficient nitrogen and phosphorous removal from natural and man-made waterways. The system is typically arranged as a downward sloping flow-way onto which a mesh matrix is attached to assist in colonization by epiphytic algae. As water flows over the developed algae mat, soluble nitrogen and phosphorus are assimilated by the algae. These systems have recently gained interest by the biofuels community due to their ability to produce large amounts of biomass at costs which are typically less than microalgae production. Our research over the past 3 years has focused on the optimization of these systems for maximal biomass production and nutrient removal.

Our current research has focused on testing different matrix types (i.e. polypropylene, polyethylene, etc.) as well as different matrix attachment structures (i.e. 1-dimensional, 2-dimensional, 3-dimensional). Current research has utilized a 12 lane, 40 foot long ATS system located on a pier at Priest Landing in Savannah, Georgia. Trials are conducted on a year long basis to assess seasonal productivity and nutrient removal. Water samples are taken daily to monitor nutrient (ammonia, nitrite, nitrate and phosphorus) removal. Solar intensity, wind speed, wind direction, air temperature, relative humidity, and precipitation are also measured daily. Twice a day (a.m. and p.m.), dissolved oxygen, salinity, temperature, and pH are measured with a portable meter and recorded for each ATS lane. Biomass is harvested once a week and analyzed for biochemical composition (protein, lipid, ash, and energy) to determine its potential as an aquaculture feed ingredient and/or bioenergy source. Samples are also collected weekly to determine the amount of algal grazers (i.e. amphipods, copepods, rotfiers, etc.) which are living on the different matrix types. Research to date has shown 3-dimensional matrices extruded from polypropylene produces significantly more biomass and has higher nutrient removal than other matrices tested. The systems have also not experienced a production "crash", commonly experienced in outdoor microalgae culture, to date. Based on this data, it is apparent that ATS systems can be easily optimized for cost efficient nutrient removal in many different commercial aquaculture settings.

Picture 1. Experimental ATS system at Priest Landing, Savannah, GA.

RETAINED WATER AND BACTERIAL LOAD FOR SILURIFORMES FILLETS BEFORE, DURING AND AFTER PROCESSING

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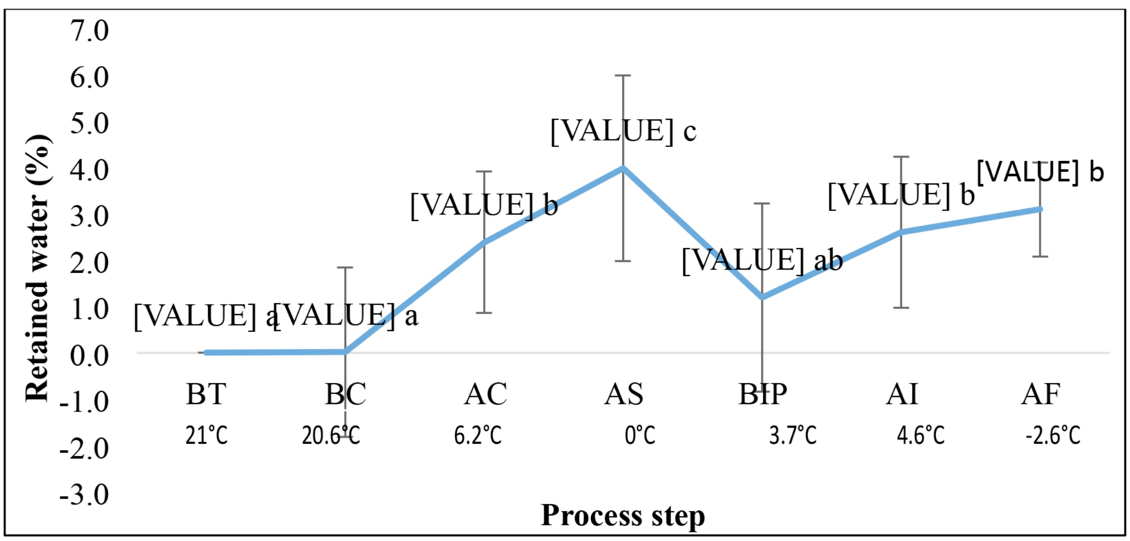
The USDA-FSIS adopted existing meat and poultry net weight and retained water labeling regulations for Siluriformes products including channel (*Ictalurus punctatus*) and hybrid (*Ictalurus furcatus x Ictalurus punctatus*) catfish. Proximate composition, retained water and bacterial load of two sizes of hybrid catfish fillets was determined. The overall mean (± SD) baseline moisture, protein and fat content of the fillets were 77.8% (1.4), 16.7% (0.5) and 5.7% (1.6), respectively. Baseline moisture (78.6 vs 76.8%) content was greater ($P \leq 0.05$) and fat content (4.7% vs 6.8%) was lower ($P \leq 0.05$) for small fillets (111 g) in comparison to large fillets (247g), whereas protein content was similar ($P > 0.05$) for both sizes. The retained water of the final fresh and frozen fillets were 1.2 ± 2.0% and 3.1 ± 1.0%, respectively and was not size dependent. PPC and TCC of the baseline fillets were ~ 4 log CFU/g and 1.6 log CFU/g, respectively and did not change ($P > 0.05$) during processing, except at injection. Moisture-protein ratio and fat content were good ($P \leq 0.05$) predictors for retained water of the catfish fillets during processing.

This work showed that market-ready catfish fillets (fresh) did not retain any water even after water and slush-ice chilling. The apparent moisture gain is lost during the process and probably after the process, regardless of fillet size.



Retained water and bacterial load for Siluriformes (hybrid catfish: *Ictalurus furcatus x Ictalurus punctatus*) from reception to final product (**BT**=Before Trimming (Baseline); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; **BIP**= Before ice packing (Fresh fillets) ; AI= After injecting (polyphosphate injection), **AF**=After freezing (Frozen fillets).

SLUSH-ICE CHILLING PROCESS COULD AFFECT SURVIVAL/GROWTH OF *Salmonella* spp. AND LOWER MICROBIAL FLORA IN SILURIFORMES

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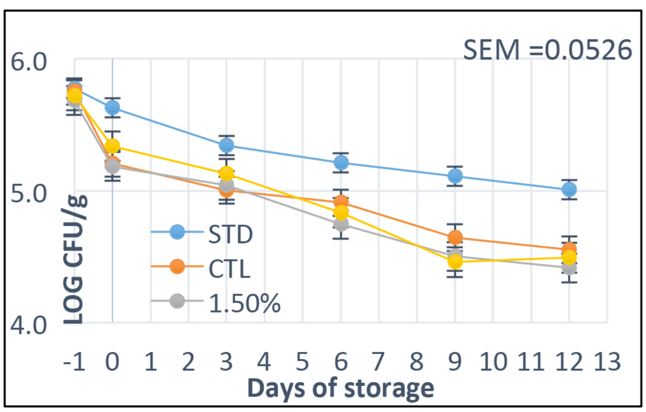
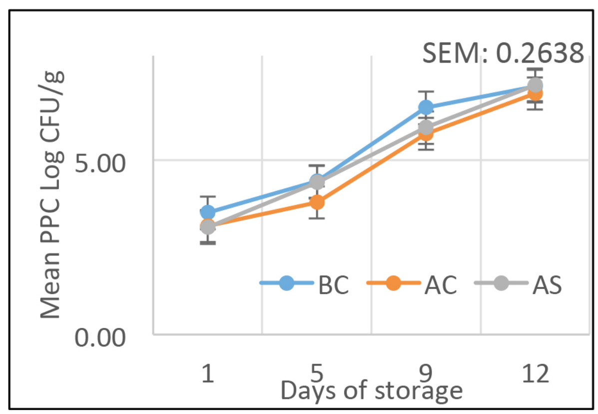
Slush-ice chilling is a process used to lower fish/fillet temperature quickly and effectively prior to packaging/freezing the product. However, little is known about the effect of this system on fillets' safety and quality. This study was to determine the fate of *Salmonella spp*. and microbial load on catfish (*Ictalurus furcatus x Ictalurus punctatus*) fillets subjected to simulated slush-ice chilling and refrigerated storage conditions. Catfish fillets inoculated with a cocktail of *Salmonella* strains were then subjected to a mixture of slush ice and brine. Samples were held in a cooler at 2°C for up to 12 days to enumerate Salmonella spp. Additionally, fillets taken before-water chill, after-water chill, and after 24-h slush-ice, were stored in a cooler at 2°C for up to 12 days to analyze psychotrophic plate (PPC), total coliforms (TCC), and generic *E. coli* counts. Salmonella counts were slightly reduced ($P \leq 0.05$) between 0.55 - 0.83 log CFU/g by slush ice treatments (0% - 4.5% salt). Salmonella reduction was similar ($P > 0.05$) among all treatments after 12 d of storage. Psychotrophs, coliforms, and *E. coli* counts were similar ($P > 0.05$) among fillets collected before and after 24 h in slush ice. Under the assessed conditions, slush-ice treatments had minimal impact on *Salmonella spp*. and did not have a residual effect during refrigerated storage. Psychrotrophic plate counts (PPC) increased similarly during refrigerated storage, regardless of chilling process.



Salmonella spp. counts on catfish fillets by salt concentration and days of storage at refrigeration temperature 2 ± 2°C. (Day from -1 to 0 indicates the time in slush ice. STD: untreated samples; without salt and ice slush. CTL: 0% salt, 1.5% salt).



Psychotrophic plate counts (PPC) of catfish fillets before and after chilling over 12 d at 2 ± 2°C. (BC: Fillets collected before water chill. AC: fillets collected after water chill. AS: fillets collected after 24 h in slush ice).

HOGFISH (*Lachnolaimus maximus*) BROODSTOCK HUSBANDRY, HAREM FORMATION, VOLITIONAL SPAWNING, AND LARVAL REARING

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Hogfish (*Lachnolaimus maximus*) are a valuable sport and food fish. Hogfish are commonly targeted by recreational spear fishermen, but also can be caught on hook and line. Recreational and commercial harvest of hogfish in the United States is most common in Florida. From 2000 to 2015, the average number of hogfish landed annually in Florida by recreational fishermen was about 145,000 fish with commercial landings averaging around 45,000 pounds. Given the dearth of information on hogfish aquaculture, we are currently in the process of evaluating methodologies to achieve two main objectives: (1.) develop and spawn broodfish and (2.) define efficient egg incubation and larval culture protocols.

In 2019, the University of Florida's Indian River Research and Education Center Aquaculture Lab began collecting wild broodstock and acclimatizing them to captivity. Recreational charter captains and ornamental fish collectors that target hogfish were used to acquire broodstock for this research. Once fish were brought to the Aquaculture facility in Fort Pierce, Florida, growth rates of three groups of (>30.5 cm) were recorded monthly. These groups were observed four times daily for behavior changes, harem formation behavior, and sex change behavior. Changes in sexual dimorphism and harem formation behavior were observed and were recorded daily. These behaviors included jaw locking, color change, aggression between assumed males, and mating displays.

Repeated volitional spawning occurred starting in December 2019. Data will be presented on hatch rates, survival to first feeding, optimum feeding protocol and water quality. Additionally, adult hogfish were subjected to anesthesia trials evaluating the effectiveness of Tricaine-S and Aqui-S 20E (INAD #11-741) on various fish sizes.

TRANSGENE DELIVERY STRATEGIES TO INTEGRATE CATHELICIDIN GENE IN THE CHANNEL CATFISH GENOME BY CRISPR/CAS9 KNOCK-IN SYSTEM

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CRISPR/Cas9-based gene knock out in mammalian cells, particularly in teleosts has proven to be very efficient in regards to mutation rates, but precise insertion of exogenous DNA or gene knock-in via the homology directed repair pathway remains elusive. While microinjection is more routinely used to introduce CRISPR components into fish embryos, it is labor-intensive and time-consuming, as embryos need to be injected individually. An alternative to microinjection is electroporation, which uses a rapid and high-voltage electric pulse to deliver DNA into embryos. Here, we utilized single electroporation, where CRISPR components were co-delivered with the donor DNA into fertilized eggs, and double electroporation, where both sperm and fertilized eggs received the CRISPR components and donor DNA. In this study, we designed double-stranded DNA constructs driven by zebrafish ubiquitin (dsDNA Ubi 40ng/μl) and carp β-actin (dsDNA β-actin 40ng/μl) promoters, as well as plasmid DNA construct driven by zebrafish ubiquitin promoter (plasmid Ubi 50ng/μl) carrying cathelicidin gene, a disease-resistance gene derived from the American alligator.

We succeeded in integrating with high efficiency an exogenous cathelicidin gene into chromosome 1 of channel catfish genome. As shown in Table 1 and Figure 1, highest integration rates were found using microinjection, both in dead fry with plasmid

Ubi as donor DNA (61.5%) as well as in alive fingerlings with sdDNA Ubi donor (31.8%). Additionally, electroporation also proved to be an efficient method to deliver transgene as indicated by 44.4 % integration rate in dead fry and 12.5 % in alive fish using single electroporation technique.

Overall, we have successfully generated a CRISPR knock-in transgenic channel catfish carrying cathelicidin gene using microinjection and electroporation strategies. In general, integration rates were higher in dead fry, indicating either off-target effects or pleiotropic effects. Additionally, we may be targeting a sensitive area of the genome.

Table 1. Integration rates of cathelicidin gene constructs in dead fry and surviving fingerlings that were microinjected and electroporated using CRISPR/Cas9 system.

Delivery Method	Donor DNA construct	Dead fry			Alive fingerlings		
		Total analyzed	Positive fish	Integration rate (%)	Total analyzed	Positive fish	Integration rate (%)
Microinjection	dsDNA β-actin	30	6	20.0	46	4	8.7
	dsDNA Ubi	31	6	19.3	22	7	31.8
	Plasmid Ubi	13	8	61.5	17	3	17.6
Single Electroporation	Plasmid Ubi	9	4	44.4	24	3	12.5
Double electroporation	Plasmid Ubi	25	5	20.0	20	2	10.0

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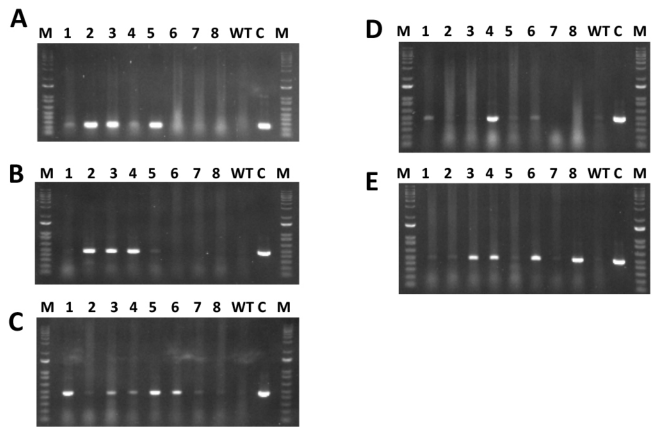

Figure 1. Genotyping of positive fish using primers specific to the different cathelicidin gene constructs. **A-C**, microinjection using dsDNA β-actin, dsDNA Ubi and plasmid Ubi, respectively. **D**, single electroporation and **E**, double electroporation. M: marker; WT: wild type; C: positive control

CATHELICIDIN ANTIMICROBIAL PEPTIDE ENHANCES PROTECTION OF CHANNEL AND CHANNEL-BLUE HYBRID CATFISH AGAINST *Aeromonas hydrophila* AND *Edwardsiella ictaluri* INFECTIONS

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Worldwide, protection of fish against infectious diseases is a major challenge in aquaculture, and economic losses due to these diseases limit profitability. The ability of antimicrobial peptides (AMPs), a class of highly conserved peptides known to possess direct antimicrobial activities against invading pathogens, was investigated for protection in channel and channel catfish female X blue catfish male hybrid catfish against infection caused by the catfish pathogens, *Aeromonas hydrophila* and *Edwardsiella ictaluri*. To identify effective peptides, the minimum inhibitory concentrations (MICs) against fish pathogens and killing kinetics against *A. hydrophila* were determined *in vitro*. Cathelicidins derived from alligator and sea snake exhibited potent and rapid antimicrobial activities against the tested catfish pathogens as compared to cecropin and pleurocidin AMPs.

When the peptides (50 μg/ml) were injected into fish and simultaneously challenged with each pathogen through immersion, increased survival rates in channel and hybrid catfish were observed for both cathelicidins (alligator and sea snake) as compared to other peptides and the infected control (Figure 1). Serum lysozyme levels also increased in channel and hybrid catfish injected with both cathelicidins then challenged with *E. ictaluri* (Figure 2). Increase in serum lysozyme is an indication of elevated immune response against bacterial infections.

These results clearly show the potential of cathelicidins to protect fish against bacterial infections and demonstrates a strategy that overexpressing the peptides in transgenic fish may provide a method of decreasing bacterial disease problems in catfish.

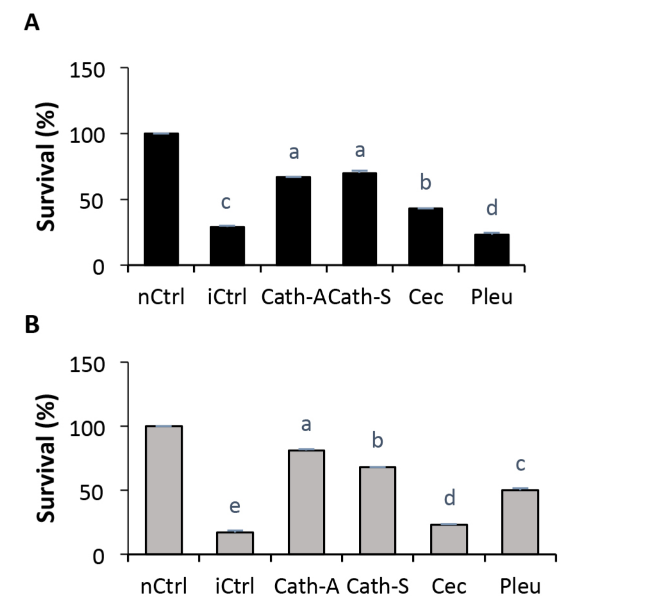

Figure 1. Effect of different antimicrobial peptides on the survival of *A. hydrophila*-infected channel (**A**) and hybrid (**B**) catfish.

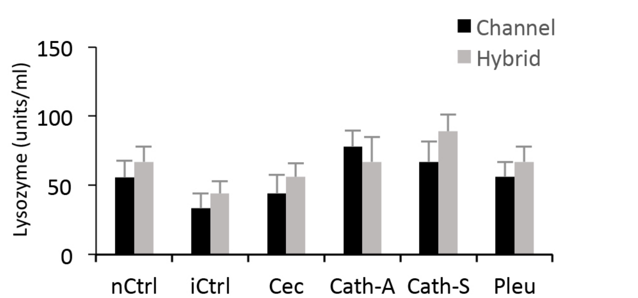

Figure 2. Effect of the different peptides on serum lysozyme level of channel and hybrid catfish following infection with *E. ictaluri*.

KAMPACHI FARMS – COLLABORATIVE OFFSHORE AQUACULTURE R&D AT THE NEXUS OF ECONOMIC OPPORTUNITY AND ECOLOGICAL IMPERATIVE

Neil Anthony Sims*, Lisa Vollbrecht, Helen Meigs, Keelee Martin and Joseph Nakoa

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Kampachi Farms, LLC, is an offshore aquaculture research and development company based in Kona, Hawaii, that recognizes and embraces the power of collaboration. We see development of offshore aquaculture as a pressing ecological imperative, and a tremendous economic opportunity. Each of our innovative breakthroughs in offshore aquaculture has only been possible through partnering with other agencies, companies, colleagues or institutions.

The Velella Beta-test (the world's first unanchored net pen, recognized as one of TIME Magazine's 25 Best Inventions of the Year in 2012) was funded by NSF SBIR, with matching support from Illinois Soybean Association; International Copper Association; Ocean Farm Technologies (now part of InnovaSea), Lockheed Martin, researchers from University of Hawaii at Hilo, with an Experimental Fishing Permit from NOAA. Our Velella Epsilon demonstration net pen, to be sited 40 miles offshore of Sarasota, Florida, in US Federal waters, is supported by National SeaGrant funding, and University of Florida SeaGrant, together with a range of private industry partners. The primary goal of Velella Epsilon is to help fishermen and other ocean users understand the minimal impacts and the abundant benefits of offshore aquaculture. In the Gulf – as in Kona - actual experience with offshore net pens should render fishermen our most vocal supporters.

In ongoing feeds research, we have grown kampachi (*Seriola rivoliana*) on zero-fishmeal diets, and tested diets including soy protein concentrate, duckweed, microalgae byproducts, and Single-Celled Proteins from ag-waste biodigestors. This work has been supported by Nebraska Soybean Board, NOAA Saltonstall-Kennedy, and USDA SBIR funding, and has partnered with University of Nebraska Lincoln, the F3 Network, USDA ARS feed mill in Montana, and companies and individuals producing a range of innovative feedstuffs.

Our offshore macroalgal and seaweed biodigestion research is supported by US Department of Energy's ARPA-E program, and has involved partnering with Makai Ocean Engineering, The Climate Foundation, University of Hawaii Hilo and UH Manoa; UC Irvine; UC San Diego; San Diego State U; Lawrence Berkeley Laboratory and the National Renewable Energy Laboratory.

We also provide tours of our Kona-based research facility, with the express goal of increasing public understanding of the need for offshore aquaculture, and the benefits that can accrue from a well-planned and -managed offshore industry. We welcome interns and other volunteers. We would be pleased to engage with other innovators to help bring offshore aquaculture to its fullest potential, as a salve for the seas and a partial panacea for the planet.

HAWAII – A HOTBED OF OFFSHORE AQUACULTURE INNOVATION

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Hawai'i faces a number of constraints to commercial aquaculture development. It is the most isolated archipelago on the planet, with 2,500 miles (or 5 hours of airfreight time) to reach the West Coast of the USA. It has a relatively small potential market (population 1.1 million; with an additional 7 million tourists per year). Land, freshwater, electricity and labor are all expensive. There is strong regulatory oversight, a cumbersome bureaucracy, and a tax structure that together have earned the state the nickname of "The People's Socialist Republic of Hawai'i". There is also a history of anti-development activism that has – in the past – thwarted both commercial and research projects, such as Deep Ocean CO_2 sequestration trials, the Superferry, and (possibly) the Thirty Meter Telescope.

Despite these odds, however, Hawai'i has become a leader nationally – and internationally – in the development of offshore aquaculture. There are several countervailing attributes to the islands: there is a strong cultural tradition of aquaculture through the ancient Hawai'ian fishpond system; in some locations, offshore waters are literally a stone's throw from the shoreline; there are protected lee waters behind most of the islands, protecting them from prevailing trade winds and seas; there is an abundance of maritime, fishing and diving expertise; there is legislative, regulatory and community support for the expansion of local seafood supplies; and there were some pioneering visionaries in the 1990's who helped bring the industry to fruition.

The Natural Energy Laboratory of Hawaii Authority (NELHA) and the surrounding waters of the Kona Coast are particularly conducive to innovative offshore aquaculture R&D and commercial development. Kona waters are relatively protected from trade winds and winter swells, yet provide access to truly offshore conditions within 200 m of the coastline. NELHA's land-based incubator and commercialization park facilities provide reticulated deep seawater and surface seawater, as well as pre-approval for most aquaculture activities.

This presentation will review the history of offshore aquaculture R&D in Hawai'i, and explore the reasons why the industry has gained a foothold here. The presentation will then discuss current and future needs for the continued expansion of both the R&D efforts, and commercial culture in the offshore realm.

ENGINEERED QUORUM QUENCHING BACTERIA ATTENUATE EARLY MORTALITY SYNDROME IN *Litopenaeus vannamei* BY SUPPRESSING TOXIN PRODUCTION

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Vibrio parahaemolyticus is a rapidly evolving pathogen of both human and seawater animals. Depending on the diversity of horizontally acquired pathogenicity factors, *V. parahaemolyticus* may cause a broad range of diseases, including food poisoning in humans and Early Mortality Syndrome (EMS) or Hepatopancreatic Acute Necrosis Syndrome in shrimp. In shrimp farms, EMS-caused mortality may reach 100% in the first 30 days after infection leading to substantial business losses for farmers.

The control of bacterial pathogens without use of antibiotics or harsh chemicals is a challenge in modern aquaculture where animal densities are often very high. In many bacteria quorum sensing molecules (QS autoinducers) regulate many pathogen traits including biofilm and toxin production. Thus, the elimination of QS molecules or quorum quenching (QQ) may be used to manage bacterial infections and virulence. Here we test the hypothesis that probiotic bacterial strains engineered to reduce QS concentrations may be an alternative approach to conventional antibacterial treatments for the control of EMS in shrimp.

As an enhanced probiotic, we selected *Enterobacter* Ag1, which is salt-resistant and capable of colonization of shrimp intestines. To create Ag1 quorum-quenching derivatives, we cloned and expressed a number of lactone-degrading enzymes under the control of a strong gene promoter. Lactonase AidH expressing Ag1 was selected as the optimal construct due to its ability to quench QS regulated luminescence in light-emitting *V. harveii* strain. To test Ag1-AidH ability to manage EMS in shrimp, we supplemented shrimp food with bacteria and monitored shrimp mortality. We showed that the experimental shrimp group had reduced mortality (25 to 55%) relative to controls. Additionally, we found that expression of the *pir*-like toxin, the primary pathogenicity factor in *V. parahaemolyticus* infected shrimp intestines, was reduced by more than 2-fold by QQ bacteria.

The level *pirA* expression increases more than 10,000 fold during bacterial growth associated with the production of QS molecules. Co-cultivation of quorum quenching Ag1 with *Vibrio* lead to a 2- to 3-fold reduction in *pirA* expression. We propose that reduction in shrimp mortality is, in part, associated with reduced toxin production mediated by QQ bacteria. QQ may provide sustainable solution to control *Vibrio*-related shrimp diseases and mortality.

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UNDERSTANDING THE ISSUE OF POOR SPAT RETENTION ON NEW ZEALAND'S GREENSHELL™ MUSSEL *Perna canaliculus* FARMS

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The early stages of Greenshell™ mussel (*Perna canaliculus*) aquaculture in New Zealand are highly inefficient, with the majority of seed mussels ('spat') lost from farming substrata shortly after seeding out. These high spat losses (conversely known as poor spat retention) are extremely costly to the Greenshell™ industry, where they constrain production and limit industry growth. The causes of poor spat retention are unclear, though it appears to be predominantly caused by secondary settlement behaviour, which is a prominent feature in juveniles of this species. However, the factors that trigger secondary settlement behaviour are poorly understood, and therefore, cannot be managed. To date, few studies have addressed the issue of poor spat retention directly, and as a result, no solutions to the problem currently exist. Through a series of field experiments, this study sought to identify some dominant causes of poor spat retention on Greenshell™ mussel farms under typical farming conditions.

Four experiments were conducted; each measuring spat retention across multiple farm sites under typical farming conditions. In each experiment a series of experimental droppers were seeded out with spat from a single cohort, and their performance was measured over time.

In each experiment, spat retention was poor (frequently < 30%), and the majority of spat losses took place early on in the production cycle, shortly after seeding out (Fig. 1). Spat losses were also consistent among farm sites, indicating that local environmental conditions likely played little role in influencing spat retention. The timing of spat losses in relation seeding substrata breakdown suggest that changes in the arrangements for seeding out could be used to reduce spat losses.

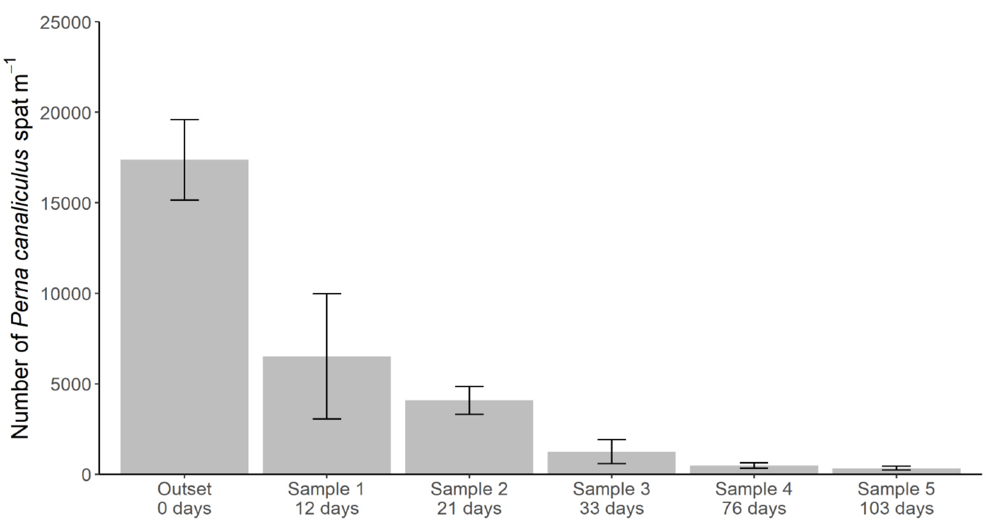

Fig. 1. Spat retention (mean number of spat remaining m^{-1} of grow rope ± SE) averaged across farm sites in one of the experiments.

GAINING AN UNDERSTANDING OF MIDWEST AQUACULTURE PRODUCERS' VIEWS OF THE NORTH CENTRAL REGIONAL AQUACULTURE CENTER THROUGH PERSONAL INTERVIEWS

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Midwest Extension, researchers, and producers are seeking to increase communication regarding North Central Regional Aquaculture Centers' (NCRAC) funded projects and the overall proposal process. Increasing communication should lead to producers further understanding university constraints as it pertains to proposal writing as well as improve research and Extension's knowledge of what the industry believes should be investigated to help advance the aquaculture sector. Several small group discussions will occur on farms with producers throughout the Midwest in the fall of 2019 as part of a NCRAC funded Extension project. A qualitative interview guide was developed to capture comments, concerns, and ideas related to NCRAC. Producers are being identified by their current and/or past involvement in NCRAC, as applicable, and will be solicited through personal e-mail and phone calls for participation in this study. On-farm small group (1-5 producers) discussions will be held to promote heavily engaged participation, which is unlikely to be found in larger group settings. The primary dissemination of the data will occur the first weekend in February 2020 during an aquaculture communication day prior to the North Central Aquaculture Conference in Columbus, Ohio. The 2020 conference will be held jointly between the Ohio Aquaculture Association (OAA) and NCRAC. A presentation and printed materials will be given at the start of the day. This information will be used to provoke discussion for the remainder of the communication day. Data results, a communications meeting synopsis, and a discussion will be presented.

LIFE CYCLE ASSESSMENT OF DECOUPLED SEMI-COMMERCIAL AQUAPONIC SYSTEM, INITIAL FINDINGS

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Aquaponics systems have great potential to improve the sustainability of food production systems by leveraging the waste of one production system (aquaculture) to feed another (hydroponics). While aquaponics is generally thought of as a "sustainable" approach to fish and vegetable production, the assumption that aquaponics systems are inherently sustainable has never been thoroughly tested. This effort will utilize the life cycle assessment (LCA) approach to rigorously test the hypothesis that decoupled aquaponics has lower environmental impacts than stand-alone recirculating aquaculture and conventional vegetable production.

Intensive data collection has been and continues to be conducted on the existing pilot aquaponic system at Auburn University. This data coupled with a process model developed in SuperPro Designer and calibrated with field data has allowed us to conduct lifecycle analysis on the following impact categories: total primary energy, climate change, consumptive water use, land use, eutrophication potential, and oxygen depletion. Environmental impacts will be quantified and specific operations within the aquaponics system that contribute the most to environmental impacts will be identified. Isolating areas of weakness within the system will allow us to develop new approaches to further reduce environmental impacts and improve overall sustainability.

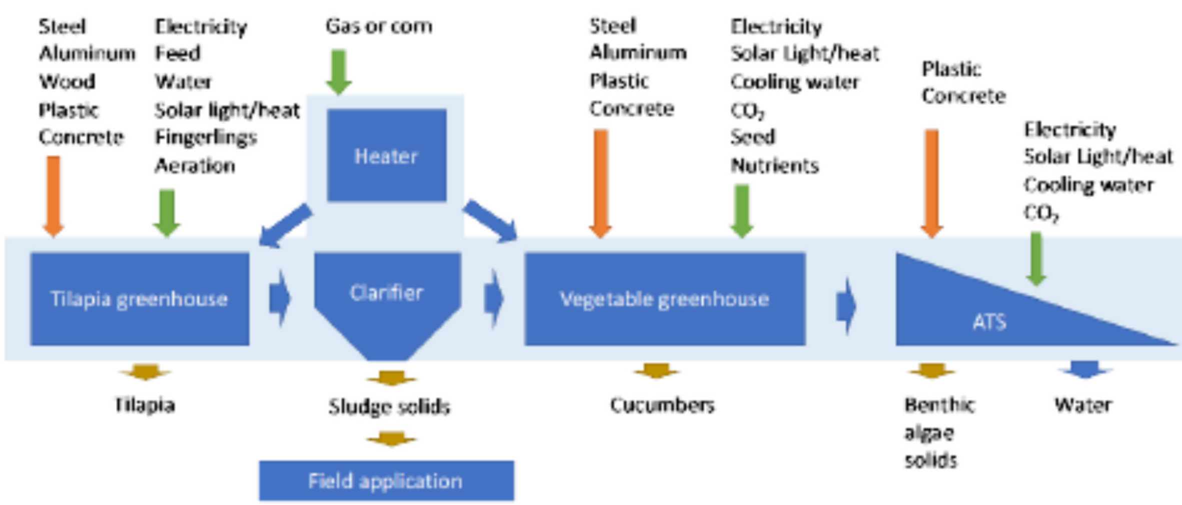

Figure: A schematic of the aquaponics system is shown with the system boundary in light blue.

ECONOMIC ASSESSMENT OF DECOUPLED SEMI-COMMERCIAL AQUAPONIC SYSTEM FROM 2015 TO 2019

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Since 2015, an aquaponics working group comprised of professors and students from Aquaculture, Horticulture, Biosystems Engineering, and Food Science have been operating a semi-commercial aquaponic system. The aquaponic system includes one standard commercial greenhouse for fish production and one standard commercial greenhouse for plant production.

In the fish greenhouse Nile tilapia are grown in one 27,000-gallon. Tilapia are housed in a standard commercial greenhouse measuring 2,880 square feet. Tilapia of various sizes are grown simultaneously and are partially harvested as the market demands at approximately one pound. Fish are fed multiple times per day with a commercial fish feed at varying protein levels depending on their size. What fish effluent is used for watering vegetables is replaced with fresh water by gravity from a rain-fed reservoir.

In the plant greenhouse cucumbers and cherry tomatoes are produced in Dutch buckets filled with a substrate, either pine bark or perlite. The plants are trellised to make use of the three-dimensional space of the greenhouse. Plants are irrigated with fish effluent using drip emitters and an irrigation pump. Environmental conditions are controlled by a thermostat and a humidity probe based on the seasonal parameters. Propane is burned as heating fuel in the winter.

All income and expenses related to the system have been carefully documented from the beginning of the operation up to the present day. That data will be presented in an economic assessment of the system as a whole, including the cash flow, enterprise budgets for both plant and fish production, and some sensitivity analysis.

Combined Tilapia/Vegetable Aquaponics Enterprise Budget, Auburn AL Oct. 2015 - Sept, 2016

Item	Unit	Quant.	Price	Amnt	Amnt
I. Receipts					
Tilapia sales					$ 8,537
- To Dining Services	lb	2,347	$ 2.00	$ 4,695	
- AU Fish Market	lb	1,921	$ 2.00	$ 3,842	
Cucumber sales					$ 15,784
- Tiger Dining Services	lb	6,409	$ 1.20	$ 7,691	
- AU Fisheries	lb	303	$ 1.00	$ 303	
- East Alabama Food Bank	lb	6,491	$ 1.20	$ 7,789	

IMPROVING FARMED FISH HEALTH WITH THE RED MACROALGAE TURKISH TOWEL *Chondracanthus exasperates*

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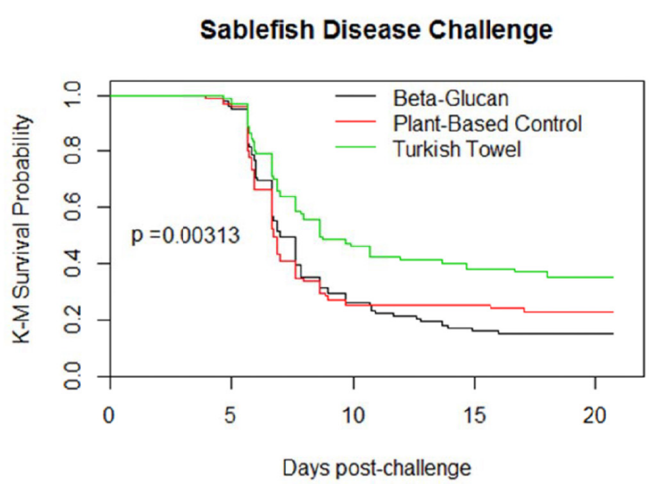
Terrestrial plant proteins have been shown to adequately replace a portion of the fishmeal used in aquaculture feeds for a number of species. Complete replacement has yet to be achieved due to nutrient deficiencies and species-specific sensitivities. Macroalgae contains many essential nutrients that are limiting in terrestrial plants (i.e. trace minerals, omega-3 fatty acids, amino acids). There also appears to be health benefits associated with the use of macroalgae in aquaculture feeds for some fish species. The combination of the two ingredient types shows great potential for the complete replacement of fishmeal and fish oil in aquaculture feeds. Recent studies at NWFSC have shown improvements in feed intake, growth, and liver histomorphology when the red macroalgae *Turkish Towel Chondracanthus exasperates* was added to plant-based feeds for sablefish (*Anoplopoma fimbria*) a cold-water marine fish of the Eastern Pacific Ocean.

A feeding and disease challenge study was conducted in 2019 to evaluate the health benefits associated with a 10% addition of Turkish Towel to an alternative, plant-based feed for juvenile sablefish. An additional experimental feed was evaluated which included the addition of 1% Macrogard (beta-1,3/1,6 glucans) to a plant-based feed. When macroalgae is added, wheat flour and soy protein concentrate are removed from the formulation to maintain similar protein content among experimental feeds. Two experimental feeds were prepared in addition to a standard plant-based feed formulation, used as a control.

The disease challenge was carried out at NOAA's Newport, OR laboratory with atypical *Aeromonas salmonicida* according to standard laboratory protocols. There were no mortalities in the no pathogen controls. When Turkish Towel was used as a supplement in the plant-based diet, sablefish had significantly greater survival than fish fed either the plant-based control or beta-glucan feeds.



EVALUATING MICROALGAE SPECIES EFFECT ON SURVIVAL AND FEEDING DURING EARLY LARVICULTURE FOR THREE MARINE ORNAMENTAL SPECIES

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The marine aquarium hobby has grown considerably in recent decades to become a multi-billion-dollar industry. Less than 10% of commercially available marine species are cultured, while the rest are wild-caught. The global marine aquarium industry is working to improve culture practices to reduce population strain from harvest on imperiled ecosystems. Species that spawn demersally dominate the aquaculture market due to the availability of established rearing protocols, and high relative parental care and larval survival. Pelagic spawning species usually provide no parental care and the newly hatched larvae have a small mouth gape and underdeveloped gastrointestinal system that limit their diet to copepod nauplii and other small, easily digestible zooplankton or phytoplankton. Challenges associated with these characteristics need to be addressed for production of pelagic species to become commercially viable. Species such as the Pacific Blue Tang (*Paracanthurus hepatus*), Melanurus Wrasse (*Halichoeres melanurus*), and Yellow Wrasse (*Halichoeres chrysus*) are of particular interest because of their popularity in the marine aquarium trade and lack of established rearing methods.

This study focuses on the manipulation of the culture environment by providing varying algae species, potentially resulting in unique environmental and nutritional profiles which can lead to varying survival and prey capture. Pelagic eggs were collected, enumerated and stocked into 15L static tanks containing either the control treatment, *Tisochrysis lutea, Chaetoceros muelleri,* or *Tetraselmis chuii*. After three days *Parvocalanus crassirostris* nauplii (<75um) were provided and the larvae can feed for three hours. The larvae were then harvested, enumerated and visually inspected for ingestion of nauplii. Results suggest inclusion of the microalgae *Tisochrysis lutea* during early larviculture of the Melanurus Wrasse aids in prey capture whereas the addition of the diatom *Chaetoceros muelleri* negatively impacts larval survival ($P < 0.001$). Trials conducted with Pacific Blue Tang show an increased feeding response with inclusion of either *Tisochrysis lutea* or *Tetraselmis chuii* ($P = 0.003$). The Yellow Wrasse showed no survival or feeding advantage to the inclusion of microalgae. These results will help to shape commercial production protocols and inform decisions about feasibility of production.

EFFECTS OF MICROALGAE DENSITY, PREY DENSITY AND POTENTIAL FEED ATTRACTANTS ON SURVIVAL AND FEEDING INCIDENCE FOR PACIFIC BLUE TANG *Paracanthurus hepatus* AND MELANURUS WRASSE *Halichoeres melanurus*

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Although the majority of the marine ornamental fish market is satisfied through the sale of wild caught individuals, ornamental aquarium consumer's preference is shifting to include more aquacultured fish due to the knowledge of declining ecosystems, the need for sustainable products and concerns related to capture methods. Melanurus Wrasse (*Halichoeres melanurus)* and Pacific Blue Tang (*Paracanthurus hepatus)* are of great interest to aquaculture due to their popularity in the marine aquarium trade and lack of established commercial rearing methods. Pelagic spawning species produce underdeveloped larvae that rely on a diet of copepod nauplii and other small, easily digestible zooplankton. Environmental parameters, such as light intensity, can influence survival and feeding success in a culture setting. Challenges associated with these characteristics need to be addressed for production of pelagic species to become commercially viable.

Here, we experimentally evaluated different larval rearing protocols from 0 to 3 dph. Algal and prey densities were evaluated to determine a suitable rearing environment that resulted in increased survival and feeding incidence during the transition from endogenous nutrition to exogenous feeding. Pelagic eggs were collected, enumerated and stocked into 15 L static tanks. For evaluation of algal density, a control treatment was included with no algae present and three treatments containing *Tisochrysis lutea* were examined at 100,000, 300,000, or 500,000 cells/ml. To investigate live feed density requirements, *Parvocalanus crassirostris* nauplii were stocked at densities of 2.5, 5, or 10 nauplii/ml. Potential feed attractants were also evaluated, comparing a control treatment with no feed attractant to the addition of taurine, tryptophan, or betaine at 10^{-5} mg/L in the rearing environment. For each experiment, *P. crassirostris* nauplii ($< 75\ \mu$m) were provided at 3 dph and the larvae were able to feed for five hours. The larvae were then harvested, enumerated and visually inspected for ingestion of nauplii using a dissecting microscope with digital camera attachment. Results suggest a *T. lutea* density of 300,000 cells/ml is optimal for both survival ($P < 0.0001$) and feeding incidence ($P = 0.02$) for Melanurus Wrasse and for survival of the Pacific Blue Tang ($P < 0.0001$). Prey density experiments resulted in no difference in feeding incidence across treatments for both species. Inclusion of feed attractants decreased survival ($P < 0.0001$) and had no effect on feeding incidence for Melanurus Wrasse. These results will help to shape commercial production protocols and inform decisions about feasibility of production for these valuable ornamental species.

INFLUENCE OF GENOTYPE ON DNA METHYLATION AMONG OLYMPIA OYSTER POPULATIONS

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An organism's epigenome refers to markers that control how genes are expressed, without altering the underlying DNA sequences. When epigenetic landscapes are heritable, the parental environment could possibly influence the phenotype of progeny. Evidence of this transgenerational epigenetic inheritance in aquatic invertebrates is growing, and may be a mechanism linking parental exposures to offspring performance.

A major question, however, is how the underlying genome mediates epigenetic plasticity. For instance, DNA methylation changes in a stress-response gene may be universal in a group of taxa. Alternatively, changes could be highly specific to a locally adapted population, therefore making species-wide predictions difficult. It is important to know whether DNA methylation patterns are consistent across genetically-distinct families and populations. This is particularly pertinent for studies attempting to link epigenetic modifications to intergenerational plasticity, and in species with high genetic variability, such as oysters.

To assess the influence of genotype on DNA methylation patterns in the Olympia oyster (*Ostrea lurida*), we raised individuals from two distinct populations in common conditions, and assessed both relatedness and methylation patterns using 2b-RAD and MBD-seq, respectively. We found distinct patterns in DNA methylation across populations and were able to associate DNA methylation with phenotype. Taken together, this study highlights the importance of considering DNA methylation in pursuing targeted phenotypes.

AN –OMIC APPROACH TO EXPANDING THE FUNCTIONAL UNDERSTANDING THE OF ACORN BARNACLE *Amphibalanus amphitrite*

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The acorn barnacle *Amphibalanus (=Balanus) amphitrite* is an established hard foulant with a global distribution in tropical and sub-tropical marine environments. Due to the robust nature of *A. amphitrite* in both the wild and in laboratory conditions, it has been studied extensively to understand several aspects of acorn barnacle function including settlement and growth, with particular attention to the formation and composition of adhesives at the substrate interface.

Our research group has been expanding our knowledge of the development of the adhesive interface, relying on several techniques ranging from con-focal microscopy to mass spectrometry (MS). The latter has relied heavily on a transcript library that enables spectral assignment and, therefore, proteomic analysis. This has allowed us to identify a wide range of proteins and enzymes at the adhesive interface. The general protein identification scheme via MS (Fig. 1) highlights the predicted and experimental approaches to gather spectra. One critical caveat of this scheme for *A. amphitrite* is the genomic information available is limited to transcripts gathered from one tissue rather than a completely sequenced genome. As a consequence, it is not uncommon that up to 80% of peptide and potential protein hits are unidentified. This presents a major bottleneck to advancing our understanding of key acorn barnacle functions.

Here, we present preliminary results on sequencing the genome of *A. amphitrite* using a combination of long- and short-read next-generation sequencing technology and discuss the challenges associated with collection of high quality genomic DNA. Building upon our existing transcript and protein knowledge, we demonstrate how complementary gene and transcript data sets are powerful tools to help unravel key functional mechanisms of *A. amphitrite* through an expanded identification of proteins associated with the formation of the adhesive interface and the adhesive itself. Collectively, these –omic results will deepen our understanding of acorn barnacles, which, as sessile crustaceans, are one of nature's more unusual creatures possessing a robust and durable underwater adhesive.

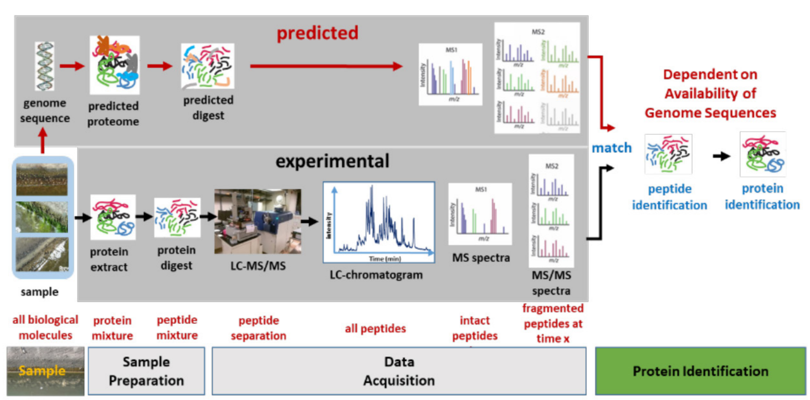

Fig. 1. Overview of protein identification scheme via mass spectrometry.

EFFECT OF PROBIOTICS ON *VIBRIO* BACTERIAL POPULATION OF *Litopenaeus vannamei* IN CULTURE PONDS AT NAUPADA, ANDHRA PRADESH, INDIA

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The current study was carried out for 147 days to assess the *Vibrio* count of *Litopenaeus vannamei* culture ponds from Naupada, Srikakulam District, Andhra Pradesh, India. Three ponds were selected, one is control and other two were experimental (Pond A and B). The physico-chemical parameters of the culture ponds were recorded by adopting standard methods. During summer crop the control ponds were harvested at 24.0 gm on 107th day and at 31.0 gm on 121st day for the year 2017 and 2018 respectively due to the incidence of *Vibriosis* disease. Where as in winter crop during 2017 the control pond was harvested at 5.5 gm on 47th day because of *Vibriosis* but in 2018 the control pond was harvested normally at 32.5 gm on 145th day, as this pond is free of *Vibriosis*. Experimental ponds were harvested normally in summer season at 30.5 gm on 124th day & 31.0 gm on 127th day during 2017 and 34.0 gm on 127th day & 35.5 gm on 127th day in the year 2018 respectively. Where as in winter season of 2017 the experimental ponds were harvested normally at 28.0 gm on 124th day as well as on 127th day. In the year 2018 winter, the experimental ponds were harvested at 36.0 gm at 146th day and 35.5 gm at 147th day respectively. This study suggests that the probiotics are the key agents which have a great impact on the reduction of total *Vibrio* count in culture ponds of *L. vannamei*.

BRIDGING THE GAP BETWEEN A LARGE-SCALE FUTURE AND THE SMALL-SCALE NOW: A HOLISTIC DESIGN FRAMEWORK FOR SEAWEED FARMING PLATFORMS

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The US Department of Energy ARPA-e program has envisioned a large-scale future for seaweed farming in the United States via the MacroAlgae Research Inspiring Novel Energy Resources (MARINER). MARINER projects have developed innovative designs for large-scale, offshore seaweed farming, with the future aim of 100 ha unit farms. Ideally, these innovative designs and components thereof would transfer directly to the existing, albeit nascent, coastal seaweed farming sector in the USA. However, there is a clear discrepancy between the proposed large-scale farms and the scale of the existing seaweed aquaculture industry. In Maine for example, the majority of seaweed farms grow sugar kelp, *Saccharina latissima*, on farm leases that are limited to 37 m^2 (400ft^2). These small operations are limited in capital and infrastructure and their needs are distinct from the MARINER-oriented design process, which is focused on maximizing offshore survivability and high production.

We have developed a holistic design framework for seaweed farm engineering to meet current industry needs and challenges. Our design uses pre-identified industry priorities categorized within three major focus points (Figure 1) to ensure functionality across the spectrum of uses. Our team at the University of New England has developed a scalable large-scale array system deployed at a 2 ha site in Saco Bay, Maine. As part of this design process, our team sought to both satisfy MARINER goals (scalability, offshore survivability, high production), and design a system to meet the needs of the growing inshore seaweed farming industry.

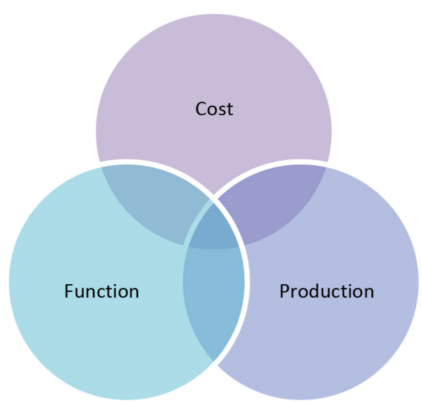

Figure 1. Major components of holistic design framework for bridging large-scale design and current small-scale state of seaweed farming industry

SHELLFISH AQUACULTURE LEASING AND PERMITTING IN VIRGINIA

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The Commonwealth of Virginia has a long history of support for shellfish culture to include both oysters and clams (and historically scallops) since the 1800's. State-owned bottomlands are held in trust for the benefit of the citizens of the Commonwealth. In the late 1800's the natural oyster beds were mapped and they were protected by the state constitution to be held for public use. Other bottomlands not considered to be natural shellfish beds were then made available for leasing for private shellfish production. Such leases were originally used primarily for seed production and/or were enhanced with shell to make them commercially viable for shellfish harvest. This served to provide large areas where wild caught oysters could be grown and sold for shucking purposes. These leases were managed by the Virginia Commission of Fisheries during much of the 1900's. The Commission of Fisheries was eventually re-named the Virginia Marine Resources Commission when authority was granted to the agency for habitat management permitting over state-owned subaqueous bottomlands.

In the late 1980's the first attempts to produce shellfish using more intensive containerized methods occurred. Many of these initial attempts were not considered successful for a variety of reasons. However, by the 2000's, aided by some technological gear improvements and an increase in the availability to obtain triploid (sterile) oysters that reached market size in less then two years, renewed interest in such containerized aquaculture methods began to be implemented successfully. Many traditional use shellfish bottom leases were converted to more intensive containerized and/or spat on shell use methods. Additionally, the wild clam industry was also transitioning to more intensive planting of aquaculture clams (using protective netting) on leases where salinity and bottom conditions are more favorable for clams than for osyters.

The Virginia Marine Resources Commission Engineering/Surveying Department is responsible for managing the shellfish leasing program for the Commission. The Habitat Management Division (under which the Engineering/Surveying Department is located) also is responsible for issuing permits for any aquaculture activity requests over or upon state-owned tidal bottomlands.

Currently, the Engineering/Surveying Department oversees the leasing of 133,722 acres of bottomlands under 5,501 individual leases. The Habitat Management Division has issued dozens of aquaculture related permits beginning in the 1990's.

MEMBRANE TO PROTECT SHELLFISH PRODUCTION TOWARD OSTREID HERPES VIRUS

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Ostreid herpes virus, OsHV-1, responsible of mass mortalities of oyster juveniles is a threat for shellfish culture. In this context means of treatment of hatcheries/nurseries have to treat inlet waters are necessary to protect oysters. The aim of the study was to test membrane filtration to eliminate OsHV-1 at the lab scale.

Ultrafiltration membranes in PES with a pore size of 0.02 μm were used to treat contaminated solutions prepared by injection of viral suspensions in oyster spats. To evaluate the retention performance of the process, *in vitro* and *in vivo* tests were carried out. *In vitro* evaluation was realized by virus concentration measurements in water before and after treatment using PCR analysis. *In vivo* tests consisted in putting in contact ultrafiltered solution with juveniles or larvae (aged 8 days) to simulate real conditions of shellfish production and injecting permeate in juveniles. Oyster mortality was then followed for 7 days and compared to the ones obtained in positive (contaminated seawater) and negative (ultrafiltered seawater) controls.

In vitro measurements highlight a retention of the virus by ultrafiltration process (Figure 1). Indeed, if the retention is not complete because DNA is detected in permeate, its concentration is bellow quantification limit of PCR analysis (10 DNA copy. μL^{-1}) and the removal was up to 4 log. For the 3 tests realized, whatever the quantity of virus in contaminated seawater, the concentration permeate was bellow this limit. In vivo tests are essential to determine the treatment efficiency. In the case of larvae (Figure 2), the test led to a total mortality of oyster in positive control and 40 % mortality in negative control. Larvae put in contact with ultrafiltered solution showed a 55 %. In the case of oyster spat, no mortality was observed in oyster permeate baths: a protection of larvae is obtained with ultrafiltration process.

To conclude, the study demonstrated the efficiency of ultrafiltration to treat OsHV-1 for hatcheries/nurseries. Indeed, the retention is not complete but allows the protection of oyster spat and larvae. Whatever the feed concentration (~10^5 UFC.mL^{-1}), (i) the virus concentration in the permeate was below quantification limit, (ii) no mortality was observed in spat in contact with permeate and (iii) mortality rates of larvae were closed to the one obtained with negative control. Ultrafiltration could be use with high efficiency to treat inlet water for shellfish production.

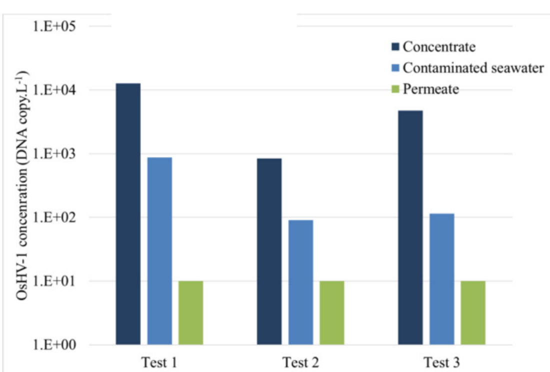

Figure 1: OsHV-1 concentrations

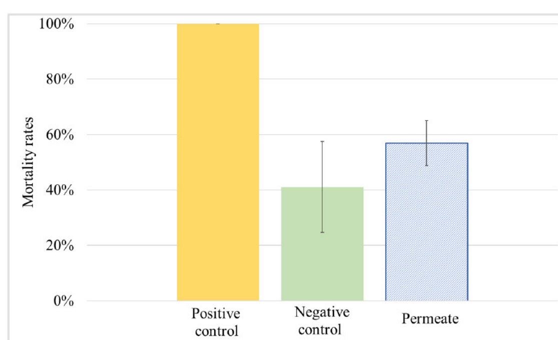

Figure 2: Mortality rates of larvae

EFFECTS OF THE MACROALGA *Asparagopsis taxiformis* ON ENTERIC METHANE EMISSION AND LACTATIONAL PERFORMANCE OF DAIRY COWS

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The macroalga, *Aparagopsis taxiformis* (AT) is a source of multiple halogenated compounds and has been shown to decrease enteric methane (CH_4) production *in vitro* and in sheep. One *in vitro* and two *in vivo* studies were conducted to determine the antimethanogenic effect of AT in lactating dairy cows. *In vitro*, 10 treatments were tested in repeated 24-h incubations with ruminal inoculum collected from two lactating Holstein cows. Treatments were: control (no additives), AT included at 0.25, 0.5, 0.75, 1.0 and 1.5% of feed dry matter (DM) and bromoform ($CHBr_3$), the active compound in AT, equivalent to $CHBr_3$ found in AT dosed at 0.25, 0.5, and 1.5% (DM basis). A short-term *in vivo* study (Exp. 1) was conducted with six lactating Holstein cows. AT inclusion rates were 0, 0.25, 0.50, and 0.75% of feed DM intake (DMI). Each experimental period consisted of 7-d diet adaptation and 3-d sampling, followed by a 7-d washout period. Experiment 2 (Exp. 2) was conducted with 20 lactating Holstein cows in a replicated 4 × 4 Latin square design with four 28-d periods. Treatments were control (basal diet), 0.25% AT, 0.50% AT, and 2.0% oregano leaves of feed DMI. The first 21 d of each period were for diet adaptation and the last 7-d for sample collection. Enteric CH_4 emission was measured 8 times over 3-d using the GreenFeed system. Data were analyzed using the REG (*in vitro*) and MIXED (*in vivo*) procedures of SAS. In the *in vitro* experiment, AT decreased CH_4 yield quadratically [$P = 0.02$; 13.3, 13.0, 13.5, 10.8, 7.3, and 1.2 mL CH_4/g feed DM (SEM = 1.56) for control, 0.25, 0.5, 0.75, 1.0, and 1.5% AT, respectively]. $CHBr_3$ decreased CH_4 yield linearly [$P < 0.001$; 9.5, 0.5, and 0.1 mL CH_4/g feed DM (SEM = 1.1) for 0.25, 0.5, and 1.0% $CHBr_3$, respectively]. In Exp. 1, daily CH_4 emission decreased linearly ($P < 0.001$) with AT inclusion rate: 392, 367, 84, and 83 g/d, (SEM = 20) for BG, 0.25, 0.5, and 0.75% AT, respectively. Methane yield was decreased quadratically ($P = 0.02$; from 17.5 to 5.7 g/kg DMI; SEM = 1.3) by AT. DMI and MY were decreased ($P \leq 0.05$) by 15 to 22 % at the highest (0.75%) AT inclusion level. In Exp. 2, daily CH_4 emission decreased ($P < 0.001$) by 35% at the 0.50% AT inclusion rate compared with the control, 351 vs. 229 g/d (SEM = 19.4). Methane yield was also decreased ($P < 0.001$) by 29% at the 0.50% AT inclusion level. Dry matter intake and MY were decreased ($P = 0.006$) by 6.9% and 5.7%, respectively, in cows fed 0.50% AT. The CH_4 mitigation effect of AT appeared to diminish from experimental period 1 (339 vs. 128 g CH_4/d, control and AT, respectively; SEM = 29.5; $P < 0.001$) to experimental period 4 (299 vs. 337 g CH_4/d, control and AT, respectively; SEM = 29.5; P = 0.35) of the study. Oregano leaves and AT at 0.25% had no effect on enteric CH_4 emission in Exp. 2. *Aparagopsis taxiformis* has the potential to decrease enteric CH_4 emission in lactating dairy cattle; however, further research needs to determine the persistency of the mitigation effect of AT.

FROM FOOD TO FUEL: KELP MARICULTURE RESEARCH IN ALASKA

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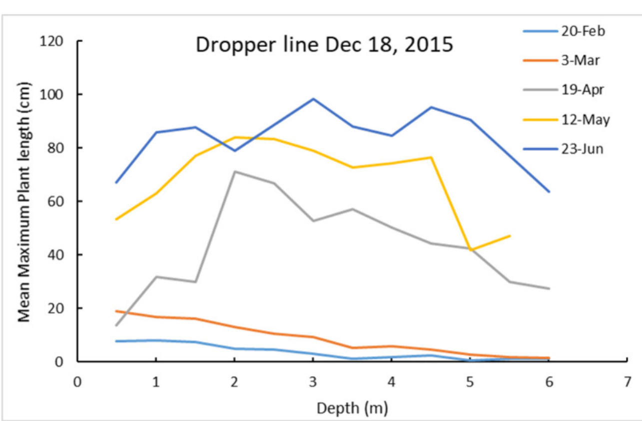
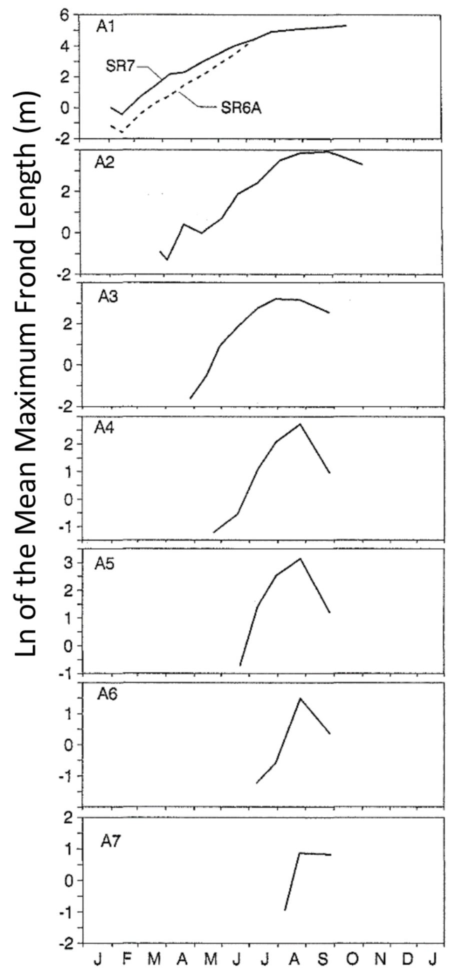
Commercial mariculture of seaweeds in Alaska is in its infancy. Currently there are only a handful of farmers producing commercial amounts of seaweeds and almost all are kelp species. In the late 1980s work on the giant kelp, *Macrocystis*, was undertaken to augment the herring spawn on kelp industry. This work was carried out in Juneau and Sitka with help from Japanese mariculture experts. Outplants grew well in the winter, but began to degrade over the summer. Fertilization appeared to reverse this degradation.

Recently we have been researching optimal ways to grow kelps such as *Saccharina latissima* (sugar kelp), *Alaria marginata* (ribbon kelp) and *Nereocystis luetkeana* (bull kelp) for commercial potential. *Saccharina latissima* growing near Juneau, Alaska behaves as an annual with growth of the juvenile sporophyte beginning in late winter. Beds of *S. latissima* near Juneau have been found mostly in low energy, shallow subtidal areas of mixed mud, shell hash, sand and small rocks. Outplants on seeded string wrapped around longlines show optimal growth when set out in October/November and harvested in April, corresponding to the start of the spring phytoplankton bloom.

We are currently working on a DOE/ARPA-E MARINER project to determine the best way to grow *Saccharina latissima* in very large, offshore arrays for the eventual purpose of biofuel production. There are three major aspects of the research: determining how to seed longlines with *S. latissima*, designing an economical outplanting structure and developing methods to efficiently harvest the product. Aside from the standard method of releasing spores from fertile fronds on cremona-like string, we have attempted to accomplish "direct seeding" on string and on longlines.



Growth of Saccharina latissima outplanted in December, 2015 set a various depths below the surface.



Growth of Macrocystis outplanted at various times of the year

ADVANCES IN APPLICATION OF ONTOGENETIC AND PHYSIOLOGICAL RESEARCH FOR OPTIMIZATION OF MARINE FISH HATCHERY PRODUCTION

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Consistent hatchery production of marine fish continues to be one of the critical bottlenecks in the growth of this sector of the aquaculture industry. This is particularly true for production of high-value, yet challenging to rear, pelagic fish species such as mahi-mahi (*Coryphaena hippurus*) and tuna (*Thunnus*). In many cases, the difference between success or failure in development of hatchery technology for marine fish species comes down to how well understood the species is at all early life stages from ontogenetic and physiological standpoints. Inadequate understanding of the species-specific differences in organogenesis, growth patterns, nutritional demands, and behavior, among other factors, serve to limit the production potential of challenging species. Put simply, some marine fish species are more forgiving than others. There are many species that have historically been identified as having high market potential, yet reliable commercial-scale hatchery production has yet to be realized. However, advances in ontogenetic and physiological research have allowed for new insights into many of these challenging fish species, thereby allowing producers to make substantial improvements in developing hatchery technology for them. Findings from utilization of these advancements in a variety of marine fish species, such as mahi-mahi, will be presented. Information that is key to improving marine fish hatchery production, such as examination of the activity levels of selected digestive enzymes during ontogeny, will also be discussed for different species. Additionally, results will be presented detailing how such information can be utilized effectively to optimize marine fish hatchery production, particularly in the case of high-value candidate species. Many of the findings from early life stage ontogenetic and physiological research have provided valuable insights that extend beyond benefits in hatchery production, leading to further improvements in nursery and grow-out stages. Species-specific examples will be presented and discussed.

GOOD AGRICULTURAL PRACTICE (GAP) FOR AQUAPONIC PRODUCE AND GLOBAL FOOD SAFETY INITIATIVE (GFSI) CERTIFICATION, 2020 UPDATE

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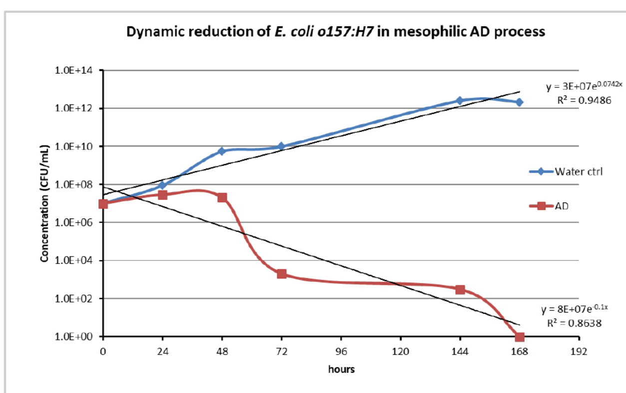
In July 2019, CanadaGAP announced it would phase out Global Food Safety Initiative (GFSI) certification of aquaponics operations no later than 1 April 2020. CanadaGAP cited concerns about chemical hazards and other contaminants, though the decision was not based on any incident. Hypothetical concerns reportedly arose from research indicating plants grown in hydroponic operations could uptake pharmaceuticals (e.g., antibiotics, hormones, opioids) and potentially human pathogens.

CanadaGAP claimed aquaponics operations would have until March 2020 to obtain GFSI certification from other GFSI certifying bodies. But individual CanadaGAP certifiers terminated GFSI certification of aquaponics operations soon after the July 2019 press release. This has caused hardship for aquaponics growers.

The paper identifies the reasons why the cited concerns are aquaponics, which is incompatible with use of antibiotics. Pathogenic risk is reduced by nitrifying bacteria.

The paper identifies Global Food Safety Initiative (GFSI) certifying bodies that continue to certify aquaponics systems, including GlobalGAP and USDA Harmonized GAP.

Finally, the paper contains recommendations aquaponic farmers can adopt to mitigation risk and reassure concerned consumers.



INVESTIGATIONS IN PRODUCING STERILE SUNSHINE BASS

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Sunshine bass are an important food fish raised in US aquaculture. Spawning of these hybrid striped bass is done by manual fertilization of female white bass eggs with male striped bass sperm. The industry grows these fish to ~1.5 to 2 lbs over ~1.5 to 2 years, during which time the females can become fertile and produce eggs. This is a major problem in the industry, as the majority of producers sell these fish whole and there is a considerable loss in weight (i.e., dollars) due to expulsion of eggs caused by handling stress; eggs prematurely released can also lead to considerable water quality issues. Therefore, the industry needs a way to produce sterile fish.

One way to produce sterile fish is through the creation of triploids. Triploidy can be induced through temperature shock applied to the embryo shortly after fertilization; so far, there has been only limited success. We chose a range of temperatures and times post-fertilization to shock the eggs during initial trials. Larvae produced were then checked for triploidy using a flow cytometer. Based on these data, we chose the highest triploid rates from the cold- and warm-shocks to continue trials. A large-scale trial was performed and larvae from both treatments and a diploid control were spawned from a single batch of eggs. Fish will be grown for two years to examine growth and gross morphology.

From this large-scale trial, flow-cytometer results on 2-day old larvae indicated triploid production was 54% in the warm- and 52% in the cold-shock treatment groups. Fish were transferred to a fertilized pond where the larvae ate zooplankton over 28 days and grew to about 2 cm. There were few survivors in the cold-shock pond; none of these were triploid when checked with a Coulter Counter (CC). There were several thousand fish in the warm-shock pond and a sample of 50 fish indicated 14% triploid (by CC). Diploid and warm-shock triploid fish were then moved to separate growout ponds and fed commercial hybrid striped bass diets for 5 months; we then determined 7% success rate (by CC) of the triploid survivors. We will grow these until spring spawning 2020 to verify maturation/gamete production vs diploid fish from the same batch of eggs.

VERAMARIS OIL AS A FISH OIL REPLACEMENT IN CALIFORNIA YELLOWTAIL *Seriola dorsalis*

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California yellowtail (CYT) *Seriola dorsalis* is a top candidate species for aquaculture in southern California. CYT is typically reared on commercial diets whose nutrient profile and ingredient composition rely heavily on fish meal and fish oil. Here we attempted to replace fish oil with Veramaris oil in the diet of juvenile CYT. Veramaris oil is a natural oil from marine algae. The treatment diets for this study included: TRT 1 – Fishmeal Free (poultry by-product meal and fish oil), TRT 2 – Veramaris Oil (poultry by-product meal and Veramaris oil), TRT 3 – Soybean Oil (poultry by-product meal and soybean oil), TRT 4 – Fishmeal Control (fishmeal and fish oil). Each treatment diet was formulated to contain 45% protein and 15% lipid. CYT juveniles (initial weight 19.95 g) were fed the experimental diets for 64 days. At the completion of the trial, TRT 4 had the fastest growth, while TRT 1 and TRT 2 had similar growth (Table 1). Feed conversion ratios (FCR) also show a similar trend. Survival and fish health were high among all treatments. Samples were taken to determine proximate composition and fatty acid analysis of both the diets and the fish. Finally, liver and gut samples from fish from each treatment tank were histologically processed. At this time the biochemical and histology results cannot be reported because analysis has yet to be completed. However, based on the growth and FCR results, we have shown that Veramaris oil has good potential to replace fish oil for juvenile CYT.

Table 1. Weight and feed conversion ratio from juvenile California yellowtail (*Seriola dorsalis*) fed four experimental diets. Values sharing lower case letters within columns are not significantly different ($P<0.05$).

Treatment	Diet	Final Weight (g ± SD)	Weight Gain (% ± SD)	FCR (mean ± SD)
1	Fishmeal-Free	151.90 ± 10.53^{b}	661.60 ± 56.41^{b}	1.27 ± 0.03^{b}
2	Veramaris Oil	146.33 ± 9.40^{b}	633.62 ± 49.30^{b}	1.33 ± 0.04^{b}
3	Soybean Oil	108.65 ± 4.40^{c}	445.48 ± 21.25^{c}	1.54 ± 0.03^{c}
4	Fishmeal Control	185.96 ± 8.98^{a}	832.68 ± 47.23^{a}	1.16 ± 0.04^{a}

NUTRIENT REQUIREMENTS: AN OVERVIEW ON MARINE FISH NUTRITION IN THE AMERICAS

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Reliable technologies in broodstock spawning fingerling production, nursery and growout cobia (*Rachycentron canadum*), pompanos (*Trachinotus carolinus),* jacks (*Seriola spp*) and snappers (*Lutjanus spp*), among others have been successfully established in recent years in the Americas. As with the development of any new industry, there are serious hurdles to overcome before commercial viability can be secured. Development of optimal feeds for the new target species is at the top of the list of problems to resolve. A lack of knowledge about the nutritional requirements and digestibility of nutrient ingredients at the various life stages of most commercially important tropical and subtropical high-value marine fish species being cultured has been identified as the major problem to be addressed and solved. Currently, economic feed conversion rates remain extremely high, ranging from 2.0-4.0. The problem is aggravated at larger sizes, when the fish reach maturation and growth and survival rates decrease remarkably. Researchers and the industry are tackling these major issues by focusing on identifying the nutritional requirements at different life stages of each species targeted, aiming at formulating and manufacturing economically viable and ecologically efficient aquafeeds. In this paper, we'll summarize the current status of nutrient requirements of tropical species of marine fish in the Americas and the Caribbean regions, focusing on cobia (*Rachycentron canadum*), jacks (Seriola spp) and snappers (Lutjanus spp).

ANALYSIS OF THE ECONOMIC PERFORMANCE OF THE QUAHOG TRANSPLANT PROGRAM IN RHODE ISLAND

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Assessing the economic performance of any management strategy is essential for analyzing its success, especially stock replenishment programs. Since most of the replenishment programs are designed for local fisheries, the economic performance of these programs varies with location and fishery. Using Rhode Island as a case study, we consider the shellfish transplant program and measure its economic performance. The transplantation is carried out in Rhode Island by collecting marketable size quahogs from prohibited fishing areas and stocking them to selected open fishing areas. The direct benefit of transplantation is increased harvest of quahogs from the transplanted fishing areas. The economic benefits from the program cannot be estimated directly, since there is no tracking mechanism for transplanted quahogs.

One way to assess the benefits of enhancement programs is to analyze their effects on the quantity of harvested quahogs. This study used a daily dealer shellfish harvest, combined with the weather data of the region and data on transplantation of quahog to identify the effect of transplant on quahog harvest. A bioeconomic model was developed to estimate the effect of transplant on harvested quantity of quahogs after controlling all other factors such as stock effect, fishing area, time, and weather. The panel data was analyzed multi-level, mixed model where the time variable was considered as fixed variable and fishing area was considered as random variable. The result from the model showed that the there is no statistical evidence to indicate that transplantation influences the harvest of quahogs from the Narragansett Bay area. However, the net returns indicate that the transplant program is profitable. However, this result is not assessing the indirect effect – the recruitment of the transplanted quahog- into the account. Thus, a further research in this area is warranted.

Table 2.4. Effect of Transplants on Quahog Harvests from the Bay Area in RI using Mixed Models

Variable	Dep. Var.= Quantity of Quahogs Harvested (lbs.)	
	(1)	(2)
Quantity of quahogs transplanted (lbs.)	0.05	0.04
	(1.12)	(1.04)
Fishing effort	162.0***	164.10***
	(25.23)	(27.34)
Fishing effort x effort concentration	19.37***	19.17***
	(8.11)	(8.51)
Cumulative catch of previous time period	–0.02	–0.03**
	(–1.01)	(–2.03)
Autocorrelation specification	AR(0)	AR(1)
Observations	120	120
Adjusted R-Square	0.98	0.98
Log Likelihood	–1431.1	–1420.45
AIC	2882.19	2862.90

EVALUATING THE DIETARY TAURINE REQUIREMENT OF HYBRID STRIPED BASS, (*Morone chrysops x M. saxatilis*)

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A dietary requirement for the sulfonic acid taurine has been documented in many marine species. However, it has not been well studied in hybrid fish with fresh water and marine genes, such as the hybrid striped bass *(Morone chrysops x M. saxatilis)*. Therefore, an 8-week feeding trial was conducted to evaluate the effects of varying levels of dietary taurine on growth performance, health, and body composition on juvenile hybrid striped bass. Six diets were formulated to contain 38% crude protein (CP) (88% of the protein provided by soy-based ingredients and 12% from menhaden fish meal) and 12 % lipid. In four diets, the sulfur amino acid dl-methionine was supplemented to achieve a level of 1% of dry weight, and taurine was supplemented incrementally at 0, 0.5, 1, and 2% of dry weight. Two additional diets were prepared to be marginally deficient in methionine (0.8% by weight) and either supplemented with or without taurine at 1% dry weight to evaluate the potential interactions between methionine and taurine. All diets were fed to triplicate groups of 17 juvenile hybrid striped bass averaging 9.75 ± 0.027 g/fish that were randomly assigned to eighteen, 110-L aquaria connected as a recirculating aquaculture system at the Texas A&M Aquacultural Research and Teaching Facility. Fish were fed twice daily at a percent of body weight which approached apparent satiation and was adjusted weekly. Diets containing increment levels of taurine were analyzed using one-way ANOVA with the JMP Pro 14 software and determined to show no significant ($P > 0.05$) differences in terms of growth performance, feed efficiency ratio (FER), whole-body composition, hepatosomatic index (HSI), intraperitoneal fat ratio (IPF ratio), and muscle yield. Responses of fish fed diets with and without supplemental taurine (0 and 1%) and methionine (0.8% or 1% total methionine) were analyzed by two-way factorial ANOVA on JMP Pro 14 software. Hybrid striped bass fed the methionine-deficient diets had significantly ($P < 0.05$) reduced percentage weight gain as well as reduced muscle yield and HSI compared to the fish fed the methionine-supplemented diet. Interestingly, taurine inclusion at 1% in the diet containing 0.8% methionine statistically increased weight gain and feed efficiency of fish compared to those fed the diet without supplemental taurine or methionine. Thus, taurine inclusion in the diet significantly improved growth performance of hybrid striped bass when methionine was limiting. Analysis did not show a statistical interaction between methionine and taurine (positive or negative) on growth performance or body composition when each nutrient was supplemented at 1%. In conclusion, the growth performance and body composition of hybrid striped bass did not respond to taurine supplementation when dietary methionine was adequate, but did respond when dietary methionine was limiting.

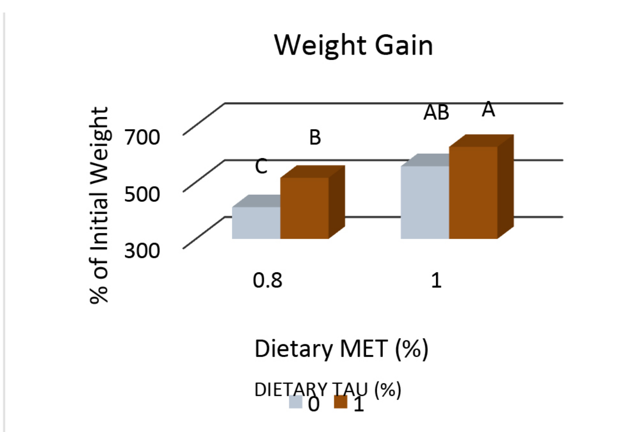

Figure 1. Weight gain of hybrid striped bass fed diets with 0.8 and 1% methionine with two levels of taurine

SEA CUCUMBER (*Apostichopus californicus)* CULTURE AND ENHANCEMENT OPPORTUNITIES IN WASHINGTON STATE

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Demand for giant red sea cucumbers (*Apostichopus californicus*) continues in Asian markets while wild harvest has declined on the west coast of North America. Multiple trials are in place in Washington state to culture this species on upland and floating structures that support other species (figures 1 and 2). Co-culture of sea cucumbers has proved to be effective with mussels, black cod, sea weed and oysters. In addition, there are current food resources from aquaculture that the wild population currently exploits but conceivably could utilize more efficiently. Efforts to further this utilization are in progress as well.

Figure 1. Dulse (*Palmaria palmate*) (left) and mussel raft (right).

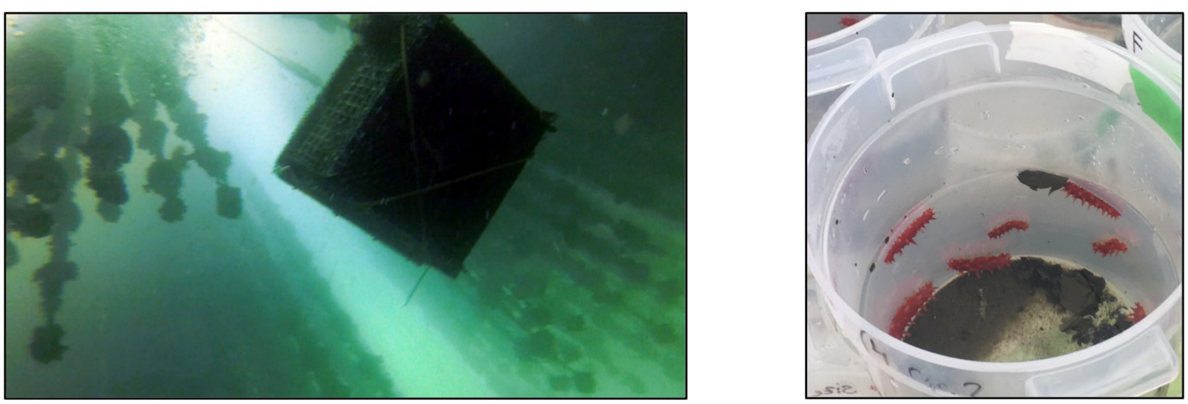

Figure 2. Sea cucumber tray deployed amongst mussel lines and juvenile feeding trial.

THE CHALLENGES AND INCREASE OF IMPORTANCE OF INDUSTRY CERTIFICATIONS TO FUNDING IN PUBLIC SECONDARY SCHOOL AQUACULTURE PROGRAMS

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The Academy of Natural Resources at Island Coast High School in Cape Coral, Florida celebrated its twelve-year anniversary last year. Over that time instructors have delivered lessons in aquaculture, hydroponics, aquaponics, and sustainability to over one thousand students. These students had the opportunity to learn in the classroom over a four-year program with a course sequence of Agriscience Foundations, Aquaculture I, Aquaculture II and Aquaculture III. The students put their skills to the test running a commercial level facility that has grown to feature two indoor vaults systems, various aquaponic systems, and multiple fiberglass finfish tanks.

Traditionally this unique program has been funded by sponsors, the school district, federal Perkins funds, and fundraising. However, in Florida, education funding is shifting, bringing changes to the classroom and funding. In 2007, the Florida Career and Professional Education Act (CAPE) was created to provide a statewide partnership between the business and education communities in order to attract, expand, and retain targeted, high-value industry and to sustain a strong, knowledge-based economy and is part of Section 1003.491 Florida State Statutes. The main objectives of this program are to create rigorous middle and high school programs that articulate to post-secondary coursework and industry certification, support economic development, respond to critical workforce needs, and provide state residents with access to high-wage and high-demand careers. There are significant financial incentives tied to this program to ensure success.

With industry certifications already in place from large companies like Adobe, Microsoft and Cisco Systems, Digital Information Technology classes have enjoyed certifications and the advantage of CAPE funds for years. In 2016, the Aquaculture Technician Certification offered by the Florida Aquaculture Association (FAA) was added and carries a 0.1 weight. The FAA utilized the Aquaculture Science, 3rd Edition by Rick Parker, PhD. and publications from both University of Florida Tropical Aquaculture Laboratory and the Southern Regional Aquaculture Center to develop the one hundred question test which requires a 60% for a passing score. To qualify to take the exam, individuals must have completed 75% of Aquaculture II or worked over 300 hours at a certified aquaculture facility. The initial cost is $95 with a $50 retake fee and three attempts allowed. With the recent introduction of the certification, high cost of administration, and few study materials, the school district required each student to pass three pretests with a score of 85% or higher before sitting for the exam. With these high stakes high quality study materials were necessary and I developed 5 different pretests and multiple quizlets covering each standard of the certification test. We experienced a 100% pass rate on the certification exam with sixteen students earning the certification.

CRISPR-BASED DIAGNOSTICS FOR RAPID DETECTION OF WHITE SPOT SYNDROME VIRUS IN SHRIMP

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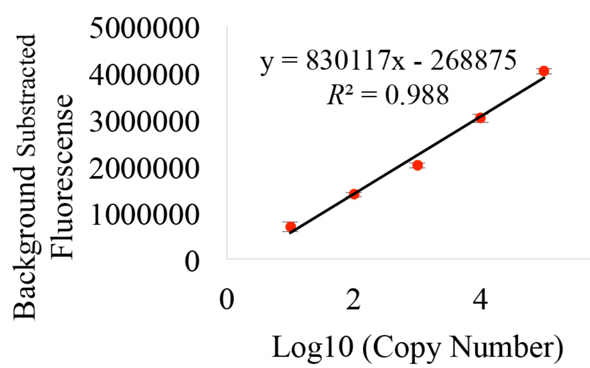
The pacific white shrimp *Penaeus vannamei* is the most profitable crustacean aquaculture organism globally. Production of white shrimp has quadrupled since 2000 and sits at an all-time high, with an estimated yearly value of ~12-15 billion dollars. While productivity is high, impediments to increased supply continue to impact shrimp aquaculture. One of the greatest impediments is the spread of diseases. Pacific white shrimp have a long history of issues related to infectious viral diseases, from Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) which emerged in 1981, Yellow Head Virus (YHV) and Taura Syndrome Virus (TSV) which emerged in 1991, Infectious Myonecrosis Virus (IMV) emerging in 2004 and White Spot Syndrome Virus (WSSV – one of the most influential of all viral epizootics) which emerged in 1992. The impact these diseases have had is best exemplified in the costs they have taken on global shrimp production, estimated at well over 20 billion dollars. WSSV itself has cost over 6 billion dollars since its emergence due to large scale rapid mortality events around the world.

Due to the rapid progression of WSSV and the inability to vaccinate crustaceans, early diagnostics are critical for limiting transmission. However, current diagnostics using histology, PCR, qPCR, and RT-PCR require sophisticated equipment, expensive reagents, specialized training and are time intensive (~ days to a week), which is outside the window where action can be taken. In contrast, we are proposing to develop new next-generation CRISPR technologies (i.e. SHERLOCK – Specific High sensitivity Enzymatic Reporter UnLOCKing) to the diagnosis WSSV infecting shrimp. This new technology will allow for diagnostic assays that are inexpensive, rapid (~1-3 hours), extremely sensitive (attomolar level), require no complex equipment or specialized training, and can be employed in the field or at the pond-site. This will enable rapid and effective management to safeguard aquaculture production by ensuring the production of genetically superior, disease free broodstock and post-larvae (PL) through captive breeding programs, by limiting movement of infected broodstock and PL, and increasing biosecurity.

We designed and evaluated a number of primers and guide RNA probes for the amplification of WSSV DNA and detection of WSSV sequences. Trials with these assays resulted in an assay that was able to detect down to 10 synthetic viral DNA copies with strong linearity in our standard curve (Fig. 1). Tests of previously challenged samples showed high accuracy and specificity, only detecting infected samples, and not detecting other OIE-listed epizootics (TSV, IHHNV, YHV, etc). Finally, a comparison of our assay to qPCR showed the SHERLOCK assay had sensitivity down to ~1-10 copies, and that both assays were highly congruent for the quantification of viral load from unknown challenged samples. We are currently evaluating the robustness of our method by testing a large number of individuals.



RNA/DNA RATIOS ALTERATION AND MEAT QUALITY EVALUATION OF FRESHWATER FISH COLLECTED FROM KOT KHAIRA, JHANG

Salma Sultana*[1], Hafsa Ambreen[1], Tayyaba Sultana[1], and Shahid Mahboob Rana[1,2]

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The study was carried out to evaluate the alterations in RNA/DNA ratios and meat quality of freshwater fish. The fish was collected from drainage canal, Kot Khaira, Jhang. The study was approved by the ethical committee of Govt. College University, Faisalabad. Nucleic acids were extracted from the muscle tissues. The RNA/DNA ratios as indicator of growth were calculated by spectrophotometer at 600 nm. The meat quality was assessed by protein profiling using SDS-PAGE for qualitative assessment after quantitative analysis. All the data was analyzed statistically using the ANOVA.

The sampling of fish was carried out during September, 2018 to December, 2018. Control fish was procured from Fish seed hatchery, Satiana Road, Faisalabad. Water quality parameters of river water were assessed at the time of sampling using multi-parameter instrument HI-9829 HANNA meter throughout the experimental period.

The color of the sample is dark brownish to brown.The water sample has pungent smell which indicates presence of pollutants. The industrial waste water entering the river usually rises the surrounding temperature. The pH of the sample was 6.7.In the present water sample the D.O value was 1.29 milligram/liter.

After the dissection of fish the muscle piece was taken and nucleic acid i.e. DNA and RNA was isolated. The DNA and RNA values were estimated and difference in DNA and RNA concentrations and RNA/DNA ratio in different fish samples were observed. It was observed that DNA & RNA concentrations were 0.093μg/ml & 0.238μg/ml, and 0.054μg/ml & 0.266μg/ml, in experimental & control fish respectively.

The RNA/DNA ratios showed the significant differences in all fish samples as compared with the control group ($P<0.0001$) The industrial effluents greatly influenced the meat quality of inhibiting that area. The protein quantity also showed significant difference..

Table 3:Comparison between Experimental and Control regarding DNA (600nm).

	Experimental	**Control**
N	15	15
Mean	0.160	0.240
Std. Error	0.007	0.003

EVALUATION OF AQUATIC POLLUTION IN SELECTED ORGANS OF WILD FISH THROUGH HISTOPATHOLOGICAL STUDIES

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istopathological assessment has been considered an important indicator of environmental pollution. Untreated sewage and industrial wastes from Faisalabad city are disposed to River Chenab through different drains. Present research was planned to investigate the effects of freshwater pollution on wild fish samples. Sample of Wild fish *Oreochromis niloticus* were collected from upstream and downstream sites to the entrance of Chakbandi Main Drain in to River Chenab.Histological studies were performed on fish tissue specimens of liver and kidney. Farmed fish and fish from upstream areas were used as control and fish collected from downstream sites were considered exposed to drain water.

Haematoxylin Eosin stained histological sections of different tissue of downstream specimen of *Oreochromisniloticus*revealed necrosis, lifting of lamellar epithelium, hemorrhage and epithelial hyperplasia of gills, liver with vacuolated cytoplasm, bile duct with proliferation and melanomacrophages, kidney withshrinked renal cortex, necrosis and dilation of renal tubules and splitting of muscle fiber and atrophy of muscle bundle. Whereas histological studies of upstream specimens of *Oreochromisniloticus*shown fusion of secondary lamellae and vacuolization in gills, liver hypertrophy, vacuolar degeneration and pyknotic nuclei Kidney, and splitting of muscle fibers and degeneration in muscle bundle. Histological sections of gills, liver, kidney and muscle of farmed fish were observed with normal architecture. This study revealed that the Chenab River is being polluted due to the discharge of industrial effluents, sewage run off from different cities and industries through different drains whose effects are reaching in inhabitants particularly in fish.

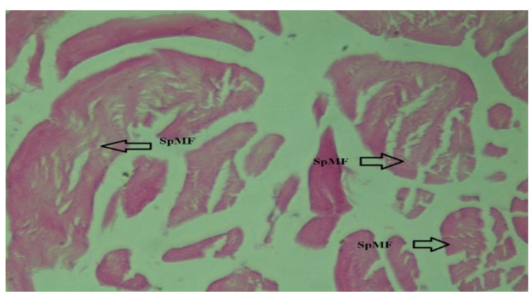

Fig.4.1: Upstream water fish *Oreochromis niloticus*. Showing degenerationin muscle tissues with splitting of muscle fibers (SpMF) (Hematoxyline& Eosin stain, 40 X).

AN ASSESSMENT OF CURRENT NOAA SEA GRANT EXTENSION CAPACITY AND IDEAS ON MEETING THE EXTENSION NEEDS OF AN EXPANDED INDUSTRY

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For nearly 50 years, the National Oceanic and Atmospheric Administration's (NOAA) National Sea Grant College Program (NSGCP) has supported the development of sustainable marine and Great Lakes aquaculture businesses through the funding of competitive research projects. The primary means of extending the research findings to the aquaculture industry is through Land Grant and Sea Grant extension services. Based on recent federal appropriations, Sea Grant will likely invest more than $120 million in aquaculture research, extension, outreach and education over the next 10 years. To take full advantage of these resources, Sea Grant will ensure there are enough extension professionals to support technology transfer and applied research for sustainable growth of the marine and Great Lakes aquaculture industry.

In mid-2019, Sea Grant extension personnel and their level of effort were identified for all 34 Sea Grant programs. Sea Grant supports just over 35 full time equivalents (FTEs) spread among 107 aquaculture extension professionals working in every Sea Grant region (Figures 1-2).

An expanded aquaculture industry will place greater demands on existing aquaculture extension personnel. Data with respect to current extension personnel and level of effort provide a baseline from which future staffing decisions can be made. Meaningful discussions among members of the industry, Land Grant and Sea Grant institutions and others with a vested interest in technology transfer should lead to methodical approach to identify extension needs at the state, regional and national levels.

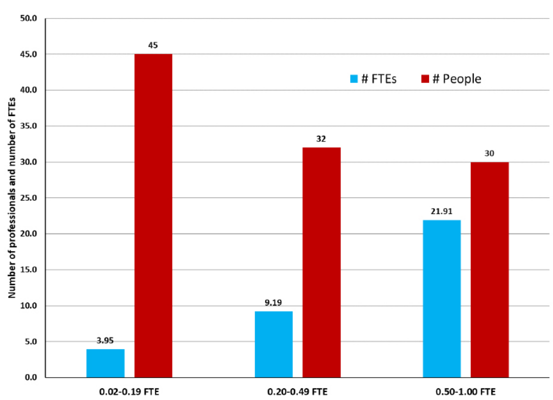

Figure 1. Number of people and number of aquaculture extension full time equivalents (FTEs) supported by Sea Grant grouped into three categories (<0.2 FTEs, 0.2-0.49 FTEs and 0.5> FTEs).

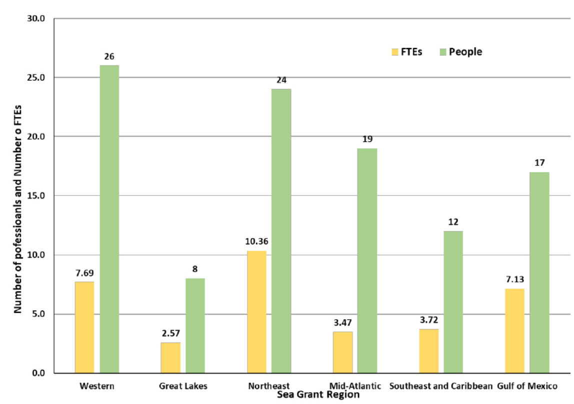

Figure 2. Number of people and number of full time equivalents for Sea Grant supported aquaculture extension professionals by region.

BLUE REVOLUTION HAWAII: PROPOSAL FOR A PACIFIC INTERNATIONAL OCEAN STATION

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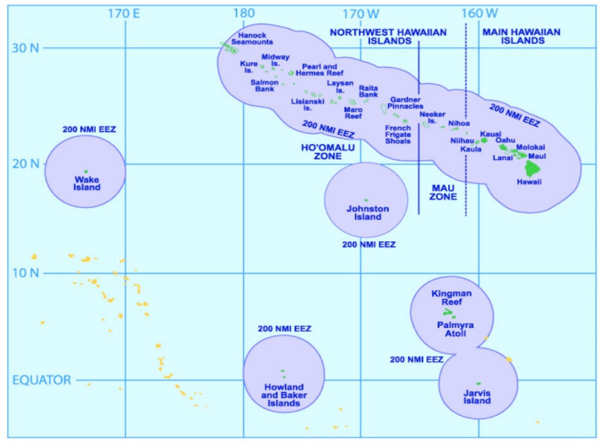
World fisheries agencies have long warned of the depletion of wild fish stocks in our oceans due to overfishing to meet rapidly growing human demands for seafood. The potential of aquaculture for providing safe, sustainable seafood supplies to the world has long been recognized. Seafood aquaculture already supplies total yields about equal to caught fish, but 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 proof of economically sustainable and environmentally protective best practices is needed for commercially viable aquaculture.

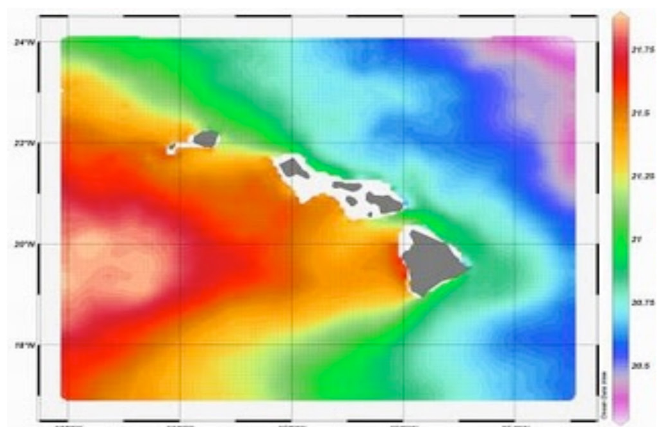
Blue Revolution Hawaii is advocating the deployment of the Pacific International Ocean Station (PIOS) in Hawaiian Exclusive Economic Zone (EEZ) waters 35-75 miles offshore on the leeside southwest from the Hawaiian Islands. PIOS would be a pilot test station for eventual deployment of commercial ocean 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. The region lies in the unused zone between existing Hawaii-based day-boat and longline fishing operations, and is situated far from near-shore usages, navigation lanes, and marine mammal and reef preserve areas.

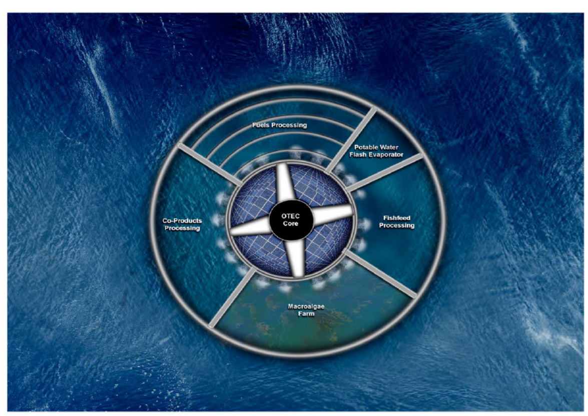
The PIOS Station would be designed to support a test fleet of submerged fish-growing cages for growing pelagic species of fish from fingerlings to harvesting. Supporting growing systems such as for seaweed and feeder fish may also be attached for testing of on-platform Integrated Multi-Trophic Aquaculture (IMTA) operations. A semi-buoyant core platform (similar to oil/gas drilling platforms) would be elevated above storm-surge wave heights and would provide housing and operations space for up to 200 scientific researchers, ocean monitoring personnel, and fishcage operations workers. An outer line of tethered ocean wind turbines can provide electricity for on-platform operations. The core platform can also have an on-board system for deep water upwelling to bring bacteria-free and nutrient-rich deep water to the surface to support IMTA growing operations.



U.S. Exclusive Economic Zone around Hawaiian Islands



Ocean thermal map of Hawaiian EEZ (ref: Gerard Nihous)



Artist rendering of Pacific International Ocean Station

(Continued on next page)

The PIOS Station research would include ongoing monitoring and data-gathering of climate, marine life and ocean environment, and would test, design and validate best practices for sustainable and environmentally protective operations for deep ocean aquaculture and other ocean resources production.

PERIPHYTON COMMUNITY DYNAMICS WITHIN AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM

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The removal of waste nutrients in aquaculture effluents remains a limitation to expansion of the industry. Fish retain 20-30% of the nitrogen in feed; the remainder is released into the water. Ammonia is the main catabolic product, but effluents also carry nitrate and nitrite. Integrated multi-trophic aquaculture (IMTA) systems incorporate plants to absorb these nutrients, improving water quality and producing a valuable by-product. Plants preferentially uptake ammonia whereas periphyton, the aquatic biota that develops as a natural film on submerged substrate with nutrients and light, is thought to deplete both ammonia and nitrate. The present study investigated the biological potential of periphyton-based biofilters for nutrient removal in fish production systems.

Nutrient assimilation trials were conducted in a marine IMTA system containing red drum *Sciaenops ocellatus* and sea vegetables *Sesuvium portulacastrum*, where wastewater from fish culture flowed into plant aquaponic raceways through a series of integrated production tanks. Eukaryotic and prokaryotic periphyton communities were characterized using sequencing technologies in two different system configurations: periphyton only (P) or periphyton and sea vegetables (P+SV). Response parameters (water chemistry, water quality, plant growth, and periphyton growth and biomass) were measured throughout a 5 week trial.

Prokaryotic communities were similar between P and P+SV systems (Table 1). The family Saprospiraceae, a group thought to break down fish wastes into nutrients used by aquaponics plants, was dominant in all communities. The cyanobacterium *Leptolyngbya*, which thrives in high organic content waters, was also abundant. Eukaryotic communities were different between P and P+SV systems (Table 2). Periphyton grew faster in P systems, perhaps due to greater abundances of the carotenoid-producing yeast *Sporobolomyces* in P treatments. P systems had greater abundances of the nutrient-rich diatom *Amphora*. No differences in terms of nutrient removal between treatments. Optimization of the IMTA system is currently underway.

TABLE 1. Average abundance of discriminatory OTUs within prokaryotic periphyton communities.

OTU ID	P+SV	P
Porphyrobacter spp. (Proteobacteria)	2.43	4.05
Flavobacterium spp. (Bacteroidetes)	2.84	2.07
Thalassobius spp. (Proteobacteria)	3.33	0.60
Leptolyngbya sp. (Cyanobacteria)	1.50	3.47
Rhodobaca bogoriensis (Proteobacteria)	1.96	1.52
Azoarcus toluclasticus (Proteobacteria)	1.99	3.40

TABLE 2. Average abundance of discriminatory OTUs within eukaryotic periphyton communities.

OTU ID	P+SV	P
Chironomus tentans (midge)	11.5	0.31
Sporobolomyces pyrrosiae (yeast)	4.41	10.3
Cancrincola plumipes (copepod)	23.0	20.5
Amphora coffeaeformis (diatom)	4.07	8.91
Amphora sp. (diatom)	2.60	6.97
Paratrichodorus minor (nematode)	7.50	5.78

BIOFLOC TECHNOLOGY AS BIOSECURITY FOR SUSTAINABLE SHRIMP FARMING

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Biofloc technology is a very promising for stable and sustainable production as the system has self-nitrification process within culture ponds with zero water exchange (Yoram, 2000, 2005a&b & Yoram, et at 2012). The technology has been successfully applied commercially with shrimp *(L. vannamei)* in Belize (McIntosh, 2000a, b & c, 2001). It also has been applied with success in shrimp farming in Indonesia, Malaysia (Taw 2004, 2005, 2008, 2010: Taw & Setio, 2014).

The effect of bioflocs on growth and immunity on *P. vannamei* has been studied by Jang, In-Kwon (2012), Kim, Su-Kyoung, et al (2013), Julie E., et al (2014). Bioflocs with unicellular protein (CP 30-50%) is natural extra nutritious feed for the shrimps. Although heterothrophic bacteria play the most import role in biofloc formation, the floc also contains microalgae and these might be important for nutritional quality of biofloc and moreover the ability of certain microalgae to interfere with bacteria cell-cell communication could serve to prevent pathogenic bacteria from expressing their virulence genes, thereby preventing infection (Natrah, F.M.I et al, 2014). Biofloc showed positive effects on shrimp immunity and disease resistance (Ekasari J, et al 2014). Recently, according to Shinn (2019) challenging *Penaeus vannamei* post larvae with AHPND in biofloc water had higher survival rates.

Main economic benefits of shrimp biofloc system are - better biosecurity (zero water exchange - RAS), stable water quality (DO & pH), low FCR (1.0 to 1.2), higher energy efficiency (680-1,000 kg/hp), maximum production of 50.0mt/ha in 0.25 ha ponds to 12 kg/m3 in Raceways. Probiotic bacteria produced in situ (better immunity) leads to sustainable production.

GENETIC VARIABILITY OF NILE TILAPIA *Oreochromis niloticus* STRAINS AS DETERMINED BY MICROSATELLITE DNA MARKERS

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Nile tilapia (*Oreochromis niloticus*) are originally from the Middle East and Africa and are the second most popular finfish produced in aquaculture systems. Genetic preservation of current Nile tilapia strains should be carefully conducted to prevent inbreeding, which could compromise productivity. The purpose of this study was to investigate genetic variability of Nile tilapia strains using microsatellite DNA markers; a total of nine strains have been analyzed. The analysis included DNA extraction from each fish sample and amplification using polymerase chain reaction (PCR). Nine microsatellite loci were analyzed and amplified products (microsatellite alleles) were separated by capillary electrophoresis using the Applied Biosystems™ Genetic Analyzer 3500 (Thermo Fisher Scientific). For each strain, the number of alleles, level of heterozygosity (polymorphism), frequency of genotypes, and deviation from Hardy-Weinberg Equilibrium were evaluated. Table 1 presents preliminary data on number of alleles and level of heterozygosity for microsatellite loci in Nile tilapia strains. More detailed data on intrastrain and interstrain genetic variability will be given in presentation. Based on results of this study the optimal schemes of tilapia crossing and selective breeding will be outlined.

Table 1: Preliminary data on number of alleles and level of heterozygosity for nine microsatellite loci in Nile tilapia strains.

Nile tilapia strain	Microsatellite loci								
	UNH 104	UNH 123	UNH 178	UNH 214	UNH 216	UNH 222	UNH 880	UNH 974	UNH 985
Lake Manzala	1/0	2/0.2	1/0	2/0.6	2/0.4	1/0	2/1.0	2/0.2	2/0.8
Miami YY	3/0.8	2/0.2	2/0.6	2/0.4	2/0.4	2/0.6	1/0	1/0	2/1.0
LSA Hybrid	5/0.8	6/1.0	2/0.2	4/0.8	3/0.6	4/0.8	4/0.8	2/0.6	6/1.0
FishGen YY Dark-1	4/1.0	2/1.0	3/0.8	3/1.0	3/0.6	2/0.6	3/0.8	3/0.6	4/1.0
FishGen YY Dark-2	2/0.4	1/0	2/0.4	1/0	1/0	2/0.4	2/0.8	2/0.2	3/0.4
Miami	4/0.6	4/0.6	4/0.8	4/0.8	2/0.4	2/0.4	2/0.4	5/0.8	3/0.6
Ismailia Canal	4/0.8	5/0.8	3/0.8	5/0.8	2/0.6	4/0.8	5/0.8	4/1.0	5/0.8
FishGen YY Red	3/0.6	1/0	1/0	1/0	2/0.6	3/0.4	3/0.8	1/0	3/0.8
GIFT	5/0.8	8/0.8	3/0.8	6/1.0	4/0.6	5/0.6	8/0.8	5/1.0	5/1.0

COASTAL HYDRODYNAMICS AND SEDIMENT ANALYSIS TO OPTIMIZE USE OF SHELLFISH FOR COASTAL INFRASTRUCTURE PROTECTION

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A unique environment is created in coastal areas and estuaries providing a habitat for shellfish species including oysters and mussels. The presence of these bivalves can affect the hydrodynamics and sediment properties in the surrounding area. Preliminary findings suggest both oysters and mussels can help enhance coastal sediment stability through protection and accretion. This study focused on analyzing the hydrodynamics and sediment properties around reefs located in subtidal and intertidal zones.

The hydrodynamics around the reef were measured using an Acoustic Doppler Current Profiler (ADCP). The ADCP gives a velocity profile of the water column showing how the presence of a bivalve reef can affect the water flow. Sediments were analyzed based on erodibility parameters obtained via Jet Erosion Tests and grain size.

The information gained from this study will help determine future coastal infrastructure. Oysters and mussels could assist shoreline protection, scour mitigation, and enhanced wild areas. However, different species tend to grow and interact with sediment in different ways; and different coastal conditions can affect the growth of reefs (artificial or natural) differently.

COLLABORATIVE COMMUNICATION STRATEGIES TO IMPROVE PUBLIC PERCEPTIONS ABOUT US MARINE AQUACULTURE

Kimberly Thompson*, Kevin Madley, Cynthia Sandoval, and Mark Rath

Seafood for the Future
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There is great potential (ecological and economic) to grow nutritious protein in U.S. waters to complement land-based agriculture and support a more climate resilient food supply. Despite this, the growth of the sector in U.S. waters is negligible relative to its capacity for responsible growth. One of the barriers to growth is a lack of "social license" from the public. Negative perceptions among small, but politically powerful public stakeholder groups can greatly complicate, and even prevent the growth and expansion of marine aquaculture projects. It is imperative that efforts are made to 1) educate the general public about what marine aquaculture is and what the sector looks like in the U.S., 2) humanize and share stories about the farmers, researchers, and others involved so the public becomes more familiar with, not just marine aquaculture, but the people who make it happen, and 3) provide accurate information that is accessible to diverse stakeholder groups for more consistent distribution.

The Aquarium of the Pacific's Seafood for the Future program, in collaboration with NOAA, Sea Grant, and others, is working on a number of projects to help familiarize the public with marine aquaculture in the U.S. and to facilitate collaborative action to disseminate accurate about marine aquaculture more consistently across diverse stakeholder groups. These efforts include a communication toolkit, a story map featuring locations and information of U.S. marine aquaculture farms, and a story map (or other public-facing platform as appropriate) to highlight case studies where marine aquaculture has affected economic and social viability (positive and negative). These tools can be used to educate the general public, as well as engage stakeholders, including policy makers, with accurate information about marine aquaculture in the U.S. The success of these projects greatly depends on quality and diversity of participation. We will need all hands on deck!

BRIDGING THEORY AND PRACTICE FOR PUBLIC PERCEPTIONS AND SOCIAL LICENSE: PANEL DISCUSSION

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Public perceptions and social license (or lack thereof) play an important role in marine aquaculture development, particularly in developed countries like the U.S. and Canada. While there is a growing consensus that responsible marine aquaculture can and should play a bigger role in a sustainable food portfolio that is more resilient to the changing climate, sector growth and expansion in the U.S. is negligible. This is in part due to the fact that public perceptions (real and perceived) have not kept pace with the state of the science. Further complicating matters is the fact that research into perceptions and social license for marine aquaculture in developed countries is relatively new, but efforts to address public perceptions and garner social license for the development of specific projects are currently underway. This panel will discuss the challenges and opportunities to bridge scientific theory and application specific to addressing public perceptions and garnering social license for responsible marine aquaculture development in the U.S. and Canada.

BRIDGING SCIENCE-BASED EDUCATION, OUTREACH, AND MARKETING TO SUPPORT RESPONSIBLE US SEAFOOD

Kimberly Thompson and Oriana Poindexter

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Responsible seafood plays an important role to support a more sustainable food supply in the changing climate. It is also an important source of jobs and economic opportunities. Seafood is one of the most globally traded commodities, and the U.S. imports the majority of its seafood, both wild and farmed, despite having strong management and adequate resources (ecological, economic, and political) to support sustainable production. Marketing plays an important role to help U.S. fishermen and seafood farmers differentiate their products from cheaper imports, but it does not address the social and political challenges associated with obtaining or maintaining permits and necessary approvals to fish or farm the seafood in domestic waters.

Social license—the implied permission that communities and society may grant for activities that rely on our natural resources, like fishing and aquaculture—is a significant barrier to growth and expansion of responsible seafood production and consumption in the U.S. One of the key variables needed to garner social license is trust. It is imperative that marketing efforts work in tandem with education and outreach efforts to build the public's trust in the U.S. seafood sector by familiarizing them with what a responsible seafood sector looks like and providing scientifically accurate information more consistently across diverse stakeholder networks, including marketing and branding efforts. The Aquarium of the Pacific's Seafood for the Future (SFF) program and NOAA Fisheries have collaborated with diverse stakeholders, including farmers, fishermen, seafood suppliers, and others to bridge science education, outreach and marketing. We are working with industry, academic, and nongovernment organizations to pair broader, science-based outreach and education with marketing efforts to educate the public about the important role responsibly produced U.S. seafood plays in our sustainable food future. These efforts provide valuable support for businesses that are working hard to produce responsibly and aim to support a more positive reception for the growth and expansion of responsible seafood availability among the public, and increasing consumption of well-managed U.S. seafood. In this session we will share examples and highlight opportunities for science-based education, outreach and marketing to join forces to support a healthy, diverse and responsible seafood sector in the U.S.

PATHOGENICITY AND ANTIBIOTICS SENSITIVITY PROFILE OF AEROMONAS BESTIARUM USED IN EXPERIMENTAL INFECTION OF DIFFERENT DEVELOPMENTAL STAGES OF CLARIAS GARIEPINUS

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Motile Aeromonas Septicaemia (MAS) known to be the commonest bacterial infection of cultured fish is mostly ascribed to Aeromonas hydrophila. This study was therefore conducted to determine the pathogenicity of Aeromonas bestiarum in fry, juvenile and post-juvenile of Clarias gariepinus, and evaluate the antibiotic sensitivity profile of the organism for effective control.

Aeromonas bestiarum was isolated from dead fry in Ijebu Ode. The organism was characterized and used for this study. Two-hundred apparently healthy fry collected from a commercial hatchery were randomly divided into four experimental groups of 50 fry. Three groups were infected with 1x108 (cfu)/ml of Aeromonas bestiarum by immersion in 2L of water, while the fourth group were not infected. Fish in the infected and control groups were monitored daily for 21 days for signs of infection and mortality. The cumulative mortality in fry, juvenile and post-juvenile were 85%, 82% and 50% respectively. Gross lesions observed in post-juvenile fish were bulgy eyes, swollen dorsal muscle caudal to the cranium, congested kidney and skin depigmentation. Histological lesions were equally recorded in the hepatic tissue, diffuse degeneration and necrosis of the tubular epithelium in the interstitium of kidney. It was re-isolated from infected fish in the different developmental stages, while the organism was observed to be sensitive to two antibiotics. Groups of survivors in the different stages were treated for five days. On the second day of treatment, there was 15%, 14% and 0% mortality in the treated fry, juveniles and post-juveniles respectively, while the mortality rate of untreated but infected group (control) were 40%, 42% and 42% for fry, juveniles and post-juveniles fish respectively.

This shows Aeromonas bestiarum causes high mortality in fry, juvenile and post-juvenile of Clarias gariepinus. It's however sensitive to Enrofloxacin and Gentamicin which can be used for treatment of infection by Aeromonas bestiarum for now

USING ALTERNATIVE LOW COST ARTIFICIAL SEA SALT MIXTURES FOR INTENSIVE SHRIMP (*Litopenaeus vannamei*) PRODUCTION

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Inland marine shrimp (*L.vannamei*) production popularity is increasing as recirculating aquaculture systems (RAS) can provide a fresh, high-valued product year-round to nearby consumer markets. If operated in a controlled environment, high animal stocking densities and limited water exchange make RAS an attractive option, minimizing space and water requirements. A complete, artificial sea salt mixture is typically used for inland marine production, which can be a substantial production cost. Developing an alternative salt mixture that includes the important ions necessary for proper shrimp growth may reduce production costs. For this experiment, shrimp production and water quality dynamics were examined using various mixtures of a low cost salt (LCS) mixture and a complete sea salt (CSS) formulation.

Six salt mixtures dictated by different inclusion levels of the LCS and CSS were used for this experiment. The LCS formulation was as follows: NaCl (75%), $MgCl_2$ (5%), $MgSO_4$ (12%), KCl (1%), $NaHCO_3$ (0.5%), $CaCl_2$ (4%), and the CSS was Crystal Sea® Marinemix (Marine Enterprises International). The mixtures that defined each treatment were as follows: 100% LCS, 97.5%/2.5% LCS/CSS, 95%/5% LCS/CSS, 90%/10% LCS/CSS, 80%/20% LCS/CSS, and 75%/25% LCS/CSS. Each treatment included four, randomly assigned 1m^3 culture tanks operated at a salinity of 15 ppt; each system included an external settling chamber and biofilter, operated continuously. The initial weight of the shrimp was 2.9 g, and all tanks were stocked at 263 shrimp/m^3. To determine if significant differences existed between treatments regarding water quality and shrimp production data, an α-value of 0.05 was used.

There were significant differences detected between treatments with regard to DO, pH, salinity, and turbidity. However, these differences in water quality were subtle and did not appear to impact overall shrimp production as no significant differences were found with average weight (g), biomass (Kg/m^3), survival (%), FCR, or growth rate (% growth/week) metrics. When analyzing the cost of salt per kg of shrimp produced, the 100% LCS, 97.5%/5% LCS/CSS, and 95% /5% LCS/CSS all were significantly lower than the 75%/25% LCS/CSS. It costs \$8.83 USD to make 1m^3 of the 100% LCS mixture at 15 ppt salinity, compared to \$12.89 to make the 75%/25% LCS/CSS mixture. The results from this study indicate that the 100% LCS formulation may reduce artificial sea salt cost by 70% when compared to increased CSS mixtures while having no significant impacts on shrimp production. Using this alternative salt formulation may reduce production costs and make inland shrimp farming a more lucrative venture.

GENE EXPRESSION OF SOLUTE CARRIER (SLC) TRANSPORTERS ASSOCIATE WITH RADIO-ISOTOPE FLUX KINETICS IN INTESTINE OF RAINBOW TROUT *Oncorhynchus mykiss*

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Methionine (Met) is an essential amino acid (EAA) playing an important role in protein synthesis and regulating various cellular & physiological pathways. In mammals, Met absorption can be achieved by sodium dependent and/or sodium independent solute carrier transporters located in apical (mucosal) and basolateral (serosal) membranes of intestinal epithelial cells. However, the knowledge of how Met absorbed in fish intestine, a critical site for animal nutrient uptakes, has not been fully understood.

This study was aimed to describe the fundamental properties of Met transport along trout gut. Specifically, Met transport in the presence or absence of sodium was assessed in three intestinal segments including pyloric caeca (PC), midgut (MG) and hindgut (HG) using ^{14}C radiolabeled DL-Met flux; and identification of Met-link transporters involved using gene expression.

The results demonstrated that Met transport was strictly dependent on sodium and obeyed saturable manners. This indicated the existence of Na^+-dependent transporters as the driving force in fish gut. This was supported with the gene expression of sodium-methionine cotransporters such as ASCT2 (SLC1A5), B^0AT1-like (SLC6A19-like) and y^+LAT1 (SLC7A7). On the contrary, the flux rate of DL-[^{14}C]Met was not a function of substrate concentration when the assays were performed in Na^+-free buffer. This was in line with the absence of Na^+-independent transporter $b^{0,+}AT$ (SLC7A9) in trout genome and no/low expression of LAT1 (SLC7A5), LAT2 (SLC7A8), LAT3 (SLC43A1). Understandings the function and expression of these transporters are informative for practical endeavor through feed formulation since DL-Met is commonly supplemented in commercial fish feed. Future direction moves towards investigating the potential interaction of Met with some other AAs. This contributes to explain AAs transport antagonism and dietary AAs balance.

EX-VIVO CHARACTERIZATION OF METHIONINE TRANSPORT IN THE INTESTINE OF RAINBOW TROUT *Oncorhynchus mykiss*

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The inclusion of crystallized methionine (Met) in plant-derived diets has been commonly practised for optimal growth and health of animals. Nutrient absorption begins in the intestine, but little has been known about the Met absorptive mechanism in fish. How much and how fast Met absorbed largely relies on transporters located in the apical and basolateral side of the intestinal membrane. Understanding the mechanism behind Met transport is important to develop a cost-effective feed formulation. The objective of this study was to characterize Met transport pathways and transporters involved in Met uptakes in rainbow trout gut.

Rainbow trout were housed in a recirculating system with optimal growth conditions (dissolved oxygen above 6 mg/L and temperature around 12^0 C). Healthy fish were sacrificed. Freshly-collected gastrointestinal tracts including pyloric caeca (PC), midgut (MG) and hindgut (HG) were mounted in experiment Ussing chambers. Radioactively-labelled substrate DL-[^{14}C] Met was used as a tracer to measure unidirectional flux from mucosal (apical) to serosal (basolateral) side of the intestine. The experiment was carried out with physiological buffer in the presence or absence of sodium to characterize Na^+-dependent and Na^+-independent transport mechanisms. Data were fitted to the classic Michaelis-Menten equation to determine the maximal flux rate (J_{max}) and affinity (K_m). Gene expression using RT-qPCR was performed to identify candidate genes associated with Met flux kinetics. The experiments were performed in both triploid (3N) and diploid (2N) trout as tools to determine the Met transport mechanism in trout gut.

The results demonstrated that DL-Met transport was Na^+-dependent and concentration-dependent at the concentration of 0.2-20 mM. Calculated K_m values around 0.6-1 mM and no significant differences found among segments, suggesting the presence of a similar Na^+-dependent low-affinity transporter along the intestinal tract. J_{max} in PC & MG of 2N trout were 0.0014 ± 0.0002 and 0.002 ± 0.0002 μmol/cm^2.hr, respectively. These were significantly higher than J_{max} in PC & MG of 2N trout with 0.0009 $\pm$ 0.0001 and 0.0013 ± 0.0001 $\mu mol/cm^2$.hr in PC & MG respectively (student's t-test, $p < 0.05$). These differences could be due to lower mRNA expression of low-affinity Na^+-dependent transporter B^0AT1-like (SLC6A19-like) in 3N trout compared to 2N trout.

Observations in this study confirmed that fish growth could be improved via ploidy and genetic manipulation. Additionally, knowledge of Met transport may contribute to understanding amino acid interaction, dietary design, and study fish diseases associated with transporter disorders.

INCREASING SURVIVAL, COMPETENCE AND POST SETTLEMENT SURVIVAL IN LARVAL *Tripneustes gratilla* THROUGH VARIOUS STOCKING DENSITIES

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In Hawaii, the sea urchin *Tripneustes gratilla* has recently been aquacultured for use as a biological control agent to help eradicate dense, rapidly growing populations of invasive algae. While these urchins have been successfully reared in hatcheries, most research thus far has focused on adult gonad development/production rather than larval production. Current aquaculture methods yield a post-settlement survival of less than 0.02% of initial larvae spawned at 46 days post fertilization (DPF). This research aimed to increase larval survival, competency and post settlement survival by testing various larval stocking densities in order to increase production yields to better utilize *T. gratilla* in biocontrol.

A stocking density trial was conducted with *T. gratilla* larvae for 29 days. Six stocking densities (1/mL, 2/mL, 4/mL, 8/mL and 16 /mL) were tested (n=5). Urchins were kept in 1.5 L conical bottles with constant aeration, 24 hr light and fed a mixed diet of *Chaetoceros mulleri* and *Tisochrysis lutea*. Each bottle was sampled daily to monitor urchin health/survival, algal consumption, and water quality. At the end of the larval period, urchins were assessed for competence and stocked into two settlement trials to monitor post settlement survival: a small scale 7-day trial to track individual urchin development and a large scale 37-day trial to better compare post settlement survival to commercial hatchery production.

After 29 days, average larval survival was highest (43.26%) in the 2/mL treatment but was only significantly greater than those in the 8/mL and 16/mL treatments (p=0.045 and p=0.005 respectively, Fig. 1). In the small-scale settlement trial, survival in the 1/mL treatment was significantly higher than other treatments (p<0.05, Table 1A). In the large-scale settlement trial, survival from initial larvae spawned was 1.11% in the 1/mL treatment (Table 1B), a ten-fold increase than what is normally observed using commercial aquaculture techniques. This research shows that stocking of *T. gratilla* larvae at densities higher than 6 urchins/mL can have detrimental effects on survival during the larval period and may have lasting effects into post settlement survival to the juvenile stage.

Figure 1. Larval Urchin Survival during the 29-day larval period. Error bars denote standard error

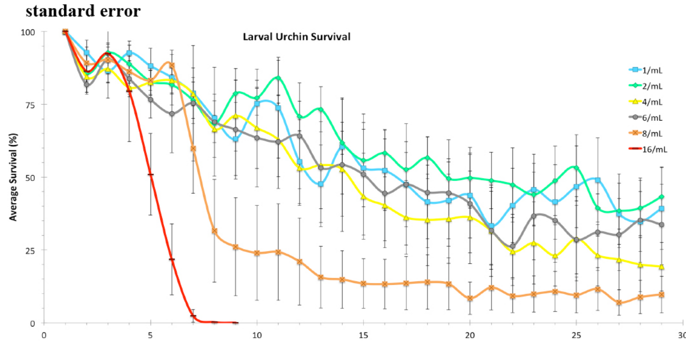

Table 1. (A) Average post settlement survival of (n=48) of *T. gratilla* 7 DPF (B) Post settlement survival in large aquariums of initial larvae spawned

Treatment	A) Small Scale Settlement Trial		B) Large Scale Settlement Trial	
	% Competency	Average % Survival Post Settlement	% Competency	% Survival post settlement of initial larvae spawned
1/mL	100.00	68.75	39.07	1.11
2/mL	100.00	41.67	20.26	0.09
4/mL	100.00	47.92	27.49	0.21
6/mL	100.00	43.75	21.21	0.04
8/mL	100.00	45.83	28.20	0.05

DESIGN CONSIDERATION FOR A 1,500 FT^2 AQUAPONIC SYSTEM

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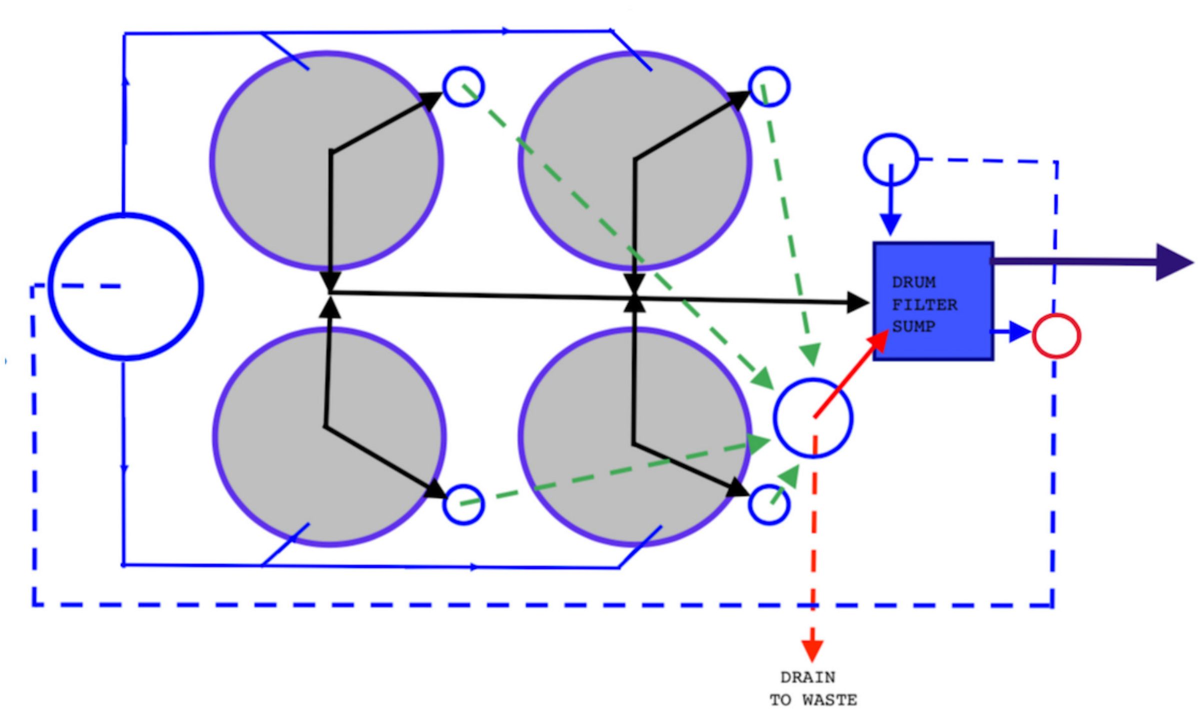
Show design, components, and parts list of a 1,500ft^2- 2,000ft^2 aquaponic system. The goal is to provide a fully functioning and proven system that is large enough for a single person to operate efficiently.

A start up aquaponic system that has been full sized to optimize the efficiency of a single owner/operator. System design includes four fish culture tanks, filtration, mineralization, aeration, DWC, media/wicking beds, water circulation pump and all associated plumbing. All calculations, has been done, including: TDH (total dynamic head), settling velocity, friction loss, pump curve, blower selection (aeration), CFM of air/kg of fish, CFM of air/m^2/in DWC, and fish to plant biomass ratio. Fish waste to plant ratio has been calculated to a wide varieties of plant crops versus greens and herbs.



DIY HOME SYSTEM AQUAPONIC SYSTEM

Huy Tran

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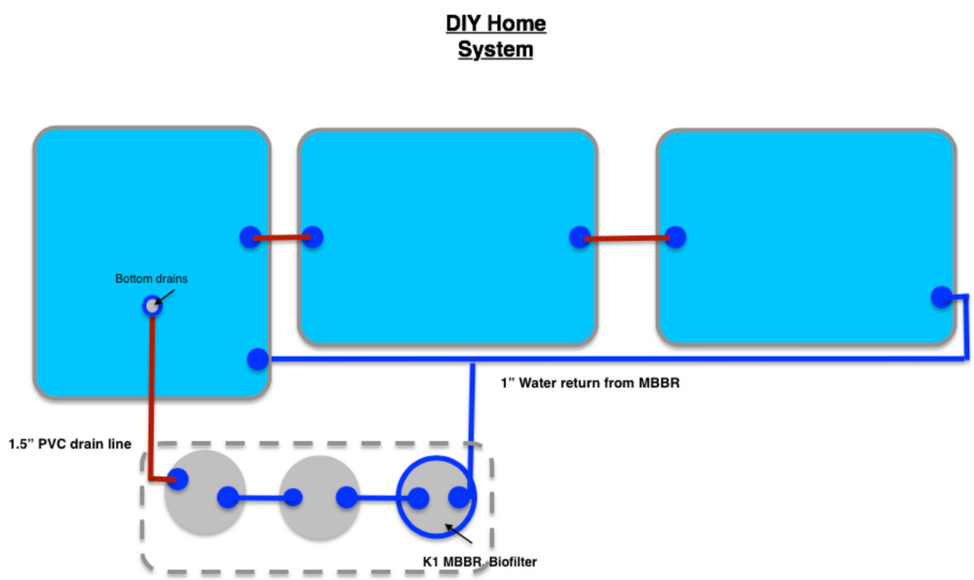
Show design, components, and parts list of a DIY home aquaponic system. The goal is to provide a fully functioning and proven system that is easy to build with local sourced common materials.

A DIY system that has been full sized to optimize the efficiency of a home scale or school system. System design includes fish culture tanks, mechanical filtration, biological filtration, mineralization, aeration, DWC, media/wicking beds, water circulation pump and all associated plumbing. All calculations, has been done, including: TDH (total dynamic head), settling velocity, friction loss, pump curve, blower selection (aeration), CFM of air/kg of fish, CFM of air/m^2/in DWC, and fish to plant biomass ratio.



THERE IS NO SUCH THING AS ZERO RISK—UNDERSTANDING AND ADDRESSING FISH HEALTH THREATS IN RAS CULTURE OF SALMONIDS

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It is a common misconception that fish health risk is negligible in RAS because they do not require the same inputs as other rearing systems and are thus protected from many of the infectious threats that come with the use of natural resources. RAS can support a level of biosecurity well beyond what is possible in open aquaculture. Some believe that adhering to a strict biosecurity plan is enough to protect their fish and investment from pathogens and the associated losses. Biosecurity is an essential element of fish health management, but to suggest that risk is minimal in biosecure RAS is to reveal a narrow view of fish health and a fundamental misunderstanding of risk assessment. Assuming environmental conditions are stable, pathogens are perhaps the most imminent threat to fish health in open aquaculture. As a result, fish health management in this context is rightly focused on the prevention, diagnosis, and treatment of infectious disease. However, in RAS-based aquaculture, a greater emphasis on physical injuries and environmentally or nutritionally driven diseases may be warranted. Of course, this doesn't mean that infectious disease is irrelevant is RAS. Some pathogens are ubiquitous and resist even the most rigorous attempts to avoid or eradicate them. Risk is a function of both the likelihood of an event occurring and the severity of the consequences should the event come to pass. Although RAS operators can strive to minimize one or both of these factors, there is no such thing as 'zero risk'. This presentation will provide an introduction to risk assessment as it applies to fish health management in RAS, highlight a few pathogens of concern in salmonid aquaculture, and underscore the need for vigilance and comprehensive fish health management in RAS culture of salmonids.

NUMERICAL ANALYSIS OF THE FLOWS AROUND FISHING PLANE NETS USING THE LATTICE BOLTZMANN METHOD

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Flow through and around fishing net cages is an important issue in aquaculture. A three-dimensional multi-relaxation time (MRT) lattice Boltzmann model was used to simulate the flow field around a plane net in a constant current. The model was validated with experimental data. The results show that this numerical model is applicable to and reliable for the investigation of the flow field around a fishing net and can provide the detailed velocity distribution in the net aperture. Based on this model, a series of simulations were carried out to analyze the influences of the net solidity and the current velocity on hydrodynamic characteristics and flow-velocity attenuation. The results indicate that the flow-velocity attenuation is mainly related to the net solidity of the plane net, with a higher velocity drop response related to a higher net solidity. These results provide fundamental information for further studies on the flow field around the fishing net cage, which has potential application in analyzing the impacts of biofouling on fishing nets.

EFFECTS OF DIETARY SUPPLEMENTATION OF A NOVEL XYLANASE-DIRECT-FED MICROBIAL FEED ADDITIVE ON THE GROWTH PERFORMANCE, WHOLE BODY NUTRIENT RETENTION, AND NUTRIENT DIGESTIBILITY OF JUVENILE NILE TILAPIA *Oreochromis niloticus* FED PRACTICAL DIETS WITH REDUCED ENERGY DENSITY

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In an effort to reduce the overall cost of production, livestock producers look to reduce feed costs by utilizing alternative feed ingredients or by replacing expensive nutrients via supplementation of various feed additives. One reformulation strategy that is gaining attention in aqua production is the reduction of dietary energy, coupled with supplementation of carbohydrases and/or direct-fed microbials (DFMs), additives that may improve the nutritive value, digestibility and utilization of feed. The combinational use of xylanase and DFMs has been shown to improve gut function and nutrient digestibility in poultry and swine, allowing for improved production performance and feed costs savings. Similar effects have not yet been fully elucidated in fish. Therefore, the aim of the present study was to evaluate the effect of an optimized blend of endo-xylanase and multi-strain *Bacillus spp*. DFMs (EP), on growth performance, whole body nutrient retention and nutrient digestibility in juvenile Nile tilapia.

600 juvenile tilapia, with mean initial body weight (BW) of 12.3 ± 0.7 g, were randomly assigned to 1 of 3 dietary treatments, with 4 replicate tanks of 50 fish per treatment, and raised to 61 days of age in 500L recirculating freshwater tanks. A standard-energy (positive control, PC) practical diet was formulated to 4326 kcal/kg gross energy (GE), 32.8% crude protein and 8.6% crude fat. In a second treatment (NC), dietary energy was reduced by 120 kcal/kg GE (2.1% reduction in crude fat), compared to the PC. In a third treatment, NC diets were supplemented with 100 g/MT xylanase-DFM blend (NC+EP). All diets contained fishmeal (5% of the diet) and plant-based ingredients as protein and fiber sources. Fish were hand-fed to satiety 3 times/day.

After 61 days, supplementing NC diets with EP significantly improved ($P < 0.05$) final BW, specific growth rate, feed conversion ratio, protein efficiency, whole-body retention of protein and energy, and apparent digestibility of energy. At 100 g/MT, EP supplemented to NC diets appears to improve growth performance, feed efficiency and digestibility of key nutrients at a rate similar to, or exceeding, standard-energy PC diets and can therefore compensate for at least 120 kcal/kg GE in practical juvenile tilapia diets.

TABLE 1. Calculated analysis of experimental diets.

	PC	NC	NC+EP
Crude protein, %	32.8	32.8	32.8
Crude lipid, %	8.6	6.5	6.5
Gross energy (kcal/kg)	4326	4206	4206
Total phosphorus, %	1.11	1.08	1.10
Xylanase activity (U/g)	-	-	10.0
Bacillus spp. (CFU/g)	-	-	1.0×10^5

TABLE 2. Tilapia growth performance, whole-body nutrient retention and apparent digestibility of energy after 61 days feeding. Means within rows with different letters differ significantly ($P < 0.05$).

Metric	PC	NC	NC+EP
Final body weight, g	70.1 b	63.9 a	72.0 b
Specific growth rate, %/d	2.86 b	2.70 a	2.89 b
Feed conversion ratio	1.07 a	1.18 b	1.08 a
Protein efficiency ratio	2.85 b	2.59 a	2.82 b
Protein retention, %	39.5 b	35.8 a	38.9 b
Energy retention, %	30.5 b	28.3 a	30.7 b
App. dig. of energy, %	87.8 a	87.1 a	89.3 b

MAKING PRESCISE GENETIC CHANGES IN THE TILAPIA GENOME

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Efficient tools that dissect gene function and enable introduction of desired genetic modifications at precise locations will radically advance existing genome improvement strategies in animal agriculture. Recently the type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas) system has been adapted to serve as a targeted genome mutagenesis tool. CRISPR/Cas9 have been used to create knock-out alleles with great efficiency in multiple organisms, including fish. Here we report expansion of targeted genome modification repertoire in tilapia. Using CRISPR/Cas9 and circular donor DNA we achieved high frequency of precise knock-in of foreign sequence and further demonstrated the possibility to replace and repair a mutant allele at equally high efficiency.

Our strategy co-targeted pigment genes and used pigment defect as selection markers to identify individuals carrying the desired modification.

We successfully generated tilapia lines where b-globin 3UTR was integrated downstream of dead-end1 (dnd1) coding sequence. We obtained close to 50% of larvae with precise homology-directed knock-in amongst selected embryos. F2 tilapia homozygous for b-globin 3UTR integration developed into sterile adult with string-like ovaries and translucid testes, revealing the essential role of dnd1-3'UTR in the maintenance of adult germ cells.

In addition to the homology directed knock-in, we attempted to swap a mutant version of the tyrosinase pigment gene for a wild type version. Here we used an albino line of tilapia carrying a 7-nucleotide deletion at the tyrosinase locus (Tyr^{alb7}). We show repair of Tyr^{alb7} in as high as 8% of injected embryos, as visualized by the appearance of mosaic pigmented melanophores. We further found germ line transmission of the corrected allele in frequency between 10% and 50%.

Our study indicates that precise genomic modification can be achieved by homology directed repair in the tilapia genome with high efficiency. These results open exciting possibilities for further improvement in breeding programs, allowing for example, rapid introgression of favorable or de novo alleles into a breeding population.

INFOGRAPHICS AS TOOLS FOR DISSEMINATING AQUACULTURE RESEARCH

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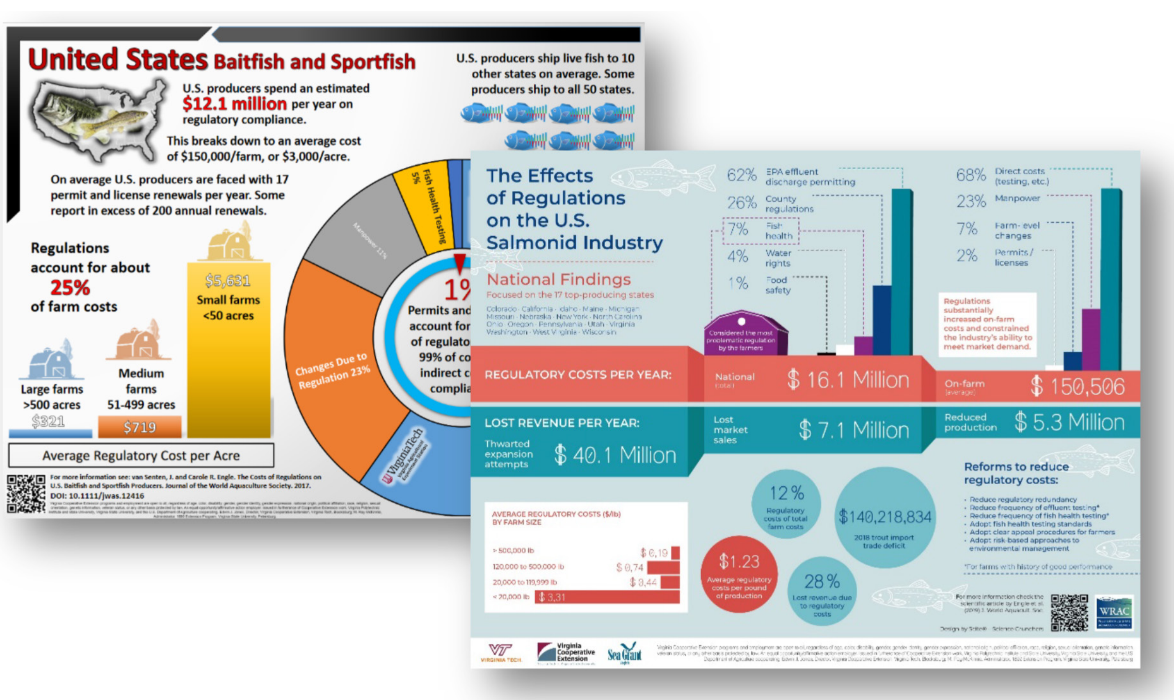
Visual representation of data is not a new phenomenon. We have always relied on graphs, charts, and images as methods of sharing scientific information and making information more accessible to our audiences. Infographics are an evolution of data visualization, that use a collection of images, graphs, charts to convey information about a particular topic in a visually attractive format. Developed in the 1980's as a means for journalists to support story telling through a visual component, infographics have proliferated throughout the business and marketing world. In this digital age, where there is near constant and fierce competition for the attention of our audiences, research and extension can also benefit from the use of infographics as tools to both capture an audience's attention and share valuable information. Integrated with social media and other digital platforms, infographics can also serve to connect audiences with additional information and materials. However, infographics should not just be a random assembly of related imagery. Careful thought and consideration should be given to the appearance and design of these documents to maximize their effectiveness as one tool in our communications efforts. Elements of infographic design will be discussed in addition to metrics on user interaction and engagement.



HETEROTROPHICALLY CONDITIONED RECIRCULATION SYSTEM FOR BIVALVE LARVAL CULTURE

Christopher D. Bentley, Richard Snyder, Reza Ovissipour, Michael Schwarz, Setareh Shiroodi, Steve Urick and *Jonathan van Senten

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Standard bivalve hatchery practices rely on good ambient water quality for reliable production, however ambient water quality can change and have caused issues for hatcheries in years past. A collaborative effort is currently underway between Virginia Institute of Marine Science- Eastern Shore Lab (VIMS-ESL) and Virginia Tech Seafood Agriculture Research and Extension Center (VSAREC) to develop a recirculating aquaculture system (RAS) to allow bivalve hatcheries to reuse production water and minimize impact from fluctuating ambient water quality. Two pilot scale systems were developed for testing. The system design consisted of a 600 L traditional moving bed bioreactor with K1 Kaldnes carrier elements (AnoxKaldnes, Lund, Sweden) for ammonia reduction with the addition of a cultured heterotrophic microbial community to reduce organic waste and remove bacteria from larval production waters. A conventional foam fractionator was also used to assist with removal of dissolved organics. Initial efforts have developed and standardized a heterotrophic conditioning protocol.

Three trials were conducted in 600 L bioreactors to test the preliminary biofilm development procedure and survival of bivalve larvae in system water. In trial 1, multiple 2-day cycle spikes of organics, at a level expected as worst-case scenario for larval culture failure, were absorbed and treated by the system. Very little perturbation of the biomass in the filter was noted. Plate counts of heterotrophic bacteria showed slow growth of colonies indicating planktonic phase bacteria were semi-dormant. Trial was conducted at both VIMS-ESL and VSAREC sites.

A replicated experiment was conducted for trial 2 at VIMS-ESL with 60 L batch cultures of *C. virginica* larvae (n=4). Larvae were reared in either water treated by the experimental 600 L RAS (treatment) or standard hatchery water (control). Larval survival was slightly lower (d2-5 post hatch =35.5%, ± SD 3.9%; d5-7 post hatch = 49.3%, ± SD 6.5%) in the RAS treatment when compared to the standard treatments (d2-5 post hatch = 48.3%, ± SD 1.0%; d5-7 post hatch = 57.0%, ± SD 5.5%). Initial qualitative assessment of larval growth was promising, but indicated a potential negative impact from the RAS rearing water that needs to be addressed.

VIRGINIA SEAFOOD AREC PROGRAMMATIC EXPANSION

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Core research and Extension programs at the Virginia Tech - Virginia Seafood AREC focus on seafood safety, seafood quality of wild caught and cultured animals and products, business and marketing support for the commercial and aquaculture industries, engineering, thermal processing, intensive saltwater recirculating aquaculture, and education/ outreach for industry and consumers. The VSAREC is in the midst of significant program expansion. Infrastructure-wise, $9 million in funding, from various sources, was completed in the fall of 2019 to build a new Center adjacent to the old and failing site. Architectural/ construction engineering review and final permitting is in process, with groundbreaking anticipated early spring, 2020. Construction is projected for completion 12 months thereafter. In addition to our team of faculty, two new Tenure-track faculty lines were added, one in 2018 (Food Quality and Safety – Dr. Reza Ovissipour) and one in 2019 (Agriculture Economics and Marketing – Dr. Jonathan van Senten), and a third Tenure-track position (Traceability and Blockchain) has been approved for early 2020. New major program area expansions are currently underway including: Sustainable Food Production Systems (aquaponics, microbiome, RAS, alternative proteins including plants, and insects, probiotics, and cellular aquaculture); Economics and Marketing (policy and regulatory impacts, farm production economics, economic impact analysis, financial benchmarking, and automation/robotics); in addition to offshore renewable energy and co-location of synergistic activities. A new Programmatic pillar for the VSAREC on Traceability (Big Data managements, Blockchain technologies, etc.,) is slated for 2020. Once construction begins on the new center, efforts in earnest will commence to develop the first of several large-scale industrially applied R&D centers.

POTENTIAL OF BLACK SOLDIER FLY LARVAE (*Hermetia illucens*) FOR ARCTIC CHAR (*Salvelinus alpinus*) DIETS IN AQUACULTURE

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Aquaculture continues to be the fastest growing animal food sector and accounts for over 50% of the world's fish consumption (FAO, 2014). Increased amounts and diversity of protein and lipid ingredients are required to support the sustainable and rapid growth of this industry. Fishmeal, fish oils, and land-based options are the current source of proteins and lipids in feeds for aquaculture, but ingredients made from insects are being considered by the industry as an alternative ingredient. However, there is still uncertainty regarding how these novel ingredients will affect fish growth and survival throughout their life cycle.

Arctic charr is an excellent choice for development of a sustainable land-based aquaculture industry given its high market demand for good quality product, a high market price, and the suitability of this species for production under high densities. However, there are challenges limiting the use of this species for land-based aquaculture, including the tendency for very low fertilization and hatch success, high mortality during transition to first feeding, and a lack of optimized rearing protocols for commercial production.

The aim of this study was to investigate the use of black soldier fly (BSF, *Hermetia illucens*) larvae meal as a feed ingredient for Arctic charr (*Salvelinus alpinus*) aquaculture. We measured growth and reproduction (fecundity and hatch rate) of Arctic charr broodstock, and growth and survival of the fry. The Arctic char broodstock were artificially mated in such a way as to also permit the determination of heritability of acceptability of BSF as an ingredient in the feed.

Two diets were produced for this trial, a control diet and a test diet. The control diet was a locally used commercial salmon feed that met or exceeded all the known dietary requirements of Arctic charr and the test diet was formulated by replacing 20% of the fishmeal with BSF (Enterra Feed Corp., Langley, BC, Canada). Diets were pelleted by extrusion by Taplow Feeds Ltd. (North Vancouver, BC, Canada). Each diet was fed to duplicate raceways of 100 fish for 3 months prior to spawning (Year 1) and 15 months prior to spawning (Year 2). Crosses were fertilized and transferred to a lab (Fisheries & Oceans Canada, West Vancouver, BC, Canada) to assess hatch rate and early life growth. At ponding, half the progeny received the control diet and half received the BSF diet. The survival and growth of the fry were tracked for 4 months to assess the impacts of broodstock diet and fertilization success on the early-life-history performance of the fry. Protein and fatty acid profiles were measured for eggs and fry.

The results show that the feed intake, survival, and growth of the BSF diet did not significantly differ from the control diet, and BSF inclusion tended to support higher survival of fry during early life history. Thus, the use of BSF for fish diets shows great potential to support the sustainable growth of aquaculture.

POTENTIAL OF BLACK SOLDIER FLY LARVAE *Hermetia illucens* AS A SUSTAINABLE ALTERNATIVE SOURCE OF PROTEIN FOR AQUACULTURE DIETS: SUPPLY, PERFORMANCE, AND FUNCTIONALITY

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As the world population grows, global food production will need to increase far beyond current volumes without depleting resources. A possible solution is to harness the power of insects paired with aquaculture which may alleviate some pressures on natural resources. This presentation discusses the capacity of a commercial insect producer: Enterra Feed Corporation, to supply a sustainable alternative source of protein to the aquaculture industry currently, and projections for the future.

Insect ingredients for aquatic nutrition have a promising future based on their feed value, differentiation to end consumers, and functional benefits for efficient fish production. The ability of insect ingredients to supplement less sustainable proteins has been tested without compromising growth or efficiency in a variety of aquatic species including Arctic charr, Tilapia, and sturgeon. Examples of possible functional benefits will be discussed in a review of current research, including effects to intestinal microbiota and gut morphology.

APPLICATION OF DIRECTED BIOTICS TO CONTROL PATHOGENS: COMPLETE PREVENTION OF WSSV-ASSOCIATED MORTALITY IN ADULT SHRIMP (*Litopenaeus vannamei)* INOCULATED WITH *Bacillus subtilis* ENGINEERED TO PRODUCE WSSV-TARGETING DOUBLE STRANDED RNA (dsRNA-VP19)

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RNA interference mediated gene silencing of essential pathogen genes has been demonstrated to be an effective means to control viral replication in shrimp both by intramuscular injection of targeted dsRNA molecules and by feeding formaldehyde-inactivated *E. coli* expressing dsRNA. The challenge, however, has been how to continuously produce and deliver RNA interference molecules in shrimp for pathogen control. Pebble Labs has developed a platform technology (Directed Biotics) for continuous production and delivery of RNA interference molecules using engineered bacteria that are naturally associated with the host. Previously, we have demonstrated that feeding shrimp an RNase III-deficient *Bacillus subtilis* strain expressing dsRNA targeting essential white spot syndrome virus (WSSV) genes was able to reduce shrimp mortality by two-fold in WSSV challenged adult shrimp. To determine which *B.subtilis* strains were most effective in controlling viral replication by delivery of RNA interference (RNAi) molecules, we compared a number of *B.subtilis* expressing RNAi molecules in shrimp-WSSV mortality trials. We found that one of the tested strains (strain X) has a probiotic effect in *Litopenaeus vannamei*. We observed that feeding shrimp unmodified *B.subtilis* strain X resulted in increased resistance in shrimp to WSSV infection (35% reduction in mortality) and also to *Vibrio parahaemolythicus* infection (20% reduction in mortality). This strain was further modified to express a dsRNA targeting an essential WSSV gene, Vp19, or for expression dsRNA-Luc targeting a non-existent Luciferase gene as a dsRNA negative control. For virus challenge experiments, shrimp were fed strain X bacteria expressing dsRNA-Vp19, dsRNA-Luc or commercial food without bacteria for 5 days prior to WSSV challenge by injection.

Full protection (zero mortality) was observed at 10 days post-infection in shrimp receiving food top-coated with dsRNA-Vp19 expressing bacteria while the control group of shrimp fed commercial food had 85% mortality. Additionally, qPCR analysis of WSSV VP19 expression indicated a 3-log fold reduction in virus titer in surviving shrimp fed bacteria expressing dsRNA-vp19 compared to the control group. Using this Directed Biotics platform technology, we have achieved complete control of WSSV in the lab and are currently carrying out field trials.

BLUE FIELDS: OPEN-OCEAN ARRAY FOR HIGH-YIELD MACROALGAE CULTURE

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Blue Fields addresses the primary challenges to commercializing offshore macroalgae cultivation in tropical environments using neither freshwater, nor land, nor fertilizer. Challenges include the nutrient-limited nature of tropical surface waters; offshore depths rendering fixed grid or multiple point mooring arrays too expensive; and expensive labor for harvesting.

The Blue Fields project is addressing these challenges by: (a) Performing land-based tank trials growing multiple tropical alga species with various Deep Seawater (DSW) and surface seawater inputs to determine nutrient requirements for scalable production; (b) Designing an upwelling system to provide pulsed DSW-nutrients (c) Modeling and designing a macroalgae array that conceptually allows for harnessing current and wind energy for harvesting, and optimizes nutrient dispersal; (d) Deploying a demonstration system that tests all these subsystems in Hawaii's offshore environment, validating performance in the field.

In addition to offshore commercial macroalgae culture's capitalization opportunities, there are potential ecological benefits to future scaling of such efforts. These include: uptake of atmospheric carbon, reduction of ocean acidification, and the creation of offshore structure/nursery-habitats for marine wildlife. Implementation of this technology in eutrophied marine zones may also provide further benefits to coastal ecosystems, preventing or mitigating the development of "dead zones".

Kampachi Farms is currently permitting the demonstration project offshore of the Big Island, and this presentation will focus on the engineering design, planning, and permit process.

MARINE PERMACULTURE TO REGENERATE OCEAN PRODUCTIVITY

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Today's warmer surface waters limit natural overturning circulation and vertical mixing by increasing density stratification in the upper ocean, particularly in the subtropics, reducing available nutrients for algae, fish habitat, fish feed and forage fish upon which other fish depend.

In order to increase food security, bolster marine ecosystems and export blue carbon, infrastructure associated with Marine Permaculture restores mixed-layer overturning circulation locally, thereby regenerating fish habitat, growing local seaweed species, and cooling surface water to pre-industrial era temperatures, preventing coral bleaching and moderating marine heat waves.

Marine Permaculture enables larger offshore open-ocean seaweed cultivation that use the vertical shear of mesoscale eddies for maneuvering. Renewable energy sources provide the power needed for seaweed irrigation and guidance, enabling cultivation across subtropical oceans, eliminating the limitations of nearshore cultivation.

Feeding growing global populations is straining diminished marine ecosystems. These resources need to be protected from overexploitation and climate disruption. Each hectare-scale Marine Permaculture has the potential to grow >3000 tons of seaweed per year, fixing a similar amount of carbon dioxide and providing local adaptive strategies for ocean warming, acidification, coral reefs bleaching protection and counteracting climate change at scale.

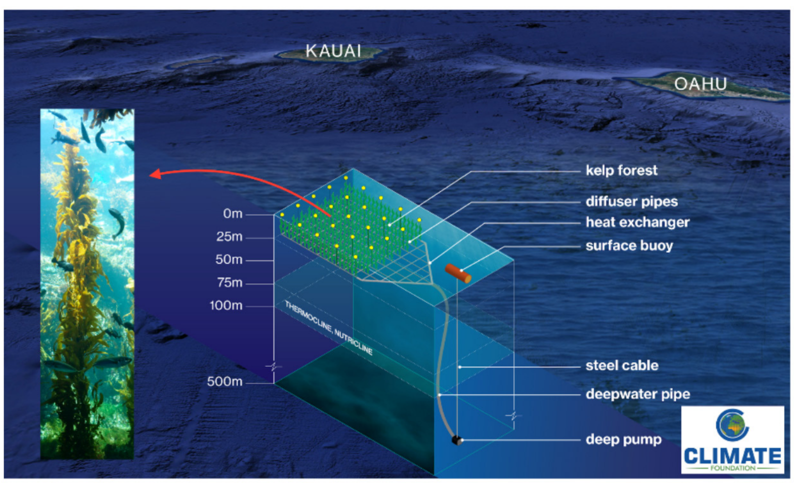

Figure 1: Marine Permaculture scalable architecture

GLUTAREDOXIN 2 FROM *Hippocampus abdominalis* (HAGRX2) AND ITS POTENTIAL INVOLVEMENT IN CELL SURVIVAL AND IMMUNE RESPONSES

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Glutaredoxins are belongs to thioredoxin super family and possess thioredoxin catalytic motif. In vertebrates, Grx1 and Grx2 consider as dithiol proteins whereas Grx3 and Grx5 are monothiols. According to previous studies, Grx2 perform critical functions in both development and redox homeostasis in fish. Therefore, HaGrx2 was characterized by bioinformatical, transcriptional and functional assays.

Insilico analysis was performed by CLC main work bench version 8.0. Tissue distribution and immune challenge experiment (LPS, Poly I:C, *E.tarda* and *S.iniea*) was conducted and performed q-PCR analysis. Further, FHM cell survival upon H_2O_2 stress was measured by MTT assay.

According to the *insilico* analysis, HaGrx2 possess typical glutaredoxin structure with CPYC thioredoxin active motif and showed highest identity (95.4%) with Grx2 from *Hippocampus comes*. *HaGrx2* exhibit highest expression in skin and brain of healthy fish. Liver *HaGrx2* showed significant upregulation toward poly I:C, *E.tarda* and *S.iniea*. Further, HaGrx2 exhibit significant antiapoptotic activity against H_2O_2.

Grx2 is an enzyme that regulate many cellular pathways. As it is a redox enzyme, directly regulates the redox signaling during oxidative stress conditions. Also, it is known as a redox switch for releasing mitochondrial ROS during innate immune responses. Further, it can regulate the mitochondrial energy producing complexes by deglutathionylation thus boost the metabolism and host defense mechanisms indirectly at normal and stress conditions. Therefore, Grx2 might play a vital role in cell survival, growth, proliferation and innate immune responses. Altogether, results in this study suggests that HaGrx2 actively involved in host innate immune responses as well as cell survival during oxidative stress conditions.

MOLECULAR IDENTIFICATION AND INNATE IMMUNE ROLE OF FIRST TELEOST MALECTIN FROM *Hippocampus abdominalis*

Sellaththurai Sarithaa, K.A.S.N Shanaka, Kishanthini Nadarajapillai, W.K.M Omeka*, Thanthrige Thiunuwan Priyathilaka, and Jehee Lee

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In this study, we have identified and characterized ER resident lectin type protein, malectin from big-belly seahorse *Hippocampus abdominalis* (HaMLEC). Lectins are carbohydrate-binding proteins have a role in cellular and molecular level recognition and also mediate bacterial and viral binding to the targets. Malectin is involving in backup glycol-protein quality control in endoplasmic reticulum (ER) and have binding specificity to Glc_2-N-glycan. Also, malectin contains CBMs like structure, which might have the carbohydrate and bacterial binding ability. Malectin indicated to have a potential role in innate immunity during some recent studies. However, the studies on innate immune role of malectin is very limited.

The ORF and protein sequence of *HaMLEC* was deduced by UGENE software and the in-silico study was performed with the deduced protein sequence of *HaMLEC* by using bioinformatic tools. We have analysed the spatial and temporal transcript level of *HaMLEC* by qPCR detection. Immune challenge was carried out with four immune stimulants (*Edwardasiella tarda*, *Streptococcus iniae,* polyinosinic: polycytidylic, and lipopolysaccharide) and the liver sample was collected at different time points for the temporal level analysis. The bacterial binding ability of HaMLEC was analysed through ELISA by using recombinant HaMLEC with different bacteria (*E. tarda*, *Lactococcus garvieae*, *V. anguillarum*, *S. iniae*, *Vibrio harveyi*, *S. parauberis*, and *E. coli*).

The cDNA sequence of *HaMLEC* consists of 1269 bp ORF, which encoding 423 amino acids. The molecular weight was predicted as 49.04 kDa and the pI was estimated as 4.37. Malectin super family, C-terminal transmembrane region, and N-terminal signal peptide was identified from *HaMLEC* sequence. The *HaMLEC* showed high sequence identity (99.0 %) and close evolutionary relationship with *Hippocampus comes* during pairwise sequence alignment and phylogenetic analysis, respectively. The transcript level of *HaMLEC* was detected in all tested tissues. The significant upregulations were observed for all four stimulants with different time points during temporal level expression in liver. Moreover, the significant bacterial binding ability was observed with wide range of bacterial species with compare to the controls. The observed significant changes in immune challenge results and the wide range of bacterial binding activity suggesting that the HaMLEC might be involved in the innate immune role of *Hippocampus abdominalis*.

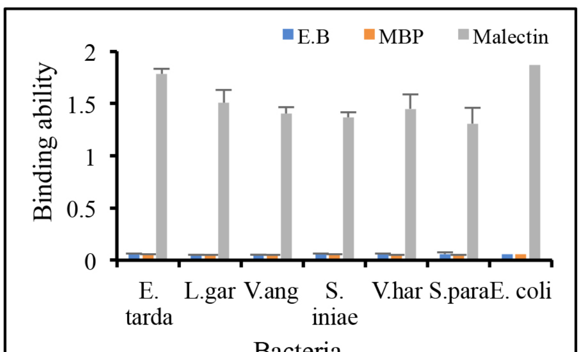

Fig. 01. Bacterial binding activity of recombinant HaMLEC by ELISA

EFFECT OF HYDRAULIC RETENTION TIME ON NFT LETTUCE PRODUCTION IN A DECOUPLED AQUAPONIC SYSTEM

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An experiment was conducted at Auburn University to determine the effects of hydraulic retention time (HRT) of aquaculture effluent containing sub-optimal nutrient concentrations on aquaponic lettuce production using nutrient film technique. Aquaculture effluent from a biofloc-type recirculating aquaculture system (RAS) containing Nile tilapia (Oreochromis niloticus) was transferred to a recirculating nutrient film technique hydroponic system and exchanged at various, pre-determined intervals. Experiment design was a completely randomized block design, containing five blocks that were each comprised of five 4-m nutrient film technique channels (FarmTek, Connecticut, USA) that held 15 plants each and were supplied with nutrient solution from one of five reservoirs. Four reservoirs were filled with aquaculture effluent and exchanged at pre-determined intervals of four, eight, twelve, and sixteen days and the fifth contained a standard hydroponic solution that was exchanged halfway through the experiment. Butterhead lettuce (Lactuca sativa 'Rex'; Johnny's Selected Seeds, Maine, USA) were germinated and grown in Oasis® Horticubes (Oasis, Ohio,USA) for 14 days before seedlings were transplanted into the nutrient film technique system and treatments were initiated.

PROC GLIMMIX was used to conduct an analysis of variance on water chemistry and plant growth data. Means were separated using Tukey's honest significance difference test (SAS Institute, Cary, NC). Plant fresh mass decreased linearly for aquaponic treatments, from 203g to 143g, as HRT increased from 4d to 16d, with the hydroponic control (HC) gaining the most fresh mass (258g). Similarly, SPAD index decreased linearly for aquaponic treatments, from 18.7 to 7.6, as HRT increased from 4d to 16d, while the HC had the highest SPAD index (28.4). Average nitrate concentrations in aquaponics treatments decreased linearly as HRT increased. Although average nitrate concentrations were higher for the HC (627 mg L), foliar nitrogen concentrations were similar for both. Differences in leaf color and lettuce growth were likely the result of sub-optimal concentrations of micronutrients. Foliar iron concentrations for aquaponic lettuce ranged from 60 mg kg to 87 mg kg as HRT decreased, but were much higher (132 mg kg) for the HC. The reverse trend was observed for foliar manganese, which increased from 399 mg kg to 692 mg kg in aquaponic treatments but was much lower (315 mg kg) for the HC. Increased manganese accumulation was likely the result of iron deprivation. In conclusion, decreasing hydraulic retention time of aquaculture effluent improved lettuce growth in a nutrient film technique system, but growth and quality were ultimately limited due to sub-optimal micronutrient concentrations in aquaculture effluent.

Table 1: Fresh Mass, SPAD, and Leaf Count taken from lettuce plants. Nitrogen Average taken from nutrient reservoirs.

Parameters ^{z}	Retention Time in Days				
	4D	8D	12D	16D	Hydro (14D)
Nitrogen Average	427.22c	433.33b	410.00e	424.44d	627.22a
Fresh Mass	203.43b	193.80b	166.17c	143.81d	257.78a
SPAD	18.723b	17.465b	17.775b	7.645c	28.373a
Leaf Count	45.90ab	43.50b	40.00bcx	36.20c	50.800a

z) Means separation by Tukey's honest significance difference test (ANOVA, $p<0.05$). Differen letters indicate that means are significantly different.

NON-TOXIC PHOTOACTIVE RELEASE COATINGS FOR BIOFOULING CONTROL

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Novel non-toxic coating technology for biofouling control developed for the aquaculture industry is presented that relies on the photoactive generation of hydrogen peroxide to reduce the settlement of biofouling organisms rather than the leaching of pesticides.

Biofouling, the unwanted growth of biological organisms on underwater surfaces, has long been recognized as a major problem for commercial aquaculture. Biofouling dramatically increases labor costs, reduces the value of product, and can harm cultured species. Fouling clogs gear, stops water flow and food delivery, can compete with culture organisms for food or space, and can directly affect the growth and survival of cultured organisms. As a result, considerable physical and economic effort is directed toward the prevention and control of biofouling at culture facilities. Cleaning of gear and use of toxic coatings are the primary methods employed by the industry to maintain biofouling-free surfaces. Time and energy expended to keep gear clean taxes aquaculturalists consuming as much as 30% of labor costs and contributing 15% to operational costs.

A novel bio-based release coating was developed that, when immersed in water, controls the settlement of biofouling organisms. Biofouling control is accomplished by the release properties of the polymer that degrades and dissolves when exposed to sunlight. Catalysts in the formula generate low levels of hydrogen peroxide when exposed to sunlight. Peroxides are oxidizing agents known to thwart the settlement of biofouling organisms. Peroxides also facilitate the gradual breakdown of the bio-based coating resulting in a release of the surface layer, and along with it any biofouling that may have attached. This solution to the biofouling problem is sustainable because peroxides quickly dissociate back to oxygen and water after leaving the coating surface, and all ingredients in the bio-based coating are generally regarded as safe (GRAS).

Results from biofouling resistance testing on PVC test panels and aquaculture gear (bags, trays and cages) performed from 2015 to 2018 are presented. Oysters and scallops grew significantly larger in treated bags and trays over a three month grow-out period. Treated gear requires less maintenance and can be reused without cleaning. Results from field testing demonstrate that photoactive release coating technology is a viable solution to the biofouling problem experienced by shellfish farmers, who rely on gear changes and cleaning to control biofouling.

EVALUATION OF GENETIC VARIATION AND GENE EXPRESSION PATTERNS OF DIFFERENT STRAINS RELATED TO SMD UTILIZATION IN LARGEMOUTH BASS

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In order to evaluate utilization variation of, and growth response to, soybean meal diets in largemouth bass for breeding programs, test diets with 0%, 35%, and 50% fishmeal having been replaced by soybean meal (SBM; S0, S35, and S50) were administered to three northern strains of largemouth bass (LMB; Jones, Marlow, and Remlinger).

In the S35 and S50 groups, the Marlow and Remlinger strains had much better growth response to soybean meal diet (SMD) than the Jones strain, indicating that these two strains can better utilize diets containing high level of SMD. The results suggest that sufficient genetic variation exists for growth and feed efficiency in LMB fed diets containing both SBM and fishmeal, and genetic improvement may be possible for the trait of feed efficiency. In all the three strains, the growth performance of individuals in the S35 and S50 groups obviously declined when compared with the S0 group, and the specific growth rate and weight gain correlated negatively with dietary levels of SBM.

Genes that were differentially expressed between dietary SBM levels were identified. Transcripts of genes related to insulin-signaling pathway and fatty acid metabolism and biosynthesis were significantly downregulated in the S35 and S50 groups compared with the S0 group. Interestingly, several transcripts of genes (*egfr*, *pla2g4*, and *ugt*) involved in GnRH-signaling pathway and steroid hormone biosynthesis were also downregulated in the S35 and S50 groups, inferring that feeding with SMD could impair the sexual development of juvenile LMB. Likewise, the genes related to insulin signaling and fatty acid metabolism and biosynthesis were downregulated in the Jones strain compared to the Marlow and Remlinger strains in all three treatments. These results also suggest that insulin-signaling pathway and fatty acid metabolism and biosynthesis play important roles in adaptation to diets with high levels of SBM in LMB. Overall, the Marlow and Remlinger strains exhibited better adaptation to SBM diets, and could be considered as potential breeding candidates.

This study provides useful information for LMB breeding programs, and it contributes to our understanding of genetic mechanisms related to growth performance of LMB fed with high levels of SMB.

AQUACULTURE BOOT CAMP (ABC): ENHANCING THE SUSTAINABILITY OF NEW AQUACULTURE/AQUAPONIC FARMERS WITH INNOVATIVE TRAINING

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The Ohio Center for Aquaculture Research and Development (OCARD) at the Ohio State University, in partner with Ohio Aquaculture Association (OAA), University of Wisconsin –SP, and other partners, has developed and operated the Aquaculture Boot Camp (ABC). ABC program offers new and next generation farmers **3-I levels, 3 areas and 3 types** of integrated training in aquaculture/aquaponic production and business management strategies. The "**3-I levels"** are**: Intensive**, an in-depth level involving immersion in a year-long hands-on training and classroom/mentoring program; **Intermediate**, a mid-level involving participation in a variety of learning activities and workshops; and **Introductory**, a general or entry level where sharing of information is the goal, and involving participation in the ABC online education and webinars. The **"3 areas"** are general/traditional aquaculture, aquaponics, and related business and marketing. The **"3 types"** are hands-on, classroom/mentoring, and internet/webinar.

The ABC Phase I (ABC-1) had been successfully completed during 2012 – 2015: By the end of the ABC 1 project, ***twenty-four new businesses/farms were created by 2013 and 2014 year-class ABC Intensive graduates***. After participation in two ABC intensive classes in 2013 and 2014, students, on a scale of 1 being strongly disagree and 4 being strongly agree, reported an average of 3.5 when asked if the program met their expectations, and they would recommend this program to their business partners or relatives. At the end of ABC-1, the ABC **Intermediate** program surpassed the projected number of participants by 186.87%, and the **Introductory** program surpassed the participation goal by 557.10%. That means that more that 700 new and beginning farmers gained knowledge of aquaculture production and new technologies by participating in ABC Intermediate workshops and bus tours, and more than 9,000 participants gained new knowledge by accessing ABC Introductory, ABC website tools and information, ABC/OAA Newsletters and magazines. In addition, some participants or potential new farmers who are interested in aquaculture training experience received ABC and OAA internships and mentoring.

ABC Phase 2 (ABC-2) has been successfully conducted during 2016 – 2019 in Midwest. A key addition to ABC-2 is the inclusion of aquaponics. This expansion is a direct result of strong interest from Ohio and the North Central Region. For ABC-2**,** thirty-six one-day **Intensive** classes were completed, and 105 highly motivated new and limited resource fish farmers and aquaponic producers have been trained through the **Intensive** program**;** 35 pilot-scale aquaculture/aquaponics practice projects were completed by the intensive students; 12 general workshops in aquaculture and related business and 4 bus tours of aqua-farms were conducted for both **Intensive** and **Intermediate** students; Five conferences were held for all the "**3I**" level students; Around 900 new and beginning farmers gained knowledge of aquaculture/aquaponic production and new technologies by participating in ABC **Intermediate** workshops and bus tours, and more than 10,000 participants gained new knowledge by accessing ABC **Introductory** and ABC website tools, and Newsletters. Fourteen internships are complete through **Internship** program. Twenty-four issues of aquaculture/aquaponics Newsletters were published. ***Twenty-one new businesses/farms have been created by the 2017 and 2018 ABC-2 Intensive course graduates***.

PREVENTION EFFICACY OF VACCINES AGAINST THE TRANSMISSION AND VIRULENCE EVOLUTION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Vaccine usage in aquaculture is rapidly expanding. Many vaccines have proven to be a powerful tool for managing clinical disease in aquatic animals. However, vaccine impacts on pathogen transmission are rarely investigated and remain largely unknown. In the few cases where studied, many vaccines have shown poor efficacy at blocking transmission, despite effectively reducing disease levels. This is problematic because vaccines that do not prevent transmission can allow for the persistence of pathogens in host populations. This is likely to greatly hinder eradication efforts and long-term disease management. Furthermore, theory predicts that vaccines that reduce clinical disease but do not block transmission, can select for the evolution of increased pathogen virulence. Such vaccines may allow hosts infected with highly virulent pathogen strains to survive longer than unvaccinated host, thereby increasing the transmission duration of the pathogen. Evidence of vaccine induced virulence evolution has been found in Marek's disease in the poultry industry. However, investigation of this phenomenon is limited.

We investigated three vaccine types, DNA, inactivated, and attenuated, against infectious hematopoietic necrosis virus (IHNV) in rainbow trout. Each vaccine was evaluated for its ability to prevent host clinical disease, viral shedding, transmission, and virulence evolution. To assess disease prevention efficacy, vaccinated and unvaccinated fish were exposed in batch to a natural immersion challenge of IHNV and mortality tracked over course of infection. To assess viral shedding, water samples were collected from individual infected fish at numerous time points then processed by quantitative PCR targeting the virus. To assess transmission, the proportion of naïve fish infected after cohabitation with infected fish was quantified. To assess virulence evolution, the virus was subjected to multiple rounds of transmission between fish in a serial passage design, then the virulence of the passaged virus was compared to the unpassaged virus.

We found that the three vaccine types differed in their disease prevention efficacy, with the DNA vaccine being the most effective, followed by the inactivated vaccine. All vaccines allowed for significant levels of viral shedding and transmission, despite strong disease protection (Figure 1). Both increases and decreases in virulence were observed after passage of virus through vaccinated fish, although changes were small. In conclusion, this work demonstrates the importance of assessing the transmission blocking efficacy of vaccines in addition to disease prevention. This is likely to result in more effective and sustainable disease management.

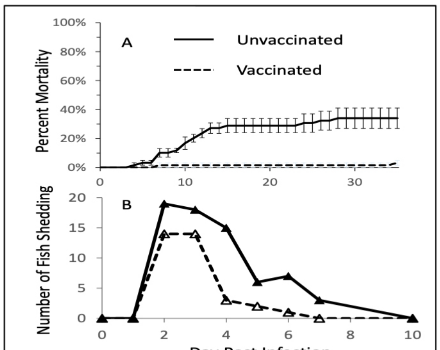

Figure 1. Mortality (A) and shedding (B) prevention efficacy of DNA vaccine against IHNV.

THE EFFECT OF DIET AND TEMPERATURE ON GONAD ENHANCEMENT OF GREEN (*Strongylocentrotus droebachiensis*) AND RED (*Mesocentrotus franciscanus*) SEA URCHINS

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When sea urchin population growth is uncontrolled, they can over-graze and decimate macroalgal beds, creating areas termed “urchin barrens”. Sea urchin gonad enhancement is a proposed method to remove sea urchins from barren grounds, promote the re-growth of macroalgal beds, and produce a highly-valued marketable product. Prepared feeds need to be developed that not only increase gonad yield, but also impart appropriate market colour and flavour. This project assessed the effects of two prepared diets (V10.1.9 and V10.1.10) and a natural feed (bull kelp, *Nereocystis luetkeana*) and three seawater temperatures (8, 12, and 16°C) on gonad yield/quality for both the green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchin held under laboratory conditions for 12 weeks. Green urchins fed the prepared diets had overall higher gonad yields and better colour than the green urchins fed kelp at all three temperature treatments; with V10.1.9 producing the highest yields for all three temperatures and V10.1.10 producing the best colour at 16°C. Red urchins fed V10.1.10 at 12 °C had the highest gonad yields out of all the treatments, while the red urchins fed kelp at 8°C had the lowest yields. The gonad colour was better overall with the red urchins fed V10.1.10, with the best colour again obtained at 16°C, which is the same trend as seen in the green urchins.

INVESTIGATING THE EFFECTIVENESS OF SEAWEED SPECIES ON METHANE PRODUCTION AND RUMEN FERMENTATION *in vitro*

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Due to implications of global climate changes, greenhouse gas (GHG) emissions coming from the livestock industries have been the topic of ongoing investigations. Several studies have looked at feeding seaweeds to assess their impact on methanogenesis in ruminants. Natural volatile halomethanes found in some seaweeds have been shown to inhibit rumen methanogenesis.

In this experiment, 6 different species of seaweed; *Sargassum horneri* (SH), *Ecklona arborea* (EA), *Ascophyllum nodosum* (AN), *Sargassum fluitans* (SF), *Mastocarpus papillatus* (MP), *and Fucus vesiculosus* (FV), were analyzed for their effect on gas production and composition, volatile fatty acid (VFA) production, and neutral detergent fiber (NDF) degradability *in vitro*. Incubations were duplicated and carried out for 24h with inoculum taken from two cannulated lactating Holstein cows fed a standard corn silage/alfalfa haylage and concentrate feeds diet. Treatments were tested in triplicate, at an inclusion rates of 0.5, 1.0, and 2.0% of feed dry weight (DW). *Asparagopsis taxiformis* (AT) has known antimethanogenic properties and was used as a positive control at 1% DW. Gas samples were collected at 12 h and 24 h and analyzed for methane (CH_4) and hydrogen (H_2) concentrations. VFA production and NDF degradability were analyzed at incubation termination. Compared with the negative control (NCON; i.e., animal feed without seaweed) at 12 h, none of the treatments decreased CH_4 production. Relative to NCON at 24 h, SH decreased ($P = 0.01$; SEM = 0.6) CH_4 production by 15% (8.9 vs. 7.6 mL/g of feed DW, respectively). Methane production was increased, compared with NCON, by MP and SF ($P \leq 0.04$, SEM = 0.6; 10.0 and 9.9 mL/g of feed DW, respectively). In line with previous research in our laboratory, AT decreased ($P < 0.01$) CH_4 production by 98% compared with NCON. Hydrogen production was negligible (average of 0.01 mL/g feed DW) for all treatments, except AT, which averaged 2.0 mL/g of feed DW ($P < 0.001$). Compared with NCON, MP and FV increased ($P \leq 0.05$; SEM = 5.3) total VFA concentration (65.2, 76.4 and 74.3 μmol/mL, respectively) and MP also increased ($P = 0.02$; SEM = 3.1) acetate concentration (34.5 vs. 40.6 μmol/mL). Proprionate concentration was increased by MP and FV ($P \leq 0.02$; SEM = 1.8) when compared with NCON (24.3, 23.9, and 20.5, respectively). There was no effect of seaweed on molar proportions of VFA, acetate: propionate ratio, and NDF degradability. Overall, except the positive control *Asparagopsis taxiformis* and *Sargassum horneri* (15% reduction at 24 h), seaweed species tested in this *in vitro* experiment did not influence enteric methane production. Some species (*Mastocarpus papillatus* and *Fucus vesiculosus*) appeared to have a positive effect on rumen fermentation by increasing total VFA and acetate and/or proprionate concentrations.

SUPPLEMENTATION OF TWO POTENTIAL PERFORMANCE INHIBITORS AND THEIR EFFECTS ON METABOLITE PROFILES IN JUVENILE RED DRUM, *Sciaenops ocellatus*

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The South Carolina DNR has utilized a natural diet of cut fish, squid, and shrimp as a positive reference diet in multiple feeding trials. Although this has mainly been incorporated as an “across-trial” reference treatment, growth performance on this treatment has always out-performed all of the >20 pelleted feeds our laboratory has evaluated. Recently, we have been able to incorporate nuclear magnetic resonance (NMR)-based metabolomics into our analyses. After profiling the metabolites found in pelleted feeds versus those observed in the natural diet feed items, we have identified multiple non-trace metabolites that are present in high soy containing feeds that are absent in the natural fish, squid, and shrimp feed items. To explore whether these metabolites may be inhibiting growth, a feeding study was carried out with a fishmeal based reference formulation and a fishmeal-free, 50% soybean meal formulation.

Two metabolites, melibiose and uridine monophosphate, that were identified as being present in the feeds and tissues of fish fed high soy containing diets, but absent from feed or tissues of fish fed natural diet items, were supplemented individually and in combination to both of the base formulations for this study. At the conclusion of the twelve week feeding trial, liver samples were taken for NMR metabolite profiling to assay differences in physiological response of juvenile red drum to the addition of these metabolites. Growth and performance metrics were also collected and analyzed. Pairwise comparisons and principle component analyses were utilized to assess the results of metabolite additions to the two base formulations.

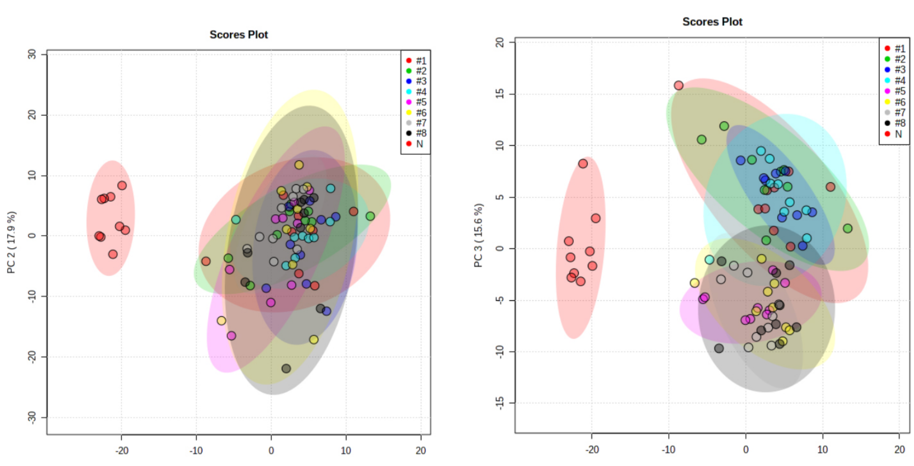

Figure 1. Principle component analyses comparing PC1 vs PC2 (left) and PC1 vs PC3 (right). Most of the variance in the data (PC1 = 29.7%) is due to differences in metabolite profiles of fish fed the Natural (N) diet and fish fed the experimental pelleted diets. However, significant differences were detected along PC3 (explained variance = 15.6%) between two main clusters: diets 1,2,3,4 (one cluster) and diets 5,6,7,8 (second cluster).

PRODUCTION AND PERFORMANCE OF TETRAPLOID AND INTERCROSS TRIPLOID RAINBOW TROUT

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Tetraploid (4N) induction rates of ~80% are routinely achieved in rainbow trout (*Oncorhynchus mykiss*) when eggs are exposed to 9000 PSI for 8 minutes starting at 62-65% of the first cleavage interval (FCI). Factors that affect FCI are not fully understood but has been found to differ among populations, year to year within the same population, and is affected by temperature and age of the ova post-ovulation. Since the FCI appears to be consistent through-out the spawning season for a genetically similar population of fish held under similar conditions, FCI only needs to be determined for a few females at the start of the season. First generation 4Ns have low survival, high rates of deformity, and poor-quality eggs compared with diploid (2N) fish, and sperm that is often too large to pass through the egg micropyle to successfully fertilize the egg.

Second generation 4Ns are much improved in all these areas except sperm size. It has been our experience that in the laboratory, all offspring of 4N x 4N crosses are 4N, and 4N x 2N crosses are triploids (3N) and sterile. We have found the intercross triploids (3NC) produced by crossing a 4N and 2N parent, to be superior to the more common pressure-shock-induced triploids (3NP) in several performance traits including growth and disease resistance. We have also found family values for growth to be more consistent with their 2N values when the families are 3NC as opposed to 3NP, supporting a greater potential to improve 3NC growth performance compared to 3NP performance when genetic selection is based on the 2N phenotype. Nevertheless, genetic gains for each trait are generally more improved following each generation of selection, then they are between 3NC and 3NP fish, and including tetraploidy into a selective breeding program likely delays availability of genetic gains to production seedstock.

Two all-female 4N broodstocks derived from our genetically improved lines are maintained at NCCCWA. The lines are enriched each generation with de novo 4N males derived from the most improved 2N broodstock. Paint Bank Fish Culture Station (PBFCS; Virginia Department of Game and Inland Fisheries) has recently produced its 3rd generation of 4Ns from its own stocks. Primary impediments are sperm size and the cost and time required for ploidy confirmation. At PBFCS, we have been using flow cytometry to confirm a limited number of males as 4N and selecting those with high fertility. Fertility is determined by taking sperm from each male and fertilizing eggs from a pool of eggs, and determining fertility within 24 hours of fertilization. The fertility test is to in theory screen for males with narrow sperm. We are concerned it may be better to evaluate 4N male fertility based on reaching later stages of development as we sometimes find there are more bad eggs sorted as good eggs by mechanical egg pickers when 4N males are used. If all males are confirmed as 4N, then the fish in the next generation will be either 4N if the female is 4N, or 3N and sterile if the female is 2N meaning no further need to test for ploidy. Nonetheless, we suggest inspecting the sperm for size before using a 4N male since it is easy to differentiate 4N from 2N milt under 400X magnification.

VIABILITY OF INTACT, PRE-KILLED ROTIFERS AS AN ALTERNATIVE FIRST FEED FOR ZEBRAFISH *Danio rerio* FRY

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Rotifers (*Brachionus plicatilis*) are an important first-feed item for zebrafish fry due to their enticing motility, gutloading capacity, and small culture footprint. Although larval fish benefit from feedings, the process for culturing rotifers can be laborious and is periodically prone to collapse which can impede survival and growth of newly hatched fry. To minimize labor costs, we wanted to see if pre-killed, enriched rotifers could yield comparable or better survival for zebrafish fry.

Wild-type zebrafish (AB and TL) were spawned to produce an F1 generation of AB and TL hybrids (ABTL). Embryos were collected and placed in 20mL of E3+MB embryo media at a density of 60 embryos/tissue culture dish and maintained at 28°C. They were inspected twice daily and received a 50% media exchange and removal of dead embryos and detritus. Fry were supplied with food daily following inflation of the swim bladder at 5dpf and transferred to polycarbonate tanks at 7dpf. Water parameters remained constant for all fish (Table 1) with no detectable levels of ammonia or nitrite. Nitrate levels were kept below 10ppm. Fry were fed prescribed diets (Table 2) to satiation three times daily with only two feedings on weekends. At 15dpf, fry received 48hr. brine shrimp and, if able to consume it, replaced their first feed diets with thrice daily feedings of brine shrimp and Gemma 75. Mortality rates and time to maturation were recorded for each feeding regime.

Preliminary findings indicate fry initially fed a mixture of Roti-Feast and Gemma 75 had comparable survival to those fed live rotifers, with both groups surpassing those fed only on Gemma 75. The lack of food motility did not hinder palatability for fry. The use of Roti-Feast required less overall cleaning and daily colony maintenance versus the use of live rotifers. Overall, using whole, pre-killed prey items may provide facilities with labor and cost savings in addition to an equally effective first feed alternative to live prey. Future studies should follow fry fed pre-killed diets through ontogeny and investigate any potential skeletomuscular deformities, fecundity problems, and life expectancy versus standard feeds.

Table 1. Zebrafish fry environmental parameters in lab.

Temperature	Flow	pH	Conductivity	Light Cycle	Fry Density
29+/-1°C	65mL/min	7+/-0.3	1000 +/- 50µS	14:10	60 fry/L

Group 1	Group 2	Group 3	Group 4	Group 5
Live Rotifers (*B. plicatilis*)	Roti-Feast (*B. plicatilis*)	Gemma 75	Gemma 75 +Roti-Feast	Gemma 75+Live Rotifers

Table 2. First-feed paradigms for zebrafish fry.

UNDERLYING MECHANISMS FOR SELECTED DISEASE RESISTANCE AND ENHANCED NON-SPECIFIC RESISTANCE IN RAINBOW TROUT

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A critical problem facing all U. S. aquaculture is loss due to disease. In the past year alone, diseases accounted for 90% of all losses of trout intended for sale, greatly impacting the livelihood of our farmers. Our proposal is directly concentrated on this problem. Previously, we focused on sustainable aquaculture efforts by evaluating the effects of dietary replacement (marine-based to plant-based protein sources) on rainbow trout. Through this effort, we developed a rainbow trout strain that thrives on an all plant-protein formulated diet (ARS/UI strain). In selecting for diet tolerance, we discovered this strain has also undergone positive selection for nonspecific disease resistance – to both Infectious Hematopoietic Necrosis Virus (IHNV) and Flavobacterium spp. Therefore, the goal of this project was to determine the mechanisms behind this nonspecific immunity by performing a multi-pathogen challenge and measuring several disease performance characteristics among ARS/UI and two other trout strains commonly used in the commercial aquaculture industry, one selected for disease resistance to these pathogens for several generations and the other completely unselected. We examined lysozyme activity changes in mucus secretions and innate gene expression using RNA-sequencing by sampling kidney, spleen, intestine, and liver at time zero and at 4, 12, 24, and 48 hours post-infection with IHNV and *F. columnare*. We then integrated innate gene expression with disease performance characteristics and made comparisons among strains to identify significant differentially expressed genes and compared with the genetic background of the fish. Through these objectives, we expect to narrow a set of candidate genes useful in the co-selection of two aquaculturally-important traits: enhanced nonspecific disease resistance with diet utilization. Preliminary results will be presented. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. USDA-NIFA-SRGP-006544 from the USDA National Institute of Food and Agriculture.

KELP FARMING IN ALASKA – EXPANSION, OPPORTUNITIES, AND CHALLENGES

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Aquatic farms submitted the first applications to produce kelp in Alaska in 2016. The following year, these farms grew 7.5 tons of product. The 2019 harvest is expected to be near 100 tons, which would make Alaska the largest producer of kelp in the United States. The acreage of Alaska aquatic farm area in current applications is more than double the total acreage of two years ago, and most of the new acreage is slated for farming kelp.

Expansion of kelp and other algae production in Alaska is certain. However, how far the expansion will go, what future opportunities exist, and how the challenges will be overcome is still largely unknown because the industry is so dynamic.

Alaska has more than 10,000 kilometers of coastline and nearly 4 million square miles in the exclusive economic zone, with fewer than 1 million residents. Much of this area is remote, nutrient rich, and free from many of the area conflicts that occur in other regions. Yet with remoteness comes added expense. Large distances from population centers mean fewer services and higher transportation and maintenance costs. The small local population means that any large-scale production will be transported to distant markets, increasing costs. And despite the small population, conflicts still arise between user groups.

In addition to the need to develop markets, decisions on which species to grow, how to grow them, where to grow them, when to harvest, and how to process the product are still in progress. As the industry grows, new factors are changing all of these questions.

Current farms are primarily focusing on food products, but the development of expertise and infrastructure may allow for expansion into new areas, such as biofuels and alginate products not currently in production.

The regulatory and political climate are also in flux, as the State of Alaska and the federal government both adjust to the new and changing needs and realities of the industry on the ground.

GETTING IT RIGHT: SHARED CHALLENGES AND NEW BRIDGES IN THE AQUACULTURE COMMUNITY

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Captive rearing, including aquaculture, has long been a tool for species production and conservation and its utility has never been more important. In fact, some conservation researchers suggest that 2,000-3,000 terrestrial species alone will need to be reared captively in the next 200 years. At the same time, the importance of efficient food production has never been more important. Applications of this tool include supporting recreational and harvest-based fish and wildlife management, restoration and recovery of declining populations, rehabilitation of displaced or injured fish and wildlife, and outreach and education. They also include providing health and sustainable food, while supporting small and large scale economic opportunities. Captive rearing takes many forms including production facilities, zoos, aquariums, hatcheries, and rehabilitation centers. Outcomes range from fresh filets, to wild supplementation or release programs, and even conservation-based refugia programs maintaining individuals or populations entirely in captivity. While numerous success stories have resulted from captive rearing efforts, the tool is often subject to criticism and these challenges exist in all sectors of aquaculture.

In order to continue success in aquaculture's varied captive rearing programs, it is critical that managers and researchers work together to identify clear thresholds for success, apply research-based methods to evaluate contemporary protocols, employ the latest available technology to meet objectives, and communicate successes and advances to the aquaculture community as a whole. This is true for programs aimed strictly at production or supplementation, to those aimed at maintaining valuable genes, and the many outcomes in between. The author provides a broad look at some of the challenges facing captive rearing in conservation aquaculture, some of the historical and contemporary efforts to overcome those challenges, and opportunities to tackle those challenges with new partners. This talk builds on a Symposia presented at the American Fisheries Society annual meeting in Reno 2019. It serves to uncover an important opportunity to build connections in the aquaculture and captive rearing communities across private, public, and non-profit institutions tackling similar challenges in this rapidly growing field.

A NOVEL RESTORATION APPROACH: THE POST-LARVAL COLLECTION AND CULTURE OF A SEA URCHIN, *Diadema antillarum*, TO CONTROL THE ABUNDANCE OF *Ramicrusta*

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In Puerto Rico, a more recent and potential threat to the coral reefs is the increasing abundance of *Ramicrusta* spp. *Ramicrusta,* an encrusting red alga, forms a thin, crustose layer that spreads over the substrate and can grow over the living tissues of corals. *Ramicrusta* was the dominant substrate category at many shallow east coast reefs (e.g., Fajardo, Culebra, and Vieques), reaching a cover (±SE) as high as 63.0 ± 5.8%. At present, herbivorous fishes, like parrotfish and surgeonfish, have not been observed eating *Ramicrusta*. However, *Diadema antillarum*, a keystone herbivore, has been witnessed eating *Ramicrusta* in the laboratory and the field. The goal of this project was to increase reef resilience by increasing the population densities of *Diadema* on reefs by restocking lab-cultured urchins. The objective was to enhance herbivory and decrease algal cover, especially *Ramicrusta*. During the summer months (May to October), *Diadema* settlers were collected on settlement plates set along the shelf edge in La Parguera, Puerto Rico. Settlers (0.4 mm to 1.0 mm test diameter) were brought back to the lab and grown out in aquaria and raceways. Lab-raised *Diadema* were transferred to reefs once the urchins reach a size sufficient to reduce mortality (2.0 cm to 4.0 cm test diameter). In August 2018, 480 *Diadema* were restocked to two reefs highly impacted by *Ramicrusta* in Fajardo, Cayo Diablo and Los Lobos. Within two months, *Ramicrusta* cover was significantly reduced by 63% at reefs restocked with *Diadema*. The substrate inside the experimental plots was characterized by more available space and turf algae, which allows the settlement and colonization of corals and other benthic organisms. In August 2019, another 510 lab-reared *Diadema* will be transferred to two more reefs in Culebra. The restocking of *Diadema* may be an effective tool to mitigate *Ramicrusta* cover, decrease algal cover and overall enhance reef health.

KEEPING INTEREST IN AQUAPONICS ALIVE: A COMPOSITE LOOK AT TRENDS, KEYWORDS AND SENTIMENT ANALYSES IN THE INDUSTRY

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With the continual growth of aquaponics, many entrepreneurs and hobbyists alike are taking on the practice. The wide range of applications and multidisciplinary designs provide mixed results as well as a vast range of successes and failures. In order to better understand the industry, a thorough investigation of the current trends and sentiments is necessitated. Aquaponics could benefit from a stronger and more comprehensive online presence to help bolster its appeal.

The research was a combination of three separate projects combined to make an integrated analysis. First, a sentiment analysis was performed in R software surrounding the keyword "aquaponics" in Twitter. The results were assessed based on frequency, associated keywords and overall positive or negative sentiments. Next, aquaponics was explored in Google Trends to find the relative popularity in the last 15 years. This was coupled with Google Ad's keyword planner feature to assess the volume of searches, associated keywords, forecasts and historical metrics. Finally, ScholarPlot^{R} software had scraped Google Scholar to find the frequency of "aquaponics" and its associated keywords in Google Scholar from 1990-present. The results were then compiled to form a comprehensive snapshot of aquaponics' online presence and overall sentiment.

Key findings indicate that there is a strong divide between commercial aquaponics producers and hobbyists. Keywords typically indicate non-technical, basic assistance with a focus on beginner instruments. There were very few references to specific producers and retailers (n=<5) and commercial aquaponics typically was associated with costs and equipment. A focus on outdoor-indoor setups was also emphasized, as well as a concern for size and scalability. The results suggested that cannabis production was a concern for hobbyists. Hydroponics was associated with aquaponics in a comparative light, although the associated keywords yielded a neutral sentiment. Although scholarly articles continue to increase, web interest peaked in 2013. The data suggests smaller growth in aquaponics than what is necessitated to continue to accelerate the industry. Continued outreach and education are necessary for creating a personalized identity for aquaponics, and not rendering it as a hydroponics alternative. The successes and failures of commercial operations yield concern for sentiments and investor confidence. Aquaponics can also benefit from increased communication between hobbyists and commercial operators, alongside a more streamlined approach to tailoring expectations of the disciplines.

PARTNERSHIPS IN RESEARCH AT HARBOR BRANCH OCEANOGRAPHIC INSTITUTE

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Since its inception in the 1970s as Harbor Branch Oceanographic Institution, Inc.'s (HBOI) Aquaculture Division, partnerships in research have played an important role in advancing research and development for the aquaculture industry. During this early period through 2007 when HBOI was a nonprofit research institution many private partners were hosted for active research and commercial enterprise in its 60+ acre Aquaculture Development Park. Some activities that took place in the Park included HBOI assisting with development of a new aquaculture industry in Florida by retraining fishers to be clam farmers after the net ban took effect. HBOI also started for profit subsidiaries in marine ornamentals, clams and shrimp. HBOI also developed and taught aquaculture industry workshops and started an aquaculture degree with a local state college. In 2007 HBOI merged with Florida Atlantic University (FAU) to become FAU's Harbor Branch Oceanographic Institute (FAU-HBOI) or Harbor Branch for short. The Aquaculture Development Park, now 30 acres, is still a vibrant and active research entity which hosts what are now called Public Private Partnerships (3Ps). The flavor of these partnerships at has changed from what it was prior to 2007. In the past HBOI hosted commercial ventures and activities to assist in development of the aquaculture industry. Whereas today Harbor Branch requires a strong research component to any 3P that is established; still with the goal to advance the aquaculture industry. How these current 3Ps are structured and examples of 3Ps that are ongoing will be discussed.

CULTURE OF THE HAWAIIAN OYSTER, *Dendrostrea sandvichensis* (G.B. SOWERBY 11, 1871), AND RESULTS FROM TRIALS FOR WATER QUALITY IMPROVEMENT

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Dendostrea sandvichensis (G. B. Sowerby II, 1871) is a small, flat oyster species with an Indo-Pacific distribution which includes Hawai`i. It is one of four native true oyster species documented as extant in Hawai`i. The Pacific Aquaculture and Coastal Resources Center (PACRC) at the University of Hawai`i Hilo has been developing culture methods for this species since 2010. Over the last two years, the Waiwai Ola Hawaiian Islands Waterkeepers, has partnered with the Pacific Aquaculture and Coastal Resources Center (PACRC) at the University of Hawaii Hilo and other partners to test the use of this species in Hawai‘i to restore native oysters and to improve water quality and clarity. Although the benefits of using suspension-feeding bivalves for water quality improvement and public engagement are well known, unique challenges have been encountered in use of *D. sandvichensis*.

Hilo Bay was the first site where aquacultured specimens of this species were first deployed in 2013, followed by tests in the traditional Hawaiian fishponds, Hale O Lono (Hilo, Hawai`i) and He`eia (Kāne`ohe). Recently a concerted effort has been made to deployed and test the growth rates and survival of *D. sandvichensis* in seven sites on O`ahu including two in Pearl Harbor conjunction with the U.S. Navy, Kāneohe Marine Corps Base, Sand Island, the Waikalua fishpond and two marinas at the mouth of the Ala Wai Canal. The latter is one of the most contaminated waterbodies in Hawai`i. We will present data on the growth rate, survival and biofouling issues at these sites.

In conjunction with the native oyster restoration project, Waterkeepers engage youth in watershed education about stormwater, wastewater, water quality, and fishing safety.

ASSESSING OFFSHORE AQUACULTURE GEAR AND POTENTIAL PROTECTED SPECIES INTERACTIONS IN SOUTHERN CALIFORNIA: A COLLABORATIVE WORKSHOP

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In recent years, a marked increase in whale entanglements in primarily Dungeness crab and California spiny lobster fishing gear has been documented along the West Coast of the United States, including California. This has heightened concerns from marine resource managers/regulatory agencies and other interested parties regarding any activities that put lines in the water, including offshore aquaculture activities.

At the same time, marine offshore aquaculture is positioned to expand in state and federal waters. Recognizing the concerns regarding potential interactions between offshore aquaculture gear and various protected marine species, particularly whales, NOAA Fisheries' West Coast Regional Aquaculture Coordinator in California convened a workshop to discuss and educate participants about the various types of offshore aquaculture gear and how it functions, how farms are managed and operated to avoid interactions, and also discuss and educate participants about the various whale species in the Southern California Bight and what we know and don't know about their behaviors, relative to lines in the water.

The workshop purpose, in addition to these educational sections, was to explore how to, in a regulatory context, address assessing potential risk of interactions with offshore aquaculture gears. Participants from many state and federal regulatory agencies were represented, as well as scientists, industry representatives, marine engineers, and stakeholders. Various tools for siting offshore aquaculture farms were also presented, as well as an introduction to species-specific 3-D models in development that can then be run through offshore aquaculture farm simulators to further inform understanding of potential whale behaviors if they encounter an offshore farm.

A number of next steps were outlined as outcomes of the workshop, one of which is convening a Southern California Offshore Aquaculture Working Group to continue these discussions and identify viable solutions in this developing industry.

ECO-MIMICRY: THE ECOLOGY BEHIND THE SUCCESS OF HAWAIIAN AQUACULTURE SYSTEMS

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For centuries Hawaiian aquaculture systems (*loko i`a*) have contributed to sustainably supporting a population level similar to the size that exists in Hawai`i today. As Hawai`i attempts to regain a state of sustainable resource abundance (*`āina momona*) and to be a scalable model of sustainability for the world, science can translate between indigenous worldviews and global worldviews to convey the underpinnings of the productivity in Hawaiian aquaculture systems. We will explore various forms of eco-mimicry utilized in the design and implementation of these systems—such as habitat engineering, trophic engineering, and trophic management—and how these concepts can be applied globally to address the needs of an ever-increasing human population. We will also discuss the role that community-based aquaculture systems play in building local-scale economies.

SYNERGISTIC EFFECTS OF DIETARY *Enterococcus faecium* AND FRUCTO-OLIGOSACCHARIDE ON GROWTH IN JUVENILE NILE TILAPIA *Oreochromis niloticus*

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An 4-week feeding trial was conducted to evaluate the effects of dietary supplementation of *enterococcus faecium* as a probiotic and fructo-oligosaccharide (FOS) as a prebiotic in juvenile Nile tilapia, *Oreochromis niloticus*. Fish averaging 8.09 ± 0.21g (mean±SD) were randomly distributed into seven groups of rectangular 45 L tanks (20 fish/tank) with three replicates, and each group of fish were fed by one of the seven experimental diets: A basal diet without probiotic or prebiotic supplementation was used as control (CON), and six other diets were formulated by adding 10^6 CFU/g *E. faecium* (EF_6), 10^7 CFU/g *E. faecium* (EF_7), 10^8 CFU/g *E. faecium* (EF_8), 10^6 CFU/g *E. faecium* + FOS (EF_6F), 10^7 CFU/g *E. faecium* + FOS (EF_7F) and 10^8 CFU/g *E. faecium* + FOS (EF_8F) to the CON diet. Growth performance and other parameters would be measured after 4 weeks of feeding trial, and the results will be presented.

INDUCED STERILITY TO BLOCK SALMON MATURATION

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Many farmed fish attain sexual maturity before reaching market size. Sexual and early maturation is associated with a substantial decrease in somatic growth due to the diversion of energy into development of massive gonads. The period of intensive gonadal growth also results in deterioration of flesh quality and an increase in susceptibility to stress and disease, all of which can have significant economic, biological and business consequences for aquaculture. In the past, the prevalence of grilsing in farmed Atlantic salmon was estimated at 20–30%. Additionally, precocious male maturation has been reported as high as 80% by harvest time in land-based, closed containment grow-out systems. As land-based aquaculture is becoming an increasingly important and popular approach to meet the demand in aquatic food production, especially for high value species, it is imperative that highly effective methods are developed to prevent and/or block sexual maturation. Triploidy often results in reproductively sterile salmon but because of associated performance issues, it is not always accepted by industry. We have developed a practicable technology to efficiently produce infertile fish. Sterilization minimizes energy input toward gonadal growth while enhancing muscle (flesh) development and promoting fish health. Our technology uses a bath-immersion to execute a transient gene-silencing method that doesn't introduce any genetic modification into fish. It will thus alleviate the concerns associated with Atlantic salmon early maturation in land-based farming. Of particular interest, we discovered that a molecular transporter, Vivo, can effectively carry the Morpholino oligomer (MO) across the chorion, enter the embryos and reach the target cells. Vivo-conjugated MO against Deadend (dnd; an essential gene for early germ cell development) effectively disrupted germ cell development and resulted in the production of reproductively sterile fish. The technology was first developed in zebrafish and, very promisingly, has also been shown to induce sterility in tilapia, sablefish, rainbow trout and Atlantic salmon. Currently, we are optimizing this sterilization technology in Atlantic salmon and studying its impacts on growth-related performance traits by comparing growth, performance and fillet yield among sterilized, normal fertile diploid fish, and triploid Atlantic salmon.

EFFECTS OF ENVIRONMENTAL SALINITY ON THE STRESS RESPONSE IN MOZAMBIQUE TILAPIA *Oreachromis mossambicus*

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Abrupt environmental challenges, such as a change in salinity, are often associated with the induction of stress responses in fish. These responses are typically mediated by the endocrine system to rapidly enable the mobilization of energy stores that are used to cope with the stressor. Plasma glucose, therefore, is often used as an indicator of a stress response. In the case of salinity acclimation however, the stress response may vary according to acclimation history. While most studies on the stress response in fish have focused on one-way transfers between fresh water (FW) to seawater (SW) or vice versa, little is known about the stress response in species which may be continuously exposed to dynamically changing salinities.

We have investigated the physiological responses of Mozambique tilapia acclimated to both steady-state and dynamically changing salinity regimes. We replicated a tidal estuarine environment by rearing male tilapia in salinities that changed between FW and SW every 6 hours. Glucose concentration was compared between fish reared in FW, SW, those transferred from FW to SW, SW to FW, or either of the salinities to a tidal regimen. The fish transferred to a tidal regimen were sampled at either the FW phase (TF) or the SW phase (TS) of the tidal cycle.

The transfer of fish from SW to FW induced an increase in plasma glucose by days 3 and 7. By contrast, transfer of fish from FW to SW did not elicit a change in plasma glucose. A transient increase in plasma glucose was observed in TF fish transferred from either FW or SW by 3 days. These results indicate that the transfer from SW to FW elicits the highest plasma glucose levels compared with other salinity transfers. These findings suggest that fish exposed to dynamically changing salinities may not be as susceptible to stress as those transferred between steady-state salinities. Further analyses of markers of stress, shall further inform on the stress effects in fish exposed to dynamically changing salinities. [Supported by HATCH (#HAW02051-H), NOAA/ UH-Sea Grant (#NA14OAR4170071, R/SS-12), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01) and NSF (IOS-1755016)]

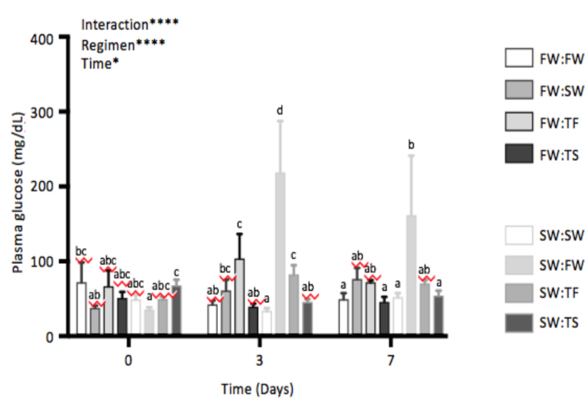

FIGURE 1. Plasma glucose levels of steady state male fish, FW to SW transfers and vice versa, and FW or SW transfers to TR, sampled at TF and TS.

MODELLING THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE PHYSIOLOGICAL-BASED HABITAT SUITABILITY OF THE SEA CUCUMBER *Apostichopus japonicus* IN NORTHEN YELLOW SEA

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Environment change is currently one of the main driving forces behind changes in species distributions. We coupled a biogeochemical sub-model, which simulates trophic resources, and an ecophysiological sub-model, which simulates growth, using mechanistic bases. The effects of environmental conditions on physiological-based habitat suitability was then examined for commercially important species in the northern Yellow Sea, sea cucumber, Apostichopus japonicus. Combining ecophysiological model with the temperature and food conditions estimated by an ecosystem model, allowed spatial differences in habitat suitability to be estimated with contrasting temperature and food conditions.

NATIONAL AQUACULTURE EXTENSION CONFERENCE PLANNING AND DEVELOPMENT FOR THE 7TH NAEC

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National Aquaculture Extension Conferences (NAECs) are interregional projects funded by the USDA-supported Regional Aquaculture Centers (USDA RACs). Conferences have been held in Little Rock, AR (1992), Annapolis, MD (1997), Tucson AR (2003), Cincinnati OH (2007), Memphis, TN (2011) and Boise, ID (2017). Both NOAA Sea Grant and the USDA RACs provided funding for the 2017 NAEC.

Held every four to six years, NAECs provide the only dedicated national aquaculture Extension event that allows participants to present programs and trainings, tour aquaculture operations, exchange information and network. Conference planning occurs at least two years in advance in order to secure a venue in the desired timeframe. Efforts are made to locate conferences in different regions of the country where commercial aquaculture is present and to reflect suggestions made in previous NAEC participant surveys.

An experienced person(s) in aquaculture education, Extension and outreach acts as the local program host and their employer as the lead institution which processes the grant funds. The planning process involves: finding a volunteer host, securing a conference location, venue and dates, developing a project proposal and venue contract, planning the program and tours, and developing the conference survey and summarizing the results. National Aquaculture Extension Steering Committee and National Conference Planning Committee members assist and advise the conference co-chairs in the various aspects of the planning process. Current efforts are underway to secure a conference location for the 7th NAEC in Portland, Maine during June 2021.

TEXT MINING OF BEST FISHING TRAITS IN ARKANSAS

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The rapid growth of the Internet has created a vast amount of unstructured text data since the birth of the Internet in 2003. Text mining, also known as text analysis, is the process to apply a set of algorithms to transfer qualitative data to quantitative data and to find patterns and discover associations hidden in the unstructured text. It is now becoming increasingly popular in many application areas including text mining of hotel customer comments, interpreting students' comments in teacher evaluations, understanding product reviews, and so on. However, text mining has not yet found many applications in Aquaculture and Fisheries.

Web based reviews of best fishing traits in Arkansas from 2008 to 2019 were collected. Sentiment analysis was applied. Sentiment analysis is the computational technique finding and classifying opinions or comments expressed in a piece of text in order to evaluate the writer's attitude towards a topic, product, etc., as positive, negative, or neutral.

GENERATION OF KNOCK-IN TRANSGENIC CHANNEL CATFISH *Ictalurus punctatus* CARRYING ELONGASE GENE FROM MASU SALMON *Oncorhynchus masou* AND DESATURASE GENES FROM RABBITFISH *Siganus canaliculatus* USING CRISPR/CAS9, TOL2 TRANSPOSON SYSTEM AND DOUBLE ELECTROPORATION APPROACHES

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Channel catfish (*Ictalurus punctatus*) is a very popular freshwater food fish consumed in the USA, but unlike marine fish, it contains low levels of n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA;20:5n-3) and docosahexaenoic acid(DHA;22:6n-3). EPA and DHA have important beneficial effects on human health, such as prevention of cardiovascular disease. To modify the HUFA metabolic pathway to produce transgenic channel catfish lines with high levels of n-3 HUFA, one elongase transgene (Elovl2 isolated from masu salmon) and two desaturase transgenes (Δ4 and Δ5/6 desaturase genes isolated from Rabbitfish) driven by the carp β-action promoter were introduced into channel catfish embryos using CRISPR/Cas9, Tol2 or double electroporation approaches. Three sgRNAs for Elovl2, Δ4 and Δ5/6 donor plasmids were designed to target at chromosome 1, chromosome 2 or chromosome 7 non-coding regions using the CRISPR/Cas9 system. CRISPR/Cas9 and Tol2 approaches were utilized with microinjection and single electroporation delivery methods. Double electroporation delivered donor plasmids to both sperm and fertilized eggs without sgRNA.

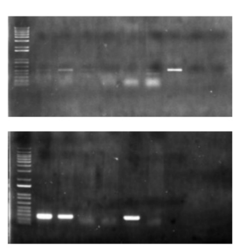
A total of 20.8% of microinjected fish using the CRISPR/Cas9 system were positive, double electroporation had a 9.3% integration rate, and there were no positive fish using Tol2 transposon system. Seven fish carrying masu salmon Elovl2 gene and 1 positive fish carrying rabbitfish Δ5/6 gene were identified by PCR and sequencing. CRISPR/Cas9 with microinjection method was more efficient than double electroporation and Tol2. The masu salmon Elovl2 gene was more amenable to gene transfer than the rabbitfish desaturase genes. These fish will play a very important role in building high producing n-3 HUFA channel catfish lines in the future.

A M 1 2 3 4 5 6 C WT H_20 n-3 HUFA channel

B M 7F 7B 8F 8B C WT H_20

Figure 1. PCR analysis of different transgenes integration. A: Masu salmon Elovl2 gene. B: Rabbitfish Δ5/6 gene. M: marker; WT: wild type; C: positive control; H_2O: negative control

Donor plasmids	CRISPR/Cas9		Tol2		Double electroporation
	Microinjection	Single electroporation	Microinjection	Single electroporation	
Masu salmon_Elovl2	2/3 (66.7%)	0/6 (0)	-	-	0/1 (0)
Rabbitfish_ Δ4	0/3 (0)	-	0/2 (0)	0/3 (0)	-
Rabbitfish_ Δ5/6	0/4 (0)	0/14 (0)	-	0/4 (0)	0/21 (0)
3 donors_mixed	3/14 (21.4%)	0/4 (0)	0/2 (0)	0/8 (0)	3/10 (30.0%)
Total	5/24 (20.8%)	0/24 (0)	0/4 (0)	0/15 (0)	3/32 (9.3%)



POTENTIAL APPLICATION OF DIETARY SUPPLEMENTATION OF VITAMIN C IN ENHANCING GROWTH AND MITIGATING IRON TOXICITY IN CATFISH

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Catfish is the leading and most successful aquaculture industry in the U.S., with channel catfish (*Ictalurus punctatus*) being the predominant species. The success of catfish aquaculture is highly dependent on the superior feed as well as the water quality of the production systems. In most parts of the U.S. the main source of water for fish culture is deep underground water which is high in iron content. However, when certain threshold levels of iron are exceeded in the water, it can exert toxic effects on fish. Nevertheless, for the profitable catfish aquaculture, it is important to find a solution that can have a dual benefit of -promoting growth as well as mitigating the toxic effect of waterborne iron.

Therefore, this work investigates the potential application of dietary supplementation of vitamin C in the diet of catfish. A total of 360 catfish juveniles (average weight: 42.72 ±0.42g) were distributed into 6 groups with three replicates (20 fish per tank). Fish fed three isonitrogenous (35% crude protein) and isolipidic (8% lipid) diets, and two different levels 143 ppm and 573 ppm of vit. C for 8 weeks. All groups were also exposed to elevated iron (9.5 mg/L Fe^{3+}, representing 25% of the determined 96 h-LC_{50} value). As such there were six groups viz (i) Control (Con; fed with commercial diet), (ii) diet supplemented with low vit. C (143 ppm; LVC), (iii) with high vit. C (573 ppm; HVC), (iv) Control exposed with elevated iron (Con+Fe), (v) LVC exposed with elevated iron (LVC+Fe), and (vi) HVC exposed with elevated iron (HVC+Fe). At the end of the feeding trial, production performance (weight gain %) of LVC+Fe group were significantly reduced compared to the control (Fig. 1), suggesting growth inhibiting effect of iron. Interestingly, fish fed with vit. C (LVC and HVC) had significantly higher weight gain (%) relative to control, signifying growth promoting effect of vit. C. Moreover, superior growth performance of LVC+Fe and HVC+Fe groups in comparison to Con+Fe (Fig. 1), indicate potentiality of vit. c in mitigating toxic effect of iron. Beside growth related parameters, blood chemistry (hemoglobin content, hematocrit), iron burden in the plasma and liver, oxidative status as well as histopathology examination of liver and gills were conducted to validate the potential benefit of dietary vit. C. Conclusively, it can be stated that vit C supplementation can be an effective approach for boosting growth as well as alleviating iron toxicity in catfish.

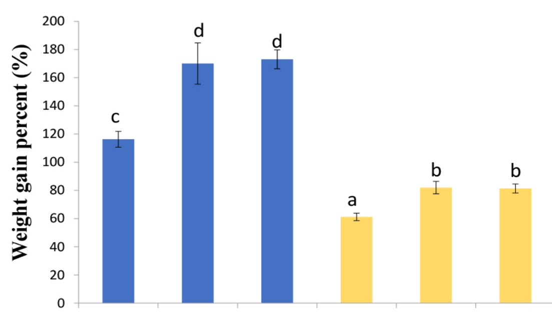

Figure 1. Weight gain (%) under the testing conditions.

DIETARY BENTONITE (A NATURAL AND LOW-COST CLAY) SUPPLEMENTATION MITIGATE THE IRON TOXICITY IN RAINBOW TROUT

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Aquaculture is the fastest growing food-producing sector and contributes over half of the total food fish consumed globally. United States is the eighth-largest producer of rainbow trout (Oncorhynchus mykiss) worldwide, and Idaho is the top producer among all states. However, in Idaho the water source for fish culture normally acquired from ground water aquifers drilled in basalt, which contains high level of iron. Although, iron is an important chemical element required by fish for several metabolic processes, elevated level of iron in fish culture units can cause growth retardation and damages to vital organ. Currently, there is no promising therapeutic agent that can efficiently mitigate this water borne issues in trout farming. Hence, finding an effective and safe compound to prevent iron build-up as well as ameliorating their toxicity on fish is a demand in the aquaculture industry. Bentonite, a natural clay with superior adsorption properties, has been suggested as a good chelating agent for different (heavy) metals including iron. Therefore, the current study was designed to evaluate the dietary effect of bentonite clay in rainbow trout following exposure to elevated iron containing diet.

Six experimental diets were formulated to contain 0% bentonite (D1: CWF/CF), 2% bentonite (D2; CLB), 4% bentonite (D3; CHB), 0.25% iron as $FeSO_4$ (D4; CFe), 0.25% $FeSO_4$ +2% bentonite (D5; CFeLB), and 0.25% $FeSO_4$ + 4% bentonite (D6; CFeHB). In this feeding trial D1 diet was fed to 2 groups of fish viz., group 1 without filter (CWF) and group 2 with filter (CF). Experimental system was used as flow through system and natural ground water contains 3 – 5 ppm iron therefore water filer was used for group 2 (CF) to remove the iron from natural ground water.

Additional 6 groups tanks were containing 3-5 ppm iron in the water throughout the feeding trial. Each diet was fed to four replicate groups of rainbow trout (2.3±0.01 g initial weight) stocked with 20 fish per tank. Fish were hand fed to apparent satiation twice daily for eight weeks.

At the end of the feeding trial, the highest weight gain (%, Fig. 1) and specific growth rate was found in fish fed control diet -reared in filter water (CF) but no significant difference was noticed in the feed conversion ratio among the various groups. Interestingly, weight gain (%) in both CFeLB and CFeHB was significantly higher than the CWF and CFe. No significant variation was noticed among the fish fed either low or high bentonite with iron and those fed with bentonite alone. Based on the growth performance indices, it can be concluded that dietary supplementation of bentonite at a dose of 2% can enhance the growth of rainbow trout when challenged with high dietary iron.

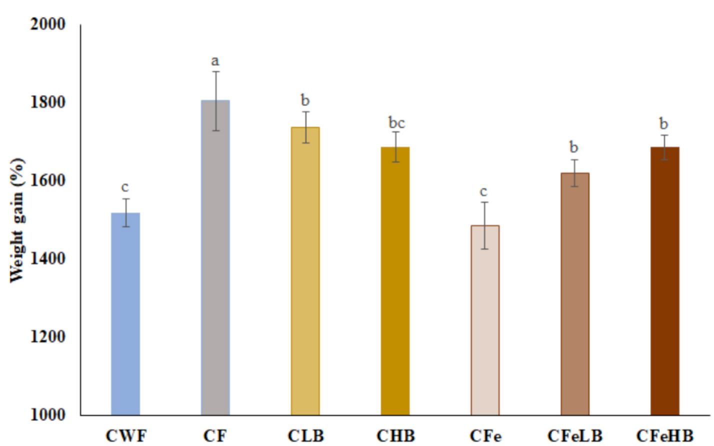

Figure 1: Growth performance (weight gain %) of rainbow trout of different treatments

IN VITRO AND *IN VIVO* EFFECTS OF BUTYRATE, PROPIONATE AND THEIR COMBINATION IN HIGH-PLANT-PROTEIN-DIETS FOR RED DRUM (*Sciaenops ocellatus*)

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Disease outbreaks are recognized as a major bottleneck for aquaculture production and trade. It is estimated that annual losses due to pathogenic diseases can reach deficits of billions of dollars to global aquaculture. Aggravating this scenario, the increased demand for alternative protein ingredients to replace fishmeal in aquafeeds has led to higher inclusion of plant feedstuffs, and these ingredients may contain anti-nutritional factors and secondary metabolites that can impair the functional competency of the gastrointestinal tract (GIT) against pathogen translocation. Organic acids or acidifiers are promising feed additives that can alleviate these issues by inhibiting the proliferation of pathogenic bacteria in the intestine. They are composed of short- and medium-chain fatty acids with bacteriostatic properties resulting from fermentation of complex carbohydrates by autochthonous bacteria. Their relatively small chemical structures are lipophilic and can diffuse across the cell membrane of gram-negative bacteria, and within the bacterial cytosol, the dissociation of these molecules acidify the intracellular environment, ultimately leading to ATP exhaustion of the bacteria which inhibits cellular growth and induces death.

The objective of this study was to investigate *in vitro* and *in vivo* the supplementation of two organic acids individually as well as in combination in a high-plant-protein diet with the carnivorous red drum. A basal diet was formulated with soybean products to provide 75% of the total crude protein, and supplemented with either 0 or 0.5 g kg^{-1} of butyrate, propionate, or their combination. In the *in vitro* experiment, digesta was aseptically collected from 16 juvenile fish and incubated under anaerobic conditions with an incomplete anaerobic media supplemented with the experimental diets. After 24 h incubation at 27°C, the samples were frozen and further processed for DNA extraction and the microbial communities were compared among treatments by denaturing gradient gel electrophoresis (DGGE). Results from this trial suggested that the supplementation of the organic acids mildly affected the bacterial community, having a 90% similarity with that of the basal diet. A 60-day feeding trial also was conducted to evaluate if the experimental diets would affect production parameters and intestinal microbial community. Performance of the fish was analyzed as a mixed model, having a 2×2 factorial design (absence or presence of butyrate or propionate as main factors) and the disposition of the aquaria serving as a covariant. Digesta contents were collected at day 30 and 60 of feeding to compare the microbial communities within treatments by DGGE. The supplementation of propionate to the diet slightly but significantly (P=0.02) impaired the growth performance of red drum, and feed efficiency was also slightly but significantly (P=0.01) impaired by the addition of butyrate. The DGGE results showed that the bacterial microbiome of the gut was significantly affected by the supplementation of the organic acids individually and by their combination, when compared to fish fed the basal diet at the two different collection points. Supplementing organic acids in high-plant-protein ingredients did not benefit the growth performance of red drum. Further investigation of the immunological parameters of fish plasma and results for the intestinal DNA for Next Generation Sequencing are pending.

CNPQ: 207141/2014-2

BIOTELEMETRY STUDIES ON POST-RELEASE BEHAVIORS OF CULTURED AND WILD JAPANESE EELS (*Anguilla japonica*) IN SHALLOW BRACKISH WATERS IN JAPAN

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Japanese eel (*Anguilla japonica*) populations have been rapidly decreasing due to overfishing and habitat loss since the 1970s in Japan. Understanding the detailed ecological aspects of eels is essential to establish effective conservation measures. To augment depleted stocks, cultured eels have been widely released by fisheries cooperatives. However, studies on the ecology and behavior of cultured eels after release are limited, and the effectiveness of stocking remains unclear. The aim of this study is to examine behavioral characteristics of wild and cultured eels released in the wild to consider appropriate management policies for eel populations in Japan.

Studies were conducted in Matsukawa-ura lagoon in Fukushima (northern Japan) and the Tonda River in Wakayama (central Japan) using ultrasonic telemetry, which estimates the position of each eel by ultrasonic signals. Twenty-eight and 13 receivers were set in the Matsukawa-ura and Tonda River, respectively. Twenty wild and 12 cultured eels were implanted with acoustic transmitters and released in Matsukawa-ura in September 2016, and 20 wild and five cultured eels were similarly outfitted and released in the Tonda River in June 2018. Temporal and spatial changes of received signal data of each eel were analyzed.

Some eels migrated to the sea, and some moved between the lagoon/ river and the sea several times after release. Migration from the Matsukawa-ura lagoon to the open sea occurred at night during ebb tide in autumn (October to November; Table 1). Out of the eight eels that migrated to the sea, seven individuals were cultured. However, it is unlikely that the seven cultured eels (2 years old) were mature enough for a spawning migration. This migration behavior is thought to be abnormal for cultured eels that were raised under constant high temperature. In the Tonda River, three cultured and one wild eel migrated to the open sea in September and October. Seasonal patterns of behavioral activity were found: high in summer and autumn, and low in winter for both eel groups. Both eel groups had nocturnal feeding activity; in particular wild eels were most active around dusk. Diel rhythms of cultured eels were less pronounced compared to wild ones. The results of the present study indicate that the behavior of cultured eels differs from that of wild eels and that this difference most likely is caused by the artificial rearing conditions implemented in the aquaculture ponds 0.5 - 2 years before release. Although some cultured eels migrated to the sea, it is difficult to assess the contribution of these eels to overall reproduction in the wild. Previous studies indicated that the release of cultured eels may negatively impact ecosystems. Therefore, the release of cultured eels may disrupt the ecosystem; and unknown effects on reproduction should be reduced as much as possible. Instead, establishing rules to prevent overfishing of glass eels and restoring habitats in rivers and coastal areas to foster the establishment of wild yellow eels as future spawning stock are important.

Table 1. Data on eight eels released on 19 Sept. 2016 that migrated to the open sea from the Matsukawa-ura lagoon.

Eel	Origin	TL (mm)	BW (g)	Last day detected	Last time detected	Tide	WT (°C)
1	Wild	571	305	10-Nov	18:30	ebb	12.4
2	Cultured	578	359	11-Nov	1:44	ebb	14.3
3	Cultured	567	325	29-Oct	22:18	flood	15.8
4	Cultured	587	358	12-Nov	19:05	ebb	12.3
5	Cultured	564	324	15-Nov	18:05	ebb	15.3
6	Cultured	564	345	25-Nov	18:13	ebb	11.3
7	Cultured	548	295	16-Nov	19:44	ebb	13.6
8	Cultured	564	356	10-Nov	1:38	ebb	12.1

DNA VACCINE TARGETING TO MATURE MYOSTATIN INCREASE GROWTH PERFORMANCES IN ORANGE-SPOTTED GROUPER *Epinephelus coioides*

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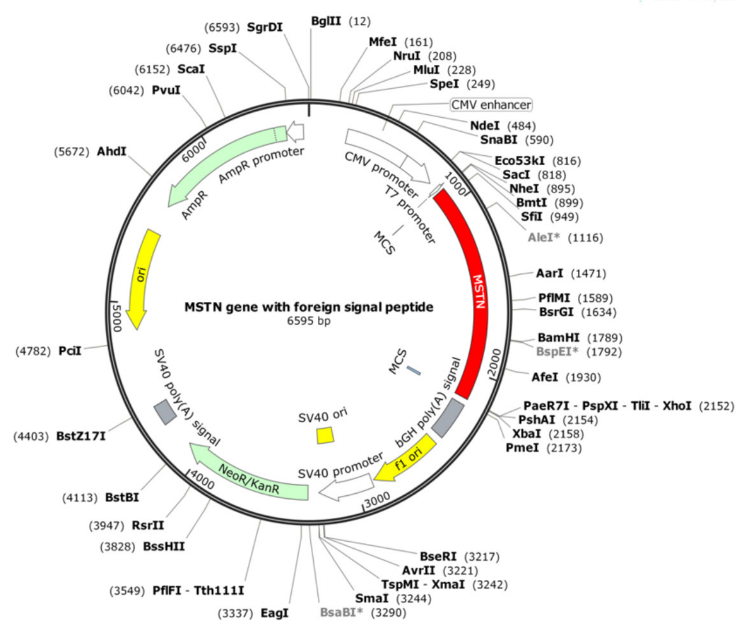
Myostatin (MSTN), also known as the growth differentiation factor-8 (GDF-8) is a member of the TGF-β superfamily, negatively regulates skeletal muscle growth. Depression of myostatin function leads to dramatic growth of muscle mass in mice, human and several livestock animals. The role of myostatin function in fish skeletal muscle growth was not consistent as there are several MSTN orthologs and its expression is not restricted to muscle. We isolated and identified myostatin DNA sequence from orange-spotted grouper *Epinephelus coioides* by using RACE technique. The myostatin cDNA of *E. coioides* consisted of 3782 nucleotides with a 99 bp 5'UTR, 1378 bp 3'UTR and an open reading frame (ORF) of 1131 bp, encoding for a protein of 376 amino acid residues. Myostatin mRNA are widely expressed in various tissues with the highest expression in the skeletal muscle. In an attempt to down-regulate myostatin, we constructed a DNA vaccine against myostatin (named PCS-MSTN) by cloning the hepatitis B surface antigen (HBsAg) S gene to *E. coioides* mature myostatin cDNA sequence in pcDNA3.1 vector. The recombinant vector PCS-MSTN was used to immunize *E. coioides* with body length 10cm and weight at approximate 25g over 3 times with interval of 2 weeks of vaccination. After 1 months of final vaccination, sera from vaccinated grouper showed the presence of specific antibodies against myostatin. In addition, the body weight of fish treated with 200 ng DNA vector was increased significantly. Myogenin mRNA expressions in skeletal muscle and brain tissue were increased in comparison with the controls. These preliminary results provide initial evidences that depression of myostatin function via DNA vaccine may promote *E.coioides* grouper growth performances.



TRADE DYNAMICS AND DURATION OF CHINESE FOOD IMPORTS

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Using a unique and rich dataset that provides information on China's food imports at the transaction level, this paper explores the determinants of the survival of trade relationships. The firm-product-country trade relationships are very dynamic, with a median of one year (Table 1). We examine the effect of processing trade, which performs only intermediate stages of production by processing imported inputs for re-exporting, on the trade duration. Processing trade is an important trade regime in many developing countries which facilitates trade by providing tariff reductions or exemptions. It is particularly important for the seafood sector, where about two-thirds of imported seafood in China are used for further processing and re-exporting.

Our findings show that ordinary imports in China are less at risk of ceasing a relationship compared with the same food products imported under the processing trade regime. An increase in tariffs will increase the hazard rate for ordinary imports significantly. We also find that prior importing experience with similar countries in terms of geographical, cultural, and economic proximity, along with experience of related products, reduces the hazard rate of the import duration. We use seafood and oilseeds as an example to account for the potentially different patterns across product groups. In the seafood sector, the effect of trade regimes is less significant, and durations are found to be less likely to be affected by a tariff change. Moreover, the advantage associated with foreign ownership, which is found in food products in general, does not exist in the seafood sector.

Table 1. Distribution of active spells across China's food imports relationships, 2008-2014

Spells across Relationships			Observed Spell length		
Total number of spells	**Number of relationships**	**Frequency**	**Spell length (years)**	**Number of spells**	**Frequency**
1	209,440	93.23%	1	167,974	69.81%
2	14,421	6.42%	2	41,423	17.22%
3	770	0.34%	3	16,137	6.71%
4	13	0.01%	4	7,416	3.08%
			5	4,047	1.68%
			6	2,315	0.96%
			7	1,306	0.54%
Total	226,304		Total	240,618	

HOW DO SEAFOOD EXPORTERS RESPOND TO THE BORDER REJECTIONS?

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Despite the increasing trend of food exports from China to the United States due to the robust demand, reduced tariff and eased restrictions on cross-border investment, there have been an increased use of non-tariff measures (NTMs) which makes access to the US market remains challenging. Seafood is the product group with the most shipments refused over 2007-2017. It accounts for 20.8 percent among all shipments of food products from China that were rejected at the US border. Using a transaction-level data for Chinese exports and US data on import refusals, we explore how heterogeneous firms respond to the border rejections in terms of entry, exit, survival, and trade deflection to other markets.

The results indicate that US import refusals have a small negative effect on the extensive margins, but a relatively significant impact on the intensive trade margins. Exporters of food tend to export less to the US if the product they export has been rejected in the last year. Our research shows this adverse effect also spills to related products and firms. At the same time, exporters are less likely to ship goods to alternative markets once those products have been refused entry in the US market, which implies a progressively global harmonization of food safety regulations. However, import refusals still yield significant trade deflection through reputational spillover effects. If firm-product pairs got rejected by the FDA, other firms selling the same products are more likely to export those products to countries other than the US.

EFFECTS OF A NOVEL BENZENESULFONAMIDE ON ANTIOXIDANT ENZYMES AND HEMATOLOGICAL PARAMETERS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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In this study, a mixture of a suitable chalcone and *p*-hydrazinobenzenesulfonamide hydrochloride in 1:1.1 mol ratio in ethanol (25 ml) in the presence of glacial acetic acid (0.05 ml) was refluxed for synthesis of 4-(3-(4-Bromophenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide**,** B4, for 7h at first experiment.

Effects of B4 on antioxidant enzymes of gill and liver, and changes in hematological parameters were determined in rainbow trout. For this purpose, rainbow trout alevins were exposed to 0, 0.25, 0.5, 1 and 2.5 mg/L of B4 and 1 ml of DMSO for 96 hours. Blood samples were collected for hematological analyzes and mortalities were recorded daily.

At the end of the study, it was determined that exposure to B4 has caused significant changes in antioxidant enzymes of gill and liver of alevins ($p<0,05$). Catalase (CAT) (EU/ mg protein), superoxide dismutase (SOD) (EU/mg protein) and glutathione peroxidase (GPx) (EU/mg protein) activity values reduced, while MDA values were increasing significantly ($p<0.05$) in gill and liver of fish compared to control group. SOD activity was time dependent, since it was reducing at initial and then increasing.

WBC, MCV, MCH and MCHC levels increased, while RBC Hb, Htc and PLT levels were decreasing by increasing the dose from 0.25 to 2.5 mg/L of B4 ($p<0.05$) compared to control. There were no differences between control and DMSO group ($p>0.05$) with respect to all tested properties.

It is suggested that B4 should be investigated further for possible usage in aquaculture industry to produce healthy fish.

HOW TO WORK WITH VETERINARIANS: A NOT-SO-SECRET SECRET TO ENHANCING AQUACULTURE'S BOTTOM LINE

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Although disease is a leading cause of lost revenue, many aquaculture facilities do not have strong working relationships with veterinarians. Several factors continue to hinder formation of these connections, including: a) availability of local veterinarians with interest or expertise in production aquaculture medicine, b) failure of many aquaculture business plans to correctly factor health and disease management costs, c) lack of understanding of how to maximize veterinary collaboration and its difference relative to current often more reactionary health and disease management. Programs to educate veterinarians in aquaculture medicine and to educate producers on the value of veterinary collaboration have increased since the 1980s. These have been provided by state agencies and universities, continuing education at veterinary conferences and fish health meetings, and most recently, a USDA NIFA grant administered through the University of Florida. Various avenues exist to facilitate locating veterinarians. Aquaculture business plans, especially those requiring investment, often inadvertently overestimate production capacity by underestimating disease incidence and costs for management. More accurate plans requires greater transparency and collaboration between economists, production managers, and health professionals. Finally, veterinarians can enhance aquaculture businesses by assisting with development of holistic health and biosecurity plans, disease diagnostics, emerging diseases, health inspections, and interpreting regulations.

SYSTEMATIC ANALYSES OF THE GENETIC RELATION AND ORIGIN OF THE CULTURED LARGEMOUTH BASS POPULATIONS IN THE USA AND CHINA

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Largemouth bass *Micropterus salmoides*, native to the Southeastern United States, has shown extreme economic and ecological significance throughout the North American continent. This species has gained access to a large area of habitats outside its native range, having been introduced to many countries in Asia, Europe, and Africa since the 19th century, and has played a great role in aquaculture, freshwater fisheries, and ecological systems of these regions. Identification of the population origin of an introduced species is a prerequisite for testing hypotheses concerning the factors responsible for its breeding and control.

In the present study, a total of 1,125 individuals from 28 sampling sites were collected. Two sites were located in the Guangdong province, where largemouth were introduced in 1980's for aquaculture in China. The genetic structure of largemouth bass distributed in the United States was evaluated, and the origin of the cultured *M. salmoides* populations in both the United States and China were inferred based on microsatellites data. The results showed that largemouth bass populations collected from native areas were mostly assigned into two genetic clusters, while some populations were admixed to some extent. Most individuals of cultured populations collected from Zhanjiang and Guangzhou, China, as well as cultured populations from Piketon (OH), North MC Fish Hatchery (Mississippi), and Lake Kickapoo (Texas) in the United States probably came from northern strains; while most individuals of populations collected from the Turcotle Fish Hatchery (Mississippi) were probably from southern strains. Interestingly, the cultured populations collected in this study showed relatively pure genetic source, while some natural populations were admixed to some extent.

FOOD, FEEDS, FERTILEERS AND BIOFUELS - OPPORTUNITIES, CHALLENGES AND FUTURE DIRECTIONS OF OPEN WATER SEAWEED AQUACULTURE IN THE USA

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The value of cultivated seaweeds worldwide exceeds $U.S. 11.0 billion with a production of more than 30 million metric tons. The most valued of the maricultured seaweeds is the red alga *Porphyra*/*Pyropia*, or nori. It is a major source of food for humans throughout the world, although it is primarily cultivated in Asia (China, Japan and South Korea). Worldwide production is approximately 2 million metric tons with an annual value of over $U.S. 1.9 billion. In addition to *Porphyra*/*Pyropia*, other edible seaweeds include *Gracilaria*, *Undaria* and *Saccharina* with their collective value exceeding $U.S. 7.3 billion. Farmed seaweeds are also the industrial sources of carrageenans (*Eucheuma* and *Kappaphycus*) and agars (*Gracilaria*). These important polysaccharides are found in food, used in biotechnological and biomedical industries and have a global value more than U.S. $1.2 billion. The increasing demand for safe, healthy, and minimally processed foods is creating new opportunities for seaweed products as functional foods, nutraceuticals, cosmeceuticals and alternative medicinal products that have enabled the growth of seaweed farming industry in US coastal waters. This developing industry is environmentally responsible, provides ecosystem services by removing excess nutrients (carbon and nitrogen) from the ecosystems, improves water quality and potentially reduces ocean acidification. With the nursery and outplanting technologies developed at the University of Connecticut and the University of Alaska for *Saccharina latissima* and other kelp species have been successfully cultivated in open water farms in the Northeast and in the Gulf of Alaska, USA. In addition to kelp cultivation, The University of Connecticut and others also have successfully cultivated *Gracilaria tikvahiae* and *Pyropia*/*Porphyra* in open water farms in the Northeast USA. Seaweed aquaculture now offers other new opportunities with the planned expansion into the Exclusive Economic Zone with the ARPA-e (US DOE) MARINER Program. With improvements in productivity, kelp species, *Eucheuma* spp. and other seaweeds could potentially be a viable feedstock for commodity applications including animal feeds and biofuels. More details about the current technologies and future directions of seaweed aquaculture in the US will be presented.

SHRIMP BROODSTOCK NEEDS OF INDIA: HORSES FOR COURSES

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Indian shrimp aquaculture has been growing at a galloping pace ever since the introduction of vannamei in 2019. India is the only country among the shrimp producing countries which imports all its broodstock required - more than 200,000 animals every year. India is also the only country in the world to take the entire broodstock through a central quarantine wherein the broodsrock is tested for all the OIE listed diseases before getting released to the respective hatcheries.

Of late, the profitability of the farmers is coming down as the emerging diseases are making the operations unviable and WSSV, the good old viral pathogen continues to play havoc every now and then. The conventional SPF stocks don't seem to be giving all that good results as there is an explosion of farming on many creeks limiting the carrying capacity. Implementing foolproof bio-security measures is also very difficult as the farmers are small with average per capita land holding less than 2 HA. What the industry needs at this juncture is tolerant stocks for both WSSV and pathogenic bacteria. We need to look at Ecuador's sustainability over the years and type of the broodstock the industry there is working with.

Apart from bio-security and carrying capacity issues, high temperature in summer months is exacerbating the bacterial problems, contributing to morbidities and mortalities. Indigenous species like monodon and indicus stand a better chance in summer. Once domesticated stocks of these two species are available, they would also help the traditional tide fed farmers -- who are presently getting very low survivals -- to improve their efficiencies. In a country like India it is very difficult to change the farming infrastructure to give better bio-security and enable high stocking densities. It makes sense to change the horses than the courses.

RECLAIMING INDIGENOUS AQUACULTURE THROUGH NATIVE ENVIRONMENTAL SCIENCE

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Indigenous people have deep and sustained connections to place. Knowledge of the environment has been and continues to be, critical in supporting and maintaining resilient and thriving communities. Indigenous aquaculture enhances seafood productivity in localized coastal areas and increases food security. Lummi Indian School of Aquaculture (LISA), founded in 1973, was a single-purpose training program to prepare technicians for employment in Tribal-owned and operated fish and shellfish hatcheries throughout the United States and Canada. LISA provided the foundation for Northwest Indian College (NWIC). Today the Bachelor of Science in Native Environmental Science (BSNES) program at NWIC focuses on curriculum that is place-based, experiential, and culturally grounded. By drawing on the deep and sustained connections to place and commitment to environmental protection, the BSNES program strengthens students understanding and development of a sense of place, relationality, inquiry, and communication skills. Upon successful completion of this program, students will value the relationality and the interrelationships between people and the environment, be able to ground and apply concepts and methodologies to place, demonstrate self-location, and use Indigenous theories and methods to conduct inquiry-based research that promotes Indigenous self-determination and the restoration and revitalization of the environment. Highlighting the BSNES Program shows how Tribal Colleges and Universities play an integral role in deepened the integration of traditional knowledge with modern aquaculture science.

A METAGENOMIC EXPLORATION OF THE FUNCTIONAL POTENTIAL OF GUT MICROBES TOWARD THE NUTRITIVE CAPABILITY OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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The nutritive role and ecology of the gastrointestinal tract (GIT) microbiota in rainbow trout remains uncertain. To improve our understanding of the rainbow trout GIT microbiome, we have performed whole shotgun metagenomic analyses on the luminal content of both the mid- and hind-GIT samples from 90 trout fed one of three dietary treatments, including a conventional fishmeal diet, or diets where fishmeal was replaced with either non-aquatic animal or a plant protein sources. In total, we observed 3,267 unique microbial species that collectively encoded 6,054 unique annotatable gene functions. By sample, 343±130 microbial species were observed in the mid- (377±142) and hind-regions of the GIT (310±111). Good's estimates of coverage indicated we had captured >96% of species and gene functions in all samples. Microbes were predominantly bacteria (74%) and largely of the phyla Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Tenericutes, and Fusobacteria. Eukaryotic (25%), and Archaea (<1%) microbes mostly from the Ascomycota and Euryarchaeota phyla were also present along with bacteriophage (1%) principally of the Caudovirales. Comparisons of species-level classifications and functional profiles revealed GIT location and location X diet relationships (Figure 1). Hind-, but not mid-GIT samples varied with diet. The hind-GIT microbes had a greater number of genes involved in carbohydrate, fatty acid, lipid, and isoprenoid metabolisms. While the mid-GIT microbes were enriched for genes involved in sulfur, potassium, and aromatic compound metabolisms. These results support a nutritive role for trout GIT microbes and indicate a potential division of nutritive function between the mid- and hind-GIT microbiomes. These differences in functional division between the mid- and hind-GIT appear to be modulated by, and in some respects, adaptable to the fish diet.

Plot of constrained canonical correspondence analysis showing the relationship of GIT location (blue, A & C) and diet (red, B & D) to the species-level taxonomic (A & B) and gene-level functional (C & D) profiles. Explanatory values on axes represent percent of total explanatory power (constrained proportion x eigenvalue x 100).

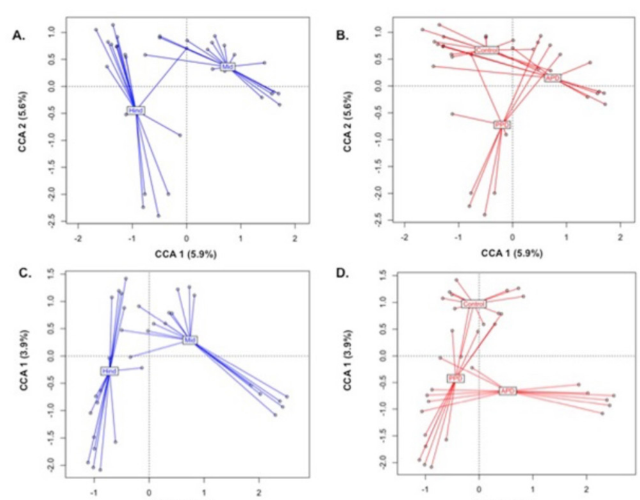

Figure 1. Contributions of GIT Location & Diet to Microbiome

USE OF DIETARY FRASS FROM BLACK SOLDIER FLY LARVAE, *Hermetia illucens,* IN HYBRID TILAPIA (Nile X Mozambique, *Oreochromis niloticus X O. Mozambique*) IMPROVES GROWTH AND BACTERIAL DISEASE RESISTANCE

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Experimental frass, a by-product of the larva meal industry, is derived from the larvae of black solder flies fed Distillers' dried grains with solubles. Frass is heterogeneous and includes larval excrement, exoskeleton sheds and residual feed ingredients along with abundant nutrients, chitin and beneficial microbes. The present study was performed to evaluate the changes in growth, feed utilization, body composition, hematology, serum chemistry, immune responses and disease resistance of hybrid tilapia, Nile x Mozambique (*Oreocromis niloticus x O. mozambique*) fed diets containing frass from black soldier fly larvae, *Hermetia illucens,* fed on dried distiller's grain with solubles (DDGS). Five diets containing frass at levels of 0, 5, 10, 20, and 30% as partial replacements of a combination of soybean meal (SBM), wheat short (WS) and corn meal (CM) on an equal protein basis were fed to juvenile hybrid tilapia (2.6 ± 0.035 g) in quadruplicate aquaria to apparent satiation twice a day for the first two weeks and once daily for rest of the feeding trail. Fish in each aquarium were group-weighed and counted at 2-week intervals. At the end of the feeding trial, blood samples were collected from fish in all groups. Final weight gain was significantly increased in fish fed the diet including the highest level of frass (30%). Feed intake and feed utilization efficiency were not significantly affected by dietary treatments. Fish fed diet with frass (5% to 30%) had higher protein utilization efficiency than the group fed diet without frass (control diet). Survival during the feeding trail was unaffected by dietary levels of frass. Similarly, whole-body composition and hematological parameters were not affected by dietary treatment. There were no significant differences observed in serum biochemistry including albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), calcium (CA), phosphorus (PHOS), glucose (GL), potassium (K), total protein (TP) and globulin (GLOB). Serum complement activity of fish fed 30% dietary frass was significantly higher than that of fish fed other treatments. Even though overall mortality during laboratory challenges was low, fish fed the diets containing frass at levels 10% and higher showed better survival against *F. columnare* challenge than that of control fish. Frass from the larvae of black solder flies fed Distillers' dried grains with solubles has potential for use as feed ingredient for improving growth of hybrid tilapia. Use of frass in the tilapia diet may prove beneficial by improving some innate serum components and the resistance of hybrid tilapia against *F. columnare* infection.

PATHOGEN DETECTION AND MANAGEMENT OF TAIWAN ZEBRAFISH CORE FACILITY AT NHRI

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Zebrafish (*Danio rerio*) has become one of the most popular animal models used in research in recent years. Their observable transparency and highly homologous genes similar to humans make them an ideal model for various research areas, including developmental biology, immunology, toxicology, and cancer studies. Most zebrafish facilities use recirculating water systems containing housing tanks, biological nitrification tanks and filtered recirculating water, so they are not sterile. Several potential effects of pathogens on zebrafish have been demonstrated. In addition to large mortality caused by pathogenic bacteria, infections in seemingly healthy fish can also lead to reduced fertility and confusion in research endpoints such as behavioral, developmental, and disease research. *Pseudoloma neurophilia* (microsporidia), *Mycobacterium marinum* (bacteria) and *Pseudocapillaria tomentosa* (nematode) are three of the most common pathogens found in zebrafish facilities. It is very important to reduce pathogens in the Taiwan Zebrafish Core Facility at NHRI (TZeNH) that provides the research community with a variety of zebrafish strains. In our facility, we utilize appearance observation, pathogen detection and molecular diagnostics to establish a zebrafish health monitoring system.

STRATEGIC AND NOVEL USE OF THE KOOTENAI RIVER NATIVE FISH CONSERVATION AQUACULTURE PROGRAM TO RESTORE WHITE STURGEON AND BURBOT

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The Kootenai River White Sturgeon *Acipenser transmontanus* and Burbot *Lota lota maculosa* were once abundant in the Kootenai/ay River Basin in Idaho and Montana, USA, and British Columbia, Canada. Historically, the native fishes provided important cultural resources for indigenous peoples, and remain so today throughout the basin despite significant population decline. Kootenai White Sturgeon are listed as endangered in both countries due to cumulative effects of habitat destruction and of Libby Dam hydro-power operations in Montana that have resulted in persistent recruitment failure since the 1970's. Due to similar causes, Burbot were functionally extirpated by the 1990's. In 1988, the Kootenai Tribe of Idaho recognized the lack of White Sturgeon natural recruitment and started an experimental aquaculture facility to determine the feasibility of using wild broodstock to artificially spawn and rear year classes to reverse population decline. The Kootenai River Native Fish Conservation Aquaculture Program (KRNFCAP) began rearing fish during 1990-1992, and has been successfully releasing annual year classes since 1997. Following the Sturgeon program, a formal Burbot program feasibility evaluation was initiated in 2003. Since its inception, the conservation aquaculture program has boosted the Burbot abundance estimates from 50 adults in 2002 up to 50,000 adults. The KRNFCAP is part of multi-agency and stakeholder collaboration, and is one component of the greater Kootenai Ecosystem Restoration.

The KRNFCAP is guided by an annual adaptive management framework. As part of a large collaboration, the conservation hatcheries are used to 1) avoid extirpation and rebuild the species abundance to jump-start natural recruitment, and support culture and recreational harvest; 2) spawn, rear, and release early life stages across habitat types/conditions to determine causes of recruitment failure; and 3) spawn, rear, and release fish in a manner that supports post-release monitoring, research, and evaluations. The presentation will provide a summary of current aquaculture practices that have succeeded in simultaneously rebuilding fish abundance while supporting post-release monitoring and evaluation that guides adaptive management of the program.

UNDERSTANDING THE IMPORTANCE OF SUBSTRATE STABILITY FOR CORAL RESTORATION AND THE PROPOSAL OF 3D-PRINTED REEF TILES FOR INCREASING RESTORATION SUCCESS

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Some have suggested that the sub-tropics will become critical habitat for coral reef development under future climate change scenarios. Currently home to marginal reefs, the subtropics are characterized by limited light penetration, extreme temperature fluctuation, salinities, or nutrient levels, conditions further exacerbated by urbanization. Nutrients accelerate bio-erosion by grazers, microborers, and macroborers which abrade corals and threaten their structural integrity. In response to a large-scale severe partial mortality event accelerated by the subsequent bioerosion from grazing by *Diadema setosum*, a restoration plan was implemented to rescue the *Platygyra* populations in Hoi Ha Wan Marine Park (HHWMP) in Hong Kong in 2016. A restoration study was initiated to make use of the healthy living tissues from thirty bioeroded *Platygyra* colonies from which they selected and reared on the nursery platform on the seabed for about a year and transplanted at three sites within Hoi Ha Wan Marine Park. We found that the survivorship of the transplanted fragments was significantly higher at sites with higher substrate stability (i.e. lower percentage cover of rubble). The results suggested that substrate stability needs to be improved prior to transplantation to secure downstream restoration success. As a pilot project on experimental scale, we propose to design and deploy 3D-printed reef tiles to stabilize substratum and create tailor-made substrate to enhance coral restoration success.

INSIGHTS INTO USE OF NON-ABLATED SHRIMP (*Litopenaeus vannamei*) IN COMMERCIAL SCALE HATCHERIES

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The Pacific white shrimp (*Litopenaeus vannamei*), is currently the most cultured marine shrimp worldwide with 75% of global shrimp production in 2016 and it represent an important marine food source for the consumers. Induction of rapid egg production in captive of this shrimp is usually induced by unilateral eyestalk ablation which involves the removal or constriction of one eyestalk through cutting, cauterizing or tying to reduce the level of hormone that inhibit reproduction of shrimp in captivity. However, due to physiological imbalance and stress caused by this practice, it has attracted attention as an animal welfare issue. As a result the development of alternatives to the use of unilateral eyestalk ablation in hatcheries has become a priority for producers, retailers and seafood certifiers.

The potential of using shrimp without unilateral eyestalk ablation (non-ablated) in modern egg production practices has been evaluated and the quality of their offspring was assessed from early development stage to edible size under commercial scale. Our current results have demonstrated that non-ablated shrimp female can have similar level of productivity (eggs and nauplii per tank/day) as ablated animals if the broodstock are supplied high quality supplementary moist feed during pre-maturation. Non-ablated animals can produce more eggs and nauplii (>20% and >16%) than conventionally ablated female. The mortality of non-ablated female over a typical breeding period is almost half the level of ablated female. The offspring produced by non-ablated females have similar growth and final survival to those from ablated female from early development stage to edible size. However, salinity stress tests indicated that offspring from non-ablated female have better resilience (5 to 10% more survival). Our findings suggest that it is possible to replace eyestalk ablation in commercial scale shrimp hatcheries and the quality of the offspring can improve in term of resilience to environmental stress.

I would like to thank the Global Aquaculture Alliance, Labeyrie Fine Foods and Seajoy for their financial support. I would like to thank co-authors for their contribution in the project.

PHYSIOLOGICAL EFFECTS OF INSECT-BASED DIETS DURING *Danio rerio* LARVAL DEVELOPMENT

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Converting land-produced organic waste in valuable biomass to be used in the aquatic environment is a good strategy to make aquaculture more sustainable. On this regard, insects represent a very promising example of alternative ingredient to fish meal and fish oil due to their nutritional profile and their bio-converting efficiency; however, their application in aquafeeds still faces possible limitations because of their lack in polyunsaturated fatty acids and the presence of chitin.

In the present study, the concept of circular economy was applied to Black Soldier Fly (BSF) (*Hermetia illucens*) rearing, improving the insect's biomass fatty acid composition by culturing them on a land-produced by-product (coffee silverskin) enriched with a 10% *Schizochytrium* sp. The insect biomass was then used to formulate five fish diets containing 0, 25, 50, 75 and 100% of insect meal with respect to fish meal, respectively. Diets were used for a feeding trial during zebrafish (*Danio rerio*) larval development (21 days) and a multidisciplinary approach including biometry, histology, gas chromatography, spectroscopy (FTIR), microbiota analyses and molecular biology was applied to better understand fish responses to the new diets. Results showed that the 50% substitution of fish meal with insect meal represented the best compromise between ingredient sustainability and proper fish growth and welfare. Fish fed with higher BSF inclusions (75 and 100%) showed a severe degree of hepatic steatosis, microbiota modification, a higher lipid content (FT-IR), fatty acid modification and higher expression of both stress and immune response markers.

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CHALLENGES IN SHRIMP NUTRITION AND SUSTAINABLITY

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In 2015, almost 60% of the global fish stocks were fully fished, since the year 2000 fish capture remained steady, around 90 million MT/Yr., close to the production limits. As result, aquaculture is growing faster than any other food production sector, contributing in 2016 to 47% of the global fish production, but with a significant consumption of non-renewable ingredients: fish meal and fish oil. If we want aquaculture to continue at such growth rates, there is an urgent need to look for and develop alternative and sustainable nutrient sources.

To meet the demand for ingredients, an aquaculture nutritionist needs to shift towards using crop-based sugar sources, a more reliable supply with lower cost than marine protein, such as soybean meal (SBM), corn stovers and other agriculture waste materials. The challenge is not simply to replace ingredients like fishmeal but also to provide disease resistance and health promoting functional nutrients (i.e. nucleotides, small peptides, etc.) required by aqua species. Here, a unique biotechnological process called CelTherm® came in, unlocking and transforming traditional crop-based ingredients, into a more ready assimilable, useful and functional nutrients.

Although plant sources such as SBM represents a sustainable crop and source of nutrients, it has anti-nutritional compounds, i.e. trypsin inhibitors, raffinose, etc. limiting to a conservative inclusion levels in aquatic diets. Through CelTherm® process, the plant material is hydrolyzed, filtered and through oligomerization is rebuilt into a higher nutritional ingredient, with plenty of small peptides, nucleotides, amino acids, readily available for marine species, enhancing the immune response, survival rate and yield in shrimp and finfish, with low fish meal diet profile.

Aquaculture nutritionists globally were challenged to produce a high performing diet, with a sustainable ingredient, fish meal free feed (F3). The table shows the results using MrFeed® Pro50 as replacement for fish meal, with outstanding results on shrimp performance, offering a viable option to the feed industry, and nourishment of mankind.

Ingredient	Fish Control	PBM	MrF	Neg Control	F3
Fish meal, menhaden	27	--	--	--	--
Poultry meal	--	24	13	--	13
MrFeed	--	--	18	--	18
Soy Protein Conc.	--	--	--	25	--
Soybean meal	26.1	26.1	26.1	26.1	26.1
Wheat gluten meal	5	5	5	5	5
Wheat flour [j]	34	35.57	28.34	30.9	26.44
Fish oil [k]	2.1	2.1	2.1	2.1	--
Canola oil	1.3	0.4	--	3	--
DHA algae	--	--	--	--	4
Dicalcium phosphate	0.8	1.93	2.8	3.2	2.8
Lecithin	2	2	2	2	2
Vitamin premix [l]	1	1	1	1	1
Lysine HCL	--	0.65	0.58	0.12	0.58
Methionine	--	0.25	0.38	0.21	0.38
Trace min premix [m]	0.1	0.1	0.1	0.1	0.1
Vitamin C [n]	0.5	0.5	0.5	0.5	0.5
Cholesterol	0.1	0.1	0.1	0.1	0.1
Calculated Analyses					
Crude Protein, %	37.1	37.8	38.1	37.9	38
Lipid, %	9.2	9.2	9.2	9.1	9.3
Phosphorus, %	1	1	1	1	1
Methionine, %	0.77	0.8	0.82	0.78	0.58
Lysine, %	2.36	2.38	2.44	2.38	2.21
Threonine, %	1.46	1.44	1.56	1.54	1.56

IDENTIFICATION OF NORTHERN SNAKEHEAD FISH-DERIVED ANTIMICROBIAL PEPTIDE GENES

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The Northern snakehead (Channa argus) is an important and staple farming fish species in China. In recent years, germplasm degeneration and intensive culture pattern have caused serious disease problems to the Northern snakehead culture industry, which hinders the green and sustainable development of the Northern snakehead farming industry. An effective way to solve the above problems is to improve the disease-resistant traits of Northern snakehead by molecular marker-assisted selection or transgenesis-based breeding techniques. To provide important candidate genes for genetic improvement of disease-resistant traits of Northern snakehead, this project aimed to screen antimicrobial peptide (AMP) genes from Northern snakehead genome, determine their functions and elucidate their action mechanisms. Three AMP-like genes have been selected by blasting the conserved amino acid (aa) sequences of teleost fish-derived five classic families of AMP against the sequence data base of Northern snakehead genome. A Channa argus-derived hepcidin-like gene (caHep) was cloned. The caHep gene contains an open reading frame of 270 bp (encoding 89 amino acids). The 89 aa mature peptide consists of 24 aa, 40 aa and 25 aa for signal peptide, prodomain and mature peptide, respectively. CaHep expression plasmid pCMS-EGFP-caHep and the control plasmid pCMS-EGFP were constructed. Transfection of pCMS-EGFP-caHep plasmid into cultured human embryonic kidney cells (HEK293) resulted in expression of EGFP and caHep. The culture supernatant of caHep-expressing HEK293 cells showed significantly increased resistance activities against *Aeromonas veronii* compared with the control. Furthermore, chemically synthesized caHep mature peptide showed significantly increased resistance activities against *Aeromonas veronii* compared with the control. Scanning electron microscope analysis indicated that treatment with caHep peptide induced significant cell membrane damage on *Aeromonas veronii* in comparison to the control.

THE INTEGRATED CULTURE OF PACIFIC DULSE *Palmaria palmata* AND ROCK WEED *Mastocarpus jardinii* IN A RECIRCULATING LAND-BASED SYSTEM WITH RED ABALONE *Haliotis rufescens*

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Producing sustainable food to meet the demands of an increasing human population will be a significant challenge in the coming decades. Integrated multi-trophic aquaculture (IMTA) is an efficient, viable and environmentally friendly method to meet these needs. Land-based recirculating systems are an excellent way to optimize food production in a small footprint; however, they do come with large associated costs. A limiting factor for an aquaculturist may be access to the constant and reliable electricity required to maintain airflow for macroalgae tumble culture. To address these limitations we compared intermittent aeration (16h on: 8hr off) with continuous aeration (24hr on) on the productivity of two macroalgae species, Pacific Dulse *Palmaria palmata* and Rock Weed *Mastocarpus jardinii* while co-culturing the macroalgae with Red Abalone *Haliotis rufescens*. This experiment was designed to replicate a commercial marine farm producing high-value species that could offset the associated costs of being land-based. The integrated system consisted of twelve 19L buckets, separated into six replicates per aeration (constant and intermittent) in a gravity-fed vertical system held under 32 W fluorescent lamps, with a 16:8-h light/dark photoperiod. Five Red Abalone *Haliotis rufescens* were held in a 190L tank, and fed 0.5 kg of bull kelp fronds each week, after a 7-day starvation period. Overflow from the abalone tank flowed into a manifold that distributed water to each of the twelve 19L buckets containing an initial stock density of 2 kg m^{-2} of Pacific Dulse *Palmaria palmata* and Rock Weed *Mastocarpus jardinii*. Air was injected through 1 mm holes drilled every 2 cm in a 1.3 cm (inside diameter) vinyl tubing that lined the bottom of each bucket in a 5-cm-diameter circle. To compare the nitrogen removal and uptake efficiency, total tissue N and C was determined at the end of the experiment. Following a seven day acclimation period, the biomass of each bucket was removed weekly, spun in a salad spinner for 20 seconds, weighed, and replaced at the initial stocking density of 2 kg m^{-2}. Water quality parameters (nitrates, nitrites, ammonia, phosphates, oxygen saturation, pH and alkalinity) were measured each week for four weeks. This experiment is ongoing, with Rock Weed *Mastocarpus jardinii* having a higher yield as of 09/2019. Producing high-value macroalgae in an integrated intermittent tumble culture with abalone may be one alternative for addressing our current and future global food demands.

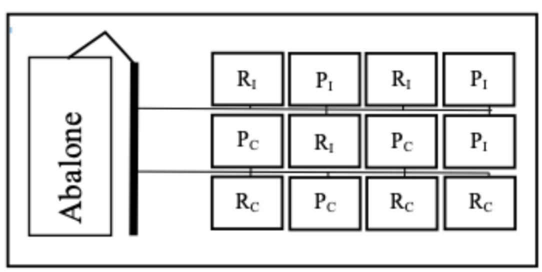

Figure 1: Experimental design: Rock Weed *Mastocarpus jardinii* R_I intermittent aeration R_C constant aeration, Pacific Dulse *Palmaria palmata* P_I intermittent aeration P_C constant aeration.

OPTIMIZING SEAWEED BIOMASS PRODUCTIVITY IN POND CULTIVATION

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For an increasing number of seaweeds species, land base pond systems are becoming a preferred choice for commercial cultivation due to the high production of biomass they can reach. These systems, commonly named “tumble culture”, consist in ponds where seaweeds are in a continuous resuspension in the water column (1m) by air supplied from the bottom of the pond. Nutrients are supplied by periodic fertilization and water renewal. Biomass densities are controlled in order to optimize biomass production. However, maximum production (biomass per unit area per time) is a combination of maximizing biomass per unit area where photosynthesis and growth are not limited. These conditions, however, are dynamic on a daily and seasonal bases. Seaweed growers usually opt for setting fixed seeding biomass densities (usually from 1 to 3 kg m^3) and harvesting period (3-4 weeks) where seaweed biomass reaches or is close to the carrying capacity of the system (7-9 kg ww). The purpose of this study was to determine the optimal production of *Ulva* sp. evaluating its physiological responses and growth during its cultivation in a land-based 40 m^3 ponds. Ponds were seeded with 1kg/m^3 with a previously selected strain of *Ulva* sp. Cultures were monitored for four weeks. Pulse fertilization and full water exchange were practiced twice a week. Photosynthesis, respiration, pigments, and nutrient content were measured. Light, temperature, pH, and dissolved inorganic nitrogen (DIN) were simultaneously monitored. Additionally, laboratory experiments were performed to assess the effects of increasing temperature and pH on the seaweed physiology (photosynthesis, nitrogen uptake). DIN (ammonium, nitrate) uptake kinetics were also determined to evaluate *Ulva* sp capacity to incorporate the fertilizer used during cultivation.

Decrease in photosynthetic capacities due to the low availability of light and inorganic carbon for photosynthesis as biomass increased was observed after the second week. Seaweed specific growth rate was also reduced, resulting in a reduction in biomass productivity. Biomass yield decreased from 300 g/m^3/d in the first two weeks to 170 g/m^3/d for the 3rd and 4th week of cultivation. Short-term (hours) increments in pH and temperature, which can occur on daily-basis, caused severe inhibition of photosynthesis and nitrogen uptake, as obtained from laboratory experiments. Also, uptake kinetics demonstrated that *Ulva* sp. incorporates ammonium more efficiently than nitrate. Generally, our results can be directly applied to optimize culture conditions of *Ulva* sp. Based on these results, biomass production could be optimized by harvesting between the first and second week of cultivation during Spring and early Summer providing a 20% net increase in biomass production in a four-week period. A similar approach can be applied to know the best harvesting frequencies in the Fall and Winter.

EVALUATION OF PROTECTION POTENTIAL OF AN ANTAGONISTIC ISOLATE OF *Pseudomonas mosselii* AGAINST INFECTION OF VIRULENT *Aeromonas hydrophila* IN CHANNEL CATFISH (*Ictalurus punctatus*)

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Motile *Aeromonas* septicemia (MAS), caused by virulent *Aeromonas hydrophila* (vAh), has severely impacted catfish farming in the southeastern United States since 2009. To date, conditions that trigger the onset of MAS outbreaks remain largely unknown and control of MAS has been a challenge issue. According to the observation of fish specialists, fish mortalities occurred in a pond from onset (≤10 fish) to outbreak (>20,000 fish) in just a few days. Consistent with this observation, laboratory experiments found that fish mortality stated at 6-8 h and peaked with 24-48 h post challenge with vAh. The rapid development of the disease makes the use of antibiotic therapy and other intervention strategies problematic.

In this study, we investigated the effect of an isolate of *Pseudomonas mosselii* (Pm) on vAh infection. Pm was originally isolated from the gill of a channel catfish that survived a lethal challenge of vAh. Assays *in vitro* showed that Pm was inhibitory to growth of vAh on LB agar plate (Fig. 1 left panel). The cell lysate of Pm produced inhibitory zone on vAh cell lawn (Fig. 1, right panel). Co-inoculation vAh and Pm in water had no significant effects on vAh growth and infection; however, Pm protected fish against vAh infection when fish were pre-immersed in Pm-propagated water for 15 min prior to challenge (Fig. 2). The relative percentage of survival of fish was approximately 79%, compared with the control. The mechanism of protection could be due to pre-occupation of infection sites by Pm and antagonistic to the establishment of infection by vAh.

Whether the isolate of *P. mosselii* can be used as a probiotic agent for control MAS is under investigation.

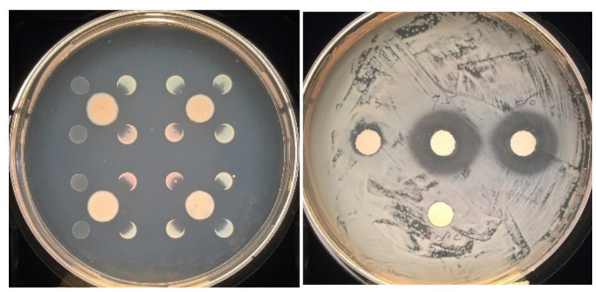

Figure 1. Inhibitory effects of *P. mosselii* on vAh

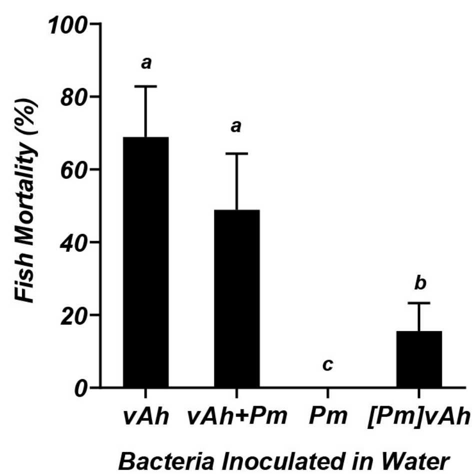

Figure 2. Effect of *P. mosselii* on vAh infection in channel catfish

EFFECT OF A COMMERCIAL PROBIOTIC ON THE CHEMICAL AND FATTY ACID COMPOSITION AND LIPID QUALITY INDICES OF EUROPEAN CATFISH Silurus glanis GROWN IN A RECIRCULATING AQUACULTURE SYSTEM

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This experiment evaluated the effect of a commercial probiotic on the chemical composition and meat nutritional quality indices of European catfish (*Silurus glanis* L.) grown in a recirculating aquaculture system. To conduct the experiment, the European catfish were placed in two separate tanks containing 45 fish each. The growing conditions for the fish in the two tanks were identical. The only difference was that 1 ml of a commercial probiotic was added to the water in one tank every other day. The meat of 6 catfish from each tank was examined.

The difference in the chemical composition of the catfish meat in the control and probiotic groups is statistically significant ($P<0.05$) (Table 1). During this experiment, 25 fatty acids were identified. Statistically significant differences in the saturated fatty acids (SFA), monounsaturated fatty acids

(MUFA) and polyunsaturated fatty acids in the two groups were observed ($P<0.05$). The group raised with probiotics had higher amounts of SFA and MUFA than those in the control group.

The fatty acid n-3/n-6 ratio was closer to the recommended value (<0.25) in the probiotic group. Indices of atherogenicity and thrombogenicity were higher in the probiotic group, while higher flesh-lipid quality and hypocholesterolemic (acid) were found in the control group. There are no specific recommendations concerning the most beneficial AI and TI values; it is only stated that lower levels are more beneficial; however, it is advised that in the human diet these indices should be lower than 1. The AI and TI values measured in this experiment were lower than 1 (Table 2).

This experiment showed that when catfish are reared in recirculating aquaculture systems containing probiotics, the meat is suitable for human consumption as a source of omega-3 and omega-6 fatty acids.

Table 1 Chemical composition (mean ± STD) of European catfish meat grown in RAS with and without probiotics

	Dry matter, %	pH	Moisture, %	Fat %	Ash, %	Protein, %
CG	21.22 ± 0.824^b	6.26 ± 0.086^a	61.51 ± 0.153^a	1.05 ± 0.023^a	0.92 ± 0.020^a	16.14 ± 0.049^a
PG	18.92 ± 0.407^a	6.28 ± 0.085^a	62.88 ± 0.250^b	1.39 ± 0.017^b	1.03 ± 0.015^b	18.60 ± 0.080^b

Means in a column followed by different letter were significantly different at $p < 0.05$.

Table 2 Health lipid indices and nutritional quality of European catfish meat grown in RAS with and without probiotics

Parameter	CG	PG
n-6/n-3	2.23 ± 0.002^a	2.70 ± 0.018^b
n-3/n-6	0.45 ± 0.0004^b	0.37 ± 0.002^a
DHA/EPA	4.22 ± 0.003^b	1.89 ± 0.0007^a
PUFA/SFA	3.31 ± 0.213^b	2.06 ± 0.099^a
EPA+DHA	10.55 ± 0.345^b	7.68 ± 0.180^a
AI	0.05 ± 0.016^a	0.07 ± 0.008^b
TI	0.09 ± 0.005^a	0.11 ± 0.004^b
FLQ	12.17 ± 0.063^b	7.98 ± 0.011^a
h/H	16.4 ± 2.139^b	8.11 ± 0.340^a

Means in the same row with different superscripts are significantly different ($p< 0.05$).

RECIRCULATING AQUACULTURE SALMON NETWORK (RAS-N): BUILDING CAPACITY OF ATLANTIC SALMON PRODUCTION IN THE US

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Fueled by the massive imports of Atlantic salmon to the US, and the challenges of expanding net-pen production of this species, we are now witnessing major investments in US land-based production of Atlantic salmon. To help build capacity for this fast-expanding industry, NOAA/Sea Grant has funded a national public-private consortium, consisting of academia, industry and federal labs in five states across the US - the Recirculating Aquaculture Salmon Network (RAS-N). The mission of RAS-N is 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. The RAS-N aims at working closely with US stakeholders to establish a 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. The main outcome of this collaborative program will be the development of a consensus road map/strategic plan and demonstration projects that will help policymakers, federal agencies and industry identify and responsibly allocate resources to promote an economically and environmentally sustainable land-based US Atlantic salmon industry. This presentation will review the current and future activities of RAS-N and solicit stakeholder engagement and input.

THE INSTITUTE OF MARINE AND ENVIRONMENTAL TECHNOLOGY (IMET) – UNIQUE MARINE AQUACULTURE PROGRAMS AND OPPORTUNITIES FOR COLLABORATIONS

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One of the major research areas at the University of Maryland Institute of Marine and Environmental Technology (IMET) is sustainable marine aquaculture. Specifically, the use of modern platforms of biology and biotechnology for the development of environmentally responsible and economically feasible recirculating marine aquaculture systems. The mission and formal charge of IMET emphasizes research, education and economic development, thus we work closely with the aquaculture industry in the US and globally. IMET's Aquaculture Research Center (ARC) is a unique 2,000 m^2 state-of-the-art, fully contained, recirculating research and development facility that focuses on aquaculture-related studies including reproduction, spawning and hatchery technologies, fish health, nutrition and alternative feeds, and the development and optimization of recirculating marine aquaculture systems. ARC contains multiple tanks of 2-20 m^3 as well as many replicated systems of smaller tanks. A computerized system maintains full control over the photoperiod, water temperature (10-30°C) and salinity (0-36 PPT) in each of the tanks. ARC also includes a hatchery wing with dedicated algae culture, live feeds and larval rearing systems (ranging from 0.5-6 m^3). The broad range of environmental conditions available at ARC, and its total biosecurity, have enabled us to conduct research and development programs with multiple fish species, including striped bass, European seabass, Mediterranean seabream, cobia, amberjack, sablefish, bluefin tuna, Atlantic salmon, coho salmon, and rainbow trout, among others. For many years, we have engaged in public-private partnerships with multiple commercial and non-academic partners in the US and beyond. Notably, IMET recently received a NOAA/Sea Grant award to lead a national public-private-government consortium titled: "Building capacity of land-based Atlantic salmon aquaculture in the US". This consortium, referred to as the Recirculating Aquaculture Salmon Network (RAS-N), integrates several academic and research institutions and major industrial partners to advance research, education, training, outreach, workforce development and the economic feasibility of this emerging industry in the US. IMET and ARC are cutting-edge operations within the University System of Maryland that are available for collaborations aimed at empowering the marine aquaculture industry in the United States.

CONVERSION OF SOLID WASTE TO FUEL-GRADE BIOGAS FROM A LAND-BASED AQUACULTURE SYSTEM FOR ATLANTIC SALMON

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As land-based production of Atlantic salmon rapidly expands, efficient life-support systems must be developed and optimized to increase water recirculation and prevent environmental pollution. Specifically, land-based salmon operations will generate 20-30 tons of sludge for every 1000 tons of fish produced. Treating these amounts of sludge in a way that is environmentally responsible is of paramount importance. Our approach has been to use anaerobic digestion bioreactors, in which methanogens convert the solid waste into fuel-grade methane. We have successfully developed this approach to convert over 90% of solid waste produced by warm-water marine fish (seabream, seabass) into combustible biogas that contains 65-70% methane. Building upon our experience, we are now studying and implementing the technology in cold water, both fresh and saline, focusing on Atlantic salmon. Sludge obtained from a large commercial Atlantic salmon smolt operation in Norway was shipped to our lab and was used to develop and enrich a methanogenic consortium that has been custom-tailored to this sludge. Experimenting with different temperatures, dry matter concentrations and C/N ratios, conditions for optimal methane production were determined. The consortium was then scaled up in a 250 liter fermenter and shipped to Norway where it was use to inoculate a 100 m^3 biogas bioreactor, with excellent results. The biogas generated is currently used on-site to operate methane-driven boilers to heat farm water, thus offsetting some of the energy cost of the operation.

Currently, we are developing and testing efficiencies of sludge-to-biogas conversion for post-smolt Atlantic salmon produced at 20 PPT salinity and 13^{o}C. Fish were stocked in two 6m^3 tanks equipped with life-support systems allowing water recirculation. Sludge collected post-drum filter was used as the initial inoculum source to develop, enrich and optimize the methanogenic consortium converting salmon waste to biogas. The optimized consortium was scaled-up in a 20 liter fermenter and inoculated into the 1000 liter anaerobic digester. As fish grew toward market size at densities of up to 65 kg/m^3, we collected increasingly larger quantities of biogas, up to 380 liters/day, with 70% methane content, which is directly combustible (right; photo of burning biogas collected from the anaerobic digester).

These studies demonstrated that Atlantic salmon solid waste produced in RAS, whether fresh-water or saline, can be efficiently converted to fuel-grade biogas to be used on-site to reduce energy costs. Complete operational parameters and results will be discussed.

ADDENDUM

USDA NIFA'S ROLE IN ENHANCING AQUACULTURE RESEARCH, EDUCATION AND EXTENSION

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National Institute of Food and Agriculture (NIFA), an extramural funding agency within the United States Department of Agriculture (USDA) invests in and advances agricultural research, education and extension. Aquaculture research and extension has been one of the areas NIFA has supported over the years. Annually, NIFA invests millions of dollars to advance aquaculture research and outreach. Recently, the agency has been 'reimagining' its values and 'customer service' as it transitions its headquarters. Similar to some of the other programs, NIFA's Aquaculture program in its current form is being evaluated for its effectiveness, efficiency, and service delivery to the aquaculture industry.

The presentation will highlight some of the changes taking place in NIFA and its implications to aquaculture programming, personnel and grant management. It will also provide a number of aquaculture research and extension funding opportunities beyond the traditional outlets. This session will also seek input from its stakeholders (researchers and industry) so as to utilize this knowledge in reimagining delivery of its mission effectively and efficiently.

ROBUST AND INEXPENSIVE TOOL DEVELOPMENT FOR MONITORING FLOW REGIME WITHIN SHELLFISH AQUACULTURE GEAR

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Despite such promising technological and economic advancements in shellfish aquaculture over the past several decades, limitations to production still exist (e.g. gear restrictions, leasing and permitting hurdles, operation costs of farms). To promote future growth in shellfish production, more research is needed to improve the gear efficiency of cultured shellfish. Among suspension feeding bivalves, optimizing flow to maximize food delivery and export waste is believed to be an important governor of shellfish growth. However, little attention has been given to these relationships relative to other aspects of aquaculture research. A major impediment for optimizing flow within gear has been the lack of tools to monitor the physical environment within different gear types. Additionally, tools must be both robust and affordable to provide research-based guidance to shellfish producers and researchers to increase production.

The goal of this research was to develop a series of inexpensive and robust tools that can accurately monitor the physical environment within shellfish aquaculture cages to understand relationships between ambient and internal physical forces generated by grow-out cages, and shellfish production. We adapted several tools with a proven track record for use in shellfish cages; specifically, clod cards (flow), ammonium peepers (residence time and waste accumulation), and accelerometers (jostling). The tools were modified for use in aquaculture and validated through a series of field trial experiments that intentionally altered the internal physical environment within oyster grow-out cages (e.g. fouling management, cage stocking density and mesh size), to assess the sensitivity of the tools and validate their use for optimizing shellfish production.

The development of reliable tools that relate shellfish growth rates to the physical environment within cages represents a major step forward towards gear optimization. Furthermore, each tool was intentionally designed to be inexpensive and user-friendly to promote use by famers and collaboration with other researchers wishing to help optimize gear at the farm scale.

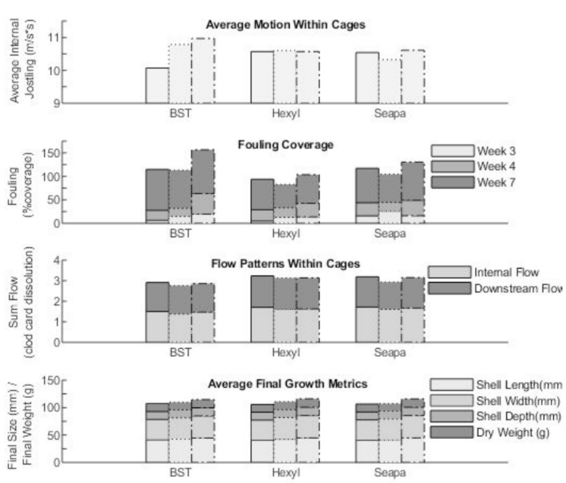

FIGURE 1: Physical characteristics of different commercial oyster cages (BST: triangular 12mm mesh, Hexyl: hexagonal 15mm mesh, SEAPA: circular 12mm mesh) affected by different weekly fouling treatments (Power Washing (-), 24-hr desiccation (. .), control (-.-.)).

ESTABLISHING A BETTER COMBINATION OF ALGAL DIETS TO IMPROVE THE SURVIVAL RATE OF MEGALOPAE IN THE LARVAL REARING OF MANGROVE CRAB *Scylla serrata* (Forsskal, 1755).

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A series of larval rearing trials using 100L capacity tanks were conducted to determine the effect of using different combinations of background algae on the survival rate of the mangrove crab megalopae. Six algal products that included frozen algae pastes (Reed Mariculture) such as the *Nannochloropsis* (Nanno 3600), *Tetraselmis* (Tetraselmis 3600), *Isochrysis* (Isochrysis 1800), *Thalassiosira* (TW 1200), were used in addition to *Spirulina* powder and live algae *Nannochloropsis* and *Chaetocerus muelleri* (CM). Treatment combinations included: treatment A (Control): Without algae; treatment B: Nanno, Tetra,TW and Spirulina powder at ratio of 8:2:2:1 grams per ton; treatment C: Nanno, Tetra, TW, Spirulina + live CM ; treatment D; live *Nannochloropsi*s alone; treatment E: live *Nannochloropsis* + live CM; treatment F: Nanno, Tetra, TW and Spirulina powder at ratio of 8:2:2:1 grams per ton (without Oxytetracycline). There were 6 replicates of each treatment. The experimental tanks were setup inside 4m x 8m rectangular concrete tanks filled with 0.5m deep water which served as water bath equipped with a heater with thermocontroller and submersible pump to allow water to recirculate. The water temperature in the water bath was set at 29°C and data loggers were placed inside and outside of the experimental tanks to monitor the temperature fluctuation throughout the experiment. Newly hatched zoeae were stocked at the density of 80 per litre. Rotifers were added immediately after stocking at the density of 5 per ml. Newly hatched Artemia were added on day 3 at the density of 0.2 per ml and gradually increased to 5 Artemia per ml by day 10. Survival rate of the larvae was determined by counting the total number of larvae at 5, 10, and 15 days after hatching and at the end of culture period, when the zoeae had completely molted to the megalopa stage. Samples of the larvae and the live feed organisms (rotifer and Artemia) were collected, flash frozen with liquid nitrogen and stored in the -80 C freezer for future determination of lipid profile. Results for each treatment were compared statistically using ANOVA. Initial results revealed that at day 15, the larvae in tanks fed with live and frozen algae mix with the addition of live diatom *Chaetoceros mulleri* have a higher survival rate than those reared without background algae. Some of the larvae in these tanks had already started molting into megalopae. The larval cultures reared without using oxytetracycline, started to collapse at day 10. At day 17, some of the larvae in tanks fed with live algae (*Nannochloropsis* and *Chaetoceros*) were still at the late zoeae stage, while many dead zoeae and megalopae were observed. This could be an indication that the larvae in these tanks have been affected by Moulting Death Syndrome. Most of the larvae in the tanks fed with frozen algae mix had already moulted to the megalopa stage. Even though neither dead zoeae nor megalopae were observed during sampling, the survival rate still dropped to less than 40%. When the culture period in all treatments was extended to 19 days the survival rate continued to drop significantly. This suggests that the larval mortality was caused mainly by the megalopae's cannibalistic behaviour. The highest production of megalopae occured at day 17. Given the same environmental and dietary conditions, it is recommended that the timing for the termination in larval rearing of mangrove crabs is at day 17.

MITOCHONDRIAL DNA METHYLATION IN NILE TILAPIA *Oreochromis niloticus*

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As the energy powerhouses of the cell, mitochondria play a fundamental role in muscle growth, which is one of most important traits in aquaculture. There is increasing evidence that epigenetic mechanisms, and DNA methylation in particular, can regulate growth. Nevertheless, methylation changes in mitochondrial DNA (mtDNA) and their potential impact on growth are currently not known in fish. Hence, the aim of this study was to determine the mitochondrial methylome in Nile tilapia (*Oreochromis niloticus*).

We isolated and sequenced mtDNA from the liver of 5 full-sib Nile tilapia females. Similarly to other cichlids, the Nile tilapia mitogenome is 16,625 bp long and contains 13 protein-coding genes involved in oxidative phosphorylation, two rRNA genes, 22 tRNA genes and the control region D-loop. We prepared libraries for whole genome bisulfite sequencing on the NextSeq platform to determine the methylation rate within CHH, CHG and CpG contexts in mitochondrial genes. Our results revealed that methylation is present in the mitochondrial genome of Nile tilapia. Unlike the nuclear genome, cytosine methylation in mtDNA occurs predominantly within a non-CpG context. Also, methylation of mtDNA was found to be strand-specific, since 68% of methylated cytosines were located on the light (minus) strand, and some tRNA genes had high methylation levels.

This is the first methylation map of the mitochondrial genome in fish. In addition to providing novel insights into mitochondrial epigenetics, our results constitute an epigenetic toolbox that may be used to modulate growth in Nile tilapia.

Acknowledgements: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreements nos 683210 and 812986) and from the Research Council of Norway under the Toppforsk programme (grant agreement no 250548/F20).

MOLECULAR CHARATERIZATION OF OMEGA AND KAPPA CLASS GLUTATHIONE S-TRANSFERASE FROM REDLIP MULLET (*Liza haematocheilus*) AND ENZYME KINETICS, OPTIMUM CONDITIONS

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Introduction

The redlip mullet (*Liza haematocheilus*) is a saltwater fish which holds paramount importance as food fish worldwide as well as in Korea. Present study, we characterized the GST kappa (LzGSTϰ) and omega (LhGSTω) from redlip mullet. Functional studies were carried out with the recombinant protein to determine its enzymatic and antioxidant properties. In addition, the transcriptional levels of *LzGSTϰ*, *LzGST*ω were determined under normal physiological conditions and immunologically challenged conditions.

Materials and methods

The transcriptome database of mullet cDNA sequences was developed using de novo assembly. Mullet fish (~100g, length 24 cm) purchased from Sangdeok fish farm in Hadong, Korea. The fish were acclimatized in the laboratory aquarium tanks at 20 °C for a week prior to the experiment. Total RNA was extracted by using RNAiso plus (TaKaRa, Japan) and cleaned with RNeasy spin column (Qiagen, Germany). Quantitative real-time PCR (qPCR), with specifically designed primers were performed to analyze the expression profile of *LzGSTϰ, LzGSTω*. The coding sequence (CDS) of the cDNA fragment was amplified by using gene-specific cloning primers. The size of the amplicon were 687 bp (LzGSTϰ) and 720 bp (LzGSTω) respectively. Digested PCR products were ligated into the pMAL-c5X vector. The ligated product was then transformed into *Escherichia coli* (*E.coli*) DH5α and the coding sequence was confirmed by sequencing. To express the recombinant LzGSTϰ, LzGSTω protein (rLzGSTϰ, rLzGSTω), the pMal-c5X/ LzGSTϰ, LzGSTω construct was transformed into *E. coli* BL21 and incubated at 37 °C in LB broth medium containing 100 μg/mL ampicillin, until the OD600 reach 0.6. Isopropyl-β-galactoside (IPTG) was then added to the culture (final concentration 0.5 mM) and incubated for 24 h at 15 °C to induce the expression of the recombinant protein. After incubation, the cells were harvested by centrifugation. The rLzGSTϰ, rLzGSTω protein was purified from the supernatant using maltose affinity chromatography. Enzyme activity was measured separately using CDNB as substrates. The absorbance of the reaction was measured, immediately and 5 min after addition of the substrate.

Result

To understand the potential endogenous functions of LzGSTϰ, LzGSTω, its relative expression was examined in different tissues. Analysis of the expression profiles from the redlip mullet revealed that *LzGSTϰ* was strongly expressed in the heart, while the lowest expression was observed in the head kidney (Fig.1.A). *LzGSTω* was strongly expressed in intestine, whereas the lowest expression was observed in the head kidney. (Fig.2.A) Activities of rLzGSTϰ, rLzGSTω and MBP against different substrates, including CDNB(2,4-Dinitrochlorobenzene), DCNB(2,4-Dinitrochlorobenzene), 4-NPB(4-nitrophenethyl bromide), 4-NBC(4-nitrobenzyl chloride), and ECA(ethacrynic acid) were then measured. Only detectable activity was observed, when CDNB used as the substrate. No significant activity was detected for MBP against any of the substrates and therefore it was treated as a control. rLzGSTϰ was shown to have GSH:CDNB conjugating activity at pH range 6 to 8 (Fig.1.B). Its highest activity was observed at pH 7. For RLzGSTω, GSH:CDNB conjugating activity observed at pH range 6 to 9 (Fig.2.B), wherein the highest activities were observed on pH 8.

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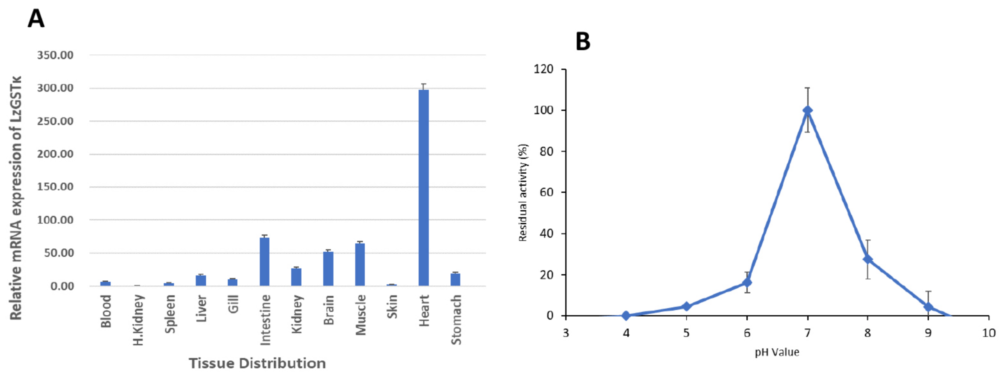

Fig. 1. (A)Relative mRNA expression level of GSTκ in different tissues. The tissues were collected from healthy mullet fish, and expression levels in each tissue were analysed using real-time qPCR. (B)Effect of pH on the GSH conjugating activity of LzGSTκ.

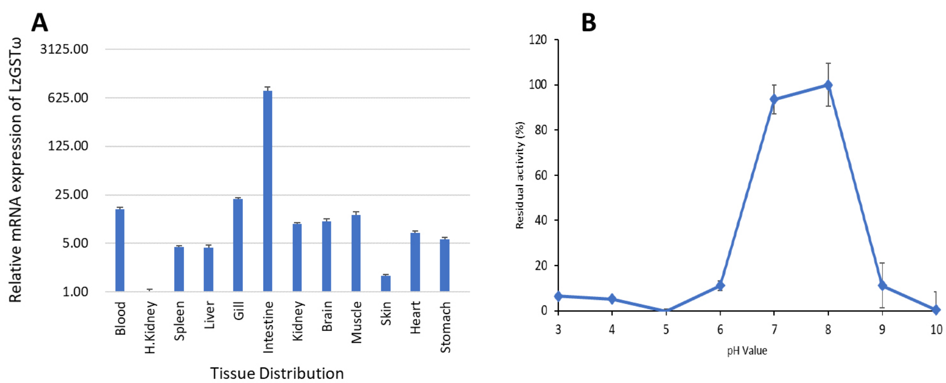

Fig. 2. **(A)**Relative mRNA expression level of GSTω in different tissues. The tissues were collected from healthy mullet fish, and expression levels in each tissue were analysed using real-time qPCR. (B)Effect of pH on the GSH conjugating activity of LzGSTω.

Reference

R. Johansen, J.R. Needham, D.J. Colquhoun, T.T. Poppe, A.J. Smith Guidelines for health and welfare monitoring of fish used in research Lab. Anim., 40 (2006), pp. 323-340

J.D.H.E. Jayasinghe, S.D.N.K. Bathige, B.H. Nam, J.K. Noh, J. Lee Comprehensive characterization of three glutathione S-transferase family proteins from black rockfish (Sebastes schlegelii) Comp. Biochem. Physiol. C Toxicol. Pharmacol., 189 (2016), pp. 31-43Comp. Biochem. Physiol. C Toxicol. Pharmacol., 189 (2016), pp. 31-43

R.H. Michel, P.E. McGovern The first J. Biol. Chem., 249 (8) (1974), pp. 7130-7139

MINERALIZATION OF FISH WASTE IN DECOUPLED AQUAPONICS – THE FIRST STEPS OF A COMBINED AEROBIC & ANOXIC PROCESS PROTOTYPE

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INTRO: In commercial aquaponics, waste mineralization is a key in eliminating aquaculture discharges while increasing horticultural yields. Mineralization is a process that allows organic matter digestion by microorganism activity. A two step process is involved; solid crushing then; organic to inorganic matter conversion. In 2017, preliminary tests (20 buckets during 2 months) focused on dissolved oxygen effects in these two processes. This revealed that solid crushing was more effective in anoxia conditions and the conversion to inorganic compounds was higher in aerobic conditions. Thus, a mineralization system prototype integrating a dual treatment phase was developped (Figure 1.).

METHOD: Ten consecutive tests were conducted with the same input of fish waste but without any system sterilization between each experiment in order to promote organisms colonisation. The first test lasted 24h, the second 48h and so on until the last test, which lasted 10 days, for a total of 65 days (24h between each test). Thus, tests are not properly comparable, but still indicate an noteworthy trend. Each input was 50L of fish sludge collected from the bottom drain of circular tanks containing 120±11 kg of rainbow trout *O. mykiss* fed daily with 1.2 kg of Skretting LP pellets. After each experiment, anoxic and aerobic tanks were cleaned manualy to collect the residual solids which were then dried in an oven. Electrical conductivity (EC) and Total Dissolved Solids (TDS) were analysed in the supernatant water that goes to plant culture.

RESULTS: These experiments indicate that the mineralization process seems to reach a threshold on EC and TDS of the supernatant water after 4 and 6 days of treatment respectively (Figure 2). The mass of dried solids collected from the aerobic tank seems constant while it tends to decrease in the anoxic tank (Figure 3). Thus, results suggest a roughly 70% in efficiency for the conversion of organic solids into inorganic ions after 10 days in this two-step mineralization system prototype.

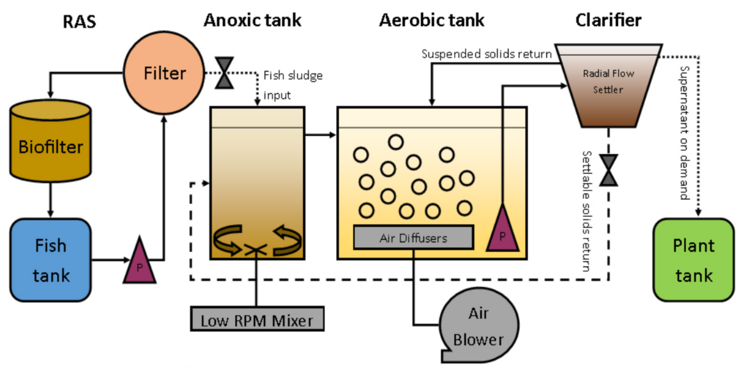

Figure 1 : Schematic view of the prototype mineralization system

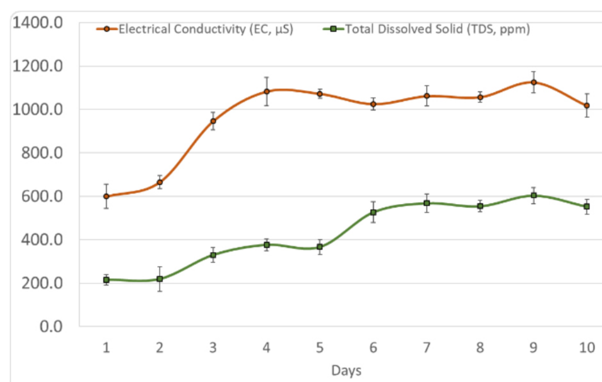

Figure 2 : EC and TDS analyzed from supernatant water

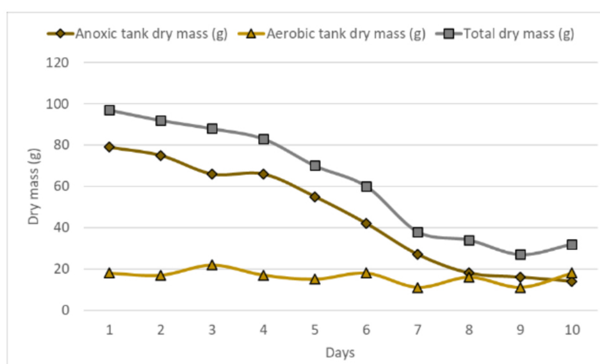

Figure 3: Dry mass collected during the ten experiments

IMPACT OF DIET AND ENVIRONMENTAL MANIPULATION ON REPRODUCTIVE PERFORMANCE IN ALMACO JACK *Seriola rivoliana* BROODSTOCK

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To develop improved broodstock management strategies for commercial-scale production of almaco jack *Seriola rivoliana*, two sets of experiments were conducted. Studies aimed to address the following: 1) variability in egg viability and the connection to brood nutrition; 2) need for environmental manipulation protocols to optimize spawn performance and gamete quality. A total of 23 mature adult *S. rivoliana* were collected in the western Gulf of Mexico (GoM) approximately 144 km offshore (90 miles) in 60 meters (195 ft) of water. Fish (2.0-4.0 kg in body weight) were transported to Mote Aquaculture Research Park in Sarasota, Florida. Two broodstock populations were established and maintained in separate photo-thermally controlled, closed, indoor recirculating tank systems ($28m^3$).

In the first experiment, a series of broodstock diet-shifts were performed. Differences in fatty acid profiles were assessed to determine which fatty acids in eggs can be predictably altered and which affect egg and larval quality. Each diet provided approximately the same amount of total fatty acids (144 mg g^{-1} dw), but differing levels of individual fatty acids. All experimental diets (fed at 3% tank biomass) included 30% squid and either 70% Atlantic mackerel (*Scomber scombrus*); thread herring (*Opisthonema oglinum*); or Spanish sardines (*Sardinella aurita*). Egg size, proximate composition, and fatty acid composition were measured for 23 spawns. Spawn performance metrics assessed for each spawn included total egg production, fertilization rate, hatch rate, and 3-day survival. Eggs from adults fed Atlantic mackerel had significantly lower levels of eicosapentaenoic acid (20:5, n-3; EPA), docosahexaenoic acid (22:6, n-3; DHA), arachidonic acid (ARA, 20:4n-6), and total fatty acids, than those fed Spanish sardine or threadfin herring. Mean three-day percent survival was significantly higher ($p<0.001$) in larvae hatched from broodstock fed thread herring (33.2 ± 1.26) and Spanish sardines (33.2 ± 1.38) when compared with those fed Atlantic mackerel (19.4 ± 1.16). In the second study, a total of 78 spawns were collected from broodstock populations subjected to a variety of photo-thermal regimes. The photoperiod (light/dark; L/D) ranged from 12 L/12 D to 9 L/15 D and temperature from 14-26°C. The total number of eggs collected from each spawn ranged from 30,000 – 1,012,000 eggs. Fertilization was recorded between 21 and 92% with hatch rates ranging from 62 – 89%. In summary, captive almaco jack sourced from the GoM are able to spawn at a wide range of photo-thermal conditions, although spawn quality is highly variable.

BIVALVE HARVESTING, TRANSPORT AND PACKAGING: HOW TO IMPROVE THE QUALITY OF PRODUCT IN CONSUMERS TABLE

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Marine bivalves consumption and production has substantially increased in recent years. However, harvesting is still carried out using artisanal techniques. Usually, bivalves are harvested to net bags and remain outside water, exposed to air and temperature fluctuations, for a few hours. Temperature fluctuations can induce thermal and oxidative stress and, ultimately, lipid peroxidation (LPO) and cellular damage. These alterations can influence the energy budget and induce mortality during the depuration and commercialization processes, reducing product quality and shelf life. Moreover, LPO is usually associated with quality losses and organoleptic alterations, which may depreciate the product.

Therefore, we aimed to evaluate the effect of different methodologies of bivalve harvesting, assessing survival, oxidative stress and damage, during depuration and shelf life of cockles (*Cerastoderma edule*): i) harvested with conventional methods –temperature variations (16 – 22 ºC); and ii) bivalve harvesting to refrigerated containers (6 ± 1 ºC) and transport (approximately 3 h) under controlled temperature conditions to depuration center. After 24 h depuration, bivalves of the two groups were packaged and stored for 6 days at 5 ± 1 ºC, to simulate the conditions during commercialization period. Samples of edible tissue were immediately frozen in liquid nitrogen and kept at -80 ºC until LPO analyses by spectrophotometry. Comet assay was performed to evaluate the DNA damage.

Cockles transported in a cooler environment presented lower levels of DNA damage (Fig. 1). Transport post harvesting in cooler conditions seemed to increase the quality of cockles until 6 days of shelf life, as lipid peroxidation (LPO) of *C. edule* was lower in these condition.

Harvested and transport of *C. edule* in a cooled environment seems to be an effective measure to minimize stress, related with handling procedures, and assure the better quality of the final product.

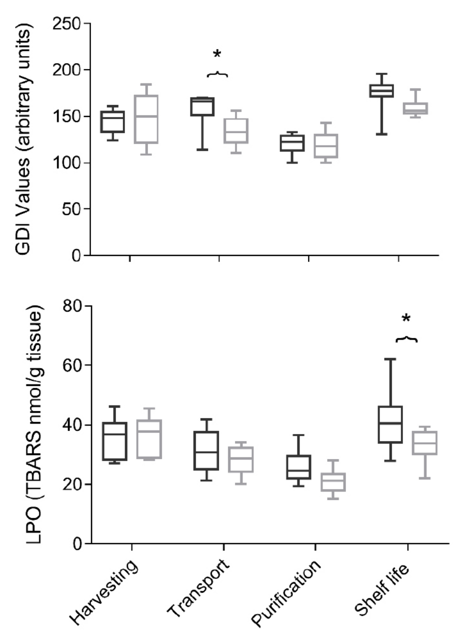

FIGURE 1. DNA damage (GDI values) and lipid peroxidation (LPO) of *Cerastoderma edule* harvested and transport in normal conditions (black) and in isothermal boxes with cool packs (grey), from harvesting to 6 days of shelf life period. *statistical significant differences between groups.

TROPICAL CORALS NUTRITION: ARE MICROENCAPSULATED DIETS SUITABLE TO IMPROVE GROWTH PERFORMANCE?

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The increasing demand of tropical corals led to the need of a sustainable supply solution without compromising natural stocks, already affected by climate change and anthropogenic activities. *Ex situ* coral aquaculture can be a suitable option to fulfill coral needs. However, it is important to consider species requirements to optimize production.

Four coral species with market value were selected (Octocorals – *Sinularia brassica*, *Sarcophyton glaucum*; and hexacorals – *Zoanthus* sp. and *Palythoa* sp.). Four feeding regimes were tested: (*i*) non feed – NF; feed with microencapsulated experimental diets (2 times per week, 580 μg L^{-1}) formulated by SPAROS, based on (*ii*) squid and fish meal – SFM, (*iii*) artemia – ART, and (*iv*) microalgae – ALG. The experiment was performed in 500 L RAS with artificial illumination, during 5 months. Before and after this period, corals were sampled to determine: survival; growth (by photogrammetry of octocorals and polyp count of hexacorals); *in vivo* chlorophyll fluorescence – maximum quantum yield of photosystem II (F_v/F_m); and biochemical biomarkers of cellular energy allocation.

Survival was 100% in all treatments. *S. brassica* growth was significantly improved with ALG, while *S. glaucum* showed better growth performance with ART (Fig. 1). Hexacorals exhibited a general growth increase when supplied with SFM. ART and ALG diets induced higher metabolism in octocorals. F_v/F_m was within healthy range for studied species in all treatments.

This study highlights that species-specific requirements must be considered for achieving a sustainable and economically viable coral aquaculture. Yet, as corals are slow-growing species, we hypothesize that differences between tested feeds may be further emphasize if experiments take place over longer periods (e.g. more than 1 year).

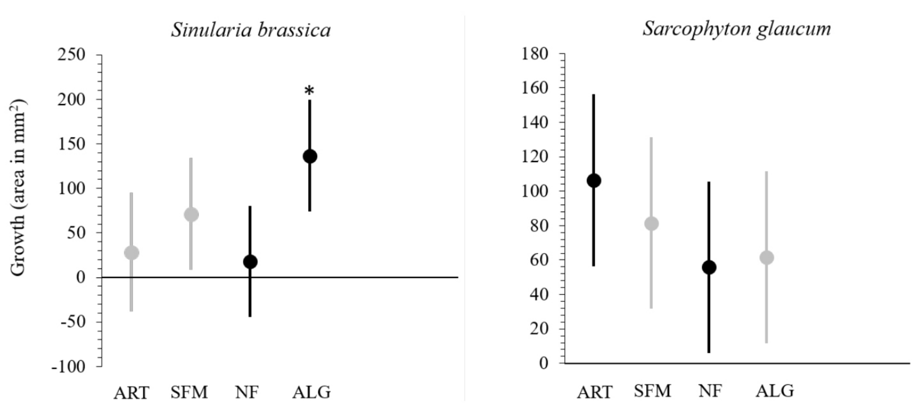

FIGURE 1 – *S. brassica* and *S. glaucum* growth in tested feeding regimes: ART – artemia microencapsulated based diet (mbd), SFM – squid and fishmeal mbd, NF – non feed, and ALG – microalgae mbd. * statistically significant differences.

TRANSMISSION ELECTRON MICROSCOPIC ANALYSIS OF *Vibrio spp.* CELLS FROM HEPAROPANCREAS OF WHITE SHRIMP *Penaeus vannamei*

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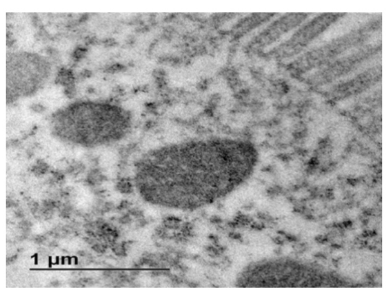
Shrimp farming has mantained an exponential growth during the last years which also has caused the spread of different viral and bacterial diseases. Vibrio is the most common genus associated with this situation. Acute Hepatopancreatic Necrosis Disease (AHPND) is caused by strains of *Vibrio parahaemolyticus* (Vp), during infection the bacteria colonize the stomach of the organisms and release a toxin that damages the functionality of the hepatopancreas and causes mortality. In 2013, this disease affected negatively shrimp production in Northwestern Mexico. The aim of this study was to analyze the morphology of Vibrio genus bacteria in white shrimp hepatopancreas *Penaeus vannamei* using the Transmission Electron Microscopy technique in samples from farms in the State of Sonora, Mexico.

Four samples of hepatopancreas bacteriology were performed on TCBS plates (MCD LAB) in order to obtain green and yellow colonies. Green colonies (presumptive to *V. parahaemolyticus*) were isolated and enriched in TSB (MCD LAB) (2% NaCl) to obtain DNA by lysis buffer and perform Real Time PCR using the IQ Real AHPND / EMS kit. For the analysis of Transmission Electron Microscopy, the protocol of Segovia and Zavala was used; where the hepatopancreas were fixed in 2% glutaraldehyde (GTA) for 24 h, after that GTA was discarded and 500 μl of sodium cacodylate (two washes) was added, then it was passed to the dehydration process where different washes were made with 70, 90 and 100% alcohol. A mixture of acrylic resin and absolute alcohol (1: 1) was added and maintained for 18 h, then a sample fragment was taken and placed in a 0.2 tube ml where acrylic resin was added, then the tube was placed in a UV lamp for 6 h and passed to the oven at 60 °C for 3 h. After this time the cuts were made in the microtome and the cut was placed on the grids, finally the sample was observed in the Transmission Electron Microscope (MET).

As results the hepatopancreas isolates presented large and green colonies with creamy consistency in TCBS, however, they also observed the presence of yellow colonies. In the Real-Time PCR analysis they were confirmed as positive for AHPND, in the hepatopancreas sections bacteria with a cell size from 0.39 to .90 μm were observed, thus showing very similar characteristics to Vp which have a cell size of between 0.40- 3 μm, however they do not have “waves” on their wall, characteristic of a Vp.



Ilustration 1. Vibrio bacteria visualized in Transmission Electron Microscope

EVALUATION OF CALIBRIN Z® SUPPLEMENTED ON DIETS FOR WHITE SHRIMP (*Penaeus vannamei*) THROUGH A CHALLENGE BIOASSAY WITH PATHOGENIC *Vibrio parahaemolyticus* (AHPND)

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Shrimp aquaculture is a profitable and important activity in the countries where it is practiced. Diseases remains as the principal limitation of the activity, generating economic losses. Since the first outbreaks in 2010, specific strains of *Vibrio parahaemolyticus* were identified as responsible for causing Acute Hepatopancreatic Necrosis Disease (AHPND) in shrimp, due to the production of the pathogenic binary toxins PirA/B. Calibrin-Z is an activated combination of montmorillonite and lepispheres used as a feed additive that binds a broad spectrum of toxins including pathogenic toxins from *V. parahaemolyticus*.

A bioassay was performed to evaluate the survival rate of juvenile white srhimp feeded with Calibrin-Z and challenged with *V. parahaemolyticus*. The trial was carried out in two phases, for the feeding phase 160 specific pathogen free (SPF) *Penaeus vannamei* (ABW 1.3 g) were divided and stocked in four fiber glass tanks (two treatments and two control groups). Each tank contained 40 shrimps, 150 L of marine water, aeration and protection mesh. Commercial feed with Calibrin-Z at two inclusion rates (0.25% and 0.5%) and feed without additive for control groups were used. During 15 days, each tank was feeded twice at day at 10% of the biomass using feeding trays. Before the challenge phase, each shrimp was individually analyzed by real time PCR and all samples were negative for WSSV, IHHNV and AHPND. Treatments were randomly assigned and evaluated in three replicate aquaria, each holding 10 shrimp (ABW 1.88 g). For the immersion challenge a bacterial broth (TSB, 2.5 NaCl %) with *V. parahaemolyticus* was added directly into the treatments and positive control groups ($7.6X10^{6}$ CFU/mL), sterile TSB was added to negative control. The trial continued for 96 hours until the mortality stopped. The survival rate for the groups resulted in T1 (0.25%) 33.3%, T2 (0.5%) 63.3%, C+ 30.1%, and C- 100%, respectively. A better performance was shown at 0.5% inclusion rate compared to the other treatments ($P<0.05$). Histopathological analysis revealed characteristic damage in hepatopancreas produced by AHPND in challenged groups, but not in negative control. Bacterial isolates obtained from hepatopancreas of moribund shrimps were positive for AHPND with real time PCR analysis. This trial demonstrate that Calibrin-Z can reduce significantly the effect and mortality of AHPND in white shrimp.

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HACCP FOR SILURIFORMES UNDER THE US FDA AND THE USDA-FSIS SYSTEMS- A COMPARISON OF HOW TO ADDRESS HAZARDS AND ENVIRONMENTAL SAMPLING FINDINGS UNDER BOTH SYSTEMS

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The USDA Food Safety Inspection Service now oversees the safety and other aspects of Siluriformes. These, as with other species of fish/seafood, were under the US FDA. This is an attempt to show potential differences between general HACCP programs under the USDA-FSIS and the US FDA. The current model for Siluriformes follows 9CFR (HACCP Regulation for Siluriformes), assuming the installation has adequate prerequisite programs in place (at minimum SSOPs to meet SCPs and Good Manufacturing Practices-cGMPs, 21CFR117B). The earlier model followed 21CFR123 (FDAs Seafood HACCP). Moreover, the model uses both the FDA's Fish and Fishery Products Hazards and Controls Guidance – 4th edition (http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM251970.pdf) and the Hazard Analysis and Critical Control Point. Training Curriculum. 5th Edition (http://seafood.oregonstate.edu/.pdf%20Links/HACCP-Training-Curriculum-5th-Ed-2011.pdf) as well as the FSIS Compliance Guideline for Establishments that Slaughter or Further Process Siluriformes Fish and Fish Products, as well as other references. Since both HACCP plans identify similar hazards associated with the product/processes, the main differences are in the ways in which the hazards are analyzed and justified. Regardless of plan, both plans identify similar potential hazards but differ in which are elevated or could be elevated to significant hazards leading to a difference in critical control points.

Environmental sampling of food contact (FC) and nonfood contact (NFC) sites in channel catfish operations under FDA and just after FSIS HACCP implementation show similar findings in terms of pre-op and post-production samples (see Table). Selected sites seemed to be persistent for *Listeria sp.* and may need additional attention in a sanitation SOP.

Time	Site	Lis sp / L.mono %	APC	*E. coli*	TCC
				Log CFU/cm^2	
Pre-FSIS	FC4	100/0*	4.0	0.0	2.7
	FC11	33/33	3.5	<1	1.4
	FC13	33/0	3.1	0.3	0.3
	FC14	33/33	1.9	<1	0.4
	NFC1	67/67	1.8	<1	<1
	NFC2	33/33	2.2	<1	0.8
	NFC4	33/0	3.5	<1	1.1
	NFC5	67/33	3.6	<1	2.0
Post-FSIS	FC2	33/33	3.6	0.6	1.4
	NFC1	33/33	1.4	<1	<1
	NFC4	67/67	4.2	0.4	<1
	NFC5	67/67	4.6	<1	0.3